Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcome in Patients With COVID-19; A Systematic Review and Meta-analysis

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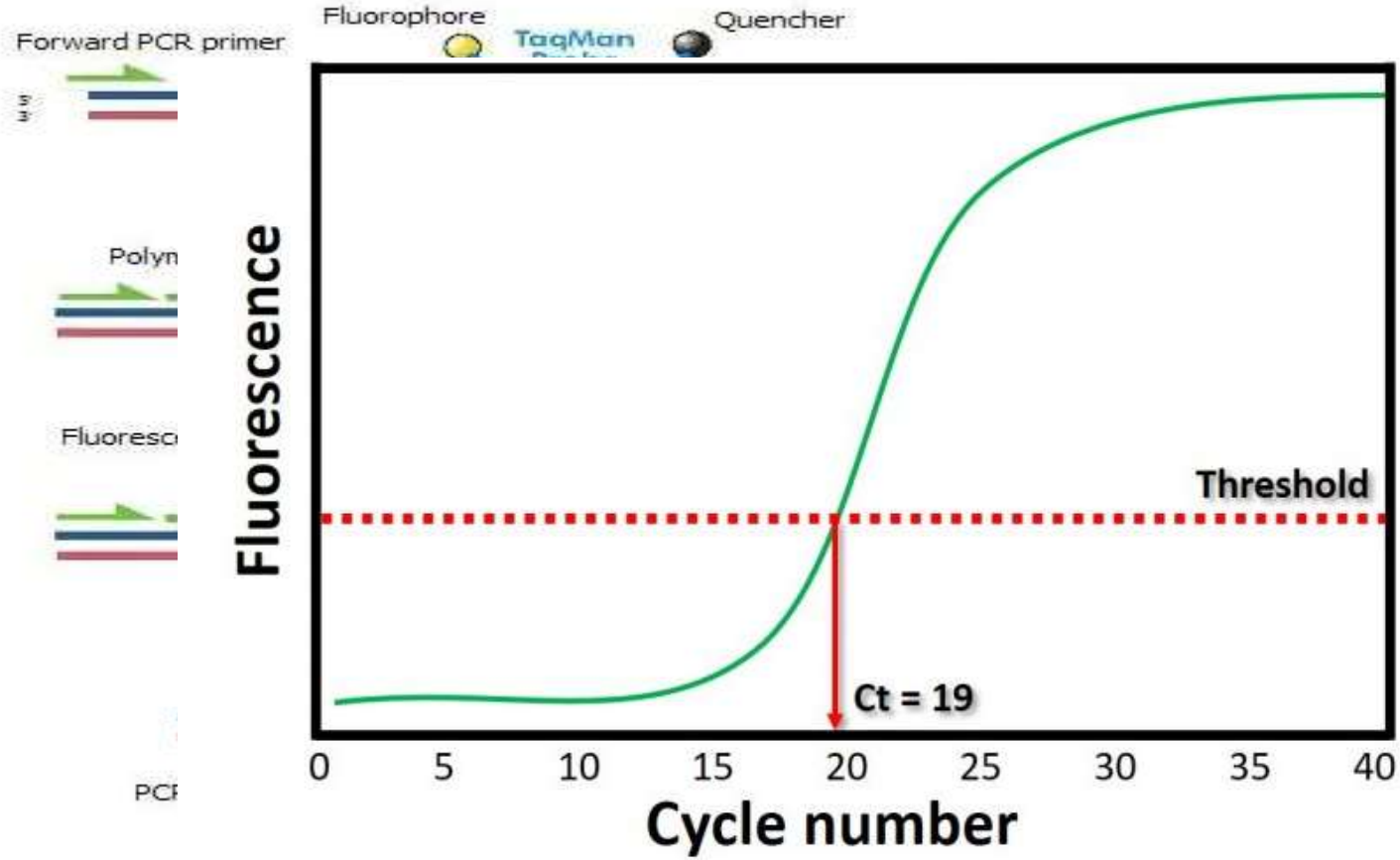
Outline

1. Covid 19核酸檢驗介紹
2. 認識實證醫學
3. 形成PICO
4. 文章評讀
5. 問題討論

COVID核酸檢驗介紹

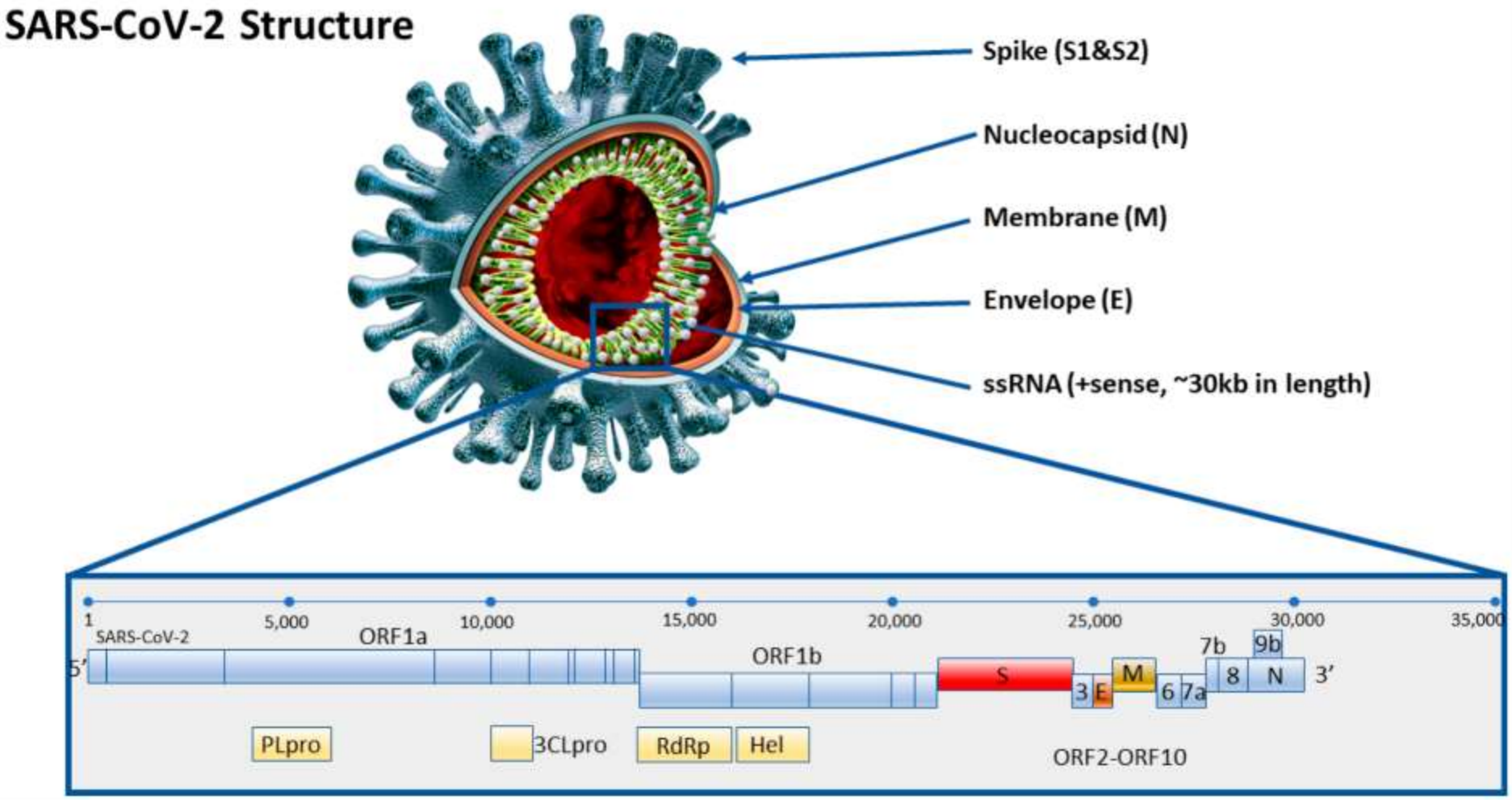
Real-time PCR(qPCR)



