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# *Sepsis associated multiorgan failure phenotypes, physiopathology and biomarkers in pediatrics: a systematic review*

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Trabalho realizado sob a orientação de:

ANDREA SOFIA DA SILVA DIAS

BÁRBARA CECÍLIA BESSA DOS SANTOS OLIVEIROS PAIVA

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# SEPSIS ASSOCIATED MULTIORGAN FAILURE PHENOTYPES, PHYSIOPATHOLOGY AND BIOMARKERS IN PEDIATRICS: A SYSTEMATIC REVIEW

Beatriz Simões[1], Bárbara Oliveiros[2,3], Andrea Dias[1,4]

[1]Faculty of Medicine, University of Coimbra, Portugal.

[2]Laboratory of Biostatistics and Medical Informatics, LBIM – Faculty of Medicine, University of Coimbra, Portugal.

[3]Coimbra Institute for Clinical and Biomedical Research, iCBR - Faculty of Medicine, University of Coimbra, Portugal.

[4]Serviço de Cuidados Intensivos Pediátricos, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

**CORRESPONDENCE:**

Andrea Sofia da Silva Dias, Serviço de Cuidados Intensivos Pediátricos, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra.

Address: Avenida Afonso Romão, 3000-602, Coimbra, Portugal.

E-mail: sofia.andrea@gmail.com

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## ABBREVIATIONS

| | |
|---|---|
| **ADAMTS** | A disintegrin and metalloproteinase with thrombospondin type 1 motif |
| **CD** | Cluster of differentiation |
| **CRP** | C-reactive protein |
| **DIC** | Disseminated intravascular coagulation |
| **ECMO** | Extracorporeal membrane oxygenation |
| **FMO** | Falência multiorgânica (Multiple organ failure) |
| **GM-CSF** | Granulocyte-macrophage colony-stimulating factor |
| **HBD** | Hepatobiliary dysfunction |
| **HLA** | Human leucocyte antigen |
| **IFN** | Interferon |
| **IL** | Interleukin |
| **IPMOF** | Immunoparalysis associated multiple organ failure |
| **LPS** | Lipopolysaccharide |
| **MALS** | Macrophage activation-like syndrome |
| **MAS** | Macrophage activation syndrome |
| **MOF** | Multiple organ failure |
| **NK** | Natural killer |
| **OFI** | Organ Failure Index |
| **PD** | Programmed cell death |
| **PDL** | Programmed cell death ligand |
| **PICU** | Pediatric Intensive Care Unit |
| **PRISM** | Pediatric RISk of Mortality |
| **RRT** | Renal replacement therapy |
| **SD** | Standard deviation |
| **SHLH** | Secondary hemophagocytic lymphohistiocytosis |
| **SMOF** | Sequential multiple organ failure |
| **SOFA** | Sequential Organ Failure Assessment |
| **TAMOF** | Thrombocytopenia-associated multiorgan failure |
| **TNF** | Tumor necrosis factor |
| **vWF** | Von-Willebrand factor |

## ABSTRACT

### BACKGROUND

Sepsis is a life-threatening condition occurring due to a dysregulated immune response to an infectious pathogen. It is a complex and multifaceted disease with important heterogeneity between affected individuals, which explains in part how challenging the management of sepsis can be and why it plays a major role in pediatric mortality. With this review we aim to compare different multiorgan failure phenotypes in septic children regarding their physiopathology, differentiated biomarkers and correlation with adverse clinical outcomes. We believe such knowledge will contribute to diagnosis accuracy, therapeutic individualization and, consequently, clinical outcome improvement.

### METHODS

Observational studies and randomized-controlled trials with a study population of children until 18 years of age, admitted to the Pediatric Intensive Care Unit with the diagnosis of sepsis, severe sepsis or septic shock and with studied variables such as biomarkers, flow cytometry, proteomics, lipidomics, mortality in Pediatric Intensive Care Unit, length of stay, requirement of mechanical ventilation, renal replacement therapy or extracorporeal membrane oxygenation were included. Studies that included only neonates or adults were excluded.

For this purpose, PubMed, Embase and Cochrane Central Register of Controlled Trials databases were searched in October 2022. Obtained articles were screened for inclusion criteria by two reviewers. Data of included studies were extracted using a predesigned and tested form. Evidence tables of the selected studies were produced regarding study and patient characteristics, outcome measurements and quality.

### RESULTS

Three studies were identified. Two studies included a total of 501 children diagnosed with severe sepsis, of which 138 had multiple organ failure with one or more associated phenotype. Immunoparalysis was the most prevalent phenotype, followed by thrombocytopenia-associated MOF and sequential MOF. Subsequent MAS development was observed mostly in TAMOF and SMOF phenotypes. Peak ferritin levels were higher among TAMOF patients. Mortality was 42% in TAMOF, 31% in SMOF and 20% in immunoparalysis subgroups. Children with TAMOF required more renal replacement therapy. SMOF subgroup had the longest length of stay.

The remaining study included the same 404 children of the second included study and identified four phenotypes (PedSep-A, -B, -C and -D) based on common characteristics between patients. Immunoparalysis was observed similarly in PedSep-B, -C and -D subgroups, but PedSep-D was the most associated with TAMOF, SMOF and MAS

development. PedSep-D patients also had the highest peak ferritin, highest mortality, required more renal replacement therapy and had the longest length of stay.

**DISCUSSION**

Hyperinflammatory phenotypes (thrombocytopenia associated MOF, sequential MOF and macrophage activation syndrome) exhibited the worse clinical outcomes in our review, but immunoparalysis remained the most prevalent clinical presentation among septic children. Moreover, children with hyperinflammation features and simultaneous immunoparalysis demonstrated to have poorer prognosis leading to a challenging therapeutic dilemma.

The clinical heterogeneity and severity evidenced by the presence of MOF with an associated phenotype, strengthen the need for validated and accessible criteria for phenotype identification in each septic child, aiming to provide personalized adjunctive therapies with the objective of immune and organ function restauration.

This review is registered in PROSPERO (CRD42022348092).

**KEY WORDS:** pediatric; sepsis; multiorgan failure; immune response; phenotype.

## RESUMO

### INTRODUÇÃO E OBJETIVOS

A sépsis ocorre devido a uma resposta imunitária desregulada contra um microrganismo infecioso. É uma doença complexa e multifacetada com importante heterogeneidade entre indivíduos, o que explica a sua gestão clínica desafiante e o seu papel na mortalidade pediátrica. Com esta revisão, pretendemos comparar diferentes fenótipos de falência multiorgânica em crianças com sépsis, nomeadamente fisiopatologia, biomarcadores e correlação com desfechos clínicos adversos. Acreditamos que esse conhecimento possa contribuir para a precisão diagnóstica, individualização terapêutica e melhoria dos resultados clínicos.

### MÉTODOS

Incluímos estudos observacionais e ensaios clínicos randomizados e controlados com uma população de crianças desde o nascimento até aos 18 anos de idade, admitidas na Unidade de Cuidados Intensivos Pediátricos com o diagnóstico de sépsis, sépsis severa ou choque séptico e variáveis estudadas que incluíssem biomarcadores, citometria de fluxo, proteómica, lipidómica, mortalidade na Unidade de Cuidados Intensivos Pediátricos e no dia 28 após admissão, necessidade de ventilação mecânica, terapêutica de substituição renal ou de oxigenação extracorpórea.

As bases de dados PubMed, Embase e Cochrane Central Register of Controlled Trials foram pesquisadas em Outubro de 2022. Os artigos obtidos foram revistos de acordo com os critérios de inclusão por duas revisoras. Os dados dos estudos incluídos foram extraídos utilizando um formulário preparado para o efeito. Foram produzidas tabelas de evidência acerca das características dos estudos selecionados, participantes, *outcomes* e qualidade.

