Low ratio of IL-10 to IFN-γ and Low IL-10 level indicate good prognosis in Salmonella enterica serovar Typhimurium triggered secondary hemophagocytic lymphohistiocytosis: a case report and literature review

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Case Report

Keywords: anti-infection therapy, hemophagocytic lymphohistiocytosis, IFN-γ, IL-10, Salmonella Typhimurium

Posted Date: April 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2766701/v1

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Abstract

Background: Secondary hemophagocytic lymphohistiocytosis triggered by *Salmonella enterica* serovar Typhimurium is rare in pediatric patients. There is a specific cytokine pattern to differentiate HLH subtypes, including IFN-γ, IL-10, and IL-6, and the ratio of IL-10 to IFN-γ, which can guide HLH treatments choice.

Case presentation: We present a pediatric 9-year old patient who presented with fever and pancytopenia for three days into our hospital, who showed positive results of *Salmonella enterica* serovar Typhimurium, Human Rhino Virus and Mycoplasma Pneumoniae infections. At the time of admission to our institution, the patient’s Th1/Th2 cytokine results showed that IL-6 was 326 pg/ml, IL-10 was 9.1 pg/ml, and IFN-γ was 246.7 pg/ml, with a ratio of IL-10 to IFN-γ was 0.04. In this study, this patient received meropenem, linezolid, and cefoperazone/sulbactam for anti-infection therapy, combined with high dose methylprednisolone therapy and anti-shock supportive treatments for twice. After careful evaluation, this patient did not receive HLH chemotherapy during the whole disease course, and he recovered well.

Conclusions: Early antimicrobial and supportive treatment would be enough for *Salmonella* triggered sHLH with a ratio of IL-10 to IFN-γ ≤ 1.33, and IL-10 ≤ 10.0 pg/ml, and/or IFN-γ ≤ 225 pg/ml on admission, and HLH-94/2004 protocol was not necessary in such condition.

Background

Hemophagocytic lymphohistiocytosis (HLH) is a potentially life-threatening syndrome leads to increased secretion of inflammatory cytokines, causing systemic inflammatory symptoms and signs[1]. HLH comprised by primary HLH (pHLH) and secondary HLH (sHLH) these two different forms[2]. sHLH could occurs in the absence of an underlying predisposing defect, typically in the setting of an infectious, malignant, or autoimmune trigger. sHLH is usually associated with viral, bacterial, fungal, and parasitic infections, such as Epstein-Barr virus (EBV), cytomegalovirus, leishmaniasis, *S.aureus, Mycobacterium tuberculosis*, and so on. *Salmonella enterica* serovar Typhimurium (S.Typhimurium), a Gram-negative species, which could be an independent trigger of sHLH and the inflammatory response is essential for this pathogen to colonize the intestinal tract, causing self-limiting gastroenteritis in human[3, 4].

Cytokine storm syndrome was associated with a variety of hyperinflammatory conditions, such as HLH[5]. In our previously study, we reported a specific cytokine pattern for HLH. When using the criteria of IFN-γ > 75 pg/mL and IL-10 > 60 pg/mL, the specificity of HLH reached 98.9% and the sensitivity was 93.0%, with a moderately increased level of IL-6 showing a median level as 51.1 pg/mL[6]. Furthermore, highly increased IL-10 (≥ 456 pg/mL) at diagnosis were independent prognostic factors for predicting early death in children with HLH[7]. Our further research demonstrated that a four-quadrant model was a useful tool for a simple evaluation of the HLH situation, as the ratio of IL-10 to IFN-γ > 1.33 combined a concentration of IFN-γ ≤ 225 pg/ml were considered to have pHLH. Distribution of these two parameters...
infection triggered sHLH (like *S.*Typhimurium triggered sHLH) were scattered, which usually showed a ratio of IL-10 to IFN-γ ≤ 1.33[8].

In this study, we present a boy who presented with fever and pancytopenia and fulfilled HLH diagnosis criteria, whose blood culture showed positive result of *S.*Typhimurium infection. Anti-infection therapy and supportive treatment were enough to cure this patient from a potentially reversible disease.