n probe

分別標定上不
光物質彼此間
此的螢光，因
是當DNA進
otide會被切
質分開，因此
強代表PCR產

Figure 1. Real Time PCR COVID-19 genetic tests detecting ORF1ab, spike (S), envelope (E), or nucleocapsid (N) gene sequences of SARS-CoV-2 coronavirus (own interpretation, based on [8]).



Target gene fragment

商品名稱	Target gene
Qiagen QIAgility	E / S
Thermo Fisher Scientific TaqMan	ORF1ab / S / N
AriMx Real-time PCR System	N1 / N2
Roche Cobas	ORF 1ab / E
Genesig COVID-19 CE-IVD real-time RT-PCR kit	E / RdRP
Biospeedy COVID-19 qPCR detection kit	RdRP
Altona Diagnostics Real Star SARS-CoV-2 kit	E / S
Hologic Panther Fusion SARS-CoV-2	兩個ORF1ab
ID now SARS-CoV-2	RdRP
Liat	N / ORF1ab
Cepheid Xpert Xpress SARS-CoV-2	E / N2
BioFire FilmArray	S / M

認識實證醫學

認識實證醫學

- 我們都處在資訊爆炸的時代，甚至無法讀完送達我們面前的所有科學文獻及其他資訊，而這些僅佔新研究資訊的一小部份：每天大約有 50 個新試驗及 2000 個新研究文章出版！
- 即使我們有時間讀完其中一些，也很難分辨哪些資料在臨床診療上最有用，同時很難在需要的時候，即時回想起最新醫療進展。但是，我們每天都會面臨一些臨床問題，為了確立最佳病患照護決策，我們必須一一解答這些問題，而這就是實證醫學的由來。
- 實證醫學教育包括四個基本步驟：提問、搜尋、評讀及應用證據。

實證醫學5步驟

步驟1
- 形成臨床問題 →PICO

步驟2
- 搜尋最佳證據 →FAITH

步驟3
- 嚴格評讀證據

步驟4
- 將臨床專業與病人價值觀相結合

步驟5
- 評估執行效果及效用

形成PICO

步驟 1：系統性文獻回顧探討的問題為何？

Question：一個問句簡潔有力表達臨床特定問題			
P	Patient/Problem	病人、臨床問題、患者族群	COVID 19 檢驗有些機器無法提供CT值
I	Intervention	關注的(實驗組)： 治療方式、劑量、頻率.... 診斷工具 暴露因子	不提供CT值
C	Comparison	對照的： 治療：ex. Placebo 診斷工具： ex. Gold standard 暴露因子	COVID 19 附CT值
O	Outcomes	臨床關注、對病患有意義可測量的結果	1. CT值是否和病人住院有直接關係(住院病患) 2. CT值是否和疾病嚴重程度有關 3. CT值是否和死亡率有關

文章評讀

步驟 2：系統性文獻回顧的品質如何？(FAITH)

F - 研究是否找到 (Find) 所有的相關證據？

良好的文獻搜尋至少應包括二個主要的資料庫 (如：Medline, Cochrane 考科藍實證醫學資料庫, EMBASE 等)，並且加上文獻引用檢索(參考文獻中相關研究、Web of Science, Scopus 或 Google Scholar)、試驗登錄資料等。文獻搜尋應不只限於英文，並且應同時使用MeSH字串及一般檢索詞彙(text words)。

在文章的方法(Methods)章節，可以找到詳細搜尋策略的說明，包括使用的名詞。

Data Sources and Search Strategies

A comprehensive search of several databases from January 1, 2019, through January 28, 2021, limited to the English language and excluding animal studies, was conducted. Given the rapid pace of publications, the search was repeated on April 19, 2021. The databases included Ovid MEDLINE and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily, Ovid Embase, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, Web of Science, and Scopus. The search strategy was designed and con-

步驟 2：系統性文獻回顧的品質如何？(FAITH)

