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Prognostic factors and outcomes in adults with secondary hemophagocytic lymphohistiocytosis: a single-center experience

Authors

Elia Apodaca[1], Sergio Rodríguez-Rodríguez[1], Elena Juventina Tuna-Aguilar[1], Roberta Demichelis-Gómez[1,*]

Affiliation

[1] Hematology and Oncology Department

"Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"

Mexico City, Mexico

[*]**Corresponding author**

Address: Vasco de Quiroga 15, Belisario Domínguez, Tlalpan, CP 14080, Mexico City, Mexico. Phone number: +52 55 54870900 ext 2700. E-mail: robertademichelis@gmail.com

Abstract

Introduction*:* Hemophagocytic lymphohistiocytosis (HLH) is a disorder caused by severe immune activation. There are no specific criteria to establish the diagnosis in adults, however the HLH-04 criteria are among the most commonly used. The HScore is a non-validated tool that can also be useful for HLH diagnosis. **Objective**: We described the prognostic factors and outcomes of 64 adults diagnosed with HLH in a reference medical center in Mexico City. **Methods:** We included patients ≥ 18 years with HLH, diagnosed and treated at our institution from 1998 to 2016. **Results:** The median age was 35 years (range, 18-77 years). The underlying cause of HLH was lymphoma in 33 patients (MA-HLH) (51.56%). Cutaneous involvement was more frequent in MA-HLH (33.33%), when compared to patients with not-malignancy associated HLH (NM-HLH) (9.68%) (p=0.022). Neurological symptoms were more frequent in NM-HLH (25.81%) vs MA-HLH (6.06%) (p=0.032). After a median follow-up of 14 months (range, 0-216 months), 30-day mortality was 26.56%. 3-year overall survival (OS) was higher for patients with MA-HLH compared to patients with NM-HLH (41% vs. 22.5%; p=0.046). Multivariate analysis showed that the presence of nosocomial infection and neurological symptoms were statistically significant predictors of inferior OS (p=0.034, p=0.033, respectively). **Conclusion:** In this series of adults with HLH, patients with nosocomial infections and neurological symptoms had a statistically significant worse OS. It is the largest series in Latin America, the most common cause of HLH was T-cell lymphoma. In our population, NM-HLH presented a higher mortality.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome (HS), is a serious clinical condition characterized by a sustained activation of the mononuclear-phagocytic system, which in turn results in an uncontrolled inflammatory response. Patients usually present with fever, cytopenias, and hyperferritinemia, ultimately leading to multiple organ failure [1,2].

HLH can be divided into two broad entities: primary HLH and secondary (reactive) HLH. Primary HLH encompasses mutations of the HLH family that are inherited with an autosomal recessive pattern. Secondary HLH is associated to diseases that can cause alterations in immune regulation, such as malignancies (particularly lymphoma), autoimmune and infectious diseases[1,2,3].

The annual incidence of HLH in the United States (US), Sweden and Italy has been estimated at 1 to 10 cases per 1,000,000 of people in pediatric populations. The national registry of Japan has reported an incidence of 1 case per 800,000 people per year, including both children and adults. Concerning adults with HLH, the available epidemiological data is very limited [4].

Additionally, information regarding HLH in Latin America is scarce. One of the few studies that reports such data, includes an article from 1998, which describes 13 patients with lymphoma-associated HLH, comparing them to non-lymphoma associated HLH. They reported a statistically significant inferior survival in the first group (7 vs. 48 months; p=0.0001) [5].

Typical clinical features in patients presenting with HLH include fever (95-96%), splenomegaly (60-79%), bicytopenia (84.9%), and hypertriglyceridemia (71-73%).

Additionally, an increased level of transaminases and total bilirubin (TB) can be observed, reaching 5 and 2.7 times the normal superior limit, respectively. Renal impairment frequency varies from 39 to 88% of the patients (acute kidney injury or nephrotic syndrome), and hyponatremia can present in up to 71%, secondary to the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Disseminated intravascular coagulation (DIC) frequency also varies, affecting from 13.9 to 86% of the patients. Lastly, neurological manifestations have been reported in up to 25% of adult patients, having a higher frequency in the pediatric population. Over half of all the HLH cases culminate in multiple organ failure, with up to 86% requiring admission to the intensive care unit [4,5,6].

An excessive inflammatory response plays the center role in the pathophysiology of HLH, mainly because of hyperactivation of T CD8+ lymphocytes, macrophages, the subsequent inflammatory infiltrate of diverse organs, and the ensued hypercytokinemia produced by uncontrolled helper T CD4+ cells [7].

