Author's response to reviews

Title: Respiratory virus in immunocompetent community-acquired pneumonia: is it real pathogen or a bystander? Comparing to influenza like illness and volunteer controls

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Author's response to reviews:

Reviewer: Tor A Strand
Reviewer's report:

1. Interesting study that needs to be presented better. The structure of the paper is good but the language is poor and there are several typos that needs to be improved/corrected before it can be considered for publication.

Reply: Thank you for your comments. We have tried our best to improve the language in the revised manuscript.

2. It is also hard to interpret the tables and which analyses the P values refer to. The analyses and statistical description should also be revised.

Reply: Most of data were listed in the tables. All data were showed as numbers (%) unless otherwise specified. Comparisons were made between groups CAP and volunteer controls in table 1, however, between virus positive patients and virus negative patients in table 3 and table 4 by non-parametric Kruskal-Wallis test for quantitative characteristics and Fisher’s exact or Chi-square test for categorical variables, respectively.

Minor concerns

1. Whether or not written consent was obtained.

Reply: Written consent was obtained from all patients. (As seen in page 4, line 111)

2. CURB-65 model needs to be explained in the abstract. There are also other abbreviations that need to be defined.

Reply: Thanks for your suggestions. CURB-65 model is explained in the revised manuscript. However, because word counts were limited within 250 words, not all variable were listed in the abstract. Other abbreviations have been defined and added in the text and abbreviation list respectively.
Reviewer: Ger Rijkers

Reviewer's report:

With interest I have studied the manuscript by Zhan et al. which deals with an important issue, namely the involvement of viral infection in community acquired pneumonia. The single, most prevalent cause of community acquired pneumonia is an infection with Streptococcus pneumoniae. When, next to conventional microbiological methods, serotype specific serology is included in making the diagnosis, over 50% of CAP patients appear to be infected with S. pneumoniae (van Mens et al., 2011). The current model is that a viral respiratory infection can cause damage to lung tissue to a degree that would favor subsequent infection with e.g. S. pneumoniae. There are many arguments that most of the victims of the 1914 Spanish flu pandemic in fact were caused by S. pneumoniae superinfection. (Klugman, Chien and Madhi, 2009: Pneumococcal pneumonia and influenza: A deadly combination, Vaccine, 27, S3, C9-C14).

Major Compulsory Revisions

1. Authors did perform bacterial cultures because in 6 cases either a pure bacterial or mixed viral/bacterial infection was reported. There is however no mentioning of S. pneumoniae, as indicated above, the number one cause of CAP. The bacteriology therefore should include cultures for S. pneumoniae (preferably with serotyping), urine analysis for S. pneumoniae and serotype specific antibodies in the paired serum samples.

Reply: Sputum bacterial culture was performed according to the clinical needs in 77 hospitalized patients with CAP in our study although bacterial culture was not mandatory in our study, which might be a drawback in our study design. However, sputum bacterial culture in daily clinical practice has a poor yield in our hospital and in Mainland of China. Positive rate of sputum bacterial culture is less than 10% in CAP patients in our daily clinical practice. However, it can be improved and raised up to 32% in reports of research (Reference, Liu YN, et al. Zhonghua Jie He He Hu Xi Za Zhi. 2006 Jan;29(1):3-8.[In Chinese]). Streptococcus pneumoniae is also rare in clinical sputum culture because specimen is not transported and inoculated in time. It is one of the limitations in our study that bacteria and atypical pathogens were not detected simultaneously.

2. The manuscript indicates that all patients were treated with antibiotics. Therefore additional clinical data should be provided (length of hospital stay, intensive care requirement, survival) comparing the patients with a "true" pure viral infection as compared with those with bacterial CAP.

Reply: Thanks for you suggestion. Additional clinical data (hospitalization rate, length of hospital stay, intensive care requirement, 30-days mortality) are added in table 4. Because only 6 patients have a positive result in sputum bacterial culture, comparisons are made between virus positive patients and virus negative patients.

Minor essential revisions:

1. These will be correction of grammar and spelling. For instance: During April and december of 2009. I assume the authors mean: Between April and
December (and not those 2 months only).

Reply: Thanks for the suggestion and we have revised manuscript according to your suggestion (As seen in page 4, line 83).

