**Peer Review File**
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**Round 1**

***Reviewer comment:***

*Goto et al. performed a retrospective monocentric study in Japan between 2015 and 2017 in patients with septic shock who received IVIG for 5g/day for three days. It is of interest to demonstrate that low dose IVIG effect is not modified by hypogammaglobulinemia, as some authors could have suggested it.*

***Major Comments***

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**Comment 1:**

*Nevertheless, the authors conclude that IVIG is effective regardless of the preexisting deficit of gammaglobulins. As IVIG are not a standard treatment for septic shock and as their beneficial effect is not consensual, the authors cannot conclude that IVIG are effective regardless of hypogammaglobulinemia. This conclusion would be appropriate if a control group was available which is not the case.*

**Response 1:**

We thank the reviewer for the pertinent comment. Controlled studies are needed to confirm the efficacy of IVIG therapy, but ethical setting is difficult in daily medical care because the prognosis may be worse in the control group (IVIG non-treated group). To suit you pointed out, we have added and changed the sentence to DISCUSSIONS and CONCLUSIONS part as follows:

***Changes in the text:***

*ABSTRACT part*

***From***: "Our results suggest that the addition of low-dose IVIG as adjunctive therapy in patients with septic shock may provide therapeutic benefit regardless of hypogammaglobulinemia status."

***To***: "Our results suggest that the prognosis of low-dose IVIG as adjunctive therapy in patients with septic shock might be no different with and without hypogammaglobulinemia."

*DISCUSSIONS part*

Added "4) IVIG treated group is not compared with IVIG non-treated group. At present, it is not clear wherever IVIG therapy have efficacy for the septic shock patients. Further controlled studies are needed to confirm the efficacy of IVIG therapy, but ethical setting is difficult because the prognosis may be worse in the control group (IVIG non-treated group)." in Page 13-14, line 18, 1-4.

*CONCLUSIONS part*

***From***: "Low-dose IVIG administration as adjuvant therapy for sepsis may be effective regardless of hypogammaglobulinemia in septic shock patients."

***To***: “The present study found that the prognosis by low-dose IVIG therapy as adjuvant therapy for septic shock might be no different with and without hypogammaglobulinemia.” (Page 14, line 7-8)

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**Comment 2:**

*Another comment is the very low mortality in patients with septic shock. The population needs then to be better described, for example with subcomponents of SOFA scores. The expected mortality would be around 40-45 %.*

**Response 2:**

We thank the reviewer for the pertinent comment. The SOFA score during the ICU stay is median 9 points in both L group and H group, and the predicted mortality may be about 40 to 45% when using subcomponents of SOFA scores. However, in our past report (Yasuda N et al., J. Crit. Care, 36, 29-34, 2016), the 28-day mortality in patients with septic DIC with a more severe SOFA score of 11 was 20.9% (27/129). The mortality (12.5%, 10/80) of this study with SOFA score of 9 points in present study is not considered to be very low.

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**Comment 3:**

*Also, from a Methodological point of view a main outcome should be clearly stated as well as a main analysis and a necessary number of patients to be enrolled calculated.*

**Response 3:**

Thank you for pointing this out. The primary outcome assessed was 28-day survival probability. We added the calculation of sample size in METHODS part as follows:
“A planned sample size of 65 patients with all examination was estimated to provide 80 % power to detect a 10 % reduction in 28-day survival probability (90% to 80%) using a two-sided type-I error of 5%.” in Page 8, Line 3-5.

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***Minor Comments***

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**Comment 4:**

*Lost to follow up/please specify that every patient is included in the primary analysis and analysed if it is the case.*

**Response 4:**

Thank you for pointing this out. All patients (80 patients) were followed up to the death. We added this sentence to RESULTS part as follows:
“All patients were followed up to the death.” in Page 10, line 11.

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**Comment 5:**

*The missing values should be better shown as well as how it is dealt with.*

**Response 5:**

Thank you for pointing this out. To suit you pointed out, we added this sentence to DISCUSSIONS part as follows:
"The lack of laboratory data to evaluate the efficacy of IVIG in patients with or without hypogammaglobulinemia, such as serum IgG, is a concern when using the IVIG. In the present study, however, none of patients lack the laboratory data." in Page 13, line 9-11.

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**Comment 5:**

*In the table 1 please beware of abbreviations.*

**Response 5:**

Thank you for pointing this out. To suit you pointed out, we have added explanation abbreviations in Table 1-3.