F - 研究是否找到 (Find) 所有的相關證據？

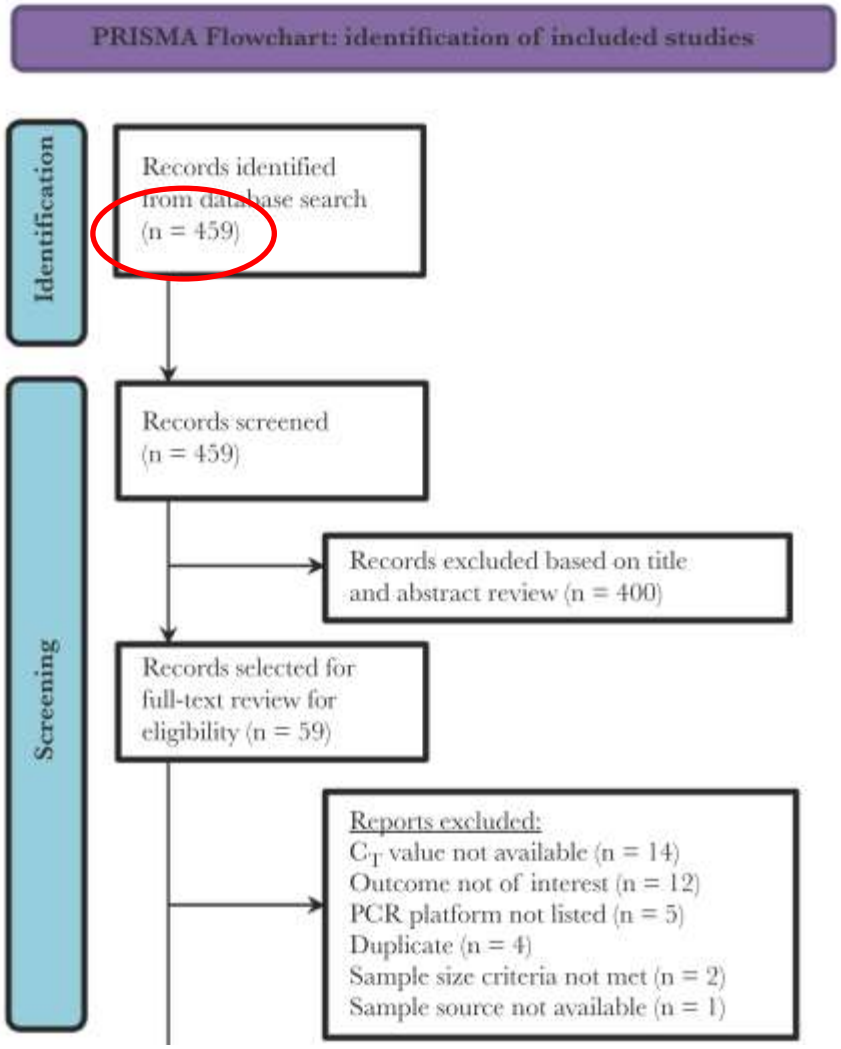
結果(Results) 章節中可以找到本篇系統性文獻回顧評估的摘要及全文文獻數目、文獻納入與排除的數量及原因。資料可能會以圖表或PRISMA的流程圖呈現。

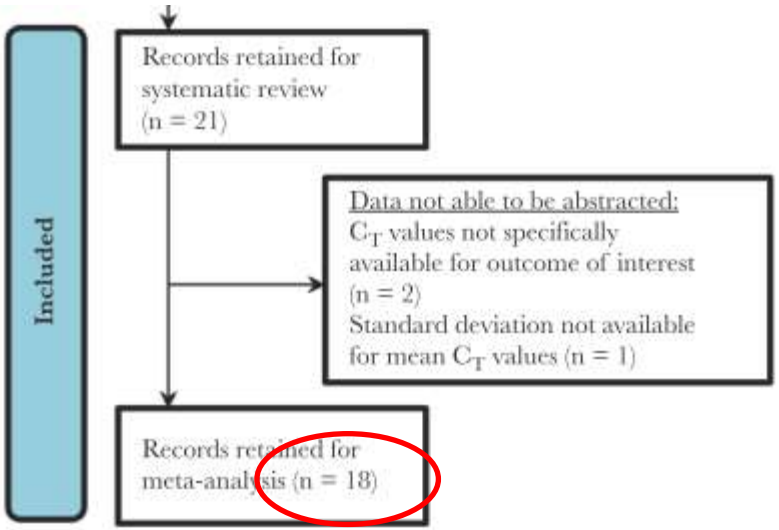
RESULTS

Study Selection and Characteristics

The search yielded 459 potentially relevant articles, of which 21 studies met inclusion criteria (Figure 1). Study characteristics are listed in Tables 1–3 for each outcome. A total of 18 studies contributed data to the meta-analysis. Overall, 8 and 10 studies reported C_T values as categorical and continuous variables, respectively, in relation to outcomes of interest and were synthesized collectively for each outcome.

