Peer Review File

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Reviewer A

This is a systematic review on the expression of interleukin 17 in children with Mycoplasma pneumoniae pneumonia (MPP), including a meta-analysis, to investigate the potential of IL-17 in diagnosing MPP (including severe vs. non-severe cases) and/or assessing treatment response.

I have some concerns that should be addressed before this manuscript can be considered for publication.

My comments, in the order of appearance:

Comment 1: Abstract, background: …is the common pathogen": I presume the authors meant "is a common pathogen".
Reply 1: Thank you very much for your kindness comments. We meant that *Mycoplasma pnuemoniae* is a common pathogen of community-acquired pneumonia. We have modified our text accordingly.
Changes in the text: We have modified our text in Page 3, line 25 /Abstract

Comment 2: Abstract, results: Please comment on the risk of bias of the 9 included studies.
Reply 2: Thank you for your comments and providing good suggestions for my manuscript. We have explained the risk of bias of the 9 included studies in abstract.
Changes in the text: We have added the risk of bias result in our text. Please see Page 3, line 41-43.

Comment 3: Abstract, results: it is unclear why the MD of 19.08 between severe and mild cases is "dramatically increased", given that the other mean differences are even larger. Please rephrase or justify why you phrase it like this.
Reply 3: Thank you for your careful review and constructive suggestions regarding our manuscript. We would like to express that IL-17 levels were significantly increased in severe pneumonia.
Changes in the text: We revised the text from dramatically increased to significantly increased. Please see Page 3, line 40.

Comment 4: Abstract, Conclusion: there seems to be a word missing in the first sentence, which sounds grammatically wrong.
Reply 4: Thank you for your comments and providing good suggestions for my manuscript. "in" was added before "pediatric general and severe MPP" to clarify that the elevation in serum IL-17 levels is observed in those conditions.
Changes in the text: Please see Page 3, line 44.

Comment 5: Introduction: I do miss a proper motivation for why studying IL17 in this context makes sense. Is this a cytokine that can be easily measured in routine care? How stable is it? What sample type is needed? Please justify why you focused on IL17 a little bit more.
Reply 5: Thank you for your comments and providing good suggestions for my manuscript. Currently, IL-17 is considered an attractive target for modulating inflammatory responses in the body. IL-17 is primarily secreted by T helper 17 cells (Th17), monocytes, and eosinophils. It plays a key role in recruiting neutrophils, activating T cells, and stimulating macrophages and epithelial cells. Ultimately, IL-17 contributes to the production of a range of pro-inflammatory cytokines, leading to inflammatory reactions within the body (Feng et al., 2021). Additionally, IL-17 has been identified as a potential biomarker for several inflammatory diseases, including acute kidney injury (AKI) (Collett et al.,) and sepsis (Zhang et al., 2022).
IL-17 levels can be measured using immunoassays such as ELISA (Enzyme-Linked Immunosorbent Assay), multiplex assays, or flow cytometry-based methods with ELISA is the standard method for detection. These tests are available in research and some specialized clinical laboratories but are not yet standard in routine

clinical practice.
The stability of IL-17 in samples is crucial for accurate measurement. Generally, IL-17 levels are stable in serum samples when stored properly. specific conditions regarding temperature and duration of storage can affect stability. For example, samples should ideally be processed and frozen promptly to maintain the integrity of IL-17 levels. The inter-assay coefficient of variation for IL-17 assays is reported to be around 6.3%, indicating a reasonable level of consistency in measurements across different assays (Sobhan et al., 2022)
Plasma or serum samples are typically used for this purpose, as they are effective in detecting circulating IL-17. Occasionally, IL-17 can also be measured in other biological fluids like cerebrospinal fluid (CSF) or bronchoalveolar lavage fluid, depending on the clinical context, but these are less common.
In healthy individuals and patients without infection or underlying autoimmune diseases, IL-17 levels are generally low. However, previous studies, including those in our meta-analysis, have reported elevated circulating levels of IL-17 in cases of pneumonia caused by *Mycoplasma pneumoniae*, both severe and non-severe. It's important to justify the focus on IL-17 in research or clinical settings by considering its relevance to the disease or condition being studied, the availability of assays, and the implications of its levels for patient care, therefore, our study aimed to investigate the circulating levels of IL-17 in these conditions.
Changes in the text: We added the information in the introduction. Please see Page 5 Line 71-74 and Page 6 Line 75-80 and 84-87.