### RESULTADOS

Três estudos foram identificados. Dois estudos incluíram, no total, 501 crianças diagnosticadas com sépsis severa, das quais 138 tinham falência multiorgânica com um ou mais fenótipos associados. A imunoparalisia foi o mais prevalente, seguido da trombocitopenia associada a FMO e FMO sequencial. Nestes dois observou-se mais frequentemente o desenvolvimento subsequente de síndrome de ativação macrofágica. Os níveis de ferritina foram superiores nos doentes com trombocitopenia associada a FMO, cuja mortalidade foi 42%, superior ao observado no subgrupo de FMO sequencial (31%) e de imunoparalisia (20%). As crianças com trombocitopenia associada a FMO necessitaram mais frequentemente de terapêutica de substituição renal. O internamento foi mais prolongado em doentes com FMO sequencial.

O terceiro estudo incluiu a mesma população que o segundo, e identificou quatro fenótipos de acordo com características comuns entre doentes (PedSep-A, -B, -C e -D). A imunoparalisia estava presente nos subgrupos PedSep-B, -C e -D. Este último estava mais associado a trombocitopenia associada a FMO, FMO sequencial e síndrome de ativação macrofágica, exibindo maiores níveis de ferritina, mortalidade, necessidade de terapêuticas de suporte e internamento mais longo.

**DISCUSSÃO**

Os fenótipos hiperinflamatórios (trombocitopenia associada a FMO, FMO sequencial e síndrome de ativação macrofágica) demonstraram piores desfechos clínicos, mas a imunoparalisia permaneceu o fenótipo mais prevalente. Adicionalmente, crianças com sépsis e características simultâneas de imunoparalisia e hiperinflamação evidenciaram pior prognóstico, tornando a decisão terapêutica desafiante.

A heterogeneidade e gravidade clínicas na presença de fenótipos associados a FMO reforçam a necessidade de critérios validados e acessíveis para a estratificação da criança com sépsis, e assim instituir terapêutica individualizada atempadamente, tendo em vista a recuperação da função imune e orgânica.

Esta revisão está registada no PROSPERO (CRD42022348092).

**PALAVRAS-CHAVE**: pediatria, sépsis, falência multiorgânica, resposta imunitária, fenótipo.

## INTRODUCTION AND OBJECTIVES

Sepsis is a life-threatening condition occurring due to a dysregulated response to an infectious pathogen.[1] The global burden of pediatric and neonatal sepsis study estimated a global incidence of 48 sepsis and 22 severe sepsis cases in children per 100 000 person-years, with an approximate pediatric mortality of 11% due to severe sepsis, highlighting the important contribution of sepsis to pediatric fatal outcomes, especially in neonates.[2]

In the presence of a microbial infection, pathogen-associated molecular patterns are recognized by pattern-recognition receptors which promptly activate the innate immune system, and when prolonged and persistent, this activation results in the “cytokine storm”, an excessive release of pro-inflammatory cytokines. Along with complement activation, in some patients, this “cytokine storm” will turn a physiological response into an exuberant and damaging hyperinflammation state.[3–5] It is known that compensatory anti-inflammatory mechanisms develop concurrently in the early stages of sepsis and when severe, a state of challenging immunosuppression might occur.[6,7]

Changes in non-immunological pathways also play a role in sepsis pathogenesis, including impairment in neuroendocrine and nervous systems[4,8], mitochondrial damage[5,7,9] and coagulation disorders[4,7]. They communicate with the immune system and modulate the inflammatory response, influencing cytokine release, apoptosis of immune cells and complement activation. These complex interactions between those multiple systems are responsible for the disease course, often reflecting a dysregulated inflammatory network[4,7,8] – a “failure of homeostasis” that leads to multiple organ failure (MOF) and death.[8]

The heterogeneity of inflammatory responses between affected individuals creates a variety of clinical phenotypes, ranging from uncontrolled inflammation to profound immunosuppression, both associated with MOF development.[3,10]

For example, secondary hemophagocytic lymphohistiocytosis (SHLH) is considered an hyperinflammation phenotype of MOF in septic children. SHLH occurs mostly in critically ill children and can be triggered by malignancy, medications, autoimmune diseases and infectious etiologies. SHLH emerges as a consequence of a qualitative and/or quantitative defect in natural killer (NK) cells, impairing their cytolytic activity, which results in pathologic activation of macrophages, histiocytes and CD8+ T cells. Persistently elevated levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-18, interferon (IFN)-γ and ferritin are the hallmark of hyperinflammation.[10–13] IL-1β is produced by tissue macrophages and has autocrine properties, perpetuating cytokine release and inflammation.[13]

According to the Histiocyte Society protocol[14], in order to make the diagnosis of SHLH, 5 out of 8 criteria must be present in the absence of a positive molecular assay. They include

clinical presentation (fever and splenomegaly), laboratory markers (cytopenias, hypertriglyceridemia and/or hypofibrinogenemia, hyperferritinemia, low or absent NK cell activity and high levels of soluble IL-2 receptor) and histopathologic changes (evidence of hemophagocytosis in bone marrow, spleen or lymph node specimens). However, these criteria were primarily designed for familial hemophagocytic lymphohistiocytosis, a rare condition, and although they are also used for SHLH diagnosis, not all are easily and rapidly accessible. Additionally, clinical presentation of SHLH and associated biomarkers are relatively non-specific adding further complexity to disease identification and management by intensivists. Authors of studies in adults[13,15,16] suggest that a more simplified approach may be used to identify septic patients with SHLH, one of that consists in the presence of hyperferritinemia (ferritin >500 ng/dL), hepatobiliary dysfunction, and disseminated intravascular coagulation (DIC).

Other hyperinflammation sepsis phenotypes have been described in pediatric patients. For example, in thrombocytopenia-associated multiorgan failure (TAMOF), uncontrolled inflammation causes activation of thrombosis and coagulation pathways with development of disseminated microvascular thrombosis – a spectrum of endothelial dysfunction, platelet aggregation and consumptive coagulopathy that results in organ ischemia and failure.[10,17]

Von-Willebrand factor (vWF) ultralarge multimers are proteins with two-fold activity: platelet adhesion and coagulation factor VIII stability. They are extremely thrombogenic and are normally cleaved to a small less thrombogenic size by A disintegrin and metalloproteinase with thrombospondin type 1 motif (ADAMTS)-13. However, the proinflammatory state of sepsis, not only enhances vWF ultralarge multimers synthesis by endothelial cells and megakaryocytes but also decreases ADAMTS-13 activity, leading to thrombotic microangiopathies.[10,18–20] Therefore, TAMOF should be considered in critically ill patients with new-onset thrombocytopenia (platelets <100 000/$mm^3$) and two or more organ failures, and reduced ADAMTS-13 activity may support the diagnosis.[17,18]

Moving to the opposite side of the spectrum, some patients will develop a state of immunosuppression due to sepsis-induced adverse modifications in immune cells from both innate and adaptive immunity,[3,5,6] describing a dysfunctional anti-inflammatory response that has been termed immunoparalysis and is related with increased risks of nosocomial infection and mortality in critically ill children.[21–23]

The immune system's impact of sepsis relies on both quantitative and qualitative modifications in effector cells that are responsible for infection clearance and homeostasis. Neutrophils exhibit impairment in chemotaxis and migration, tool-like receptor signaling, phagocytosis and cytokine production.[24] Human leucocyte antigen (HLA)-DR are major histocompatibility complex class II molecules presented in innate immune cells surface

involved in antigen presentation to lymphocytes. Reduction of HLA-DR expression in circulating monocytes has an immunosuppressive effect and can occur in the setting of sepsis,[5,22,25] as well as monocyte reprogramming, that is characterized by lower capacity of releasing pro-inflammatory cytokines by monocytes (e.g., tumor necrosis factor (TNF)-α) while maintaining anti-inflammatory cytokine production (e.g., IL-10). [21,23]