PRISMA 流程圖

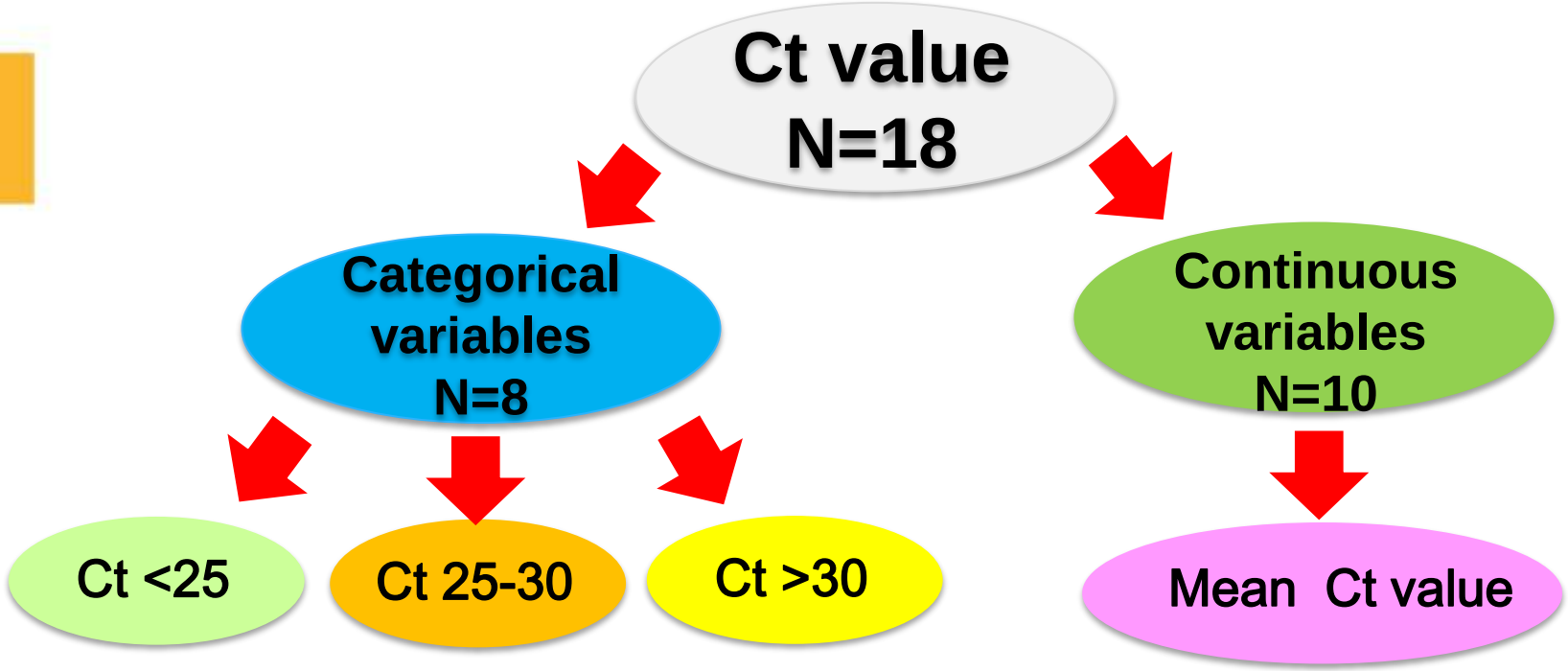


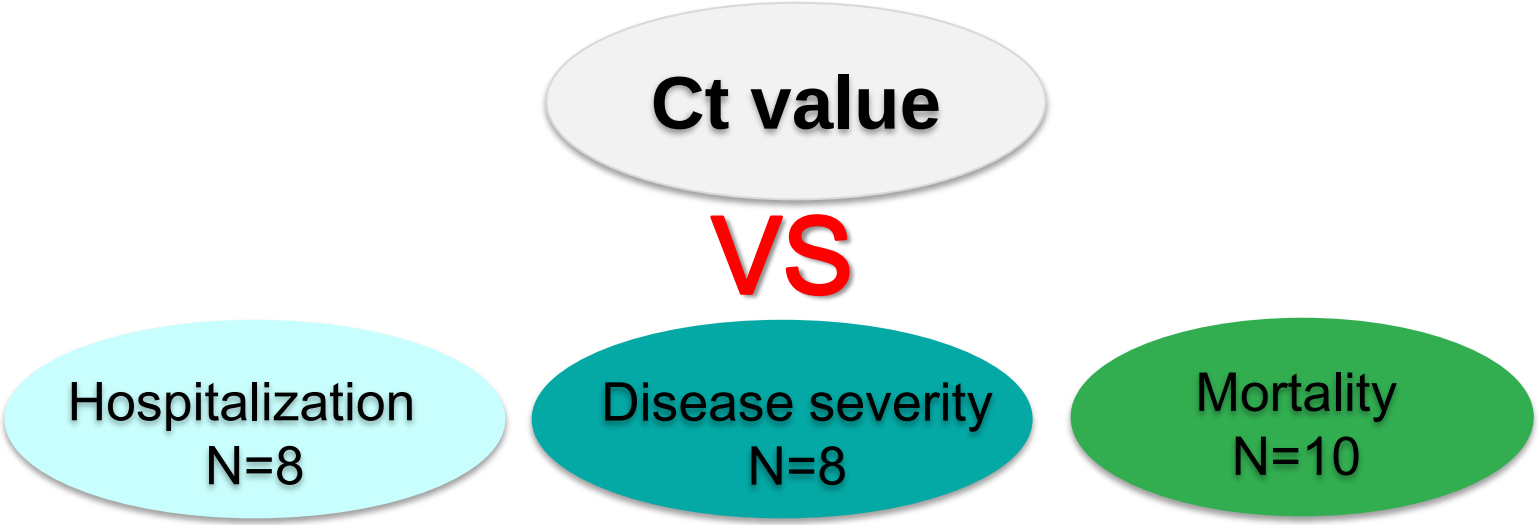


Criteria:

1. Adults ≥18 years
2. Publication in English
3. Reported Ct value data
4. Specified sample source
5. Specified rtPCR assay
6. Minimum 5 study subjects with specified outcome of interest
7. Full manuscript available

評讀結果： ☑ 是 ☐否 ☐不清楚


Ct value
N=18
Categorical variables
N=8
Continuous variables
N=10
Ct <25
Ct 25-30
Ct >30
Mean Ct value


Ct value
VS
Hospitalization
N=8
Disease severity
N=8
Mortality
N=10

步驟 2：系統性文獻回顧的品質如何？(FAITH)

A - 文獻是否經過嚴格評讀 (Appraisal)？

應根據不同臨床問題的文章類型，選擇適合的評讀工具，並說明每篇研究的品質(如針對治療型的臨床問題，選用隨機分配、盲法、及完整追蹤的研究類型)。

方法章節，可以找到所使用的文獻品質評讀標準的描述

結果章節則會列出每篇研究品質的評讀結果。

Risk of Bias Assessment

Risk of Bias assessment was performed using a modified Newcastle-Ottawa scale by 2 independent reviewers (V.P.S. and W.H.F.) (Supplementary Data). Disagreements were resolved by discussion and consensus. We assessed the representativeness of the study population, selection of the nonexposed cohort, comparability, and outcome assessment. A quantitative score for risk of bias was not used, but we focused on the most critical element of bias in this specific context, which was adjustment for confounders [22].

評讀結果： ☑是 ☐否 ☐不清楚

結果章節則會列出每篇研究品質的評讀結果

NP: nasopharyngeal
NR: not reported
OP: oropharyngeal

Table 1. Summary of Studies Evaluating Association Between CT Values and Hospitalization

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Hospitalization
Ade et al. [37]	2021	Western Germany	March 15–September 2020	1077	NP	Qiagen QIAgility	Hospitalized patients	Nonhospitalized patients	No
Amodio et al. [38]	2020	Palermo, Italy	February 24–April 8, 2020	381	Nasal, pharyngeal, or NP	Thermo Fisher Scientific TaqMan (custom)	Hospitalized patients	Nonhospitalized patients	Yes
Faico-Filho et al. [39]	2020	São Paulo, Brazil	March 17–June 17,	875	NP	Thermo Fisher Scientific	Hospitalized patients	Nonhospitalized patients and health care workers	No
Koureas et al. [40]	2021	Thessaly, Greece					pitalized atients	Nonhospitalized patients	Yes
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece	2020			real-time RT-PCR kit or Thermo Fischer Scientific TaqMan	ents with $_T$ <25 and 25–30	Patients with C_T >30	No
McEllistrem et al. [41]	2021	Pennsylvania, USA	June 24–August 23, 2020	53	NP	Cepheid Xpert Xpress	Hospitalized patients	Nonhospitalized patients and health care workers	No
Seeni et al. [42]	2021	Nevada, USA	April 1–October 30, 2020	134	NP	Cepheid Xpert Xpress	C_T <34	C_T >34	NR
Yagci et al. [43]	2020	Istanbul, Turkey	March 22–May 20, 2020	730	NP	Biospeedy COVID-19 qPCR detection kit, version 2	Hospitalized patients	Nonhospitalized patients	No

較高的 Ct 值有較低的住院率(8篇)

- 2篇支持
- 1篇沒有結論
- 5篇不支持

結果章節則會列出每篇研究品質的評讀結果

NP: nasopharyngeal
NR: not reported
OP: oropharyngeal

Table 2. Summary of Studies Evaluating the Association Between CT Values and Disease Severity Among Hospitalized Patients

