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Benefit of Anakinra in Treating Pediatric Secondary Hemophagocytic Lymphohistiocytosis

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Objective. To assess the benefit of the recombinant human interleukin-1 receptor antagonist, anakinra, in treating pediatric patients with secondary hemophagocytic lymphohistiocytosis (sHLH) / macrophage activation syndrome (MAS) associated with rheumatologic and non-rheumatologic conditions.

Methods. We performed a retrospective chart review of all anakinra-treated sHLH/MAS patients at Children's of Alabama from January 2008 through December 2016. Demographic, clinical, laboratory, genetic, concurrent treatment, and outcome data were collected and analyzed by appropriate univariate statistical approaches.

Results. Forty-four sHLH/MAS patients treated with anakinra were identified in the electronic medical record. Median duration of hospitalization was 15 days. The mean pre-treatment serum ferritin level was 33,316 ng/mL and dropped to 14,435 (57% decrease) within 15 days of starting anakinra. The overall mortality for the cohort was 27%. Earlier initiation of anakinra (≤5 days hospitalization) was associated with reduced mortality (p=0.046), whereas thrombocytopenia (<100,000/μL) and *STXBP2* mutations were both associated with increased mortality (p=0.008 and p=0.012, respectively). Considering the underlying diagnosis, systemic juvenile idiopathic arthritis (sJIA) had the lowest mortality rate, with no deaths among the 13 sJIA patients included in the study (p=0.006). In contrast, underlying hematologic malignancy had the highest mortality rate at 100% (n=3).

Conclusion. These findings suggest anakinra appears to be effective in non-malignancy associated pediatric sHLH, especially when given early in disease course and in patients with an underlying rheumatic disease etiology.

Secondary hemophagocytic lymphohistiocytosis (sHLH) is a life threatening condition diagnosed among severely ill, febrile hospitalized patients (1). Familial, or primary, hemophagocytic lymphohistiocytosis (pHLH), presents in infancy and is often due to homozygous or compound heterozygous mutations in lymphocyte cytolytic pathway genes (2). sHLH, termed macrophage activation syndrome (MAS) when associated with rheumatic disorders (3), affects children and adults

with various disorders. sHLH and MAS can be associated with heterozygous defects in pHLH genes, and thus the distinction between pHLH and sHLH/MAS is becoming blurred (4, 5). sHLH is often associated with conditions that cause chronic immune dysregulation such as rheumatologic diseases and certain hematologic malignancies. Infectious agents, particularly members of the herpes virus family (*Herpesviridae*), may also serve as a trigger to overcome a "threshold" of disease (6). Thus, sHLH/MAS is not a diagnosis of exclusion, and many authors consider MAS, pHLH, and sHLH to be on the same spectrum of disease (1, 7).

Initial symptoms of sHLH/MAS are usually non-specific. The cardinal feature is unremitting high fever, though it is not an absolute finding in all cases (8). Other features include hepatomegaly, splenomegaly, central nervous system involvement (e.g., seizures, coma), rash, hepatic dysfunction, pancytopenia, and, ultimately, multi-organ failure (8). Disseminated intravascular coagulopathy (DIC) features are often present and are partly explained by liver dysfunction, fibrinogen consumption, and thrombocytopenia (9). To combat this highly fatal disorder, both pHLH and sHLH are frequently treated with etoposide-based protocols, which are associated with significant toxicity and mortality (10). Alternative, less toxic and more targeted therapies are currently being explored for sHLH/MAS.

Pathologically, sHLH/MAS develops as part of a 'cytokine storm' (11). The proinflammatory cytokines that have been associated with sHLH/MAS include interferon-gamma (IFN-γ), interleukin-1 (IL-1), IL-6, IL-12, IL-18, and tumor necrosis factor (TNF) (12). Anakinra is a recombinant human IL-1 receptor antagonist that blocks IL-1 function. While the safety and benefits of IL-1 blockade in the inflammatory process have been demonstrated in many diseases, including its role in treating systemic juvenile idiopathic arthritis (sJIA) and sJIA related MAS (13-16), its role in non-sJIA related MAS has not been widely explored. Nevertheless, anakinra has been reported to improve survival in several cases of MAS in non-sJIA rheumatologic disorders (17), including systemic lupus erythematosus (SLE) (15). In this current study, we have retrospectively analyzed the benefit of anakinra in treating a cohort of pediatric patients with sHLH/MAS due to various underlying diseases, including rheumatologic and non-rheumatologic conditions.