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**Comment 6:**

*Please state for categorical variables n (%) also in the legend please state that Median and IQR are shown.*

**Response 6:**

Thank you for pointing this out. To suit you pointed out, we have added categorical variables n (%) and that continuous variables are shown as median and IQR in Table 1-3.

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**Comment 7:**

*In this table the criteria for septic shock need to be presented.*

**Response 7:**

Thank you for pointing this out. To suit you pointed out, we have added the criteria for septic shock in table 1 and modified our text in Page 5, line 18 and Page 6, line 1-3.

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**Comment 8:**

*I would also suggest to present a table with the outcomes, such as surgery, nosocomial infections and their types, use of catecholamines, use of artificial ventilation.*

**Response 8:**

Thank you for pointing this out. To suit you pointed out, we have added the type of nonsocomial infections, use of catecholamines, use of artificial ventilation in Table 1 and modified our text in Page 6, line 18, Page 7, line 1, and Page 9, line1-2.

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**Comment 9:**

*Figures are in my opinion not mandatory and could be summarized in a table. The survival curve is appropriate in my opinion*

**Response 9:**

Thank you for pointing this out. To suit you pointed out, we have changed Figure 2 and 3 to Table 2 and 3, respectively.

**Round 2**

***Reviewer A comment:***

**Comment 1:**

*First, the title must clearly indicate the comparison between those with and without hypogammaglobulinemia. In fact, the title did not accurately the purpose of this study, to examine whether the presence of hypogammaglobulinemia would influence the efficacy of low-dose intravenous immunoglobulin therapy. Please revise the title and make the clinical research design clear in the title. "Retrospective analysis" can not suggest the research design, which only indicate the data were collected retrospectively.*

**Response 1:**

We thank the reviewer for the pertinent comment. We changed the title as follows:

***Changes in the text:***

***From***: "Effects of low-dose intravenous immunoglobulin therapy in septic shock patients with hypogammaglobulinemia: a retrospective analysis"

***To***: "Effects of low-dose intravenous immunoglobulin as the adjunctive therapy in septic shock patients with and without hypogammaglobulinemia: a retrospective cohort study"

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**Comment 2:**

*Second, the abstract is inadequate. In the background, please explain the clinical needs for this research topic, and limitations and knowledge gaps in relation to the prognostic role of hypogammaglobulinemia in the adjunctive use of low-dose intravenous immunoglobulin. The title also needs to indicate add-on use of low-dose intravenous immunoglobulin. In the methods, the authors need to describe the inclusion of subjects, the assessments of hypogammaglobulinemia, the measurements of treatment efficacy outcomes, and main statistical methods for making the comparisons. In the results, please first report the number of subjects included in the analysis. Please report the outcome measures of the two groups and accurate P values. The comparability of the two groups should also be described. The conclusion should have comments on possible reasons for the negative findings.*

**Response 2:**

We thank the reviewer for the pertinent comment. To suit We added these sentence to the abstract parts as follows:

***Changes in the text (underline):***

Background: Intravenous immunoglobulin (IVIG) therapy has a reported adjunctive effect in the treatment of sepsis, but in light of results from a large-scale randomized control trial, evidence for improved prognosis with IVIG therapy is currently deemed insufficient. In recent years, there have been many reports of low serum immunoglobulin G (IgG) as a poor prognostic factor in septic patients. Under Japan's national health insurance system, IVIG is administered for severe infections at a dose of 5 g/day for three days (total 15 g or 0.3 g/kg). At present, IVIG administration is

not specifically formulated for septic patients with hypogammaglobulinemia. It is clinically significant to investigate whether serum IgG levels can serve as a biomarker to predict the efficacy of IVIG treatment for septic shock and help to identify those patients who might benefit from an adjunctive IVIG treatment. The purpose of this study was to compare the efficacy of this low-dose IVIG as an adjunctive therapy in septic shock patients with and without hypogammaglobulinemia.

Methods: In this retrospective cohort study, patients who received low-dose IVIG (5 g/day for 3 days) as adjuvant therapy for septic shock were enrolled. These patients were divided into two groups based on a median serum IgG level of 829 mg/dL prior to IVIG administration (< 829 mg/dL defined as hypogammaglobulinemia). To assess the efficacy of low-dose IVIG administration (0.3 g/kg), 28-day survival probability as the primary outcome, and the lengths of artificial ventilation and ICU stays as the secondary outcomes were compared using the Kaplen-Meyer method, the log-rank test, the Wilcoxon or Mann-Whitney U test.

