PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Paediatrics Open. The paper was subsequently accepted for publication at BMJ Paediatrics Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease.
AUTHORS	Yang, Shuai; Song, Ruixia; Li, Xiaohui; Zhang, Ting; Fu, Jin; Cui, Xiaodai

VERSION 1 - REVIEW

REVIEWER	Gong, Fangqi Children's Hospital, Zhejiang University School of Medicine China Competing interests: no
REVIEW RETURNED	23-Aug-2017

GENERAL COMMENTS	In this manuscript entitled "Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease", the authors have performed a case-control study to detect the level of plasma TSP-2 and TSP-1 in children with KD, and to determine whether TSP-2 can serve as biomarkers to predict the response to IVIG treatment. A small sample was enrolled and the statistical methods were relatively simple in the present study. The conclusions have clinical meaning to some extent. However, this manuscript has several shortcomings which the authors should make some revises before publication. 1. The parameters of the day to receive IVIG treatment and the day to obtain blood specimen after KD onset should be added up to in the section of result, because these parameters were also the key factors to IVIG response. In order to better analyze the role of TSP-2, the two factors should be compared between IVIG responders and IVIG non-responders. For the same reason, if the parameters of inflammatory indicators such as WBC, CRP, neutrophils and platelets were also compared, the conclusion will be more persuasive. 2. The figures should be improved for readers to better understand, what did the ring and asterisk in the figure stand for?

REVIEWER	Hilliam, Rachel Mary The Open University UK Competing interests: None
REVIEW RETURNED	26-Sep-2017

GENERAL COMMENTS	This is a well written paper with the statistics, in general, well

	explained and correctly applied. When comparing the treatment with 2 control groups it would help the reader to state that the results given in the paper always refer to the control groups in the same order. For example in this sentence it is clear " There were no differences in age among the KD group, febrile group and healthy group (2.08 (1.00, 3.33) vs 3.54 (1.50, 4.34) vs 3.00 (2.00, 3.00) years, $\chi 2=5.21$, P=0.074). " However in the next section the first sentence states: "The concentration of TSP-2 in KD group was significantly higher than febrile group and healthy group (TSP-2: 31.00 (24.02, 39.28) vs 21.93 (17.00, 24.73) vs 16.23 (14.00, 19.64) ng/ml, $\chi 2$ $=50.24$, $P<0.001$). " I'm assuming the same ordering is used, ie febrile group and healthy group, but this should be made explicit throughout the paper to avoid confusion. What is less correct is the use that has sometimes been made of multiple testing of pairs, rather than using the Kruskall Wallis test. Why has this been done? For example "The age also had no significant difference between IVIG response group and IVIG non-response group (2.21 (1.17, 3.42) vs 1.75 (0.92, 3.59) years, Z=-0.63, P=0.526). " Such multiple testing increases the danger of reporting a significant effect when one doesn't exist. It would be useful to go through the paper and check that 95% confidence intervals are always given in the form (LL,UL) - this is the lower limit; comma; upper limit all surrounded by curly brackets. Sometimes this isn't the case. The section on ROC seems to end rather abruptly with no conclusion reached. It seems to leave the reader wondering if this was clinically important or not. With a few small tweaks this paper would be a useful addition to the literature.

REVIEWER	Male, Cristoph Department of Paediatrics, Medical University of Vienna, Austria Competing interests: none
REVIEW RETURNED	26-Sep-2017

GENERAL COMMENTS	Yang et al: Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease. Summary: The study showed that children with Kawasaki disease (KD) had higher Thrombospondin-2 (TSP-2) and Thrombospondin-1 (TSP-1) levels than children with febrile illness and healthy children. Among KD patients, those who were eventually unresponsive to intravenous immunoglobulin (IVIG) treatment had significantly higher TSP-2 levels than those who responded to IVIG treatment. The authors conclude that elevated plasma TSP-2 might be a useful predictor for IVIG-resistance in acute KD.

	Major critique: 1. The study is not a case-control study as stated in the manuscript but rather a cohort study: a cohort of KD patients is identified at diagnosis when risk factors, including laboratory testing for TSP, are assessed. Patients are then followed and assessed for response to IVIG as outcome parameter. The association between risk factors and outcome is analysed. Additionally, there are two external control groups (children with fever and healthy children) as reference for TSP levels in other conditions. 2. The most appropriate analysis would be logistic regression with TSP levels as determinant(s) and IVIG response as dependent variable. Other clinical factors, particularly gender which shows different distribution in non-responders (table 1), should be analysed as well by multivariate analysis. Potentially, a score composed of several variables could achieve an improved prediction of non-response. A limitation will be the rather small sample of patients. 3. The ROC analysis shows only a moderate predictive value of TSP-2 with an AUC of 0.75. At the chosen cut-off criterion for TSP-2, the specificity is about 65% (thus, about one third of non-responders would be misclassified). Previous studies have assessed various predictive scores composed of clinical parameters and cytokine levels that have achieved superior predictive values than TSP-2 levels in this study (ref. 3, 4, 13,14; Sato et al, Int J Rheum Dis 2013, etc.). The authors should discuss their results in comparison to previous studies. 4. The authors should expand on the clinical implications of a predictive score for IVIG non-response. What would be alternative treatment approaches if non-response could be predicted with reasonable certainty? Would the authors suggest withholding IVIG, or using additional or other treatment? Given the low specificity of TSP-2, would it be justified to take a different approach at initial treatment? Minor critique: 5. The Youden index used for the ROC analysis is mentioned in the discussion only. It should be presented in the methods section. 6. Page 10, line 11: ‘Firstly, the amount of patients …’ change to ‘ … the number of patients…’