Significance and innovation:

- Recombinant human IL-1 receptor antagonist, anakinra, appears effective in treating pediatric sHLH/MAS associated with non-oncologic diseases.
- Thrombocytopenia is a risk factor for mortality in children with sHLH treated with anakinra.
- The presence of an *STXBP2* heterozygous mutation is a risk factor for mortality in children with sHLH treated with anakinra.
- Children with sJIA associated MAS have good outcomes with anakinra therapy.
- In the absence of malignancy, early initiation of anakinra therapy for sHLH is associated with decreased mortality.

PATIENTS AND METHODS

An institutional review board (IRB) approved retrospective chart review was conducted of consecutive pediatric sHLH/MAS [identified by ICD-9 (288.4) or ICD-10 (D76.1) code in the electronic medical record (EMR)] patients older than 1 year of age (to avoid pHLH), who were treated with anakinra at Children's of Alabama (CoA) from January 2008 through December 2016. There were no patients older than 1 year of age identified with homozygous HLH mutations. A diagnosis of sHLH or MAS was confirmed by using at least 1 of 6 different sets of criteria: HLH-2004 (10), HLH-2009 (18), SLE MAS (19), sJIA MAS-2016 (20), the HScore (21), or the MH score (22). Demographic, clinical, laboratory, genetic, concurrent treatment, and outcome data were collected and analyzed by appropriate univariate statistical approaches (see below). Soluble IL-2 receptor-α chain (sCD25) and natural killer (NK) function assays were performed at Cincinnati Children's Diagnostic Immunology Laboratory and tested by chromium release assay. Four different effector to target cell ratios were analyzed, and normal NK function was defined as ≥2.6 lytic units. Genetic analysis for pHLH associated genes was performed via Cincinnati Children's Genetics, and results from a subset of these patients have been previously reported (5).

Statistical analysis. Data analysis was performed using SAS software (version 9.3, Cary, NC), and descriptive statistics were used where appropriate. Patients' demographics, clinical and laboratory data, and the day of starting anakinra treatment were examined against outcome measures (duration of hospital stay, days to defervescence, drop in ferritin, and mortality) to identify any predictors of outcome. Paired t-tests were used to compare laboratory changes before and after anakinra treatment. Chi-square analysis and ordinal univariate regression analysis were used to determine the association between patient variables and outcomes. For Tables 1 and 2, the comparisons were considered hypothesis-generating, and no adjustment for multiple comparisons were made. For Table 4, chi-square analyses were performed.

RESULTS

Diagnoses. Forty-four sHLH/MAS pediatric patients treated with anakinra at CoA were identified from the EMR by ICD-9 and ICD-10 codes over the 9-year span. Forty-two children (95%) met the new sJIA MAS classification criteria (Supplemental Table 1). The more restrictive HLH-2004 and HLH-2009 criteria identified 70% and 75%, respectively. The HScore (selecting a probability score of >90%) identified 66%, and the new discriminatory MH score only identified 27% of the patients as not having pHLH. All patients, including all eight with SLE or mixed connective tissue disease (MCTD), met the SLE MAS criteria, but these criteria were not specific to SLE or MCTD. The underlying diseases of the sHLH cohort were sJIA (n=13), SLE (n=5), MCTD (n=3), malignancy/acute lymphoblastic leukemia (two with pre-B cell, one with T cell) (n=3), gastroparesis (n=3, one also had sJIA), uveitis with spondyloarthritis (n=2) [both previously reported (23, 24)], other autoimmune conditions (Sjogren disease, vasculitis, Crohn disease, sarcoidosis, anti-phospholipid antibody syndrome) (n=5), mitochondrial disorder (n=1), and unknown (n=10). Thus, sixteen (36%) of the sHLH patients did not have an identifiable rheumatic disease. Six of the 10 (60%) without any known underlying disease had identified infections, and infections were associated with 13 of the 34 patients (38%) with underlying disorders (Supplemental Table 1). Nine of the 19 total infections (47%) were members of the Herpes virus family.