Currently, there are no specific diagnostic criteria for HLH in adults, diminishing the probability of timely recognition and complicating the possibility of an accurate differential diagnosis (such as sepsis or advanced-stage lymphoma). Additionally, the limitations associated in using the 2004 pediatric criteria for HLH (HLH-04) in adult population have been reviewed, reporting the lack of accessibility to molecular testing, and the availability of the NK cell and soluble IL-2 receptor function, among others, as well as the wider spectrum of clinical manifestations in adult population[4,8,9].

The objective of this study was therefore to describe the etiology, clinical characteristics, prognostic factors and survival outcomes of HLH when associated to a malignant disease (MA-HLH), and comparing them to those not-malignancy associated (NM-HLH).

MATERIAL AND METHODS

Our study was designed as a single-center comparative retrospective analysis, performed at the National Institute of Medical Science and Nutrition "Salvador Zubirán" in Mexico City. Study subjects included patients diagnosed and treated from May 1998 to October 2016. Inclusion criteria included patients ≥ 18 years of age who fulfilled 5 out of the 8 diagnostic criteria from the HLH-04[8]: 1) fever ≥ 38.5 ºC; 2) splenomegaly; 3) bicytopenia (hemoglobin ≤ 9 g/dl, platelets ≤ 100 000/µl or neutrophils ≤ 1000); 4) triglycerides ≥ 265 mg/dl or hypofibrinogenemia ≤150 mg/dl; 5) ferritin ≥ 500 µg/L; 6) hemophagocytosis in bone marrow or spleen or liver or bone or lymph nodes; 7) soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/ml; 8) low or absent NK cell activity. The last two parameters were not measured because of its unavailability, and therefore the diagnosis was based on 6 criteria, modifying the standard for establishing a diagnosis to include patients with at least 5 of the 6 diagnostic criteria from the HLH-04.

Demographic, clinical and biochemical characteristics of HLH patients were described. Two subgroups were created for the outcome analysis, regarding the association to malignant disease: MA-HLH vs. NM-HLH. Specific disease diagnosis made according to the following criteria: patients with lymphoma were diagnosed according to the 2008 World Health Organization (WHO) classification [10]; patients with rheumatic diseases were diagnosed according to the American College of Rheumatology parameters; infectious diseases were diagnosed with agar cultures (blood or other body fluids), polymerase chain reaction (PCR), and/or histopathology reports. We did not realized studies for the detection of genetic alterations for the diagnosis of familial HLH, since they were not available. In cases where a cause could not be firmly established the etiology was classified as

idiopathic. DIC was defined as per the International Society on Thrombosis and Haemostasis score [11]. Therapeutic response was determined by evidenced clinical improvement, as well as per biochemical parameters (ferritin, liver function tests, fibrinogen) [8]. The HScore was calculated, and a cutoff value > of 169 points was considered as the best value for diagnosis (sensitivity: 93%, specificity: 86%, accuracy: 90%) [9].

All statistical analyses were performed using the SPSS software (version 21). Results are presented in terms of medians and ranges for quantitative variables, as well as percentages for categorical variables. For comparisons between groups, we used Fisher´s exact test and Chi-square test for the categorical variables; regarding quantitative variables, we compared them with the Mann-Whitney U test and the Kruskall-Wallis test. Overall survival was defined as the time relapsed from diagnosis to last follow-up or death. We used the Kaplan-Meier method and the Cox regression model in order to determine survival and prognostic factors.

RESULTS

Clinical characteristics, biochemical parameters and HLH etiology

A total of 64 patients diagnosed with HLH were included in this study. Median age at diagnosis was 35 years (range, 18 - 77); in terms of gender, 37 patients (57.81%) were male. Clinical presentation varied considerably: among the total population, 63 patients (98.4%) presented with fever, with 47 individuals (74.6%) presenting a body temperature higher than 39.4 °C. Signs indicating activation of the reticuloendothelial system were prominent and included hepatomegaly (n=46; 71.9%), splenomegaly (n=50; 78.1%) and lymphadenopathy (n=47; 73.4%). Other clinical manifestations included: skin involvement

(n=14; 21.8%), lung involvement (n=21; 32.8%), and unspecified gastrointestinal symptoms (n=17; 26.6%). Renal injuries occurred in 25 patients (39.1%), and were all catalogued as acute kidney injury (AKI).