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Disease Severity
de la Calle et al. [44]	2021	Madrid, Spain	March 1–March 18, 2020	455	NP	Thermo Fisher Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes
Faico-Filjo et al. [39]	2020	São Paulo, Brazil	March 17–June 17, 2020	376	NP	Thermo Fisher Scientific AgPath-ID	Patients requiring the ICU	Hospitalized patients	Yes
Fukushima et al. [45]	2021	Tokyo, Japan					quired chanical died	Hospitalized patients	Yes
Gaston et al. [46]	2020	Connecticut, USA					quired positive tilation, entilation, died	Hospitalized patients	No
Guo et al. [47]	2020	Guangdong Province, China	February 28 2020				g mechanical ventilation or ICU, or with shock	Hospitalized patients	Yes
Magleby et al. [20]	2020	New York, USA	March 30–April 30, 2020	678	NP	Roche Cobas	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece	February 26–May 3, 2020	518	NP or OP	Genesig COVID-19 CE-IVD real-time RT-PCR kit; Thermo Fischer Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	No
Seeni et al. [42]	2021	Nevada, USA	April 1–October 30, 2020	88	NP	Cepheid Xpert Xpress	C_T <34	C_T >34	NR

較低的 Ct 值有較高的疾病嚴重度(8篇)

- 5篇支持
- 1篇沒有結論
- 2篇不支持

結果章節則會列出每篇研究品質的評讀結果

NP: nasopharyngeal
NR: not reported
OP: oropharyngeal

Table 3. Summary of Studies Evaluating the Association Between CT Values and Mortality Among Hospitalized Patients

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Mortality
Bryan et al. [48]	2020	Washington, USA	NR	109	NP	Hologic Panther Fusion; LDT	Patients with C_T <22	Patients with C_T >22	Yes
Choudhuri et al. [49]	2020	New York, USA	March 26–August 5, 2020	1044	NP	Hologic Panther Fusion	Patients with C_T <22.9 and 23–27.9	Patients with C_T >32.9	Yes
de la Calle et al. [44]	2021	Madrid, Spain	March 1–18, 2020	455	NP	Thermo Fisher Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes (No for C_T 25–30 compared with >30)
Faico-Filjo et al. [39]	2020	São Paulo, Brazil	March 17–June 17,	376	NP	Thermo Fisher Scientific	Nonsurvivors	Survivors	Yes
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece					atients with C_T <25 and 25–30	Patients with C_T >30	No
Seeni et al. [42]	2021	Nevada, USA	October 30, 2020			Xpress	atients with C_T <34	Patients with C_T >34	NR
Shah et al. [29]	2021	Mumbai, India	March 23–June 30, 2020	111	NP	Altona Diagnostics Real Star SARS-CoV-2 kit	C_T of 11–20	C_T of 31–40	Yes
Westblade et al. [24]	2020	New York, USA	March 15–May 14, 2020	3914	NP	Roche Cobas and Cepheid Xpert Xpress	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes
Yagci et al. [43]	2020	Istanbul, Turkey	March 22–May 20, 2020	284	NP	Biospeedy COVID-19 qPCR detection kit, version 2	Nonsurvivors	Survivors	No
Zhao et al. [50]	2021	New Jersey, USA	March 12–April 8, 2020	722	NPa	LDT	Nonsurvivors	Survivors	Yes

較低的 Ct 值有較低的死亡率(10篇)

- 7篇支持
- 1篇沒有結論
- 2篇不支持

Ct value

VS

	Hospitalization N=8	Disease severity N=8	Mortality N=10
☺	2	5	7
😐	1	1	1
☹	5	2	2

步驟 2：系統性文獻回顧的品質如何？(FAITH)

I - 是否只納入 (Included) 具良好效度的文章？

僅進行文獻判讀是不足夠，系統性文獻回顧只納入至少要有一項研究結果是極小偏誤的試驗。

在文章的方法章節，可以找到文章評估的方式，以及是由誰完成評估的

Data Collection

Two reviewers abstracted data from each included study (V.P.S. and W.H.F.). Disagreements were resolved by discussion. When multiple studies from the same data set were reported, we included only the largest data set. If a study reported the use of multiple rtPCR assays, data were abstracted and synthesized separately for each assay.

評讀結果： ☑是 ☐否 ☐不清楚

MOOSE Checklist for Meta-analyses of Observational Studies[1]

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	3
2	Hypothesis statement	-
3	Description of study outcome(s)	4
4	Type of exposure or intervention used	5
5	Type of study designs used	5
6	Study population	5
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	5
8	Search strategy, including time period included in the synthesis and key words	5
9	Effort to include all available studies, including contact with authors	6
10	Databases and registries searched	5
11	Search software used, name and version, including special features used (eg, explosion)	-
12	Use of hand searching (eg, reference lists of obtained articles)	-
13	List of citations located and those excluded, including justification	8-9
14	Method of addressing articles published in languages other than English	-
15	Method of handling abstracts and unpublished studies	5
16	Description of any contact with authors	6

Supplemental File - Systematic review and meta-analysis of the association between SARS-CoV-2 cycle threshold values and clinical outcomes in patients with COVID-19

Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Tables 1-3
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	6
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	6
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Supplemental Table S3
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	Supplemental Table S3
22	Assessment of heterogeneity	Figures 2-4
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	7
24	Provision of appropriate tables and graphics	Tables 1-3, Figures 1-4
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	Figures 2-4
26	Table giving descriptive information for each study included	Tables 1-13
27	Results of sensitivity testing (eg, subgroup analysis)	Figures 2-4
28	Indication of statistical uncertainty of findings	8-9

Supplemental File - Systematic review and meta-analysis of the association between SARS-CoV-2 cycle threshold values and clinical outcomes in patients with COVID-19

Item No	Recommendation	Reported on Page No
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	12
30	Justification for exclusion (eg, exclusion of non-English language citations)	12
31	Assessment of quality of included studies	12
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	11-12
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	13
34	Guidelines for future research	12-13
35	Disclosure of funding source	14

步驟 2：系統性文獻回顧的品質如何？(FAITH)

T - 作者是否以表格和圖表「總結」(Total up) 試驗結果？

應該用至少1個摘要表格呈現所納入的試驗結果。若結果相近，可針對結果進行統合分析(meta-analysis)，並以「森林圖」(forest plot)呈現研究結果，最好再加上異質性分析。

在文章的結果章節，可以找到摘要的圖表，以及作者對系統性文獻回顧結果的解釋。

步驟 2：系統性文獻回顧的品質如何？(FAITH)

H - 試驗的結果是否相近 - 異質性 (Heterogeneity)?