In the adaptative field, modifications include apoptosis of lymphocytes and antigen presenting cells,[26–28] anergy and exhaustion of effector T cells due to higher expression of programmed cell death (PD)-1 and programmed cell death ligand (PDL)-1[29] and increased suppressive effect by regulatory T cells that seem resist to apoptosis.[5,30]

Some biomarkers and immunologic assays have been studied to diagnose immunoparalysis and quantify the degree of immune dysfunction. Measuring whole blood TNF-α production in response to *ex-vivo* stimulation with lipopolysaccharide (LPS) is a functional immune test that reflects the capacity of innate immune cells to attack invading pathogens and lower results have been associated with MOF, nosocomial infection and mortality in septic children.[21,22,31,32] Similarly, *ex-vivo* stimulation of whole blood with phytohemagglutinin reflecting low IFN-γ production capacity may demonstrate adaptative immunity dysfunction that relate with adverse clinical outcomes in these patients.[31,32]

As previously described, in the setting of immunosuppression, monocytes internalize their HLA-DR molecules, directly affecting the mechanism of antigen presentation.[22] The quantification of monocyte HLA-DR expression using flow cytometry can be used as a diagnosis tool for immunoparalysis. A prolonged reduction in monocyte HLA-DR expression has been correlated with mortality in pediatric septic patients, [3,10,21–23] although a cut-off value has not been established in children yet.

Additionally, lymphoid depletion (<1000 cells/$mm^3$) in children with sepsis-induced MOF as a consequence of apoptosis and/or diminished production, demonstrated a higher risk for developing nosocomial infections and, when persistent, it was associated with mortality.[27,28] Evidence of increased PD-1/PD-L1 expression in lymphocytes/monocytes of adult patients with septic shock suggest that these could be used as biomarkers of sepsis-induced immunosuppression. [29,33,34]

Sepsis is a broad syndrome with multiple biological and clinical features that combine differently in each patient, creating clusters or phenotypes that may respond differently to therapies and may have different risks for poor outcomes. The current knowledge states that inflammation and immunosuppression occur concurrently in the early stages of infection and the balance between them will determine each patient's pathway throughout the disease. They can develop hyperinflammation, immunosuppression or a mixture of both.[3,5] This heterogeneity

of phenotypes may be the justification for the failure of clinical trials[35–37] using anti-inflammatory therapies in septic patients and points towards new directions for the management of sepsis.

The basis for characterizing sepsis phenotypes is to understand the key components of immune dysfunction that lead to poor outcomes and then develop sensitive, specific, affordable and quickly obtained biomarkers to help differentiate patients and to determine which immunomodulatory interventions best fit each patient. This individualization will facilitate future trials on sepsis targeted therapy.[3,5,17,22,38]

Lesser information is available regarding sepsis phenotyping in children. Crucial developmental differences between the child and the adult influence their response to infectious pathogens turning pediatric sepsis into this unique entity. Differences in hemodynamics, coagulation cascade, inflammatory response and immune system are described and highlight the importance of studies directed to the pediatric population.[39]

Therefore, the objective of this systematic review is to compare different multiorgan failure phenotypes in septic children regarding their physiopathology, differentiated biomarkers and correlation with adverse clinical outcomes. We believe such knowledge will contribute to diagnosis accuracy, therapeutic individualization and, consequently, clinical outcome improvement.

## METHODS

We conducted this systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis checklist. It is registered in PROSPERO with registration number CRD42022348092 where the protocol and the search strategy can be found.

We defined our research question using the Population, Intervention/Exposure, Comparison and Outcome principle. The population object of this review was children from birth to 18 years of age, admitted to the Pediatric Intensive Care Unit (PICU) with the diagnosis of sepsis, severe sepsis or septic shock. Differently from what is stated in the initial protocol, we chose to include studies also, but not exclusively, including neonates (from birth to 28 days of age) because it might would have limited the search. The exposure and comparison objects of this review were children with sepsis, severe sepsis or septic shock and, respectively, associated hyperinflammation phenotypes, for example SHLH, or associated immunoparalysis. The main outcome was to identify differentiated biomarkers for each phenotype. Additional outcomes were mortality in PICU, length of PICU stay, secondary infection, requirement of mechanical ventilation, vasoactive support, renal replacement therapy (RRT) or extracorporeal membrane oxygenation (ECMO).

### ELIGIBILITY CRITERIA

We included studies that fulfilled the following inclusion criteria: (1) study population of children until 18 years of age, admitted to the PICU with the diagnosis of sepsis, severe sepsis or septic shock; (2) studied variables such as biomarkers, flow cytometry, proteomics, lipidomics, mortality in PICU, length of PICU stay, requirement of mechanical ventilation, renal replacement therapy or extracorporeal membrane oxygenation. By design, we included randomized-controlled trials and observational studies. Reviews and studies that included only neonates or adults were excluded.

### DATA SOURCES AND LITERATURE SEARCH

We performed the search in Pubmed, Embase and Cochrane Central Register of Controlled Trials databases in October 2022 using a search strategy developed for the effect. The search strategy is available within the protocol and contains several database-adapted Medical Subject Headings terms and their respective synonyms, including, for example, “pediatric”, “sepsis”, “multiple organ failure”, “hyperinflammation”, “immunoparalysis”, “biomarkers”, “mortality”, “secondary infection” and more.

Additionally, we applied the following filters when searching the databases: language in English, Portuguese or Spanish; studies in humans; age from birth to 18 years (child) and

publication date from 2005 to present because the most recent update for sepsis definition in children is from 2005.[40]

### STUDY SELECTION

The articles found through the search strategy were downloaded and checked for duplicates using an excel tool designed for the effect. The remaining articles were independently assessed for inclusion criteria by two reviewers (B.S. and A.D.), based on titles and abstracts, including justification when the decision was to exclude. This process was conducted in individual spreadsheets for each reviewer and compared automatically to evaluate concordance through Cohen's kappa (B.O.) and to solve eventual inclusion/exclusion divergences. When agreement not reached, the opinion of A.D. was considered.

We repeated the process under the same criteria for the remaining included articles, but in this step, we read and analyzed their full text.

Reference lists of obtained articles were hand searched.

### DATA EXTRACTION AND MANAGEMENT

Data were extracted separately by two reviewers (B.S. and A.D.) using a predesigned form and then introduced in tables to check for discrepancies. We extracted data on study characteristics (study year, design and country), patients characteristics (age, gender, comorbidity and severity of sepsis according to Pediatric RISk of Mortality (PRISM) score) and outcomes measured (biomarkers, length of stay, mortality in PICU, requirement of organ support therapies). The quality of selected articles and the overall risk of bias was determined using the Newcastle-Ottawa Scale.

## RESULTS

We identified a total of 42 studies from database searches, of which 11 from Embase, 10 from Pubmed and 21 from Cochrane Central Register of Controlled Trials. Among these, seven articles remained for full text screening, resulting in three studies[41–43] that met all inclusion criteria and were eligible for synthesis. Reasons for exclusion can be found in the flow diagram presented in Figure 1.

Characteristics of included studies, participants and methods are summarized in Table 1.

Details about individual study quality are presented in supplementary material I.

