Peer Review File

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Reviewer A

This is a very interesting topic that needs further investigation in the pediatric population. The following are some recommendations to improve the content and quality of this manuscript.

Line 60: Milrinone is described as a "medication most frequently used for children with sepsis is milrinone (a type 2 III phosphodiesterase inhibitor), which has been considered to increase contractility and improve ventricular diastolic function".

References (1) : S. L. Weiss, M. J. Peters, W. Alhazzani, M. S. D. Agus, H. R. Flori, D. P. Inwald, et al.. Pediatr Crit Care Med 2020 Vol. 21 Issue 2 Pages e52-e106. DOI: 10.1097/PCC.0000000000002198 explains:

Reply: Thank you very much for this comment. The reviewer's recommendations regarding consensus were adjusted in the text according to the recommended texts.

Changes in the text: Line 77-81 and 87-88.

33) We were unable to issue a recommendation about adding an inodilator in children with septic shock and cardiac dysfunction despite other vasoactive agents. However, in our practice, we sometimes use inodilators in children with septic shock and evidence of persistent hypoperfusion and cardiac dysfunction despite other vasoactive agents.

Rationale: There are no RCTs of inodilators (including milrinone, dobutamine, or levosimendan) in children with septic shock with persistent hypoperfusion and cardiac dysfunction. A report of two children described improvement in cardiac output with addition of inodilators. A case series of 10 children with meningococcal septic shock treated with milrinone described improved core-to-peripheral temperature gradient, with stable blood pressure and no change in acidosis. These data were not sufficient to formulate a recommendation. However, in our practice, 77% of panel members reported at least sometimes using inodilators in children with septic shock who had evidence of persistent hypoperfusion and cardiac dysfunction despite other vasoactive agents, typically in a PICU with advanced hemodynamic monitoring available.

Reference (2). Latin American Consensus on the Management of Sepsis in Children: Sociedad Latinoamericana de Cuidados Intensivos Pediatricos [Latin American Pediatric Intensive Care Society] (SLACIP) Task Force: Executive Summary J. Fernandez-Sarmiento, D. C. De Souza, A. Martinez, V. Nieto, J. Lopez -Herce, V. Soares Lanziotti, et al.

J Intensive Care Med 2022 Vol. 37 Issue 6 Pages 753-763. DOI: 10.1177/08850666211054444

This reference recommends the following management. This reviewer could not find a description for the use of milrinone.

Reply: Thank you very much for this comment. We corrected the reference on this line and reference 2 was eliminated. Only references 1 and 4 are cited, which specifically mention milrinone in sepsis.

Changes in the text: Line 77.

7. VASOACTIVE MANAGEMENT

7.2 We recommend using adrenaline as the drug of choice, reserving noradrenaline for cases with clinical or monitoring evidence of low peripheral vascular resistance. (Strong recommendation, low level of evidence.)

For the third reference after this statement, the authors refer to "Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. Hoffman et al. Circulation 2003 Vol. 107 Issue 7 Pages 996-1002. DOI: 10.1161/01.cir.0000051365.81920.28.

This paper refers to post-cardiac patient developing low cardiac output syndrome. It is a different population compared to patients with sepsis.

The authors also refer to the 77% of the committee members who ocassionally use milrinone under certain hemodyamic circumstances.

I will recommend to re-write this paragraph to prevent confusion among the readers related to the current sepsis guidelines, and to highlight that milrinone is occasionally used for sepsis, particularly severe sepsis associated to meningococcemia as described in your bibliography (References 6 and &).

Reply: Thank you very much for the valuable comment. We re-wrote the paragraph to make it less confusing. Reference 8 was replaced by a recent meta-analysis of the systemic vasodilatory effects of milrinone.

Changes in the text: Line 77-81 and 87-88.

The authors mimic the word "inotrope" which includes epinephrine for example, to vasopressor, needed for low blood pressures, particularly in septic shock (Low dose epinephrine has a beta agonistic effect and works as an inotrope, while at higher doses has an alpha effect, working as a vasopressor, similar to norepinephrine, whose main activity is on the alpha receptors. Milrinone is considered an inodilator, having an effect at the cGMP intracellularly in the myocytes and also inducing a degree of hypotension.