Results: A total of 155 patients with septic shock that underwent IVIG treatment in the intensive care unit (ICU) were enrolled. These patients were divided into two groups based on a median serum IgG level. Survival probabilities at 28 days were 90.0% and 85.0% in the high- and low-level groups, respectively, and there was no significant difference between the two groups (P=0.457). There are not also significant differences in median lengths of artificial ventilation (9 vs. 12 days, P=0.215) and ICU stays (9 vs. 12 days, P=0.199) after IVIG administration. Logistic regression revealed that these clinical outcomes were not associated with serum IgG after adjusting for confounding factors as the antibiotics.

Conclusions: Serum IgG levels was not associated with the clinical outcomes by low-dose IVIG treatment. Our results suggest that the prognosis of low-dose IVIG as adjunctive therapy in patients with septic shock might be no different with and without hypogammaglobulinemia.

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**Comment 3:**

*Third, in the introduction, it is poor to use "has not been compared in septic shock patients with and without hypogammaglobulinemia" as the rationale of this study. The authors need to clearly describe the clinical significance of this research topic and analyze the limitations of previous studies on the prognostic role of hypogammaglobulinemia and knowledge gaps, to support the clinical needs of this study.*

**Response 3:**

We thank the reviewer for the pertinent comment. We added the calculation of sample size in INTRODUCTION part as follows:

"Current guidelines (11,12) do not recommend IVIG use as a routine part of the management of sepsis, however this recommendation is not specifically formulated for septic patients with hypogammaglobulinemia. It is not reported whether the presence of hypogammaglobulinemia require IVIG administration in septic shock patients. Here we thought to investigate whether serum IgG levels can serve as a biomarker to predict

the efficacy of IVIG treatment for septic shock. Initial levels of serum IgG might help to identify those patients who might benefit from an adjunctive IVIG treatment." in Page 5 , Line 3-10.

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**Comment 4:**

*Assessments of efficacy outcomes should be detailed. The sample size estimation was written in a confused way: why there was a reduction survival rate? The focus of this study is to compare the survival rates of two groups, not the reduction in rates after and before in a same cohort. In statistics, the authors should ensure P<0.05 is two-sided. Please specify the test of the comparability between the two groups and necessary adjustment analysis if the two groups are not comparable. Because this is an observational study, I suggest the authors to examine the hypogammaglobulinemia-outcomes association in multiple regression analysis, not direct comparisons, which can answer whether the association is significant and independent from other clinical covariates. Because of this, possible clinical covariates, in particular the main antibiotics, should be collected and adjusted in the analysis. The negative findings may result from the lack of adjustment analyses.*

**Response 4:**

Thank you for pointing this out. The sample size estimation was described in the wrong way. There is a 10% difference in survival rate compared with both groups, not the reduction in rates after and before in a same cohort. We changed this sentence in Page 8, line 12. We also assessed possible influencing factors in the hypogammaglobulinemia-outcomes association, such as the antibiotics using logistic regression modeling. In the results, 28-day survival, the duration of artificial ventilation and ICU stays, and the main antibiotics (carbapenem, anti-MRSA and anti-fungal) were not associated with serum IgG levels. We added these results in Page 8, line 7-8, 12, Page 10, line17-18, Page11, line1-5 and Table 4.

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***Reviewer B comment:***

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**Comment**

*Goto et al. is a retrospective study for ICU patients who received low-dose IVIG as adjuvant therapy for septic shock. Patients with septic shock were divided into two groups based on a median serum IgG level, the duration of artificial ventilation and ICU stays of the low-dose IVIG administration in the two groups was compared. The results showed that there was no significant difference between the two groups. There are not also significant differences in lengths of artificial ventilation and ICU stays after IVIG administration. The study concluded that the prognosis of low-dose IVIG as adjunctive therapy in patients with septic shock might be no different with and without hypogammaglobulinemia.*

*There is a clear concern from the authors that the lack of lab data to evaluate efficacy of IVIG therapy in patients with/without hypogammaglobulinemia, such as serum IgG,*

*is a concern when using the IVIG. In this study, however, none of patients lack the lab data. The four limitations of the study are clearly presented in the discussion section. The study included 2027 patients who were admitted to the ICU during the study period of these patients 155 had septic shock were included in the study and was approved by ethical review board and individual consent was waived. Statistics are well presented in tables and figures and are easy to follow.I find this article informative and of educational value.*

**Response:**

*Thank you for your evaluation of this paper.*