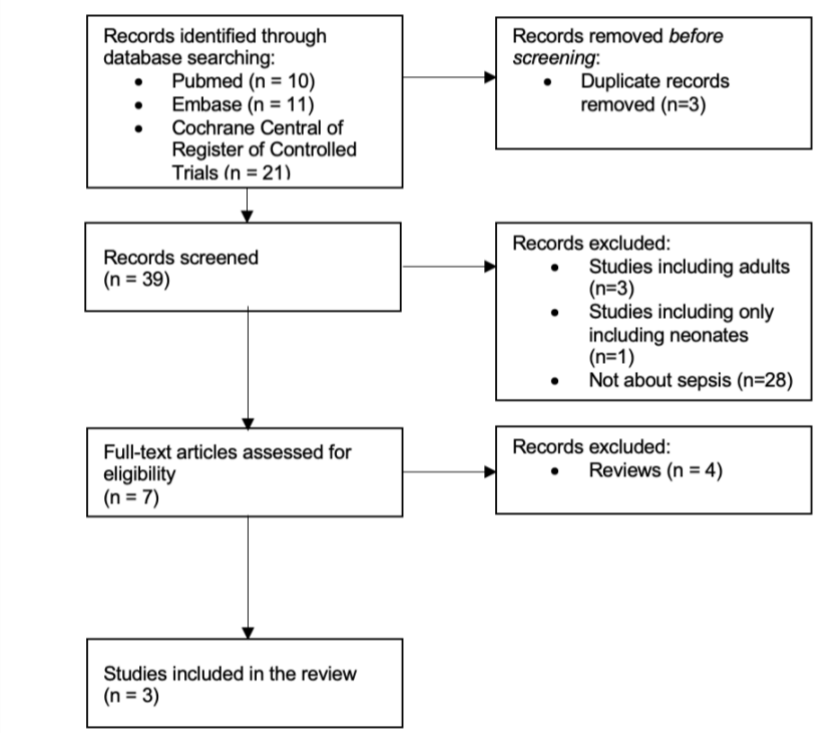


***Figure 1*** - Flow diagram of record screening and included studies according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

All studies were conducted in the United States of America and all are observational studies. Carcillo et al developed a single center prospective study[41] to compare differences between septic children who developed MOF with associated phenotypes, including immunoparalysis associated MOF (IPMOF), TAMOF and/or sequential MOF (SMOF), with septic children with none of these empirical phenotypes. One year later, the authors used the same study design with the objective of extending the findings to a multicenter assessment in nine PICUs, not including the patients from the previous study.[42]

***Table* 1** - Study and participant characteristics. Methods used.

| Characteristics | Study ID | | |
|---|---|---|---|
| | **Carcillo et al 2017** | **Carcillo et al 2019** | **Qin et al 2022** |
| **Study** | | | |
| **Publication year** | 2017 | 2019 | 2022 |
| **Country** | United States of America | United States of America | United States of America |
| **Study design** | Single center prospective observational study (Performed between 2009 and 2014) | Multicenter prospective observational study including nine PICUs (Performed between 2015 and 2017) | Retrospective observational study |
| **Quality*** | 8 | 9 | 8 |
| **Participants** | 100 children with severe sepsis | 401 children with severe sepsis | 404 children with severe sepsis |
| **Mean age (SD)** | 5.8 years (5.7) | 6.8 years (5.72) | 7 years (6) |
| **Gender** | 47% female; 53% male | 46% female; 54% male | 44.6% female; 55.4% male |
| **Comorbidity** | 59% with previous chronic illness; 41% previously healthy | 56% with previous chronic illness; 44% previously healthy | 44.6% previously healthy |
| **PRISM score** | Mean 10.7 (SD 8.7) | Median 8 (IQR [3-14]) | - |
| **Methods** | Patients with MOF were divided in subgroups according to their empirical phenotype (IPMOF, TAMOF and SMOF). Outcomes were measured and compared to patients with MOF without a diagnosed phenotype, and to patients without MOF. | | Patients were clustered in 4 phenotypes (PedSep-A, -B, -C or -D) according to 25 clinical variables, using machine learning. Correlation with IPMOF/TAMOF/SMOF/ MAS development, cytokine biomarkers and short-term outcomes was assessed. |

*PICU* - pediatric intensive care unit; *SD* - standard deviation; *IQR* - interquartile range; *PRISM* - Pediatric RISk of Mortality; *MOF* - multiple organ failure; I*PMOF* - immunoparalysis associated MOF; *TAMOF* - thrombocytopenia associated MOF; *SMOF* - sequential MOF; *MAS* - macrophage activation syndrome.

*Quality of the studies was assessed using the Newcastle-Ottawa scale in which the maximum score is 9.

Qin et al[43] applied machine learning approaches to previously obtained clinical and laboratory data and derived four computable sepsis phenotypes to facilitate the identification of children at risk for developing hyperinflammation features and that could benefit from personalized anti-inflammatory therapies. For this purpose, the authors considered the blood samples and clinical data of patients enrolled between 2015 and 2017 from the multicenter study by Carcillo et al,[42] also included in this review. Nevertheless, we chose to include this study due to the different statistical methods applied as groups were formed based on their characteristics instead of being defined *a priori*, and due to the results, which reinforce the first study.

A total of 501 different children were enrolled in both Carcillo et al studies[41,42], while Qin et al used the same patients of the multicenter study by Carcillo et al.[42] All were PICU patients with the diagnosis of severe sepsis. Sepsis, severe sepsis and MOF diagnosis criteria were the same among studies (supplementary material II).[40] More than half of the children were male and more than half had previous chronic illnesses, cancer, organ transplant or other (Table 1). Two studies[41,42] used the PRISM score to assess the severity of sepsis and all studies quantified the number of organ failures using the Organ Failure Index (OFI). Clinical criteria and confirmatory biomarkers for each phenotype (IPMOF, TAMOF, SMOF and MAS) were the same among studies and can be visualized in Table 2.

Detailed information about considered outcomes in each study is stated in Table 3.

The single center study by Carcillo et al included a total of 100 severe sepsis cases of which 37 developed MOF with one or more phenotype. This group had higher mean OFI comparing to the MOF group without any specific phenotype (mean OFI (standard deviation (SD)) = 3.4 (1.4) *versus* 2.3 (0.5)) (*p*-value not available). Twenty-four patients out of 37 developed IPMOF, 15 developed TAMOF and six developed SMOF, being seven patients with phenotype overlapping. IPMOF was significantly associated with increased age ($p<0.05$), chronic illness ($p<0.05$) and cancer ($p<0.05$) (supplementary material III). Seventy-three percent of TAMOF and 67% of SMOF patients were chronically ill, although a significant association was not found.

Peak ferritin ($p<0.001$) and CRP ($p=0.009$) levels were higher in children with MOF and one or more phenotypes comparing with children with MOF without any of the phenotypes. Between the considered phenotypes, TAMOF subgroup exhibited higher ferritin and CRP measures comparing with IPMOF subgroup (Table 3). Considering IPMOF-related biomarkers, the authors found that in the studied cohort, a low *ex-vivo* TNF production by monocytes was associated with longer length of stay, comparing with an absolute lymphocyte count less than 1000/microliter ($p<0.05$), and with a tendency to increased mortality, although not statistically significant ($p=0.505$).

***Table 2*** - Clinical criteria and confirmatory biomarkers used to diagnose each phenotype among studies.

| | Phenotype | | | |
|---|---|---|---|---|
| | **IPMOF** | **TAMOF** | **SMOF** | **MAS** |
| **Clinical criteria** | (1) Beyond 3 days with OFI* of 2 or more | (1) Thrombocytopenia (platelet count < 100 000/mm$^3$)<br>(2) Renal failure[a]<br>(3) OFI* of 3 or more | (1) Pulmonary failure[b]<br>(2) Followed by hepatic failure[c] seven days or more later | (1) Thrombocytopenia (platelet count < 100 000/mm$^3$)<br>(2) INR > 1,5<br>(3) Hepatic failure[c] |
| **Confirmatory biomarker** | Whole blood *ex-vivo* TNF-α response to LPS < 200pg/mL | ADAMTS 13 activity < 57% of control | sFasL > 200 pg/mL | Ferritin > 500 ng/mL |

*MOF* - multiple organ failure; *IPMOF* - immunoparalysis associated MOF; *TAMOF* - thrombocytopenia associated MOF; *SMOF* - sequential MOF; *MAS* - macrophage activation syndrome; *OFI* - organ failure index; *TNF* - tumor necrosis factor; *LPS* - lipopolisaccharide; *ADAMTS* - A disintegrin and metalloproteinase with thrombospondin type 1 motif; *sFasL* - soluble Fas ligand; *INR* - international normalized ratio; *pg* - picogram; *mL* - milliliter; *ng* - nanogram; *mm* - millimeter.