Reference: Pharmacotherapy update on the use of vasopressors and inotropes in the intensive care unit. J. C. Jentzer, J. C. Coons, C. B. Link and M. Schmidhofer. J Cardiovasc Pharmacol Ther 2015 Vol. 20 Issue 3 Pages 249-60. DOI: 10.1177/1074248414559838

Reply: Thank you for this valuable appreciation. We clarify the word inodilator in lines 90 and 91 to avoid confusion.

Changes in the text: Line 90 and 92

Line 73: the authors are using sepsis, severe sepsis, and septic shock interchangeable. It is unlikely to find myocardial depression in sepsis, it is more common in the progressive phases of septic shock. The terms keep changing. This reviewer is attaching a recent publication defining sepsis and pediatric shock. Please refer to table of the Phoenix Sepsis Score.

Reply: Thank you so much. Throughout the text we clarify that our study was in patients with septic shock. We clarify the term to avoid reader confusion.

Thank you very much for sharing Phoenix Pediatric sepsis score. We had the opportunity to participate and give our appreciation from Colombia to the author (Nelson Sanchez-Pinto) and a co-author from Colombia (Juan Camilo Jaramillo). Since Phoenix Pediatrics criteria had not been published when we did our research, the previous definition was used.

Changes in the text: line 145,146 is sepsis definition.

Between line 82 and 84, I recommend include a paragraph of the current findings at the microvascular level for pediatric patients in septic shock so that it can introduce the main objective for this study.

Reply: We add this comment in lines 105 to 108. This paragraph talks about microcirculation and thus we maintain the common thread of the message. We seek to make it clearer for the reader. Additionally, we added reference 13 where this important topic is reviewed.

Changes in the text: Line 105-108.

Line 111: What vasoactive medications and doses were the patients receiving prior to enrollment? Why did patients only received 10 ml/kg when guidelines recommend 40 cc/kg after admission to the PICU?

Reply: Norepinephrine at the discretion of the treating doctor (line 130 was clarified). They only received 10 cc/kg because that is what the Latin American consensus recommends (ref 2). The objective is to avoid water overload. A recent investigation by our group found that fluid overload is associated with higher mortality in children with sepsis (BMJ Paediatr Open. 2023 Nov;7(1):e002094. doi: 10.1136/bmjpo-2023-002094.), as other groups had previously found.

Changes in the text: Line 130

Line 112: Please define what SCC stands for in this paper.

Reply: Surviving Sepsis Campaign (SSC) Changes in the text: Line 132

Line 93: Sentence is incomplete

Reply: We make the correction

Changes in the text: Line 93

Line 167: What are the MVHS values for pediatric patients? Do they vary according to age? Are those available? The randomization process is not described and Table 1 demonstrates that younger patients received milrinone while older patients did not.

Reply: Pediatric MVHS values and its variation with age are not available. We rely on the reference values 19 and 20 as described on line 186-188. Non-probabilistic convenience sampling was used, taking patients who consecutively entered the ICU with a diagnosis of septic shock. (line is clarified 119-121). No randomization was done due to the type of study design. As the younger patients received milrinone (Table 1), age was considered a confounding co-variable and we sought to control it from the multivariate analysis (line 205,206).

Changes in the text: 119-121 lines

Could your statement in line 214 be associated with the age of the patient and not to the fact they received milrinone?

Reply: We do not consider because the group without milrione had a wide age range. In fact, the distribution of the variable was non-normal (medians-extreme values) and we had an age range from 0.75 years (9 months) as seen in Table 1. Changes in microcirculation in children do not modify with age as has been recently demonstrated (ref 33). We add this reference and explanation to the discussion, so that the reader understands that changes in microcirculation are not explained by different ages (line 305-308).

Changes in the text: Line 305-308

How many times and when were the patients evaluated for microcirculatory changes?

Reply: On line 129 the times are specified. It was taken upon admission to the PICU, 6 and 24 hours after starting milrinone.

Changes in the text: Line 129,139,140

Were all the patients endotracheally intubated?

Reply: We clarify in the materials and methods section (line 159-163) ventilatory support and in results (line 221,222).

Changes in the text: Line 221,222.

Line 223-228: Did all patients had similar microcirculatory findings prior to milrinone or did it vary by age?

Reply: Correct. Baseline findings in microcirculation were similar (without statistical differences) in the two groups (Figure 1). There were no changes with age. We clarify on the line 230.

Changes in the text: Line 230.