Demographics and clinical presentation. The cohort as a whole included 25 females and 19 males with a mean age of 10 years (range, 1 - 19) (Table 1). Clinical presentations included persistent fever in 43 of the 44 patients (98%), hepatomegaly (30%), and splenomegaly (34%). Laboratory features in those tested included leukopenia (<1,000/μL) (55%), anemia (hemoglobin <9gm/L) (68%), thrombocytopenia (<100,000/μL) (71%), hypertriglyceridemia (>3mmol/L) (73%), hypofibrinogenemia (<1.5g/L) (44%), low NK cell function (54%), elevated soluble IL-2 receptor-α chain (sCD25 >1,105 U/mL) (89%), and hepatitis (AST >30IU/L) (98%), (H-score criteria for HLH diagnosis) (21). Thirty-five of 44 patients underwent bone marrow biopsy, of whom fourteen (40%) had hemophagocytosis (Table 1). Treatments other than anakinra that the patients received either during their sHLH/MAS presentation or prior to sHLH/MAS diagnosis are listed in Supplemental Table 2. Concomittant therapies included corticosteroids (73%), cyclosporine A (25%), intravenous immunoglobulin (9%), etoposide (9%), tocilizumab (5%), and one patient each for abatacept, rituximab, cyclophosphamide, and plasmapheresis.

HLH Genetics. Genetic testing for 5 of the pHLH associated genes (*UNC13D, STXBP2, STX11, PRF1, RAB27A*) was carried out for 38 of the 44 patients, and there was a heterozygous mutation noted in 13 of those tested (34%) (Table 2). Six patients had an *UNC13D* (MUNC13-4) mutation (16%), one of whom died. Five of the six *UNC13D* mutations resulted in amino acid substitutions (only one considered benign), and one was a predicted splice variant. Five patients had at least one *STXBP2* (MUNC18-2) mutation (13%) (Supplemental Table 3). Two had *STXBP2* amino acid substitutions, and 3 had one or more non-coding nucleotide mutations (intron, 3' untranslated region, and just upstream of the transcription start site), some resulting in splice variants. Four of the 5 (80%) patients with *STXBP2* mutations died. One patient had a perforin gene (*PRF1*) mutation, and one other patient had a *RAB27A* mutation, both of whom survived, both with amino acid substitutions.

Regarding demographics, and baseline clinical, genetic, and laboratory data, those with thrombocytopenia <100,000/μL and those with an *STXBP2* mutation were associated with increased mortality (p=0.008 and p=0.012, respectively) (Tables 1 and 2). There were no other significant differences between the patients who survived or died for any other measures.

Hospital course and mortality. The overall mortality for all patients was 27%, and the median duration of hospitalization was 15 days. Presumed causes of mortality are listed in Table 3. Five of 12 patients died with the diagnosis of shock and 6 of 12 with the diagnosis of multi-organ system failure (MOSF). Six of the 12 patients who died had confirmed systemic infections, and 5 of these patients had positive fungal cultures. Of the 12 patients who died, 11 were on concurrent immunosuppression which included corticosteroids (Table 3). There was no association with the timing of anakinra administration and infection. Survival of rheumatic/autoimmune patients was 86% (100% for sJIA and 70% for SLE and related conditons), and 50% for all other sHLH patients, including those with cancer. Survival for non-rheumatic and non-leukemic patients was 62%.

Not surprisingly, an earlier start of anakinra (≤5 days) was associated with reduced mortality (p=0.046) (Table 4). Considering the underlying diagnosis, sJIA had the lowest mortality rate with no deaths at all among the 13 JIA patients included in the study (p=0.006, Chi-square). On the other hand, underlying malignancy had the highest rate with 100% mortality (n=3). Two of these patients were in remission on dexamethasone complicated by disseminated fungal infection, and one patient who was undergoing induction therapy died of MOSF, presumably sHLH. Nevertheless, one of the pre-B cell leukemia patients (#37) presented with MAS and was effectively treated with anakinra. It was only on his third bone marrow evaluation that the leukemia was identified (6 months after presentation, long after he had been discharged from the hospital). There had been a heightened concern for leukemia from presentation. Upon making the diagnosis of leukemia, the anakinra was stopped and he quickly succumbed to infection upon initiation of induction chemotherapy for leukemia.