Comment 6: Discussion: there remains a huge limitation, which the authors also acknowledge, and that is the unclear time of sampling within the original studies. This is a major limitation and threat to the internal validity of each study (and thereby to the meta-analysis and the study herein, as well). This should be stressed more, especially when the authors speculate about future studies. Not only larger sample sizes, but a standardized approach regarding classification of patients and sampling could be something to be added.
Reply 6: Thank you very much for your thoughtful comments. We apologize for omitting the information about sampling times. After revisiting and rechecking the original studies, we found that most of them did report the timing of sample collection. In the majority of studies, samples were taken on the day of admission or within 24 hours of hospitalization. However, one study reported blood collection within 48 hours after admission, and another did not specify the timing of blood collection. Additionally, the original studies classified patients as M. pneumoniae positive before conducting IL-17 detection. The methods used for classifying MPP included ELISA IgM titers, PCR, and clinical features of MPP, which we believe provides a standardized approach for the study. Your feedback has significantly strengthened our manuscript.
Changes in the text: We have added and elaborated in the discussion part. Please see Page 14-15 Line 268-275. Also, we amended Table 1 by adding the timing of sample collection.

Comment 7: Discussion: Another limitation is the fact that most studies compared MPP to healthy controls. Please also discuss the need to distinguish MPP from other causes of pneumonia, as they need different management strategies (different antibiotics or no antibiotics in viral pneumonia, isolation/cohorting, …). There is no discussion of existing or other biomarkers that are in use or have been reported recently with high potential (e.g. PMID 38092364), and what IL-17 would add on top of these biomarkers.
Reply 7: Thank you for your comments and valuable suggestions for my manuscript. Classifying patients is crucial for determining appropriate therapies. According to a study by Choi et al., patient classification was based on respiratory symptoms, positive radiographic findings, and polymerase chain reaction (PCR) analysis of nasopharyngeal secretions that tested positive for respiratory viruses. In contrast, the diagnosis of *Mycoplasma pneumoniae* (MP) pneumonia was based on clinical symptoms, such as fever, paroxysmal dry hacking cough with dyspnea, and crackles, as well as radiological findings and serological tests (IgM titer). Patients were then grouped accordingly before treatment. For cases of MP with viral co-infection, patients typically received macrolide antibiotics.

Since IL-17 levels are not elevated in viral pneumonia cases (Choi et al., 2019), this cytokine could serve as a potential biomarker for distinguishing between pneumonia caused by MP and viruses. Moreover, IL-17 is significantly correlated with the severity of MPP, aiding in the differentiation between mild and severe pneumonia, whereas other biomarkers such as C-reactive protein (CRP) and interferon-γ inducible protein 10 (IP-10) do not show the same correlation (Papan C. et al., 2023). Additionally, a recently identified potential biomarker, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), has been found to significantly increase in cases of pneumonia with *M. pneumoniae* positivity.

Therefore, IL-17 could assist in identifying and differentiating the causes of pneumonia, helping to guide the selection of appropriate therapies or initial treatments, such as steroids and antibiotics, and enhancing risk stratification. IL-17 also has predictive value for severity, and regular monitoring of IL-17 levels during treatment could provide insights into the effectiveness of therapeutic interventions, allowing for adjustments based on the patient's inflammatory status.

In conclusion, IL-17 serves as a valuable biomarker in the context of *Mycoplasma pneumoniae* pneumonia, offering insights into disease severity, potential complications, and guiding management strategies. Integrating IL-17 with other clinical markers can improve the overall approach to treating pediatric patients with MPP.

Changes in the text: We have amended in the text. Please see Page 13-14 Line 246-262.

Comment 8: Acknowledgments: please explain what is meant by "supported by XXX".
Reply 8: The Reinventing University Program is funded by the Ministry of Higher Education, Science, Research, and Innovation (MHESI). This program has facilitated our collaboration with international scholars. In this current work, we are collaborating with Prof. Nathorn Chaiyakunapruk, who is affiliated with the Department of Pharmacotherapy at the University of Utah College of Pharmacy, United States.
Changes in the text: We did not change in the main text.

Reviewer B
1. City and country are required information in each affiliation.
Reply: We amended in the text.

2. Please provide the information of the corresponding author, including name/academic degree (MD, PhD)/ affiliation/detail address (including road names etc.)/email address.
Reply: We added in the text.

3.
"This manuscript is written following PRISMA-ScR checklist."
"This systematic review and meta-analysis are reported following the guideline for reporting systematic reviews and meta-analysis (PRISMA)"
The reporting of checklist is repetitively shown in the Introduction and Methods section (see above), which is unnecessary. And different checklist was reported.
Reply: We removed "This manuscript is written following PRISMA-ScR checklist."
in the introduction. As well as we amended the PRISMA flowchart as attached in the e-mail.

4. Please use the round brackets ( ) throughout the article.
Reply: We amended in the text.

5. Table 1: The reference number of each study is suggested to be added in the table.
Reply: We added references in the Table 1.

6. Figure 1:
- The numbers in the figure should be double-checked.
- Please review "treatment 4".
- Please replace the figure with the attached version.
- Reply: We double-checked numbers in the figure, amended and replace the figure.

7. Figures 4-7
Please present the names of the two groups in each forest plot.
Reply: The names of the two groups were stated in each forest plot
Figure 4-5 were Pneumonia and Heathy control
Figure 6-7 were Severe MPP and Mild MPP

8. All the abbreviations in the figure(s) and table(s) should be defined in the explanatory legend.

Reply: We amended accordingly.