Table 1: please describe the reason why the age groups with and without milrinone are so different? Could that influence your findings?

Reply: As we mentioned previously, there are no differences in the different pediatric age groups in microcirculation findings. As it was a non-probabilistic sampling (known as convenience) this type of imbalance can be observed (Setia MS. Methodology Series Module 5: Sampling Strategies. Indian J Dermatol. 2016 Sep-Oct;61(5):505-9). The way to handle this situation from a statistical and research point of view is to carry out a multivariate analysis as was actually carried out. In line 210 (statistical methods) it is explained that age was included as a confounding co-variable. In the multivariate analysis (Table 2) we indicate that the age variable was included. We consider that these age differences did not affect the results from a statistical or clinical point of view (as shown in Table 2).

Changes in the text: line 210 and Table 2

Line 253: This sentence is confusing. This reviewer assumes all patients with septic shock included in the study prior to milrinone had changes in the microcirculation. Needs to be rephrased.

Reply: We clarify this phrase. Due to the new structure with the adjustments of the manuscript, it remained on line 249,250.

Changes in the text: Line 249,250.

Line 259: Was that a finding related to age and not disease process? How was the "higher inflammation" diagnosed by the authors?

Reply: Higher inflammation was defined with ferritin greater than 500 mg/dl as an inflammatory phenotype (clarified on line 166) and is described in Table 2.(Carcillo JA et al. Why and How Is Hyperferritinemic Sepsis Different From Sepsis Without Hyperferritinemia? Pediatr Crit Care Med. 2020 May;21(5):509-512). We add this reference.

Changes in the text: line 166 and reference 16.

Line 285: The authors refer to the milrinone effects in children, but the reference describes an adult population.

Reply: We clarify that this DeBacker study is in adults.

Changes in the text: Line 286

Line 354: Is there data in the pediatric population to make this statement?

Reply: We believe it refers to line 376. We eliminate the inflammatory response statement.

Changes in the text: Line 376

Line 364: It will be ideal to initially understand the microcirculatory differences between ages in sepsis and septic shock among pediatric patients that can lead us to understand the effects of different medications.

Reply: Correct. However, in healthy children, no changes have been observed in microcirculation at different ages (ref 33). In children with sepsis, it has been described by our group that there are changes in microcirculation associated with sepsis but not related to age. . (BMC Pediatr. 2024 Jan 20;24(1):68. doi: 10.1186/s12887-024-04524-5.)

Changes in the text: N/A

Why are the starting points in Figure 1 so far apart? Seems the patients were not at the same stage of infection.

Reply: As described in line 228 "Capillary density (4-6 CD) at baseline in the group that received milrinone was 22.5 mm/mm2 (IQR 15.4-43.8) and 36.8 mm/mm2 (IQR 20.3-51.7) in the group without milrinone (p=0.17)." These differences in capillary density were not significant. The interesting thing about the figure is to observe how the group with milrinone modifies the natural history of CD 4-6 in sepsis. While in the group without milrinone a slow decline continues. Additionally, Table 1 shows no differences in the PELOD-2 scale at baseline

Changes in the text: N/A.

Table 1 requires further work. How many times were the patients assessed? The lactate was not different at the beginning or after treatment (was it 24 hours later). Please clarify in the method section. The PELOD score was similar for everyone, however, it is not clear how many patients had other vasoactive medications, what types, how many were endotracheally intubated. Were the patient with GI disease dehydrated when they started the study?

Reply: Patients were evaluated at the time of admission, 6 hours and 24 hours (line 129,130). Table 1 only describes the characteristics at baseline (descriptive or univariate analysis). The other data are described in the text and figure 1, 2, and 3. We clarify in the results section that lactate was taken only upon admission and that it was related to capillary refill time (line 261). The information about other vasoactive agents was clarified in line 132. Intubation was clarified in line 159-164 and in the results section (line 221-222). Patients with gastrointestinal disease were not dehydrated.

It is important to specify that Table 1 are the baseline characteristics of the patients only.

Changes in the text: Line 129,130, 221, 222, 261.

Figure 3: Is that data represented at 24 hours?

Reply: Correct. It was clarified when reading figure 3 (line 555).

Changes in the text: Line 555

This is a very interesting topic. This reviewer recommends expanding on the initial assessment of the microcirculation in patients meeting criteria for septic shock. That would be valuable information for the readers.