**Case Presentation**

A previously healthy 9-year old Chinese boy presented with fever and pancytopenia for three days was admitted to our hospital on September 25, 2022, and discharge on October 17. During his hospitalization, the boy experienced three febrile periods: from September 22 to September 25, from October 1 to October 2, and from October 4 to October 11. At the time of admission, this boy had fever for three days, no enlargement of the spleen below his costal margins, and laboratory data were recorded: complete blood count (CBC) showed white blood cell count (WBC) was 2.33×10⁹/L; Hb was 96 g/L; platelet count was 25×10⁹/L; ferritin > 1500 µg/L; ALT was 99 IU/L, AST was 122 IU/L; fibrinogen was 2.23 g/L, D-dimer was 2.95 mg/L, triglyceride was 1.03 mmol/L; soluble CD25 was 2646.9 pg/ml; the bone marrow biopsy revealed some hemophagocytic histiocytes and decreased megakaryocytes. Later, the fibrinogen of this patient decreased to 1.12g/L on September 30, and he was suspected for a diagnosis of HLH. His parents were not consanguineous, and he did not have pseudoalbinism.

We detected bacteria and viruses from different pathogenic locations of this patient. Nucleic acid detection of 13 pathogens from nasopharyngeal swab tested on September 26 showed that Human Rhino Virus (HRV) and Mycoplasma Pneumoniae (Mp) were positive in this boy, and the left 11 pathogens included Human Coronavirus, Influenza A Virus, Influenza A Virus (H1N1 2009), Influenza A Virus (H3N2), Influenza B Virus, Human Parainfluenza virus, Human Adenovirus, Chlamydia, Human metapneumovirus, Respiratory Syncytial Virus and Human Boca Virus were all negative. A T-cell spot of tuberculosis assay (T-SPOT) tested on September 26 showed a negative result. EBV antibodies testing showed that EBVCA-IgM was 1.26U/ml, EBVCA-IgG was 10.9U/ml, EBNA-IgG was more than 600U/ml, EBEA-IgG was negative, and EBEA-IgM was 0.06COI. On September 28, our blood culture laboratory reported a positive *S.*Typhimurium result, and we diagnosed this boy was infected with *S.*Typhimurium. The culture results of stool and cerebrospinal uid reported on October 1 were both negative. The second sampling test of blood culture reported on October 4 was negative. The culture results of urine and sputum reported on October 7 were both negative, too. The bone marrow culture was negative reported on October 11. Widal test was an agglutination reaction demonstrating *S.*Typhimurium in the serum, and the FDSO, FDSH, FDSA, FDSB, and FDSC were all less than 1:40 in this patient reported on September 30. The second sampling test of Widal test reported on October 17 showed that FDSO and FDSH were both 1:40, while FDSA, FDSB and FDSC were less than 1:40.

Th1/Th2 cytokine levels, including IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ (Fig. 1) were quantitatively determined by Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Jose, CA, USA), and CBC were
successively monitored during the course of the disease in this patient, seven controls infected by S.Typhimurium, and three EBV-HLH patients on admission (Fig. 2). After 5-day, samples from this patient, one S.Typhimurium control and three EBV-HLH patients were tested for Th1/Th2 cytokine levels, and CBC were tested in this patient, five S.Typhimurium controls, and three EBV-HLH patients. Two four-quadrant analysis about ratio of IL-10 to IFN-γ, IL-10 level, and IFN-γ level of this patient on his admission were shown (Fig. 3).

From September 25 to September 26, this patient received meropenem and linezolid anti-infection therapy. From September 26 to September 28, this patient received meropenem and high dose methylprednisolone therapy. From September 28 to October 11, this patient received cefoperazone/sulbactam anti-infection therapy which was sensitive for S.Typhimurium. During the inpatient period, this boy experienced two shocks, which happened on September 25 and October 3, respectively, with blood pressure decreased to 78/40mmHg and 72/44mmHg. Both shocks were happened after a fever span and the body temperature just went back to normal level. After anti-shock therapy, his vital signs back to stable. From October 11 to October 17, this patient received meropenem therapy again. After careful evaluation, this patient did not receive HLH chemotherapy during the whole disease course, and discharged on October 17. During the non-hospitalized period, he was followed-up mainly by telephone for four months, and he recovered very well.

Discussion And Conclusions

The mechanism of infection progressing into sHLH is still unclear these days. S.Typhimurium is one of these pathogens which can trigger sHLH. Diane E. Brown et al used S.Typhimurium by oral gavage to mimic naturally-occurring infection, and after three weeks the most severely infected mice met six of eight of the HLH diagnostic criteria, which succeed an sHLH, demonstrating that S.Typhimurium alone in this animal model was an independent trigger of sHLH[9]. However, in pediatric patients who infected with S.Typhimurium, most of them did not progress into sHLH. In this study, we checked eight children infected with S.Typhimurium, and only one of them fulfilled the diagnosis criteria of HLH. At the same time, in addition to S.Typhimurium infection, this patient was infected by both HRV and Mp. The mechanism between S.Typhimurium infection and sHLH may includes multiple immunoreactions, which needs further study.