在理想情況下，各個試驗的結果應相近或具同質性，若具有異質性，作者應評估差異是否顯著(卡方檢定)。根據每篇個別研究中不同的PICO及研究方法，探討造成異質性的原因。

在文章的結果章節，可以找到研究結果是否具異質性，及造成異質性可能的原因探討。森林圖中可以找到異質性的卡方檢定結果。

Systematic review 異質性-
文獻結果是否合併，適當與否？

- 主要原因在於，並不是所有的系統性回顧 (systematic review)都適合把結果合併成一個值，變成統合分析 (meta-analysis)，例如研究彼此間研究設計差異太大、異質性 (heterogeneity)太大時。

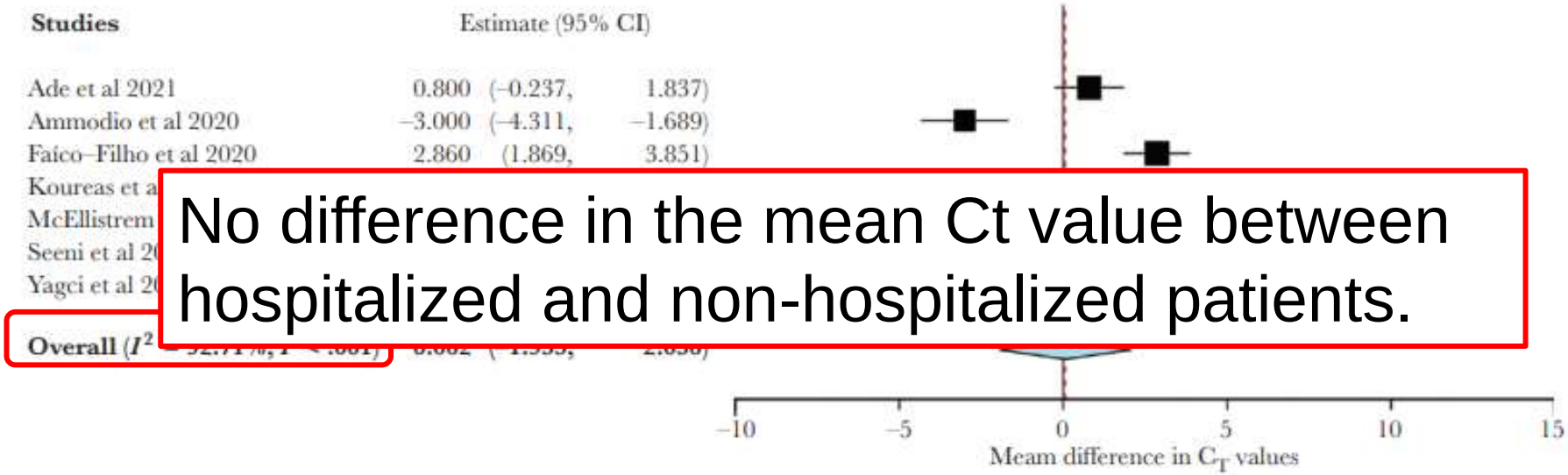
- 通常可以從文章尋找方法 (Methods)中統計分析 (statistical analysis)的描述部分判斷合理性。常用來闡述研究間異質性的統計方法如下：

I^2值：通常介於0-100%，數字越大異質性越大，但事實上 I^2 值低，不代表研究間異質性低，也有可能是檢定力不足。

Low <50%、Moderate 50-75%、High >75%

Ct value vs hospitalization

N=3291 patients



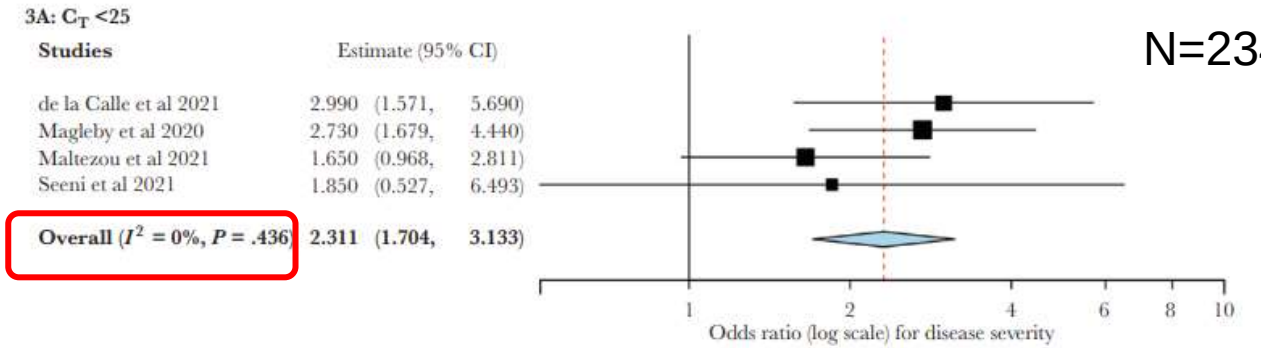
No difference in the mean Ct value between hospitalized and non-hospitalized patients.

Figure 2. Forest plot of mean C_T value difference between hospitalized and nonhospitalized patients. Abbreviation: C_T, cycle threshold.