Reply: Thank you very much for the comment. The term septic shock was clarified throughout the manuscript. Comprehensive information on microcirculation is described: capillary density, blood flow and structural damage (glycocalyx degradation). We consider this information to be very valuable for readers.

The comments you gave us have been very valuable and have made our manuscript clearer and more robust in all aspects. Thank you so much

Reviewer B

Authors are to be congratulated for their diligent efforts and work in studying microcirculation dynamics following milrinone using direct observation, which has not been implemented in conventional plasma biomarker studies. Furthermore, as the authors stated, the results from this study aid in identifying novel phenotypes in critically ill children, which is a quite relevant contribution to critical care. However, it is imperative to address several methodological shortcomings inherent to the study design and analytical approach.

1. The primary outcome was stated as “the association between microcirculation and endothelial glycocalyx changes related to the use of milrinone”. However, although the perfused boundary region (PBR) observed by sublingual video microscopy is correlated with the thickness of the glycocalyx, sublingual video microscopy does not directly observe the thickness of the glycocalyx. This raises a concern for the reliability of the outcome/objective statement.

Reply: Thank you very much for these valuable comments. It's right. The video microscope indirectly measures the thickness of the glycocalyx. It doesn't do it directly. The PBR is the distance between the red blood cell and the endothelial glycocalyx. We did not have funding to measure biomarkers such as syndecan-1 or endocan in all patients. However, the video

microscope software has several factors that provide reliability: 1. It processes the data obtained in the software without influence from the researcher. 2. Take more than 300 images, selecting the best ones for analysis. 3. It has high inter- and intraobserver agreement (ref 17). In the limitations section we added a comment in this sense (line 350-353).

Changes in the text: Line 350-353.

2. L109, all consecutive pediatric patients admitted to an ICU were included in the analysis, for whom, sublingual video microscopy was used to measure microcirculation dynamics. In the following L117, it states that "Patients who received milrinone within the first six hours after admission (considered as the baseline measurement) were included". This makes it unclear about the targeted population. As the primary objective is comparing those who received milrinone and those who did not receive it, it contradicts the statement in L117.

Reply: Thank you so much. The second sentence confuses the reader because all the patients were included consecutively. This second sentence of L 117 was deleted.

Changes in the text: Line 139 ne w peer-reviewed version

3. As this is a cohort study, inclusion and exclusion criteria may give an additional concern about selection bias. A patient flow chart should be described as transparent documentation of the sample size of the targeted population, study population, and those who were excluded from the analysis.

Reply: Thank you so much. We add Supplementary material where it is included to the patient flow chart.

Changes in the text: Supplementary material. Line 219

4. L190 describes that confounders were controlled from the design, considering the inclusion and exclusion criteria, particularly disease severity and age. However, Table 1 describes incomparable age distributions between those who received milrinone and those who did not receive it. It is unclear how the authors' aim in controlling confounders was achieved for controlling for age as a potential confounder.

Reply: Thank you so much. The phrase was confusing. Age was a covariate that we considered had confounding behavior. He sought to control not from the design but from the analysis with the multivariate model that was applied. Logistic regression was performed with the forward method (ref 21).

Changes in the text: Line 211-213

5. For a secondary analysis, pediatric patients with sepsis were further stratified by 2 years old. The result section describes that this 2-year-old threshold is based on the median age in the

studied population. If so, this should be described in the method section with biological assumption on why this 2-year-old may act as a confounder and it needs stratified analysis.

Reply: Thank you. It was clarified in the methods section, line 211-213

Changes in the text: Line 211-213

6. For univariate analysis against demographics, it is stated that the Chi-square test or Fisher exact test was used for PBR value. As the PBR value is an interval value, these tests cannot be applied.

Reply: Thank you so much. It was a transcription error. It was corrected on line 206.

Changes in the text: Line 206

7. Microcirculation estimates were compared from the baseline (upon admission), post-6 hours, and post-24 hours after milrinone administration. As the values in each three groups are correlated across these time points, paired analysis or time-series model adjusting for individual correlation is appropriate, and an independent t-test or Mann-Whitney U test is inadequate test for this case. It is unclear how these groups were compared. This concern also applies to other instances, such as comparing the CBV-Rel between at admission and at 24 hours.