There is no consensus about how to treat S.Typhimurium triggered sHLH. Eight-week of dexamethasone only treatment and HLH-94/2004 protocol was not necessary in all sHLH patients. Early intervention are needed to improve outcomes in HLH[10]. Some researchers showed that antimicrobial and supportive treatment alone were enough for Salmonella typhi (S.typhi) related sHLH. Navneet et al reported a sHLH patient triggered by S.typhi was treated by ceftriaxone along with supportive care[11]. Juanita Uribe-Londono et al reported a sHLH patient triggered by S.typhi was treated by piperacillin-tazobactam, intravenous immunoglobulin, and ciprofloxacine, who completed 14 days of antibiotic treatment and discharged from hospital[12]. Arslansoyu Çamlar et al reported an early diagnosis of S.typhi, treatment with parenteral ceftriaxon and supportive treatment prevented progression of HLH[13]. Caksen et al
reported a sHLH patient associated with typhoid fever was given oral chloramphenicol for 14 days, who was symptom-free during a 7-month of follow-up[14]. Awais Abbas et al reported a sHLH patient triggered by *Salmonella enterica* serotype typhi (*S. enterica* serotype typhi) was started on ceftriaxone, later switched the regimen to tazobactam and vancomycin, and discharged after 32 days[15]. Lemuel et al reported a patient with *S. enterica* serovar typhi related sHLH who started empirically on intravenous vancomycin, cefepime, and doxycycline, and eventually de-escalated to intravenous ceftriaxone, who discharged 2 weeks later[16]. Our clinical practice with this patient was consistent with these similar findings. By receiving meropenem, linezolid, and cefoperazone/sulbactam for anti-infection therapy, and combing with high dose methylprednisolone therapy, this patient recovered very well.

However, many researchers used both antimicrobial treatment and HLH protocol to treat sHLH triggered by *Salmonella* infections at the same time. Currently, dexamethasone, etoposide, cyclosporine A, and ruxolitinib are main choice in HLH treatment[17]. Tomoya Iwasaki et al reported a sHLH patient caused by *S. enterica* serovar Typhi, and after the completion of treatments of meropenem, acyclovir, intravenous immunoglobulin and dexamethasone, who was discharged on day 16 of hospitalization[18]. Nimisha et al reported a sHLH patient caused by *S. typhi* and progressed into *Salmonella* meningitis, after given ceftriaxone, third-generation cephalosporins, dexamethasone, and etoposide therapy, that girl eventually gave a good prognosis[19]. Akkız Şahin Yaşar et al considered a sHLH patient caused by *S. typhi*, used antibiotherapy ceftriaxone for 14 days, dexamethasone, and intravenous immunoglobulin G, and the clinical findings available were dramatically improved[20]. In this study, after careful evaluation, our patient did not receive HLH chemotherapy during the whole disease course. The clinician’s decision of whether HLH-94 treatment protocol should be standard therapy for treatment of patients with *Salmonella* triggered sHLH is required in these situations. Most of the current research is empirical, the decision making process is relevant to time point of positive culture result and clinician’s experience. Reliable laboratory markers which can differentiate subtypes of HLH at early stage would supply tremendous help for such condition.

In our previous research, different cytokine patterns of various subtypes of pediatric HLH were simple evaluation tools for early initiation of HLH treatment, and the result of Th1/Th2 cytokines can be obtained within 5 hours[8]. Patients with IFN-γ level ≤ 225 pg/ml and a ratio of IL-10 to IFN-γ ≤ 1.33 would have the best outcome in all HLH subtypes, and part of them used dexamethasone only, some patients used dexamethasone and cyclosporin A together, some other part used HLH-94/2004 protocol, and the rest chose other treatment options. In this study, the patient’s IFN-γ level was 246.7 pg/ml on admission, with a ratio of IL-10 to IFN-γ was 0.04, which mean the ratios of IL-10 to IFN-γ in this patient, the seven controls with *S. Typhimurium* infection, and three EBV-HLH patients were all lower than the 1.33 dividing line. Meanwhile, five controls with *S. Typhimurium* infection showed IFN-γ level lower than 225 pg/ml, and another control with *S. Typhimurium* infection and this patient showed slightly higher IFN-γ level than the 225 pg/ml dividing line. These results were consistent with our previous study.