At the time of diagnosis, 63 patients (98.4%) presented with cytopenias: thirty (46.9%) had hemoglobin (Hb) ≤ 9 g/dl; nine (14%) had a total neutrophil count of ≤ 1000 cells and forty-three (67%) had thrombocytopenia with ≤100 000 platelets.

The most frequently affected organ was the liver, in 34 patients (53.1%); alterations in liver function tests (LFTs) included: total bilirubin ≥ 2 mg/dl in 30 patients (47%), AST and ALT levels ≥ 3 times over the higher limit in 47 patients (74%). All patients (100%) had a ferritin level > 500 µg/L, while 49 patients (77.2%) had a ferritin level ≥ 2000 µg/L. Hypofibrinogenemia (≤150 mg/dl) was present in 20 patients (32%), and triglycerides ≥ 256 mg/dl were reported in 33 individuals (52%).

Regarding lymphopenia (≤ 200 lymphocytes), 31% of the patients presented it at diagnosis. Among the alterations of the coagulation system, we identified a prolonged partial thromboplastin time (PTT) (>50%) in 43 patients (67.2%), and the occurrence of DIC in only 7 patients (11.2%). Hemophagocytosis was identified in the bone marrow aspirate of 49 patients (76.6%); bone marrow biopsy was performed in 48 patients (75%), of which 32 (66.7%) showed hemophagocytosis.

Table 1 describes the diverse etiologies associated to HLH, whereas **Table 2** describes the characteristics for the entire population, as well as the comparison between MA-HLH vs NM-HLH. Skin involvement was more common in MA-HLH vs NM-HLH (33.3% vs. 9.7%; p=0.022). The opposite was true for neurological involvement (coma, seizures, altered state of consciousness, cerebral hemorrhage, delirium and psychosis, only if the symptoms were referred to in the medical file or if they were confirmed by lumbar puncture

and/or image), which was more common in patients with NM-HLH compared to those with MA-HLH (25.8% vs. 6.1%; *p*=0.032).

Not-malignancy associated HLH (NM-HLH)

From the 64 patients included in this study, 31 patients (48.4%) did not have an associated malignancy. In this subgroup, the most frequent etiology was infection, (n=17; 54.8%). Among these, the most common infectious agent was the human immunodeficiency virus (HIV) (n=8; 47%), followed by Mycobacterium tuberculosis (n=4; 23.5%), and Epstein-Barr Virus (EBV) (n=1; 5.9%). The second most common etiology was autoimmune disease (n=3 patients (9.7%); 100% of the patients had systemic lupus erythematosus (SLE). In just one case, a familial etiology was suspected (3.2%). The remaining NM-HLH cases were classified as idiopathic (32.3%).

Among patients with NM-HLH, 13 individuals (41.9%) had received immunosuppressive therapy prior to the HLH diagnosis. In terms of HLH therapy, 15 patients (48.4%) received corticosteroids, of which 22.6% corresponded to dexamethasone (10 mg/m^2). Nine patients (29%) were treated with the HLH-2004 protocol, whereas 8 individuals (25.8%) only received treatment for the underlying disease. Four patients (12.9%) were treated for the underlying disease in addition to corticosteroids, and 2 patients (6.5%) only received steroids. In terms of response to therapy, 16 patients (51.6%) achieved a complete response (CR) for HLH, while 12 patients (38.7%) continued to live in CR.

There were 15 deaths (48.4%) directly attributed to HLH. During hospitalization 16 patients (51.6%) presented a nosocomial infection, the most common being pneumonia (n=9; 29%). A total of 45.2% of patients presented septic shock and required invasive mechanical ventilation.

An HScore >169 was reported in 22 of the 31 NM-HLH cases (70.96%), with a median score of 224 points (range, 119 – 293).

Malignancy-associated HLH (MA-HLH)

A total of 33 patients were diagnosed with MA-HLH, 100% of which corresponded to lymphoma. These patients had the following behavior: 18 patients (54.6%) presented with T-cell non-Hodgkin lymphoma (T-NHL); 9 patients (27.3%) presented with B-cell non-Hodgkin lymphoma (B-NHL); and 6 patients (18.2%) were diagnosed with Hodgkin lymphoma (HL).