Heterogeneity
Low <50%
Moderate 50-75%
High >75%

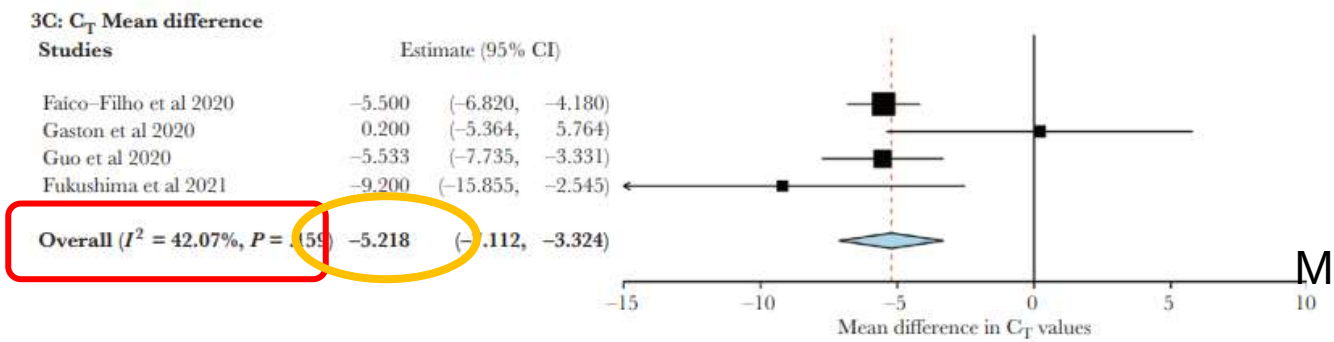
Ct value vs disease severity

N=2347 patients



3B: C_T 25–30

1. Ct <25 or 25-30 had a increased risk more severe disease.
2. Mean Ct different of -5.22.



Heterogeneity
Low <50%
Moderate 50-75%
High >75%

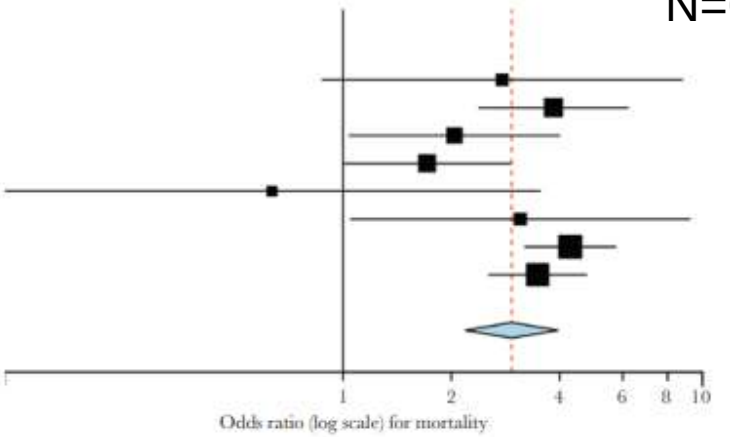
Figure 3. Forest plots of disease severity outcome among hospitalized patients. A, C_T value <25 vs >30. B, C_T value 25–30 vs >30. C, Mean C_T value difference between patients with severe and nonsevere disease in subgroup analysis by sample source. Abbreviation: C_T, cycle threshold.

Ct value vs mortality -1

N=6053 patients

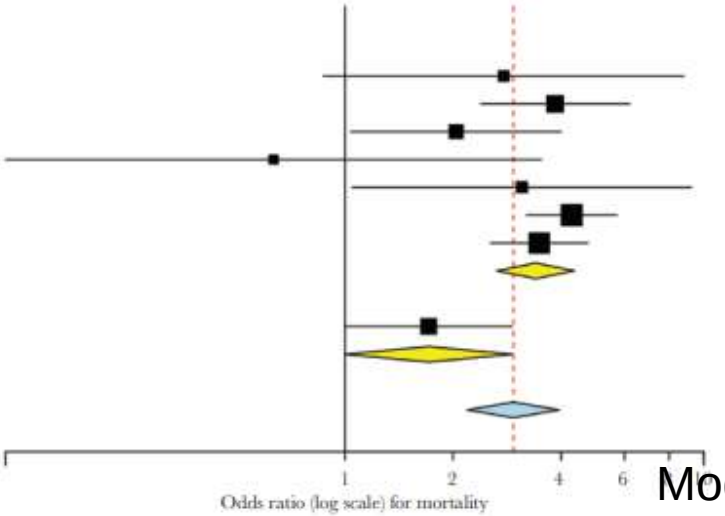
4A: C_T <25

Studies	Estimate (95% CI)
Bryan et al 2020	2.769 (0.873, 8.783)
Choudhuri et al 2020	3.850 (2.391, 6.200)
de la Calle et al 2021	2.040 (1.040, 4.000)
Maltezou et al 2021	1.713 (1.002, 2.928)
Seeni et al 2021	0.633 (0.114, 3.528)
Shah et al 2021	3.111 (1.049, 9.225)
Westblade_Roche 2020	4.285 (3.207, 5.725)
Westblade_Xpert 2020	3.479 (2.543, 4.757)
Overall (I^2 = 53.25%, P = .036)	**2.945 (2.192, 3.958)**



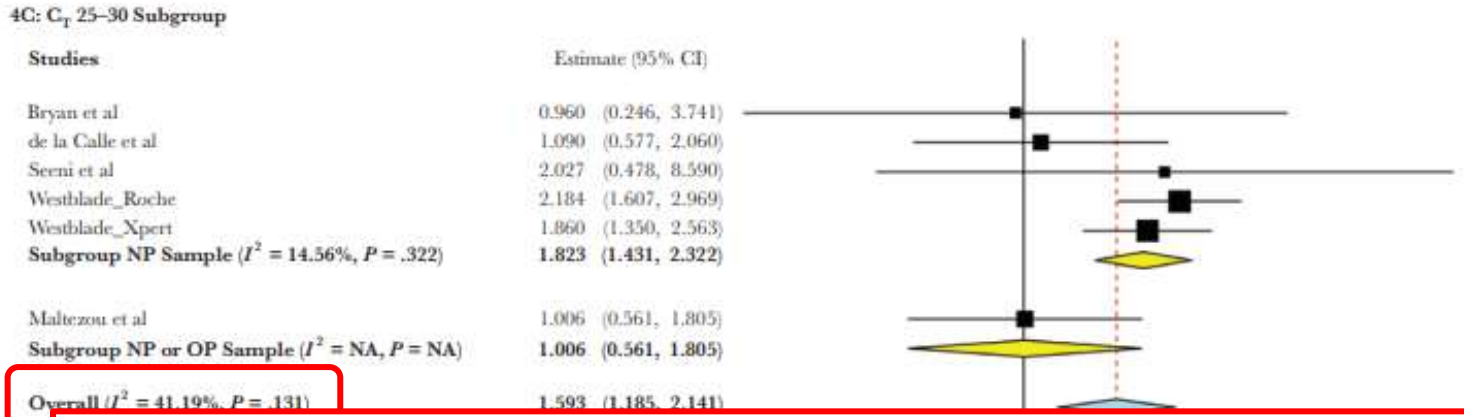
4B: C_T <25 Subgroup

Studies	Estimate (95% CI)
Bryan et al	2.769 (0.873, 8.783)
Choudhuri et al	3.850 (2.391, 6.200)
de la Calle et al	2.040 (1.040, 4.000)
Seeni et al	0.633 (0.114, 3.528)
Shah et al	3.111 (1.049, 9.225)
Westblade_Roche	4.285 (3.207, 5.725)
Westblade_Xpert	3.479 (2.543, 4.757)
Subgroup NP Sample (I^2 = 28.9%, P = .208)	**3.399 (2.654, 4.351)**
Maltezou et al	1.713 (1.002, 2.928)
Subgroup NP or OP Sample (I^2 = NA, P = NA)	**1.713 (1.002, 2.928)**
Overall (I^2 = 53.25%, P = .036)	**2.945 (2.192, 3.958)**

