Author's response to reviews

Title: Yersinia pseudotuberculosis infection in Kawasaki disease and its clinical characteristics

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Author's response to reviews: see over
Reviewer comments
Reviewer #1

Major Compulsory Revisions
None

Minor Essential Revisions
Pag. 2, Line 6-7: consider revising the structure of the sentence, is hard to understand

We have changed the text as follows:

We conducted a prospective study of 108 patients with newly diagnosed KD in one year to determine how many KD patients have positive anti-YPT antibody titers and/or positive anti-YPT-derived mitogen (YPM) antibody titers. In addition, we tried to identify clinical differences between KD patients in whom YPT infection was not a contributing factor.

Pag. 8, Line 25: Not all the abdominal symptoms in Kawasaki Disease are enterocolitis, you should change this, is better not to say enterocolitis, only abdominal symptoms (you don't specify them anywhere else in the article)

We have changed the text as follows:

Third, there were no clinical differences between the serologically positive and negative groups, except for the incidence of abdominal symptoms and CS.

Pag. 9, Line 4: consider revising the structure of the sentence, is hard to understand

We have changed the sentence as follows:
In 1983, Sato et al. reported that 2/12 patients with YPT fulfilled the criteria for KD [4]. Since then, several other groups have confirmed an association between YPT infection and KD.

Pag. 11, Line 2: I think this is a really hard affirmation, antibiotics never have been proved to be effective for Kawasaki Disease, with so many patients I would be more
We have changed the texts in both sections as follows:

**Abstract**: Additionally, further research is needed to determine whether early diagnosis of YPT can prevent KD from developing and reduce the incidence of CS.

**Discussion**: Antibiotics for Group A streptococcal infection reduce the incidence of acute rheumatic fever [31]. In the future, we should aim at conducting research to clarify if antibiotic treatment for YPT infection could prevent the development of KD or reduce the incidence of CS after rapid diagnostic techniques for YPT are developed, such as the loop-mediated isothermal amplification method [32].
Reviewer #2

In this interesting submission, authors demonstrated KD patients with YPT infection have cardiac sequelae (CS) more frequently. Furthermore, they found the treatment protocol with RAISE study could not decrease the frequency of CS. It is generally well-written, but there are some points that need further explanations.

Thank you so much for your positive comments and suggestions.

Major point
As authors described, the limitation of this study is sample size. In particular, the number of the patients with CS is too small, only 3. Therefore, in my opinion, it might be difficult to conclude KD patients with YPT infection have CS more frequently and the treatment protocol with RAISE study did not decrease the frequency of CS. It is necessary to perform larger-scale studies to confirm their data and draw firm conclusions.

We agree that our sample size is not large enough; however, we have already finished our prospective study and we cannot increase the sample number any further. Instead, we have added the following sentence to the abstract and conclusion sections.

However, our sample size is overly small to draw such conclusions. Further investigation in a larger cohort is necessary to confirm our findings.

Other points
It might be better to discuss the reason why KD patients with YPT infection have CS more frequently.
It might be better to discuss the role of YPT in the pathogenesis of KD. Previous studies revealed KD is closely associated with the activation of the innate immune system. Interestingly, recent study showed KD specific molecules in the sera are linked to MAMPs in the biofilms of some microbes including YPT (Kusuda T et al. PLOS ONE 2014;9:e113054).

We have added the following sentence to the discussion section and cited the above-mentioned reference as suggested (Ref.23).
In 2014, Kusuda et al. reported that serum KD-specific molecules were mostly derived from biofilms and possessed molecular structures common to microbe-associated molecular patterns from *Bacillus cereus*, *Bacillus subtilis*, YPT and *Staphylococcus aureus*. They also reported that extracts from *Bacillus cereus*, *Bacillus subtilis*, YPT and *Staphylococcus aureus* induced proinflammatory cytokines by human coronary artery endothelial cells [23]. This pathogenesis can contribute to the higher incidence of CS in cases of KD associated with YPT.

Page 2

Line 24 was the repetition of line 22 and superfluous.

We have removed the repeated sentence.
Reviewer #3

Minor Essential Revisions

Conclusions: line is repeated.

We have removed the repeated sentence.

RAISE study group: First sentence is too long and hard to follow: please revise.

We have changed the text as follows:

The treatment strategy was changed during the study period. At first, we started the KD treatment with the conventional IVIG protocol. However, we decided to change the treatment protocol, that is, we adopted the RAISE protocol for one of our patients, based on the newly published RAISE study, which reported clear evidence that steroids prevent CS in KD patients [15].

Discretionary Revisions

Background: Please elaborate on which YPT symptoms satisfy clinical criteria for KD

We have added the following sentence to the Background section.

_Yersinia pseudotuberculosis_ (YPT), an enteric pathogen, causes a variety of clinical symptoms such as fever, rash, desquamation, strawberry tongue, lymphadenopathy, and conjunctivitis that sometimes satisfy the clinical criteria for KD.

Overall: This paper is quite interesting, very well-written, and shows thoughtfulness in the planning of comparison clinical data.

Thank you so much for the positive comment.

My only real concern was that there are two separate issues being compared: First, the incidence and clinical characteristics of YPT infection in KD patients, and second, efficacy of RAISE vs conventional protocols. There are several parts of the paper which refer only to clinical efficacy between the two in CS, but which don't seem to fit into a paper about YPT. It is confusing to go back and forth between
those two separate issues and I would recommend two separate papers addressing this.
However, I do believe the discussion of differences in the protocols IN RELATION TO PATIENTS WITH YPT is very salient to this paper. In Table three, perhaps addressing how many of the high risk patients in each group were also found to have YPT and CS (rather than looking at the total number treated) would also give more information regarding characterization of these patients...

We completely agree with your comments. We should continue to use the same treatment regimen throughout the study period. However, as I described above, we experienced a patient with CS with conventional IVIG regimen. It was based on that patient that we decided to change our treatment strategy and adopt the RAISE protocol. The RAISE protocol was newly found to be effective for preventing the development of CS for KD patients. Thus, for the safety of the patient, we decided to change our treatment regimen to the RAISE regimen. We admit that this treatment change further complicated this study. As you describe above, we have added information regarding the number of high-risk patients in each group that were found to have YPT and CS in Table 3. We really appreciate your understanding regarding this matter.

In that same vein, I am not sure that the Figure 1 is very clear as there are many competing variables with your n. Perhaps splitting this figure into two (RAISE vs conventional) would also be helpful.

Following your suggestions, we have added the following text to the RAISE study group section, modified Figure 1 and added Figures 2 and 3.

Figures 2 and 3 show the medications and the number of patients overall, whose risk scores were ≥5 points, in the conventional group and the RAISE group, respectively.