Among patients with T-NHL, the most frequent subtype was peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) (n=9, 50%), followed by extranodal NK/T-cell lymphoma (n=6, 33.3%). Treatment-wise, 20 patients (61.1%) received dexamethasone, with 50% of them receiving an initial dose of 40 mg per day, during 4 days. Thirteen patients (38.9%) received chemotherapy: 50% received etoposide as part of the therapeutic regimen, and only 1 patient (5.6%) as part of the HLH-2004 protocol. Regarding treatment response, only 11 patients (33.3%) achieved a CR to HLH. From the 18 patients diagnosed with T-NHL, 11 individuals (61.1%) had a nosocomial infection, the most common of being pneumonia. In terms of outcomes, during the last follow-up 11 patients (61.1%) had died, with 38.7% of these deaths attributed to HLH.

Regarding patients with B-NHL, 7 patients (77.7%) were classified as diffuse large B cell lymphoma (DLBCL). In terms of therapeutic approach, 7 patients (77.8%) received dexamethasone at diagnosis, with 50% receiving an initial dose of 40 mg per day, during 4 days; the other half received 10 mg/m^2. Most patients underwent chemotherapy (44.4%); among these, 33.3% received etoposide as part of the chemotherapeutic scheme, and only 1

patient (11.1%) received the HLH-2004 protocol. Concerning treatment response, 6 patients (66.7%) achieved a CR to HLH. At the last follow-up, 4 patients (44.4%) were alive and in CR, and only 1 patient (11.1%) had died secondary to HLH complications.

Finally, patients with HL mostly included the mixed cellularity subtype. Dexamethasone was administered to 6 patients (33.3%) at the time of diagnosis, and 5 individuals (83.3%= underwent chemotherapy. Regarding treatment response, 4 patients (66.7%) achieved CR to HLH. At the last follow-up, 2 patients (33.3%) were alive and free of illness, while other 2 indivuals (33.3%) had died from HLH complications.

An HScore > 169 was reported in 28 of the 33 MA-HLH cases (84.8%) with a median score of 221 points (range, 115 – 312).

Survival outcomes and prognostic factors

Median follow-up was 14 months (range 0-216 months). Of the 64 patients included in this study, 31 (48.4%) died. Among the deceased patients, 17 (26.6%) died during the first 30 days after diagnosis. Median OS in all HLH patients was 4 months (range 0-24.92 months). When comparing NM-HLH to MA-HLH patients, the first group had a significantly inferior 3-year OS, compared to the second one (22.5% vs. 41%; p=0.046) (**figure 1**).

Chemotherapy regimens including etoposide did not have an impact in terms of OS, regardless of HLH etiology. Univariate analysis showed several factors associated to a worse prognosis, including neurological symptoms (p=0.039), DIC (p=0.016), hypofibrinogenemia (p=0.021), nosocomial infection (p=0.010), septic shock (p=0.003), and the need of invasive mechanical ventilation (IMV) (p=0.024). However, the

multivariate analysis only reported nosocomial infections (p=0.034) and neurologic symptoms (p=0.033) as independently factors associated to a worse prognosis (**table 3**).

DISCUSSION

HLH represents the final stage of an uncontrolled inflammatory process [12,13]. An increased recognition of the cytokines involved in the pathogenesis of HLH, together with the fact that the presence hemophagocytosis lacks both sensitivity and specificity, leads us to propose that HLH is an obsolete term, and that it should rather be known as an hyperinflammatory syndrome and/or hypercitokinemia [13,14].

The diagnostic criteria described by the Histiocyte Society, which was created in 1991 and updated in 2004, based on prospective studies involving pediatric patients with primary HLH [15]. Although these criteria are widely employed, their sensitivity and specificity in adult HLH are unknown, a particularly important trait since HLH in adult patients is mostly secondary to other entities, including malignancies and infections [15,16].

Our study describes the experience of our Institution in the diagnosis and treatment of adult HLH, and is the largest case series in Latin American. Similar to previous reports, our patients presented with a variety of clinical manifestations, including fever, splenomegaly, and altered LFTs in over 85% of the cases [6,16-18]. Ramos Casals et al. described in a recent review the presence of DIC in 40% of the patients; in contrast, our study reported the presence of this event in only 11.3% of the patients, resembling the data described by Jing Li et al., which reported the presence of DIC in 13.6% of 103 patients [6,17].

In terms of other accompanying manifestations, Valade et al. previously reported the main coagulation alterations in 117 patients with HLH. In their study, the most frequent alteration was hypofibrinogenemia (≤150 mg/dl), present in 52% of patients. This study also reported factors associated with a poor OS, including fibrinogen ≤200 mg/dl (p=0.04)

and prothrombin time >50% (p=0.03) [19]. In our study the presence of DIC and its association with a poor OS in the multivariate analysis showed only borderline significance (p=0.058); additionally, 67% of the patients with HLH had an increased PTT at diagnosis; however they did not have unexplained bleeding, an interesting finding which had not been previously reported. Intriguingly, the pathophysiology of these alterations remains unknown to the date. Fibrinogen decrease is not only associated with DIC and/or liver alterations, but can also be explained by the hypothesis that plasminogen levels increase secondary to the macrophage activation, resulting from the constant stimulus of IL-1 and TNF produced by the activated lymphocyte [6,19].