2. With regard to the Volunteers control group: I assume these are healthy adults, but then there is substantial comorbidity, in particular COPD and cardio/cerebrovascular.

Reply: In our study, volunteers control group were defined as individuals that didn’t experience acute illnesses within one month. In order to balance the impact of comorbidity in CAP patients, individuals with stable comorbidities was also enrolled. Phrase “healthy volunteers” is replaced by “volunteer controls” in the revised version.

3. A minor point is that the study was performed in 2009. Why did it take so long to submit this manuscript?

Reply: The manuscript was finished in early 2013. It was submitted to Respiratory Research, which is another journal of BMC serial on May 2013. However, it was rejected and we accepted the advice of the editorial board of Respiratory Research, and transferred the manuscript to BMC Pulmonary Medicine on July 2013.

Reviewer: Chunxue Bai

Reviewer's report:

Recommendation: Major Compulsory Revisions

The article entitled “Respiratory virus in immunocompetent community-acquired pneumonia: is it a real pathogen or a bystander? Comparing to influenza like illness and healthy volunteers” investigated the role of virus in CAP and found that virus is a common etiology of CAP which accounted for about one third of the etiology. Recently, virus and atypical pathogens become increasingly common in CAP, suggesting that we should pay more attention to these pathogens. This article has provided similar results and would be useful in clinical environment. Nevertheless, I have some comments:

1. In the article, there are 49 patients with community acquired pneumonia caused by viruses but only a few of them received anti-viral treatment. How did the rest of the patients get cured? Did they received antibiotics and if so, what were the roles of antibiotics in these patients? Did they incur undetected bacterial infection?

Reply: Thanks for your important comments. Our study was a prospective observational study. Treatments were decided by the clinician, but not the member of staff in our study. We gave the results to clinician once a positive result comes out. However, viral culture was conducted only twice to three times weekly. A fastest positive influenza result need about 4 to 5 days. Nucleic amplification test was not routinely performed for clinical use. Swab samples were frozen and tested by RT-PCR/rRT-PCR until 2010 when all samples were collected. Hence, the results of viral detection were not used for guide of use of antibiotics or anti-viral agents. Ultimately, seven patients (two of which was virus
positive) received short course of intravenous ribavirin before their admission to our hospital. Only one patient with pandemic (H1N1) 2009 influenza A virus infection received a long course of oseltamivir and influenza virus turn negative later (As seen in page 7, line 174-178).

All patients in our study were prescribed antibiotics according to the guideline of CAP in China by clinician (Reference, Diagnosis and therapy guideline of community acquired pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2006; 29:651-655[In Chinese]). It was hard to evaluate whether the patients with virus positive got cured by antibiotics or by himself? There was not enough information to illustrate the real role of antibiotics in the treatment of viral pneumonia in our study yet. The real role of antibiotics in pure virus infected patients is still unknown and, in my opinion, is controversial, though there is no evidence to support the idea and to make a decision to discontinue the use of antibiotics in clinical (As seen in page 9-10, line 263-275). It needs to be further studied.

On the other hand, bacteria co-infection or super-infection is common in influenza virus infected patients. Antibiotics play an important role in these patients.

However, sputum bacterial culture for clinical use has a poor yield in our hospital and in Mainland of China. Positive rate of sputum bacterial culture is less than 10% in CAP patients in our daily clinical practice. However, it can be improved and raised up to 32% in reports of research (Reference, Liu YN, et al. Zhonghua Jie He He Hu Xi Za Zhi. 2006 Jan;29(1):3-8.[In Chinese]). It is one of the limitations in our study that bacteria and atypical pathogens were not detected simultaneously. Three out of 52 patients had a positive bacterial culture in our study. The remaining 49 patients may have a positive result when more attention was paid to bacterial culture.

2. Patients have recovered even without receiving anti-viral therapy yet no false positive results have been reported in the NAT test. Please specify how the test should be used and when anti-viral drugs should be administered.