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author

In this manuscript entitled “Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease”, the authors have performed a case-control study to detect the level of plasma TSP-2 and TSP-1 in children with KD, and to determine whether TSP-2 can serve as biomarkers to predict the response to IVIG treatment. A small sample was enrolled and the statistical methods were relatively simple in the present study. The conclusions have clinical meaning to some extent. However, this manuscript has several shortcomings which the authors should make some revises before publication.

1. The parameters of the day to receive IVIG treatment and the day to obtain blood specimen after KD onset should be added up to in the section of result, because these parameters were also the key factors to IVIG response. In order to better analyze the role of TSP-2, the two factors should be compared between IVIG responders and IVIG non-responders. For the same reason, if the parameters of inflammatory indicators such as WBC, CRP, neutrophils and platelets were also compared, the conclusion will be more persuasive.

Answers: We added and compared the parameters of the day to receive IVIG treatment and the day to obtain blood specimen after KD onset in the section of methods (Page 5 line 11, Page 6 line 3) and results (Page 7 lines 6, Page 17 table 1). We also added and compared the inflammatory indicators such as WBC, CRP, neutrophils and platelets (Page 6 line 8-11, Page 8 line 4-6, Page 8 line 12-15, Page 18 table 2, Page 19 line 22-23, Page 20 line1-4).

2. The figures should be improved for readers to better understand, what did the ring and asterisk in the figure stand for?

Answers: We added the explanation in the figure legends (Page 19 line 6-7 and line 15-16).

Reviewer: 2

Comments to the Author

This is a well written paper with the statistics, in general, well explained and correctly applied.

When comparing the treatment with 2 control groups it would help the reader to state that the results given in the paper always refer to the control groups in the same order. For example in this sentence it is clear

" There were no differences in age among the KD group, febrile

group and healthy group (2.08 (1.00, 3.33) vs 3.54 (1.50, 4.34) vs 3.00 (2.00, 3.00) years, $\chi 2=5.21$, $P=0.074$). "

However in the next section the first sentence states:

"The concentration of TSP-2 in KD group was significantly higher than febrile group and healthy group (TSP-2: 31.00 (24.02, 39.28) vs 21.93 (17.00, 24.73) vs 16.23 (14.00, 19.64) ng/ml, $\chi 2 =50.24$, $P < 0.001$). "

I'm assuming the same ordering is used, ie febrile group and healthy group, but this should be made explicit throughout the paper to avoid confusion.

Answers: We revised this sentence (Page 7 line 14-15).

What is less correct is the use that has sometimes been made of multiple testing of pairs, rather than using the Kruskall Wallis test. Why has this been done? For example, "The age also had no significant difference between IVIG response group and IVIG non-response group (2.21 (1.17, 3.42)

vs 1.75 (0.92, 3.59) years, Z=-0.63, P=0.526). " Such multiple testing increases the danger of reporting a significant effect when one doesn't exist.

Answers: We removed the multiple testing of pairs (Page 7 line 11-12).

It would be useful to go through the paper and check that 95% confidence intervals are always given in the form (LL,UL) - this is the lower limit; comma; upper limit all surrounded by curly brackets. Sometimes this isn't the case.

Answer: We went through the paper and checked the expression as the form mentioned (Page 2 line 14, Page 8 lines 10-11, revised figure 3).

The section on ROC seems to end rather abruptly with no conclusion reached. It seems to leave the reader wondering if this was clinically important or not.

Answers: We added the explanation in the text (Page 10 line 1-6).

With a few small tweaks this paper would be a useful addition to the literature.

Reviewer: 3

Comments to the Author

Yang et al:

Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease.

Summary:

The study showed that children with Kawasaki disease (KD) had higher Thrombospondin-2 (TSP-2) and Thrombospondin-1 (TSP-1) levels than children with febrile illness and healthy children. Among KD patients, those who were eventually unresponsive to intravenous immunoglobulin (IVIG) treatment had significantly higher TSP-2 levels than those who responded to IVIG treatment. The authors conclude that elevated plasma TSP-2 might be a useful predictor for IVIG-resistance in acute KD.