Lymphopenia (≤200 cells) was reported in 31% of our patients, without finding any significant differences between MA-HLH and NM-HLH. Lymphopenia had not been previously described in the reported literature available for adult HLH, and it likely plays an important role in the pathophysiology of HLH.

Different lymphocyte subpopulations had been described by Bakul et al. in a series of 21 pediatric HLH patients. Among their relevant results, they found that patients with an elevated CD8+ lymphocytes count had a less aggressive disease and a better OS (140 months [16-1228] vs. 26 days [6-148]; p=0.0385); on the other hand, patients with a decrease in CD3+ lymphocytes presented with a more aggressive disease and a decreased OS (35 [9-100] vs. 89 [3-1228] days, p=0.041) [20]. It is therefore of paramount importance to recognize different subpopulations which might have varied disease outcomes. Further research in this area is warranted as it might be useful in terms of diagnosis and therapeutic targets.

Among the clinical presentation of our group of HLH patients, the presence of skin affection stands out (presence of erythematous rash, edema, purpura, and subcutaneous

nodules), together with neurologic symptoms. Skin lesions are more common in patients with MA-HLH compared to NM-HLH (33.3% vs. 9.7%; p=0.022). In a previous study by Ramos-Casals et al., researchers found that skin lesions were present in up to one fourth of patients. The likely explanation for the higher frequency of skin affection in MA-HLH might rely on the fact that most neoplasms correspond to PTCL-NOS, which characteristically presents with skin lesions at the time of diagnosis [6,21,22]. The affection to the central nervous system (CNS) has been described in previous studies with pediatric patients, being reported as a frequent manifestation associated to poorer prognosis. Seldom studies have described this affection in adult HLH patients, mainly due to the fact that such clinical manifestations are not typical, reporting a frequency of up to 25% [6,18,20]. Gou et al. reported a series of 47 patients with HLH, describing in their multivariate analysis that the presence of CNS symptoms had poor prognosis (p=0.045), which is similar to the results from our study (p=0.033). Another study that reports the same findings is the one by Lim et al., which reported a higher frequency of neurologic symptoms in the group of patients with NM-LHL (25% vs. 13%; p=0.025) [23,24]. It is not yet clear why our study reports a higher frequency of neurologic affection in the NM-HLH group (25.81 vs. 6.06%, p=0.032); a likely explanation is the fact that NM-HLH diagnosis in our Institution was usually performed when at more advanced stages of the disease.

Regarding HLH diagnosis, ferritin is one of the most relevant serum markers. A retrospective study by Allen et al. studied pediatric HLH patients, reporting that ferritin levels ≥10 000ug/L have a sensitivity of 90% and a specificity of 98%; in contrast, Alison el al. suggest that an increase in ferritin 5 times above its baseline level is associated to diverse pathologies, including kidney failure, hepatocellular damage, infections and hematological malignancies, suggesting that this cannot predict HLH [25,26]. In our

population, the median ferritin value was 4500 µg/L (range 15-27000 µg/L); 77.7% had a serum ferritin ≥2000 ug/L and 33.3% ≥6000 ug/L. The wide range in ferritin levels presented in our study might be a consequence of kidney or liver failure, secondary to HLH. The cutoff value for ferritin level according to the 1991 HLH diagnostic criteria was ≥500 µg/L, which had a sensitivity reported at 84% (specificity was not reported). A study by Kai et al. determined that a cutoff of 2000 µg/L had a sensitivity of 70% and a specificity of 68%; they employed a control group consisting of patients with elevated ferritin levels from causes other than HLH, comparing them to pediatric HLH patients [27].

The ferritin level in a state of hyperinflammation was 4799.2 µg/L, while cases of extreme hyperferritinemia (≥10 000 µg/L) were associated with HLH [28]. Among our patients diagnosed with HLH, 18.8% have ferritin levels ≥15 000 µg/L. We believe that a ferritin cutoff value of 500 µg/L allows the inclusion of other entities that may mimic the behavior of HLH in our population, suggesting the modification of the cutoff value to 2000 or even 4500 µg/L (our median) to improve the specificity of the diagnostic criteria for HLH. [27]

An important marker currently under research is glycosylated ferritin. Fardet et al. found a decreased level of glycosylated ferritin in patients with HLH compared to those with inflammatory syndrome who lacked HLH criteria (10% vs. 36%; $p<0.001$) [29].