*OFI reflects the number of organ failures, being 0 or 1 for cardiovascular, hepatic, hematologic, respiratory, neurological and renal. OFI ranges from 0 to 6.

According to OFI: [a]serum creatinine > 1 mg/dL and oliguria (urine output < 0.5 mL/kg/h); [b]need for mechanical ventilation support with the ratio of the arterial partial pressure of oxygen and the fraction of inspired oxygen (PaO2/FiO2) < 300 without this support; [c]total bilirubin > 1 mg/dL and alanine aminotransferase (ALT) > 100 U/L.

Eight children died in the study, six of which with diagnosed TAMOF, five with IPMOF and one with SMOF (table 3). TAMOF patients had higher proclivity to develop MAS (Table 3). Of the 11 children that developed MAS, seven died.

The subsequent multicenter study by Carcillo et al, in 2019, comprised 401 patients with severe sepsis from nine PICUs, of which 101 had MOF with one or more phenotypes. This group had higher median OFI scores comparing with the group with MOF without any of the phenotypes (OFI median [IQR] = 3 [2-4] *versus* 2 [2-3]) (*p*-value not available). Eighty-five patients developed IPMOF, 37 developed TAMOF and seven developed SMOF. Differently from the single center cohort, the authors found a significant association between IPMOF and TAMOF phenotypes with older age, increased illness severity and cancer (supplementary material III).

Similarly to the previous study, peak ferritin ($p$<0.001) and CRP ($p$<0.001) levels were higher among patients with MOF and one or more phenotypes and were specifically higher in TAMOF and SMOF subgroups comparing with IPMOF subgroup (Table 3).

MAS was diagnosed in 24 children, being more common in SMOF and TAMOF subgroups (seven out of seven in SMOF subgroup, 14 out of 37 in TAMOF subgroup and 15 out of 85 in IPMOF subgroup) (Table 3). Development of MAS was found to increase mortality in the group of children with MOF and one or more phenotypes (relative risk of 2.16, 95% confidence interval, 1.09-4.29), and particularly in IPMOF subgroup (relative risk of 2.55, 95% confidence interval, 1.12-5.80).

There were 44 (10.9%) deaths in the multicenter study, of which 43 occurred in the MOF group. TAMOF and SMOF patients accounted for the highest mortality rates comparing to IPMOF patients (43.2% and 42.9% *versus* 20.0%) (Table 3). PICU deaths occurred sooner for IPMOF (Median time [IQR] = 16 [8-35] days) patients and later for TAMOF (Median time [IQR] = 22.0 [13.5-44.0] days) and SMOF patients (median time [IQR] = 34 [14-42] days). IPMOF subgroup survivors exhibited earlier recovery of the whole blood *ex-vivo* TNF-α response to LPS above 200 picogram/milliliter.

The additional outcomes studied in this cohort revealed that the PICU length stay was higher in SMOF subgroup, followed by TAMOF (Table 3). The majority of patients with MOF and one or more phenotypes required mechanical ventilation support. The need for renal replacement therapies and extracorporeal membrane oxygenation was higher among TAMOF and SMOF patients comparing to IPMOF patients (Table 3).

In the last included study, Qin et al clustered 404 septic children with common findings in four different phenotypes (PedSep-A, -B, -C and -D) by analyzing 25 variables (e.g. age, gender, vital signs, CRP, ferritin and more). They assessed the correlation of those four clusters with cytokine measurements, IPMOF/TAMOF/SMOF/MAS development and with clinical outcomes that included mortality, requirement of organ support therapies and length of stay.

One-hundred and thirty-six patients belonged to PedSep-A, 102 to PedSep-B, 110 to PedSep-C and 56 to PedSep-D. PedSep-A patients were younger ($p$<0.05), mostly previously healthy ($p$<0.05) and had more viral infections ($p$<0.05), while PedSep-C and -D patients were less previously healthy (supplementary material III). PedSep-D children had more organ dysfunctions comparing with the others (mean OFI (SD): PedSep-A = 1.4 (0.5); PedSep-B = 2.1 (0.6); PedSep-C = 1.4 (0.6); PedSep-D = 3.1 (1.0), $p$<0.05).

The degree of inflammation increased from PedSep-A (the lowest cytokine levels) to PedSep-D (the highest cytokine levels), with PedSep-B and C in between. PedSep-D cluster

had the highest peak ferritin median overall, which was statistically significant when comparing to -A and -B, but not -C (Table 3).

Details of development of IPMOF/TAMOF/SMOF/MAS by derived phenotype can be visualized in Table 4. Of the 85 children that developed IPMOF, 29 (28.4%) belonged to PedSep-B, 22 (20%) to PedSep-C and 22 (39.3%) to PedSep-D. Most patients that developed SMOF ($p$<0.05), TAMOF ($p$<0.05) and MAS ($p$<0.05) belonged to PedSep-D group. MAS was diagnosed in 24 patients, 19 of which belonged to PedSep-D.

Forty-five patients died in the population studied, of which 19 (33.9%) belonged to PedSep-D cluster – the group with the highest mortality (mortality, *n* (%): PedSep-A = 3 (22%); PedSep-B = 12 (11.7%); PedSep-C = 11 (10.0%); PedSep-D = 19 (33.9%), $p$<0.05) (Table 3). These children also required more RRT ($p$<0.05) and ECMO and had the longest length of stay (Table 3).

***Table* 3** - Studied outcomes according to phenotype.

| Variable | Study ID and phenotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Carcillo et al 2017** *n*=100 | | | **Carcillo et al 2019** *n*=401 | | | **Qin et al 2022*** *n*=404 | | | |
| | **IPMOF** *n*=24 | **TAMOF** *n*=15 | **SMOF** *n*=6 | **IPMOF** *n*=85 | **TAMOF** *n*=37 | **SMOF** *n*=7 | **A** *n*=136 | **B** *n*=102 | **C** *n*=110 | **D** *n*=56 |
| **Peak ferritin, ng/mL Median [IQR]** | 885 [285-3205] | 1100 [390-4770] | 1025 [150-8930] | 663.9 [260.7-4049] | 1705 [694-13000] | 1097.1 [634-13000] | 125 [69.8-207.8] | 223 [116.5-544.2] | 405 [176.2-1485.7] | 610 [221.1-2482.0] |
| **Peak CRP, mg/dL Mean (SD)** | 15.8 (14.1) | 17.4 (15.3) | 5.4 (3.9) | 26.7 (28.54) | 41.6 (57.85) | 35.7 (19.74) | 7.3 (7.3) | 13.2 (11.5) | 15.2 (10.4) | 13.1 (11.2) |
| **Death in PICU, *n* (%)** | 5 (21) | 6 (40) | 1 (17) | 17 (20) | 16 (43.2) | 3 (42.9) | 3 (2.2) | 12 (11.7) | 11 (10) | 19 (33.9) |
| **Length of stay, days Median [IQR]** | - | - | - | 17.9 [9.2-29.6] | 23 [15.9-42.4] | 41.5 [14.4-42.4] | 9 [5.8-15] | 10.5 [5.3-17] | 6 [2.3-15] | 12.5 [7-26.5] |
| **MV, *n* (%)** | - | - | - | 80 (94.1) | 35 (94.6) | 7 (100) | 134 (98.5) | 101 (99) | 79 (71.8) | 52 (92.9) |
| **RRT, *n* (%)** | - | - | - | 23 (27.1) | 34 (91.9) | 5 (71.4) | 1 (0.7) | 7 (6.9) | 7 (6.4) | 37 (66.1) |
| **ECMO, *n* (%)** | - | - | - | 12 (14.1) | 8 (21.6) | 3 (42.9) | 5 (3.7) | 9 (8.8) | 6 (5.5) | 10 (17.9) |
| **MAS development, *n* (%)** | 6 (25) | 7 (47) | 2 (33) | 15 (17.6) | 14 (37.8) | 7 (100) | 0 (0) | 3 (2.9) | 2 (1.8) | 32 (57.1) |

*ng* - nanogram; *mg* - milligram; *mL* - milliliter; *dL* - deciliter; *IQR* - interquartile range; *SD* - standard deviation; *CRP* - C-reactive protein; *PICU* - pediatric intensive care unit; *MV* - mechanical ventilation; *RRT* - renal replacement therapy; *ECMO* - extracorporeal membrane oxygenation; *MOF* - multiple organ failure; *IPMOF* - immunoparalysis associated MOF; *TAMOF* - thrombocytopenia associated MOF; *SMOF* - sequential MOF; *MAS* - macrophage activation syndrome.