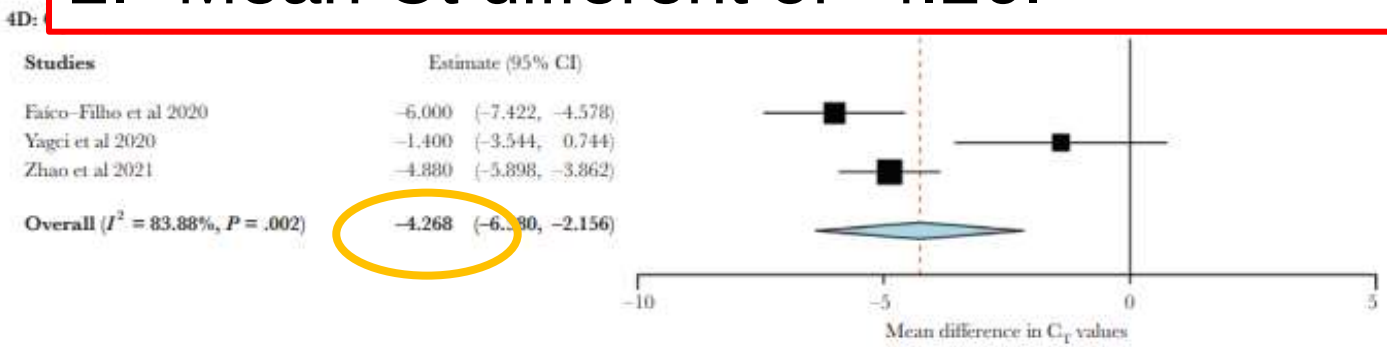


Heterogeneity
Low <50%
Moderate 50-75%
High >75%

Ct value vs mortality -2



1. Ct <25 or 25-30 had a increased mortality.
2. Mean Ct different of -4.26.



Heterogeneity
Low <50%
Moderate 50-75%
High >75%

Figure 4. Forest plots of mortality outcome among hospitalized patients. A, C_T value <25 vs >30. B, C_T <25 vs >30, subgroup analysis by sample source. C, C_T value 25–30 vs >30. D, Mean C_T value difference between survivors and nonsurvivors. Abbreviations: C_T, cycle threshold; NP, nasopharyngeal.

步驟 2：系統性文獻回顧的品質如何？(FAITH)

T - 作者是否以表格和圖表「總結」(Total up) 試驗結果？

評讀結果： ☑是 ☐否 ☐不清楚

步驟 2：系統性文獻回顧的品質如何？(FAITH)

H - 試驗的結果是否相近 - 異質性 (Heterogeneity)?

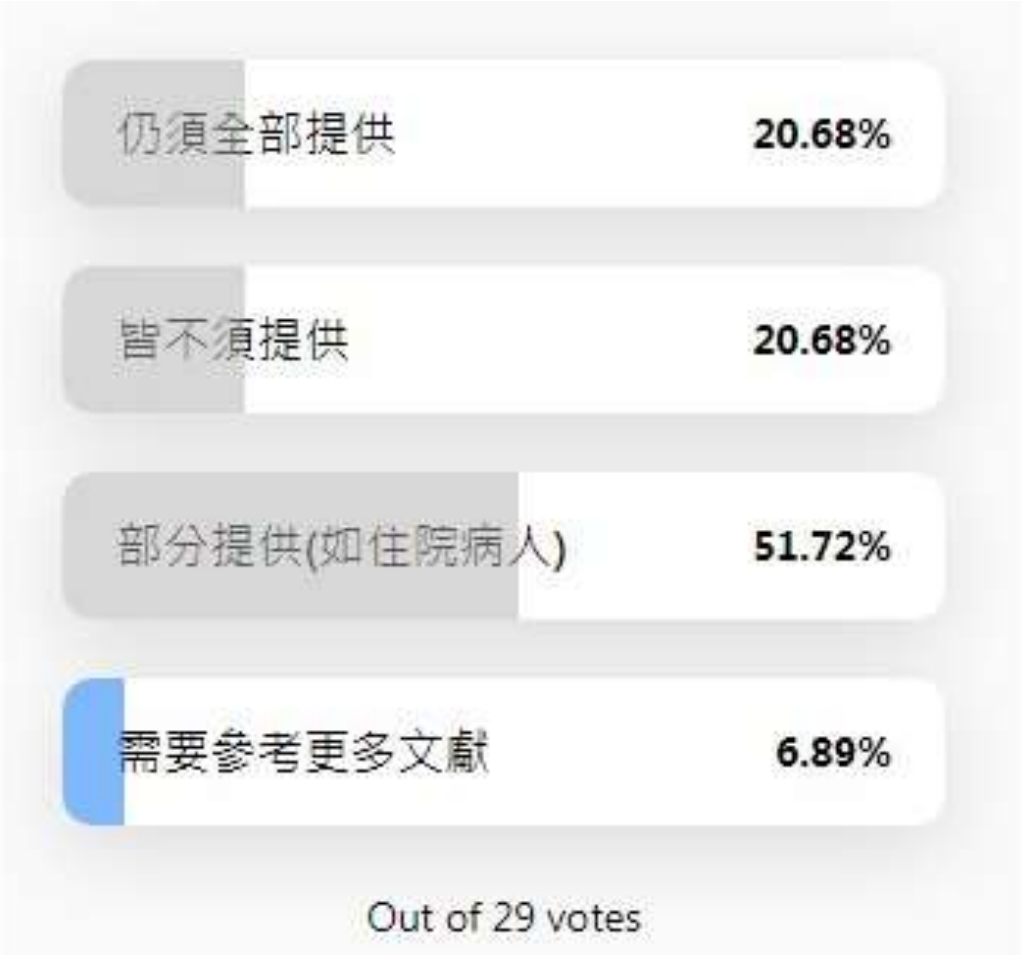
評讀結果： ☑是 ☐否 ☐不清楚

結果為何？

- 針對18篇研究共 6053 名參與者的觀察結果：
  1. CT值和病人是否住院沒有直接關係
  (住院病患)
  2. CT值和疾病嚴重程度有關
  3. CT值和死亡率有關
- 但Ct值受到 (1)檢體類別 (2)採集方法 (3)採集量 (4)採集時間點 (5)運送儲存方式 (6)檢驗方法 影響
- Ct值本身並無法直接比較，作者使用平均Ct值來消除偏差，但各分組研究結果仍具有非常高的異質性(>75%)。
- Ct值僅能輔助判斷，臨床上仍以病人實際的症狀來進行治療

問題討論

討論：COVID 19 RT-PCR不提供Ct值是否可行？



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感謝聆聽