Hemophagocytosis is among the diagnostic criteria for HLH, though it is not an absolute parameter since it is not always detected in the first bone marrow aspirate, requiring serial aspirates if the suspicion of HLH remains[15]. In our study, like others previously reported, the percentage of HLH patients who present with hemophagocytosis on bone marrow aspirate is 76.6%. Other reported series have found hemophagocytosis in 76.5%-87.4% of patients [6,30,31].

The most common cause of HLH reported in our study was malignancy (51.6%), followed by infection (26.6%), autoimmune diseases (4.7%), and suspected familial etiology (n=1, 1.6%, suspected from the clinical course of the disease, though not genetically confirmed); 10 patients were regarded as HLH of an unknown etiology (15.62%). Similar to the distribution reported by Riviere et al, who published a series of 162 HLH patients, 56.8% of the cases were associated with a hematological malignancy; additionally, Schram et al performed a multicenter study in which 68 adult HLH cases were reported, 49% were associated to malignancy, 33% to infection, and 22% were considered idiopathic [30,31]. These results contrast those reported by Otrock et al, who found a higher association with infection (41.1%) followed by malignancy (28.8%), autoimmunity (6.8%) and idiopathic (17.8%) [18].

In most reported series, the most common cause for NM-HLH is viral infection, commonly EBV, as described by the Mayo clinic (28% of cases). In our series only one case was associated with EBV infection [15]. In the present series, like the one reported by Otrock et al, HIV infection was reported in 47% [18]. The second most common infection in our study is tuberculosis (23.5%), compared to the series reported by Riviere et al where tuberculosis was described as the most common infection associated with HLH; the rest of the series currently reported have only 1 or 2 cases of tuberculosis [18,30,31]. The association between tuberculosis and HLH is not common, and there are few cases reported in the literature. However these reports highlight the difficulty of establishing a diagnosis in these patients due to the presence of fever of unknown origin, a delay in the onset of therapy and a mortality of up to 50%. In these patients the best course of action seems to be antituberculous therapy together with immunomodulatory drugs, their joint administration is key due to the fact that the antituberculous therapy will likely take some time to establish

its effect, while the early stages of HLH disease require an efficient control of the cytokine storm [32,33].

When comparing 3-year OS in MA-HLH vs NM-HLH, we found that OS in the latter group is inferior (41% vs. 22.5%; p=0.046). This is contradictory to what has been published in previous studies, where OS in MA-HLH is usually inferior to NM-HLH (2.8 vs. 10.7 months; p=0.007) [16-18,30,31]. There are several ways to explain this discrepancy, including the fact that the diagnosis for patients with NM-HLH occurred during a later disease stage. These patients are usually with a vague diagnosis (septic shock, without documented infection) and therefore HLH is not usually sought in the first days or even weeks. Consequentially, the disease is diagnosed at a later stage when the patients have already suffered a number of complications and organ failure. In our series, the group of patients diagnosed as NM-HLH presented septic shock in 45.2% of the cases.

A review by Machowicz et al. tried to seek factors which could help establish a differential diagnosis. They concluded that the current standard of 5 of the 8 HLH-04 criteria is not requisite in order to establish a diagnosis. Rather, parameters that should derive in a greater suspicion include hyperferritinemia, profound cytopenia without apparent cause, hypofibrinogenemia without DIC, hypertriglyceridemia, decreased c-reactive protein and cytokine profile. It is therefore proposed that a septic patient who fails to respond to antibiotic therapy and who presents a progressive decline in his medical condition should be suspected of having HLH, without the need to fulfill 5 out of the 8 criteria [34-37]. Applying this recommendation would allow for a more opportune diagnosis, early therapeutic intervention, and a decrease in mortality at least for the NM-HLH group. The second reason why we report a shorter OS in this group is the fact that one third of the patients in the NM-HLH group did not have a clear underlying condition causing HLH

(idiopathic NM-HLH). This particular group has no etiologic diagnosis, and their treatment is usually delayed.