*A, B, C and D refer to the computable derived phenotypes (PedSep-A, -B, -C or -D, respectively) according to clinical variables that were analyzed to form 4 different clusters of patients in this study.

***Table* 4** – Development of subsequent phenotypes in each derived cluster in the included study by Qin et al 2022.

| **Subsequent phenotype** | **Derived cluster** | | | | |
|---|---|---|---|---|---|
| | **Total *n*=404** | **PedSep-A *n*=136** | **PedSep-B *n*=102** | **PedSep-C *n*=110** | **PedSep-D *n*=56** |
| **IPMOF, *n* (%)** | 85 (21.0) | 12 (8.8) | 29 (28.4) | 22 (20) | 22 (39.3) |
| **SMOF, *n* (%)** | 7 (1.7) | 0 (0.0) | 0 (0.0) | 1 (0.9) | 6 (10.7) |
| **TAMOF, *n* (%)** | 37 (9.2) | 0 (0.0) | 6 (5.9) | 3 (2.7) | 28 (50.0) |
| **MAS, *n* (%)** | 24 (5.5) | 0 (0.0) | 3 (2.9) | 2 (1.8) | 19 (33.9) |

*MOF* - multiple organ failure; *IPMOF* - immunoparalysis associated MOF; *TAMOF* - thrombocytopenia associated MOF; *SMOF* - sequential MOF; *MAS* - macrophage activation syndrome.

## DISCUSSION

This systematic review included a total of 501 different children with severe sepsis. In both studies by Carcillo et al,[41,42] 28% percent developed MOF with one or more associated phenotype which led to increased organ dysfunction. Immunoparalysis-associated MOF was observed in 79% percent of those patients, representing the most prevalent phenotype, and was associated with older age, chronic illness, cancer and earlier death. Thrombocytopenia-associated MOF was the second most prevalent phenotype, occurring in 38% percent of children with MOF with one or more associated phenotype, followed by sequential MOF, which was the least observed and accounted for nine percent of the cases. The authors considered macrophage activation syndrome as a synonym of SHLH, but most literature defines MAS as a subtype of SHLH caused by autoimmune and rheumatological diseases (e.g. idiopathic juvenile arthritis), whereas SHLH includes other etiologies for the syndrome, like infection and cancer.[12,44] Therefore, from now, as we are discussing infection-associated SHLH, we will substitute the term MAS for SHLH. SHLH occurred in 35 patients, of which most cases had a previously diagnosed phenotype and the most frequent were TAMOF and SMOF.

Considering the derived clusters by Qin et al,[43] immunoparalysis was frequently observed in PedSep-B, -C and -D patients. TAMOF and SMOF were almost confined to PedSep-D children. PedSep-D subgroup was the most associated with phenotype development and overlap (50% had TAMOF, 39% had IPMOF, 11% had SMOF and 34% percent developed SHLH).

Peak ferritin and peak CRP were higher among septic children who developed TAMOF, followed by SMOF. PedSep-D patients also had the highest peak ferritin comparing to the other subgroups. Hyperferritinemia (ferritin greater than 500 ng/mL) is a marker of hyperinflammatory states such as SHLH, MAS and severe sepsis. Ferritin itself perpetuates inflammation by enhancing cytokine production, through activation of nuclear factor (NF)-κB which leads to pro-inflammatory cytokine release.[11,12,16] Ferritin alone cannot be considered a specific marker, but rather an indication that an hyperferritinemic syndrome is present, and the differential diagnosis must be made by adding other clinical and laboratory data. Nevertheless, some authors suggest that the higher the level of ferritin (for example above 4420 ng/mL), the more it gives a clue that SHLH is present in septic patients, predicting early mortality.[16]

CRP is an acute-phase protein synthetized in the liver in response to inflammation, infection or tissue damage. CRP levels rise at 12 to 24 hours after infection, reaching the peak after two to three days.[45] Because many inflammation processes can rise CRP levels, it lacks specificity for sepsis, but some studies evidenced the role of CRP in predicting mortality, both in septic adults [46] and children[47], suggesting that may be beneficial to incorporate this biomarker in risk

stratification models. Although not disease-specific, ferritin and CRP are two economical and readily available markers of systemic inflammation that can give clues about the presence of hyperinflammatory conditions and the risk of poor prognosis in these patients.

There were 52 deaths among the population included. Thirty-two had MOF with one or more phenotype, meaning that the development of an inflammation phenotype raises mortality in septic children. TAMOF subgroup had the highest mortality (42% of children died), followed by SMOF (31%) and IPMOF (20%). Of those who developed SHLH, 51% percent died. In the study by Qin et al,[43] PedSep-D cluster, which was strongly associated with TAMOF and SHLH development, exhibited the highest mortality (34% percent of patients died) and the longest length of stay.

The need for organ support therapies can reflect the degree of organ dysfunction and illness severity. Most included children, with or without MOF, required mechanical ventilation. The need for extracorporeal membrane oxygenation was observed only in children with MOF, with no significant difference between phenotypes. Children with MOF and an associated phenotype required more renal replacement therapies, specially the TAMOF subgroup where 92% percent of patients required RRT. Sixty-six percent of PedSep-D subgroup patients also required RRT.

The development of thrombotic microangiopathies in the spectrum of TAMOF may be the explanation of why this subgroup of patients require more RRT. As mentioned earlier, TAMOF can be triggered by the uncontrolled inflammation of sepsis and is characterized by endothelial dysfunction with new-onset thrombocytopenia and acquired ADAMTS-13 deficiency.[18,48] Thrombotic microangiopathies emerge in the setting of TAMOF due to platelet aggregation and intravascular thrombosis. Microthrombi formation in circulation will cause ischemia and organ damage, leading to acute kidney injury and, therefore, the need for RRT.[18–20,49]

Early identification and treatment of TAMOF among septic patients is imperative because, as we demonstrated, the development of this phenotype is highly related with adverse clinical outcomes. This syndrome must be suspected in the presence of new-onset thrombocytopenia (platelets <100 000/mm$^3$) and two or more organ failures. ADAMTS-13 deficiency supports this diagnosis as it plays a major role in physiopathology. In septic patients, ADAMTS-13 synthesis by endothelial cells is affected and the remaining enzyme is consumed by the persistent release of vWF ultralarge multimers.[20] ADAMTS-13 levels below 57% was the cut-off used in this review included studies to describe ADAMTS13 deficiency in sepsis and has been used by other authors as well.[10,50]

The treatment approach for TAMOF includes plasma exchange therapy, which removes thrombogenic vWF ultralarge multimers and restores ADAMTS-13 levels, being capable of improving survival in septic children.[18,48,50]

Sequential MOF was the least observed phenotype but was associated with important mortality and the longest length of stay overall. SMOF is characterized by acute respiratory distress syndrome followed sequentially by liver and renal failure. This phenotype is more common in children under immunosuppressant therapy after organ transplant that have concomitant viral infections.[51] Virus have the ability to increase shedding of Fas ligand (FasL), a membrane protein of T and B lymphocytes, and NK cells. The interaction between Fas and FasL triggers apoptosis of these cells, but soluble forms of Fas (sFas) and FasL (sFasL) suppress the mechanism of Fas-FasL apoptosis, perpetuating immune cell proliferation and causing liver cytotoxicity.[10,51,52] The expansion of immune cells causes further organ damage through cytokine release with higher inflammation.[52] Increased levels of sFas and sFasL aid the diagnosis of this condition and proper treatment must be given, including immunosuppressant tapering, antiviral therapy and rituximab.[51]

TAMOF and SMOF were intimately related with subsequent macrophage activation syndrome development in our review, a clinical entity that we demonstrated to increase mortality.