Reply: The paragraphs we had written were confusing. Thank you very much for this observation. We clarify that testing three or more measurements of dependent variables was microcirculation. In these cases, repeated measures ANOVA or the Friedman test was used for non-normally distributed variables. It was clarified in the line 207-209. The laboratory tests were measured at admission and 24 hours later, which was where t-student or Mann-Whitney U was applied.

Changes in the text: Line 207-209.

8. Microcirculation estimates were compared at 24 hours and at admission, indicating a 28% reduction in the recruitment of these vessels in the group that did not receive milrinone. The method section describes that sublingual microcirculation was measured with video microscopy on admission, and 6 and 24 hours after beginning milrinone. It is unclear how these time stamps for measuring microcirculation were applied for those who did not receive milrinone.

Reply: Thank you very much for the comment. The sentence we had written is not clear. It was also measured in the group without milrinone at admission, 6 and 24 hours. It was clarified in materials and methods (line 130) and in results (line 234).

Changes in the text: Line 130, line 234

9. L216 states that “The 4-6 CD in the group that received milrinone was higher in children under two years old (48% 23/48; 24% 8/33; aOR 0.33; 95% CI 0.12-0.89; p=0.026) compared with older children.“ The 4-6CD is a density metric. It is unclear how proportion was applied. If so, proper documentation is necessary for describing the threshold to separate groups according to the 4-6CD and its biological assumption. In addition, the use of the odds ratio should be described in the method section.

Reply: The proportion of patients with a value greater than the median obtained for each age group (younger and older than two years) at 24 hours was indicated. But the reviewer's comment is very valid. This approach can be confusing for the reader. We prefer to report values in numerical terms. That sentence was deleted and rewritten reporting the medians in each age group (lines 238-241).

Changes in the text: Lines 238-241.

10. L242-246 describes the correlation analysis between vital signs and microcirculation metrics. However, this correlation analysis was not described in the method section. In addition, it is unclear if the distribution of the interested variables satisfied the linear assumption.

Reply: Thank you. Apologies for this omission. Spearman's rank or Pearson correlation was performed according to non-normal or normal distribution of the continuous variable respectively. It was added in the statistical methods section, line 210-211.

Changes in the text: Line 210-211.

11. Some words contain typing errors or need to be spelled out. L116, 2mmol lacks the denominator; L159, D; IQR; L204, 0.5812.1; L234, 2.65.7.

Reply: We review and correct typographical errors. Thank you so much.

Changes in the text: Line 190, 239,242.

12. L162 describes that a higher figure in CBV-Rel indicates better blood volume arriving for exchange. As CBV-Rel is calculated by taking the blood volume in the larger capillaries divided by the smaller capillaries, a large value in CBV-Rel may indicate diminished blood flow to smaller capillaries. It is unclear what “better blood volume” means.

Reply: Thank you. We clarify the sentence on line 184-187.

Changes in the text: Line 184-187

Reviewer C

The introduction is well written, and given what is known about the subject from pre-clinical and clinical adult studies, the hypothesis is pertinent to providers who care for children with sepsis.

The methods used are sound and described thoroughly. My only minor suggestion for the authors is to consider a separate analysis that includes children who received crystalloids before admission to the PICU. Doing so might show potential benefits or harm from using milrinone in that group. Furthermore, in the clinical setting, most children admitted with sepsis to the PICU receive crystalloids before admission. Therefore, the study's findings might be more applicable to a broader group of patients with sepsis cared for in the PICU.

Reply: Thank you very much for this valuable suggestion. Unfortunately, all patients had received a bolus of crystalloid, at least 10 mL/kg (line 131). Additionally, we excluded all patients who had received a crystalloid bolus before admission to the PICU to avoid causing confusion (line 146). We added a comment on limitations regarding this topic.

Changes in the text: Line 358,359

In line 233, it is stated that the children in the group remained at levels similar to admission, but the p-value suggests that there was at least a trend of improvement. Can you please look into this and elaborate? This might further change the discussion arguments made further down below.

Reply: Thanks for the comment. More details were given on lines 238 and 239. Indeed, we observed a trend towards improvement with the milrinone group. Modified the natural history of microvascular damage in sepsis. We believe that this improvement in microcirculation was achieved by milrinone indirectly (systemic vasodilator effect on macrocirculation) and directly by improving capillary recruitment capacity and reducing damage to endothelial structures (less degradation of the glycocalyx).

Changes in the text: Line 238, 239.