Among these idiopathic NM-HLH cases, many could have EBV-associated NM-HLH, since only 3 patients in the idiopathic group had a viral load solicited, despite the fact that it is well known that EBV is one of the most frequent etiologic agents described in the literature [6,38]. Diagnosing an active, chronic EBV infection requires the following: 1) symptoms resembling infectious mononucleosis; 2) unusual antibody patterns against the viral capsid, early antigens and/or detecting an increase in EBV genomes in tissue, including peripheral blood; 3) the disease cannot be explained by a different diagnosis [39-41]. In our series, patients presented an antibody pattern consistent with previous infection; however, this is insufficient to discard an active, chronic EBV infection, which requires a viral load test. This would allow the establishment of an opportune and accurate diagnosis; therefore we suggest that a viral load (EBV) be requested for all patients suspected of having HLH at our Institution.

On the other hand, within the group of patients classified as having idiopathic HLH, some of them might have had lymphoma; this due to the fact that patients presented without lymphadenopathies and due to the gravity of their condition obtain a biopsy was not of dire importance. Generally, lymphoma-associated HLH is established at the same time of the diagnosis, presenting with torpid evolution and a continuously progressive decline. These aspects highlight the importance of having a well-founded clinical suspicion, together with the timely deploy of diverse tools which can prove useful in order to establish a diagnosis for both diseases and administer the correct therapy. The third reason that can explain the lower OS in this group is the fact that we had a high number of tuberculosis-associated HLH, a deadly association which results fatal in 50% of the cases [32,33].

As an attempt to solve the problematic surrounding HLH diagnosis, Farder et al. created an HScore, constituted by 9 variables, with the score > 169 points (best sensitivity 93%, best specificity 83%) [9]. However, the HScore does not include hypoalbuminemia, and coagulation system alterations, which can be present in >80% of adults with HLH [42]. Debaugnies et al found the HScore to be more efficient compared to the HLH-2004 criteria in terms of suspecting HLH, with a sensitivity and specificity of 100% and 80%, respectively, in pediatric patients, and of 90% and 79%, respectively, in adult patients [43]. In the current study, we found that 50 out of the 64 patients had an HScore >169. When divided into subgroups, 84.8% of MA-HLH had an HScore >169, compared to 70.96% of NM-HLH patients (p=0.188). A prospective study is warranted in order to validate and evaluate the usefulness of this score as per HLH etiology.

The data presented highlights the importance of improving strategies to accurately and timely establish HLH diagnosis. The current outlook must include identifying biomarkers (i.e. cytokine levels), imaging studies, changes in T lymphocytes. Amman et al. proposed diagnosing HLH by considering the different phenotypes of T cells; however studies are needed in order to strongly support the utility of these tests in a clinical setting [44].

This study has several limitations, including its retrospective nature and the impossibility of carrying out genetic studies for the diagnosic of primary HLH, which may be assorted as a diagnostic access bias. However, as HLH is a rare condition a prospective study is difficult to implement and unpractical. Another common limitation in HLH studies is the use of the HLH-2004 diagnosis criteria, which were described for pediatric population and still lack validation in adult patients. It is therefore imperative to develop and validate a diagnostic method which can accurately identify HLH patients, particularly those with secondary

HLH. We suggest the inclusion of hypertransaminasemia and hypoalbuminemia in such scores, since the previous alterations are present in 60 to 70% of the cases.

The main contribution of our study lies on the fact that it is the largest series of HLH patients in Latin America. Additionally, we report two strong independent factors implicated in a lower OS: the presence of neurological symptoms and nosocomial infections. As a final point, it is important to continue searching for differential diagnostic strategies for patients who present with septic shock. It is also highly relevant to correctly classify the etiology of HLH in order to offer targeted therapy and improve survival in this subset of patients.

Table 1. Etiology distribution for patients with HLH

Cases	N(%)
Malignant disease	33 (51.6%)
T-NHL	18 (54.5%)
PTCL-NOS	9 (50%)
T/NK NHL	6 (33.33%)
AITL	1 (5.55%)
ALK- T-NHL	2 (11.11%)
B-NHL	9 (27.27%)
LDBCL	7 (77.77%)
Plasmacytoid	2 (22.22%)
HL	6 (18.18%)
Infectious	17 (26.56%)
HIV	8 (47.05%)
Tuberculosis	4 (23.52%)
EBV	1 (5.88%)
Other	4 (23.52%)
Autoimmune	(n=3, 4.68%)
Systemic erythematous lupus	3 (100%)
Familial	(n=1, 1.56%)
Idiopathic	(n=10, 15.6%)

Abbreviations: NHL: Non-Hodgkin lymphoma; PTCL-NOS: peripheral T-cell lymphoma not otherwise specified; T/NK NHL: T-NK Non-Hodgkin lymphoma; AITL: angioimmunoblastic t-cell lymphoma; ALK- t-NHL: ALK(-) Non-Hodgkin T cell lymphoma; B-NHL: B cell Non-Hodgkin lymphoma; DLBCL: Large diffuse B cell lymphoma; EBV: Epstein-Barr virus.