When pro-inflammatory stimulus in sepsis become intense and dysregulated through overactivation of immune cells, it is crucial that NK and cytotoxic T cells have the ability to shut down the hyperinflammatory response through cell apoptosis. If the functioning of these cells is affected, stimulation of macrophages, histiocytes and T lymphocytes is maintained and amplified, perpetuating hyperinflammation with marked cytokinemia.[10,11,13,16] TAMOF and SMOF are phenotypes associated with predominant pro-inflammatory mechanisms, which may explain why these patients had higher proclivity to develop sepsis-associated SHLH - the final common pathway of exaggerated systemic inflammation.

Hyperinflammation causes rapidly progression of organ dysfunction that leads to hepatobiliary disfunction and DIC with hypercytokinemia (IL-1β, IL-18, IL-6, IFNγ) and elevated ferritin. Therefore, the presence of hepatobiliary disfunction, DIC and hyperferritinemia may be a more simplified diagnostic approach for sepsis-associated SHLH, instead of the Histiocyte Society protocol criteria.[11,13,16] Recognizing sepsis-associated SHLH can improve clinical outcomes by introducing early immune modulation therapies, including methylprednisolone, plasma exchange, intravenous immunoglobulin and IL-1 receptor antagonist (anakinra).[11,15,51,53,54]

On the opposite side of the spectrum, immunoparalysis-associated MOF was the most prevalent phenotype in our review and had better outcomes when comparing with other MOF phenotypes, but worse when comparing with children with MOF without any phenotype.

Immunoparalysis is the consequence of immune cell dysfunction caused by the hostile environment of sepsis, affecting the host ability to combat the primary infection and

predisposing to secondary infections.[21,22] When children have dominant immunosuppressive mechanisms at a given time of sepsis progression, the use of anti-inflammatory therapies will even worse the course of disease. Therefore, identifying immunoparalysis is crucial to restore immune function and prevent the development of opportunistic infections.

In our review, authors of included studies considered a functional immune testing to diagnose IPMOF, which was measurement of whole blood TNF-α production in response to *ex-vivo* stimulation with LPS and used a cut-off of less than 200 pg/mL. Carcillo et al[41] also concluded that comparing to lymphopenia, a lower TNF-α production was associated with longer length of stay and with a tendency to increase mortality in IPMOF subgroup of patients, suggesting that this functional immune test may be more predictable of illness severity. HLA-DR expression in circulating monocytes below 30% using flow cytometry has been used as a biomarker of immunoparalysis in adult studies, but a cut-off has not been stablished for children yet.[21,22,55]

Targeting the immunoparalysis phase of sepsis is a field in expansion. The use of granulocyte-macrophage colony-stimulating factor (GM-CSF) in these patients have demonstrated to improve immune function and prevent nosocomial infections and death in critically ill children with immunoparalysis.[21] The benefits of GM-CSF are widely known among adults[56,57] and the knowledge is expanding to the pediatric population. The GRACE trial (clinicaltrials.gov: NCT03769844) is currently on-going and investigating the use of GM-CSF to reverse immunoparalysis in children with sepsis-induced MOF. Other potential immunostimulatory treatments include recombinant IFN-γ[58], recombinant IL-7[59] and anti-PD-L1 therapy.[60]

The goal of the pediatric intensivist towards sepsis is to accurately phenotype each patient in order to add therapies that potentiate the control and resolution of the infectious insult. A patient might develop a dominant hyperinflammatory response, move to the pathway of a dominant immunosuppressive phase or rely between both mechanisms.

In Qin et al[43] study that was included in our review, immunoparalysis was observed among PedSep-B, -C and -D subgroups, but PedSep-D had the highest prevalence of SMOF, TAMOF and SHLH and the highest mortality, longer length of stay and required more RRT. Because in PedSep-D many patients had concomitantly immunoparalysis and hyperinflammatory phenotypes, this subgroup may be an example of children with co-existing pro- and anti-inflammatory features that might not be stable and fluctuate toward one another over disease course. The broad differences in clinical outcomes between subgroups suggest that when immunoparalysis develops along with hyperinflammation worse outcomes should be expected, comparing to immunoparalysis alone. Nevertheless, the authors concluded that PedSep-D membership identified children with increased inflammation biomarkers and that could benefit

from early anti-inflammatory treatments targeting TAMOF and SHLH, which appear to be the main culprits for worse outcomes.

PRECISE (clinicaltrials.gov: NCT05266001, NCT05267821) is a prospective, double-blind, randomized controlled trial that started enrolling in May 2022 and aims to improve outcomes in children with sepsis-induced MOF. Participants will have their immune function assessed at baseline and based on the results they will be placed in three distinct groups. The first group includes children with immunoparalysis and mild to moderate inflammation (serum ferritin level below 2000 ng/ml) and these children will be randomized to receive placebo or GM-CSF. The second group includes children without immunoparalysis and moderate to severe inflammation (serum ferritin level of 500-2000 ng/ml or a CRP above 4 mg/dl) or with immunoparalysis and severe inflammation (serum ferritin level of 2000–10 000 ng/ml) and these children will be randomized to receive either anakinra (IL-1 receptor antagonist) or placebo. The third group is the observational cohort and includes participants that do not qualify for the first and second groups and, therefore, will not receive the study drug. The degree of organ dysfunction will be evaluated through 28 days post-randomization, as well as health related quality of life and functional three months post-randomization.

A similar study in adults was published in November 2022. PROVIDE[61] is a randomized clinical trial with 240 septic adult patients that were classified in three groups according to their immune state (immunoparalysis, intermediate and macrophage activation-like syndrome (MALS)). Patients were randomized to administration of specific therapy (recombinant human IFN-γ for immunoparalysis subgroup and antagonist of the IL-1 receptor – anakinra – for MALS subgroup) or placebo. They compared survival between groups and assessed whether personalized treatment would be of benefit. However, they had to prematurely stop the study because the cut-off defined to identify immunoparalysis patients was found to be inappropriate. Nevertheless, they had interesting findings. The immunoparalysis and MALS subgroups had increased mortality (66.9% and 79.1% respectively) and patients treated with anakinra had survival at day seven with decreased Sequential Organ Failure Assessment (SOFA) compared with placebo.

The ImmunoSep trial, a new similar but optimized trial developed by the same authors is currently on-going (clinicaltrials.gov: NCT04990232). Additionally, the ImmunoSep project promises to take steps towards precision medicine by setting up a platform to help future clinical trials in patient stratification and treatment allocation based on a multidimensional analysis of omics-based datasets that will support biomarker identification, endotyping and therapeutic targets.