Reply: Most of respiratory viral disease is self-limited. However, in individuals with comorbidities, elder, infants and children younger than 5 years old and pregnancy, influenza infection is easy to turn to severe and cause death. Hence, clinician should pay more attention to these populations. It was pointed out in the manuscript (As seen in page 9, line 257-262) that “Routine laboratory detection should be taken in hospitalized CAP patients at admission for an adequate diagnosis of respiratory viruses, especially influenza virus in severe individuals. This might be sub-group of subjects demanding antiviral treatment. The clinical value of early identification and early anti-viral treatment is crucial question demanding further investigation”

3. In the CAP groups, only 3 patients had pure bacterial infection, a really low percentage compared with the previous study about the etiology of CAP. In this regard, I think you should include the test about bacterial infection too.

Reply: Sputum bacterial culture was performed according to the clinical needs in 77 hospitalized patients with CAP in our study. The results were recorded by our researcher. However, sputum bacterial culture for clinical use has a poor yield in
our hospital and in Mainland of China. Positive rate of sputum bacterial culture is less than 10% in CAP patients in our daily clinical practice. However, it can be improved and raised up to 32% in reports of research (Reference, Liu YN, et al. Zhonghua Jie He He Hu Xi Za Zhi. 2006 Jan;29(1):3-8.[In Chinese]). It is one of the limitations in our study that bacteria and atypical pathogens were not detected simultaneously.

Reviewer: Bin Cao
Reviewer's report:
The report is interesting, which is designed to answer whether RVs are bystander of pathogenic in CAP patients by etiological comparison of CAPs, ILIs and healthy controls. Authors found RVs in 34.9% of 149 CAP patients, 81.5% of 27 ILIs. Previous studies included outpatients without respiratory complaints or asymptomatic controls, while, 75 healthy volunteers served as controls in the study, and no RV positives. These results are encouraging, which suggests that combination of RV detection and clinical judgment could be helpful for the diagnosis of viral CAP.

1. However, although three methods have been used for RV detection, throat swab sampling might reduce the sensitivity, which should be discussed in limitation. On the other side, 58 RVs have been identified (18 by culture, 47 by NATs and 20 by serological tests), what is the possible reason for the inconsistent results between different detection methods?

Reply: Thank you for your comment. Single sample type is one of the limitations in our study.

NATs are known to be more sensitive than viral culture and serological techniques. It can improve overall prevalence of respiratory viral infection with additional 15% to 56% positive in etiology studies of pneumonia, when compared to studies that NATs was not implemented (Reference, Ruuskanen O, et al. Viral pneumonia. Lancet 2011; 377:1264-1275.). It is the same in our study. However, virus shedding is decreasing along with the course of illness and will go beyond the detectable limit of NATs. Additionally, virus shedding is shorter and viral load is less in the elder. Hence, NATs is more likely to be negative when the sample is taken about 7-10 day after onset of illness. On the other side, serological test is not strictly limited by time course of illness. However, pair sera with a two to three weeks interval are necessary in serological test. But not all individual response well to virus infection and not all of them has a four fold elevation of IgG titre (Reference, Gencay M, et al. Single and multiple viral infections in lower respiratory tract infection. Respiration 2010,80:560-567). Some of patients in our study only had a two fold elevation of IgG titre (it was not listed in the manuscript). Adjusting the time interval between acute serum and convalescent serum or adding the point-in-time of convalescent serum will improve the sensitivity. Hence, it is possible that there are inconsistent results between different detection methods.

2. No routine detection of bacterial and atypical pathogen is another big limitation, since the authors could not answer whether RV is main cause of CAP, or exclude the prolonged shedding of RV.
Reply: Thanks for your comments. It is one of limitation of our study that bacteria and atypical pathogens were not studied.

3. In my opinion, “virus strain” could be isolated by culture system, but not by NATs or serological tests, which should be rephrased.

Reply: Thanks for your suggestion. We have revised the manuscript according to your suggestion.

4. It is reported that leukocytosis is less common in viral infections compared to bacterial infection, however, only comparison between viral infections and viral negative patients has been conducted, therefore, the authors could not say “It was the same in our study” in discussion.

Reply: Thanks for your suggestion. We have revised the manuscript according to your suggestion. (As seen in page 9, line 235-237)

5. Comparison of RV etiological pattern with the results previously reported from the nearby places (eg: Hong Kong) should be discussed.

Reply: Thanks for your suggestion. We have revised the manuscript according to your suggestion. (As seen in page 8, line 225-226)