Table 2. Clinical and biochemical characteristics

	HLH (n=64)	MA-HLH (n=33)	NM-HLH (n=31)	P-value
Age (years), median (range)	35 (28.25-51.00)	37 (27.50-58.00)	54 (39.75-63.25)	0.554
Sex				
Male (%)	37 (57.81)	21 (63.64)	16 (51.61)	0.448
Female (%)	27 (42.20)	12 (36.36)	15 (48.39)	
Fever (%)	63 (98.43)	33 (100)	30 (96.77)	0.484
Hepatomegaly (%)	46 (71.87)	27 (81.82)	19 (61.29)	0.061
Splenomegaly (%)	50 (78.12)	27 (81.82)	23 (74.19)	0.332
Affected organs:				
Skin (%)	14 (21.87)	11 (33.33)	3 (9.68)	0.022
Kidney (%)	25 (39.06)	6 (18.18)	10 (32.26)	0.156
Gastrointestinal tract (%)	17 (26.56)	9 (27.27)	8 (25.81)	0.560
Liver (%)	34 (53.12)	17 (51.52)	17 (54.84)	0.494
Lung (%)	21 (32.81)	8 (24.24)	13 (41.94)	0.107
Nervous system (%)	10 (15.62)	2 (6.06)	8 (25.81)	0.032
Biochemical parameters (range)				
Hb (g/dl)	9.10 (7.40-11.00)	9.10 (7.30-11.60)	12.40 (10.27-14.32)	0.783
Leucocytes ($x10^9$/L)	2.40 (3.00-16.90)	2.10 (1.35-3.70)	7.10 (5.00-8.42)	0.250
Total Neuotrophils ($x10^9$/L)	1.61 (0.70-11.25)	1.26 (0.89-2.68)	4.42 (3.01-6.19)	0.218
Total lymphocytes ($x10^9$/L)	2.84 (0.21-4.14)	0.43 (0.20-0.79)	1.12 (0.65-1.64)	0.277
Platelets ($x10^9$/L)	61 (11.00-3.87)	49 (35.5-114)	241.5 (172-337)	0.788
Total bilirubin (mg/dl)	1.40 (0.34-25.74)	1.13 (0.67-3.44)	0.62 (0.44-0.82)	0.601
AST (U/L)	80 (10-1244)	73 (33-114)	24 (16-38)	0.139
ALT (U/L)	48 (6-608)	38 (18-125)	18.5 (13-28)	0.409
Alkaline phosphatase (mg/dl)	207 (21-3299)	209 (109-451)	93.5 (72.5-118.2)	0.705
Albumin (mg/dl)	2.4 (0.9-3.8)	2.6 (1.85-3.25)	3.6 (2.80-4.12)	0.053
Fibrinogen (mg/dl)	215 (29-647)	236 (109-371)	522 (435-711)	0.348
Triglycerides (mg/dl)	281 (52-804)	298 (220-426)	141.50 (198.5-202)	0.242
Ferritin (ug/L)	4500 (15-27000)	4547 (2621-13520)	274.20 (65.5-1628)	0.143
B_2microglobulin (mg/dl)	4.6 (2.2-19.7)	4.3 (3.41-6.23)	2.97 (1.85-3.85)	0.529
LDH (U/L)	663 (176-2124)	643 (308-2061)	203 (153-380.25)	0.885

Abbreviations: HLH: hemophagocytic lymphohistiocytosis; NM-HLH: no malignancy associated to HLH; MA-HLH: malignancy associated to HLH; AST: aspartate transaminase; ALT: alanine transaminase; LDH: lactate dehydrogenase.

Tabla 3. Prognostic factors associated with HLH

	Univariate		Multivariate	
Variable	OR (95%CI)	p	OR (95%CI)	p
DIC	3.66 (1.41-9.48)	0.016	2.65 (0.96-7.28)	0.058
Nosocomial infection	2.70 (1.23-5.91)	0.010	2.80 (1.08-7.24)	0.034
Neurological symptoms	2.53 (1.12-5.74)	0.039	2.76 (1.08-7.05)	0.033

Figure 1. OS for MA-HLH vs. NM-HLH