Genetic and epigenetic variations can impact the host inflammatory response and predict the existence of sepsis subclasses according to different patterns of gene expression.[62,63]

Genomic and transcriptomic studies have proved their potential to identify those classes, help patient stratification and discover new therapeutic targets. Through genome-wide expression patterns, Wong et al[64] identified three distinct endotypes (A, B and C) of children diagnosed with septic shock. The endotypes differed from each other in clinical phenotype and mortality. Illness severity and the degree of organ failure were higher among endotype A patients, which exhibited repression of adaptative immunity and glucocorticoid receptor signaling corresponding genes. With these findings, the authors developed a clinically feasible approach to pediatric septic shock subclassification based on transcriptomic profiles.[65,66] The knowledge of metabolomic and proteomic alterations in sepsis is also expanding, contributing to a better disease comprehension and stratification.[67–69]

There are limitations to consider in our review. The field of sepsis immunophenotyping is recent and children have lower incidence of sepsis comparing to adults, which affects the number of observational studies and trials about differences concerning biomarkers and clinical outcomes between phenotypes. Our database search only retrieved 39 different studies, and only three articles remained after double screening. The results rely in three articles, so conclusions must be taken cautiously. For this reason and because the third included study[43] used the same patients as the multicenter study by Carcillo et al,[42] we chose not to pursue a meta-analysis. Otherwise, results would be based mainly in the multicenter study that included significantly more patients (401 children) than the single center study[41] (100 children).

Nevertheless, with this review, we strengthened the knowledge about how the behavior of the immune system can impact the host responses during sepsis, generating a wide variety of clinical presentations. Immunoparalysis is a prevalent phenotype among critically children and although the medal for worse clinical outcomes appears to be given to hyperinflammatory phenotypes in this review, we also demonstrated that the presence of immunoparalysis and hyperinflammation simultaneously can lead to poor prognosis and earlier death, challenging the decision whether to apply anti-inflammatory or immunostimulatory strategies.

The pathway to precision medicine in sepsis has already started with the expansion of the role of functional immune testing and omics to allow patient immunoprofiling and stratification, not forgetting the contribution of age, gender and comorbidities. It is imperative to have validated and accessible criteria to identify each phenotype and then provide personalized adjunctive therapies to improve immune responses and restore organ function.

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## SUPPLEMENTARY MATERIAL

***Supplementary material I*** - Quality assessment of the studies using the Newcastle-Ottawa Scale.

| Study ID | Newcastle-Ottawa Scale | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Selection | | | | Comparability | Outcome | | | Total score (Max. 9) |
| | Representativeness of exposed cohort (Maximum: ★) | Selection of non-exposed cohort (Max. ★) | Ascertainment of exposure (Max. ★) | Demonstration that outcome of interest was not present at start of study (Max. ★) | Comparability of cohorts on the basis of the design or analysis (Max. ★★) | Assessment of outcome (Max. ★) | Was follow-up long enough for outcomes to occur (Max. ★) | Adequacy of follow-up of cohorts (Max. ★) | |
| **Carcillo et al 2017** | ★ | ★ | ★ | - | ★ | ★ | ★ | ★ | 7 |
| **Carcillo et al 2019** | ★ | ★ | ★ | - | ★★ | ★ | ★ | ★ | 8 |
| **Qin et al 2022** | ★ | ★ | ★ | - | ★★ | ★ | ★ | ★ | 8 |

***Supplementary material II -*** Sepsis, severe sepsis and multiple organ failure criteria used in included studies.

| Condition | Sepsis | Severe sepsis | Multiple organ failure |
|---|---|---|---|
| **Definition** | Infection (suspected or proven) plus the presence of two or more of the following:<br>- Tachycardia (heart rate >90$^{th}$ percentile for age in absence of stimulation);<br>- Tachypnea (respiratory rate >90$^{th}$ percentile for age);<br>- Abnormal temperature (<36ºC or >38.5ºC);<br>- Abnormal leukocyte count (>12 000, <4 000 or >10% immature neutrophils). | Sepsis plus one or more organ failures.[a] | Two or more organ failures.[a] |

[a]Organ failures were defined using the Organ Failure Index criteria: cardiovascular - need for cardiovascular agent infusion support; pulmonary- need for mechanical ventilation support with the ratio of the arterial partial pressure of oxygen and the fraction of inspired oxygen (PaO2/FiO2) < 300 without this support; hepatic – total bilirubin > 1.0 mg/dL and alanine aminotransferase (ALT) > 100 units/L; renal – serum creatinine > 1.0 mg/dL and oliguria (urine output > 0.5 mL/kg hr), Hematologic - thrombocytopenia < 100 000/mm$^3$ and prothrombin time INR > 1.5 x above normal, and central nervous system - Glasgow coma scale < 12 In absence of sedatives.

***Supplementary material III -*** Additional variables by phenotype among included studies.

| Variable | Study ID and phenotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Carcillo et al 2017** *n*=100 | | | **Carcillo et al 2019** *n*=401 | | | **Qin et al 2022*** *n*=404 | | | |
| | **IPMOF** *n*=24 | **TAMOF** *n*=15 | **SMOF** *n*=6 | **IPMOF** *n*=85 | **TAMOF** *n*=37 | **SMOF** *n*=7 | **A** *n*=136 | **B** *n*=102 | **C** *n*=110 | **D** *n*=56 |
| **Age, Mean (SD) or Median [IQR][a]** | 7.8[b] (5.97) | 7.7 (5.73) | 4.2 (3.02) | 7.7 [3.2-14.0][b] | 7.9 [2.7-15.0][b] | 5.5 [2.3-15.6] | 3 (4)[b] | 8 (6) | 10 (5) | 8 (6) |
| **Infection type, *n* (%)** | | | | | | | | | | |
| **-Bacterial** | 15 (62) | 10 (67) | 3 (50) | 38 (45) | 15 (41) | 3 (43) | 43 (31.6) | 33 (32.4) | 45 (40.9) | 20 (35.7) |
| **-Viral** | 5 (21) | 3 (20) | 1 (17) | 20 (24) | 12 (32) | 1 (14) | 60 (44.1) | 21 (20.6) | 24 (21.8) | 9 (16.1) |
| **-Fungal** | 2 (8) | 0 (0) | 1 (17) | 3 (4) | 3 (8) | 0 (0) | 0 (0) | 1 (1) | 0 (0) | 3 (5.4) |
| **-Culture negative** | **Total**: 25 (25)[c] | | | **Total**: 176 (43,9)[c] | | | 47 (34.6) | 52 (51) | 50 (45.5) | 28 (50) |
| **Comorbidities, *n* (%)** | | | | | | | **Previously healthy, *n* (%)[§]** | | | |
| | | | | | | | 96 (70.6)[b.] | 28 (27.5) | 37 (33.6) | 19 (33.9) |
| **-Chronic illness** | 20 (83)[b] | 11 (73) | 4 (67) | **Total:** 223 (56)[c] | | | - | | | |
| **-Cancer** | 10 (42)[b] | 2 (13) | 1 (17) | 20 (24)[b] | 10 (27)[b] | 2 (29) | - | | | |
| **-Previous Transplant** | 8 (33) | 4 (27) | 1 (17) | 4 (5) | 2 (5) | 0 (0) | - | | | |

*MOF* - multiple organ failure; *IPMOF* - immunoparalysis associated MOF; *TAMOF* - thrombocytopenia associated MOF; *SMOF* - sequential MOF.

*A, B, C and D refer to the computable derived phenotypes (PedSep-A, -B, -C or -D, respectively) according to clinical variables that were analyzed to form 4 different clusters of patients in this study.

[a]Carcillo et al 2017 and Qin et al 2022 used Mean (SD), Carcillo et al 2019 used Median [IQR].

[b]Statistically significant association between phenotype and variable (p value <0.05).

[c]Not discriminated by phenotype in the study.

[§]In the study by Qin et al, the authors characterized the patients as previously healthy and in the additional file, they described comorbidities individually (e.g. leukemia, liver disease and more) for each phenotype, not in categories (e.g cancer, chronic illness) like the other two included studies.