In comparison, 3 of the 6 patients with an underlying infectious etiology died from shock or MOSF. Of the 28 patients with rheumatic or autoimmune disease, only 4 (14%) died following anakinra administration. One of these patients with underlying Crohn disease had confirmed *Clostridium difficile* infection, and the other 3 died of MOSF and/or shock (Table 3). Four patients who died (3 from systemic fungal infections) also received concurrent etoposide as part of the HLH-04 protocol (10). In terms of timing, 2 patients received the HLH-04 protocol at the time of sHLH diagnosis. One patient (#24) received anakinra as a last ditch effort during the last 4 days of her life and 4 months

into the diagnosis. The other patient (#27) received anakinra 4 days after the HLH-04 protocol as adjunctive therapy because the patient's condition dramatically worsened. Another patient (#41) received anakinra one day after diagnosis, and started the HLH-04 protocol the next day. He eventually died 7 months later following stem cell transplantation. The fourth patient (#36) to receive etoposide had received the HLH-04 protocol previouisly and then relapsed T cell leukemia and sHLH. Dexamethsone was started but etoposide held for severe cytopenia from recent chemotherapy. Anakinra was added 11 days after admission, and etoposide 2 days later. In this retrospective study, all patients who received etoposide as part of the HLH-04 protocol died.

Thrombocytopenia at any point was also significantly associated with mortality, as all patients who died had platelet counts of <100,000/μL (p=0.025). Having a higher HScore for the cytopenia lineage was also associated with higher mortality (p=0.033).

In terms of a biomarker, the mean pre-treatment serum ferritin level was 33,316 ng/mL and dropped to 14,435 ng/mL (57% decrease) within 15 days of starting anakinra, with an increase nearly associated with death (p=0.075) (Table 1). However, a drop of ferritin within 15 days of hospitalization was significantly associated with survival (p=0.0202) (Table 1). Regarding the drop in ferritin level, an earlier start of anakinra (≤5 days) was associated with a greater drop in ferritin level (p=0.001) (Table 4). Specifically, anakinra given within 5 days of sHLH/MAS diagnosis led to a mean 90% reduction in ferritin compared to a mean 54% reduction if anakinra was given after 5 days of hospitalization. Anakinra given <3 days of hospitalization did not show further benefits. However, an earlier start of anakinra was associated with earlier defervescence (p=0.004).

DISCUSSION

Recombinant human IL-1 receptor antagonist (anakinra) has been shown to be highly effective for sJIA (13). MAS and sJIA flare share many clinical and laboratory features. Moreover, in addition to the 10% risk of developing overt MAS as part of sJIA, another 30-40% of sJIA patients may have occult or subclinical MAS during a disease flare that can eventually lead to overt MAS (25). This association of MAS with sJIA suggested that anakinra would also be a valuable treatment for sJIA-

MAS. There are several reports of dramatically successful use of anakinra in cases of sJIA-MAS (13, 14, 16), in addition to other rheumatologic conditions, such as SLE with or without antiphospholipid antibody syndrome (15, 26), acute rheumatic fever, ANCA positive vasculitis, eosinophilic granulomatosis with polyangiitis, Kawasaki disease (17, 27), and autoinflammatory conditions (14).

MAS and severe sepsis also share many clinical and laboratory features such as elevated serum ferritin, hepatic dysfunction, cytopenias, coagulopathy, CNS dysfunction (28), tissue hemophagocytosis, and elevated expression of CD163+ macrophages in the reticuloendothelial system (29, 30). These shared features are initiated and perpetuated by a cytokine storm in which IL-1 plays a major role (31, 32). IL-1 receptor blockade was associated with significant improvement in survival of patients with sepsis and concurrent hepatobiliary dysfunction/disseminated intravascular coagulation (33), and also in a series of patients with a sHLH/sepsis/multiple organ dysfunction syndrome (MODS)/MAS clinical picture which occurred in the setting of a variety of rheumatologic and non-rheumatologic diseases (34). Thus, based on the association of IL-1 signaling pathway dysfunction in these diverse conditions, we were encouraged to use anakinra in cases of MAS complicating a variety of rheumatologic and non-rheumatologic conditions. However, we should acknowledge that our study is limited by its retrospective nature, non-uniform approach to therapy, lack of treatment controls, and variable follow-up period. In particular, selection bias to use anakinra for those less severely ill may have played a role.