Major critique:

1. The study is not a case-control study as stated in the manuscript but rather a cohort study: a cohort of KD patients is identified at diagnosis when risk factors, including laboratory testing for TSP, are assessed. Patients are then followed and assessed for response to IVIG as outcome parameter. The association between risk factors and outcome is analysed. Additionally, there are two external control groups (children with fever and healthy children) as reference for TSP levels in other conditions.

Answers: We agreed with the reviewer's opinions and revised the paper (Page 2 line 4, Page 4 line 19).

2. The most appropriate analysis would be logistic regression with TSP levels as determinant(s) and IVIG response as dependent variable. Other clinical factors, particularly gender which shows different distribution in non-responders (table 1), should be analysed as well by multivariate analysis. Potentially, a score composed of several variables could achieve an improved prediction of non-response. A limitation will be the rather small sample of patients.

Answers: Because this is the first study to explore the predictive value of TSP for IVIG response, and the sample size is small as the reviewer mentioned, it is difficult to choose an appropriate cut-off point to transfer the continuous data of TSP to categorical data when using logistic regression. We are still accumulating specimens and trying this method when the number is large enough. Thank you for your advice.

3. The ROC analysis shows only a moderate predictive value of TSP-2 with an AUC of 0.75. At the chosen cut-off criterion for TSP-2, the specificity is about 65% (thus, about one third of non-responders would be misclassified). Previous studies have assessed various predictive scores composed of clinical parameters and cytokine levels that have achieved superior predictive values than TSP-2 levels in this study (ref. 3, 4, 13,14; Sato et al, Int J Rheum Dis 2013, etc.). The authors should discuss their results in comparison to previous studies.

Answers: We added the TSP-2 as a predictive marker in comparison to previous studies in the section of discussion (Page 8 line 20-21, Page 9 line 1-12, Page 9 line 21, Page 14 reference 14, Page 14 reference 16).

4. The authors should expand on the clinical implications of a predictive score for IVIG non-response. What would be alternative treatment approaches if non-response could be predicted with reasonable certainty? Would the authors suggest withholding IVIG, or using additional or other treatment? Given the low specificity of TSP-2, would it be justified to take a different approach at initial treatment?

Answers: We added clinical implications and alternative initial treatment approaches for IVIG non-response in the discussion section (Page 10 line 1-6, Page 11 line 13-14, Page 15 reference 18).

Minor critique:

5. The Youden index used for the ROC analysis is mentioned in the discussion only. It should be presented in the methods section.

Answers: “Youden index” has been presented in the methods section (Page 6 line 20).

6. Page 10, line 11: ‘Firstly, the amount of patients …’ change to ‘ … the number of patients…’

Answers: We revised this description (Page 11 line 7).

VERSION 2 – REVIEW

REVIEWER	Hilliam, Rachel Mary The Open University UK Competing interests: None
REVIEW RETURNED	20-Nov-2017

GENERAL COMMENTS	This paper is much improved and the responses to my comments have all been addressed. The authors have produced a useful paper of what is essentially a preliminary study. With further data a more thorough analysis can be undertaken, but this paper serves as a useful starting point.

REVIEWER	Gong, Fangqi Children's Hospital, Zhejiang University School of Medicine, No. 3333, Binsheng Road, Hangzhou 310052, PR China. Competing interests: No competing interests
REVIEW RETURNED	23-Nov-2017

GENERAL COMMENTS	In this manuscript entitled "Thrombospondin-2 Predict Response to Treatment with Intravenous Immunoglobulin in Children with Kawasaki Disease", the authors have performed a case-control study and determined that the plasma TSP-2 level was elevated in acute KD and it might be a novel predictor for IVIG-resistance, although the study sample is relatively small and the predictive value of TSP-2 for IVIG resistance in patient with KD should be further validated in clinical practice. The whole workflow is correct, the statistical methods are reasonable and the conclusion has clinical meaning to some extent. However, this manuscript has several weaknesses which the authors should be made some revises before publication. 1. TSP-2 and TSP-1 were compared between febrile control and KD patients. The samples from patients with KD were obtained before receiving IVIG treatment, because the fever duration is a key factor for the levels of inflammatory parameters, it would be more persuasive if the fever duration in both KD group and febrile group were compared when the sample was collected. 2. As shown in the Table 2, both TSP-2 and WBC were significantly increased in IVIG nonresponse group. To properly evaluate the role of increased TSP-2 in IVIGRKD, multivariable regression analysis should be performed in this paper. If the WBC and TSP-2 were all independent risk factors for IVIGRKD, the authors might optimize the ROC curve by jointing the two parameters.

VERSION 2 – AUTHOR RESPONSE