At the time of admission to our institution, the patient’s Th1/Th2 cytokine result showed that his IL-10 level was 9.1 pg/ml, and decreased to even lower level of 2.9 pg/ml. The lower level of IL-10 in
Salmonella Typhimurium associated sHLH may demonstrate good prognosis, as some researchers reported that elevated IL-10 levels were associated with poor prognosis in adult HLH[21, 22]. Compared to our classical HLH cytokine pattern, the IFN-γ, IL-6, and the ratio of IL-10 to IFN-γ in this patient were consistent with our previous study, while his IL-10 was much lower than other sHLH subtypes. Low IL-10 level may demonstrate the good prognosis of this boy. In our previous study, the median level of IL-10 in infection-associated HLH triggered by pathogens other than EBV was 72.4 pg/ml, in EBV-HLH was 492.4 pg/ml, and in malignancy-associated HLH was 361.3 pg/ml, confirming that low levels of IL-10 in sHLH subtypes indicated good prognosis. Meanwhile, the seven controls with S.Typhimurium infection in this study all showed similar low levels of IL-10, all of which were ≤ 10.0 pg/ml. Pancytopenia did not occur in any of these seven control children, and they recovered well after anti-infection therapy. The role of cytokines patterns in the evaluation of HLH treatment was important, as IL-10 ≤ 10.0 pg/ml was a good prognosis marker in sHLH.

There were some limitations in this study. First, there was the impossibility to precisely differentiate pHLH from sHLH, as this patient did not take pHLH related gene examinations during this study period. Second, the seven controls infected with S.Typhimurium recovered well and only part of them agreed to re-check the cytokines and CBC for the second time, which led to missing data. Finally, there was only one case infected with S.Typhimurium progressed into sHLH, and we could not do cohort analysis of specific cytokine pattern.

In summary, if a Salmonella triggered sHLH patient showed a ratio of IL-10 to IFN-γ ≤ 1.33, and IL-10 ≤ 10.0 pg/ml, and/or IFN-γ ≤ 225 pg/ml on admission, early antimicrobial and supportive treatment would be enough. Eight-week of dexamethasone and HLH-94/2004 protocol was not necessary in such a patient.

**Abbreviations**

HLH  
hemophagocytic lymphohistiocytosis  
pHLH  
primary HLH  
sHLH  
secondary HLH  
EBV  
Epstein-Barr virus  
S.Typhimurium  
Salmonella enterica serovar Typhimurium  
CBC  
Complete blood count  
WBC  
White blood cell count
Declarations

Ethics approval and consent to participate

This case report was approved by the Institutional Review Board and Ethics Committee of Children's Hospital of Zhejiang University School of Medicine, and was conducted in accordance with the tenets of the Helsinki Declaration. Written informed consent was obtained from the guardian of this patient for publication of this case report and any accompanying images.

Consent for publication

Written informed consent is obtained from the parents for publication of the case report.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by as supported by Zhejiang Medical Health Technology Project (No.2022497122, No.2019KY437), Natural Science Foundation of Zhejiang Province (LQ21H080003). These founders did not take part in the study.

Authors’ contributions

YYC and XJX designed the study. YYC, LFL, and XZX did the acquisition and analysis of data. YYC and SPL drafted the manuscript. XJX revised the manuscript. All authors approved the final version to be published.
Acknowledgement

We thank Prof. Yongmin Tang for his hematology instructions.

References

5. Randy Q, Cron GGAW. Cytokine Storm Syndrome. ANNU REV MED; 2023.


**Figures**
Figure 1

Th1/Th2 cytokine levels in this patient, seven controls infected with *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), and three EBV-HLH patients on admission and 5-day later. Th1/Th2 cytokines includes including IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ.
Figure 2

Complete blood count (CBC) in this patient, seven controls infected with *Salmonella enterica* serovar Typhimurium (S.Typhimurium), and three EBV-HLH patients on admission and 5-day later. Th1/Th2 cytokines includes including IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ. CBC includes white blood cell count (WBC), absolute lymphocyte count (ALC), absolute neutrophil count (ANC), Hemoglobin (Hb), platelet count, and C-reactive protein (CRP).
Four-quadrant models to differentiate sHLH patients with different features. (a) Distribution of this patient, seven controls infected with *Salmonella enterica* serovar Typhimurium (S.Typhimurium), and three EBV-HLH patients in a four-quadrant diagram based on the ratio of IL-10 to IFN-γ (> 1.33 or ≤ 1.33) and the IFN-γ level (> 225 pg/ml or ≤ 225 pg/ml). (b) Distribution of this patient, seven controls infected with S.Typhimurium, and three EBV-HLH patients in a four-quadrant diagram based on the ratio of IL-10 to IFN-γ (> 1.33 or ≤ 1.33) and the IL-10 level (> 10 pg/ml or ≤ 10 pg/ml).