In this study, anakinra treatment of pediatric sHLH/MAS was associated with improved overall survival (73%) in comparison to etoposide-based protocols for pHLH and sHLH (10) (55% survival, 82% sHLH), perhaps due in part to its more acceptable safety profile relative to chemotherapy. Moreover, the earlier initiation of anakinra (≤5-days of hospitalization) was associated with further reduced mortality in all patients (Table 4). Of note, it worked best in patients with underlying rheumatologic conditions, particularly sJIA (Table 5), but it has also shown to be beneficial in other conditions such as infection related sHLH/MAS (33, 34). Patients with underlying malignancy showed poor survival, regardless of remission status, suggesting IL-1 blockade may be ineffective in treating patients with an oncologic history. Survival rates within this cohort were also lower for EBV-induced sHLH/MAS, similar to a recent report of 44% survival in a pediatric sHLH cohort, with

the worst survival in those with both EBV and CMV infections (35). Percent survival in this current study cohort was 73% as opposed to 53% and 65% in other studies (36, 37), respectively. In a nationwide registry of pediatric patients with HLH in Korea, the 5-year overall survival rate was 68% (38% in the familial group and 81% in the presumed secondary group). The 5-year overall survival rate among 32 patients who underwent allogeneic hematopoietic stem cell transplantation was 64% (38). Similar or even higher mortality rates were reported in adults with sHLH (39). This could potentially be explained by the anakinra safety profile and avoiding the need for bone marrow transplant and the pre-transplant myeloablative regimen, both of which have their own associated significant morbidity and mortality. Nevertheless, if a patient does not have a marked response to high-dose anakinra, then cytotoxic agents are probably warranted. Interestingly, prior to the introduction of anakinra, sJIA related MAS was previously reported to have a mortality rate as high as 28% (40), yet all the sJIA patients in our cohort did well, likely attributable to the use of IL-1 inhibitors (41). In this study, MAS associated with sJIA had the best outcome with 100% survival, while sHLH associated with malignancy had the worst outcome with 100% mortality. This is consistent with a reported 85% mortality in adults with hematological malignancy associated sHLH (42).

In addition to underlying disease associated mortality, other features associated with outcomes were explored. In this study, the mean level of ferritin within the first 15 days of treatment was higher in patients who died, approaching statistical significance ($p=0.075$). Moreover, the percentage drop in ferritin at 15 days was significantly associated with survival ($p=0.0202$) (Table 1) in this cohort. This is in accordance with other studies that have associated the rate of decrease in ferritin concentration with mortality in children (43). A more compelling laboratory feature from the current study was the platelet count. Seventy-one percent of the patients described herein had clear thrombocytopenia (<100,000/μL) at presentation, and this was associated with mortality ($p=0.008$) (Table 1). This is comparable to adult studies that reported disseminated intravascular coagulation in 40% of cases and reported an association with high mortality rates, especially in patients with severe thrombocytopenia (44). In the current study, thrombocytopenia at any point during hospitalization was also associated with a significant mortality, such that all patients who died had platelet counts of <100,000/μL at

some point (p=0.025). Thus, thrombocytopenia in the setting of an inflammatory state that often leads to thrombocytosis is a poor prognostic finding.

In addition to laboratory findings in peripheral blood, pathologic specimens can be informative in the setting of sHLH/MAS. Hemophagocytosis observed on bone marrow biopsy, although considered a hallmark of sHLH, was found in only 14 (40%) of the 35 patients who were tested in the present cohort. Other studies have reported hemophagocytosis in 64% (45) to 84% (46) of sHLH/MAS patients who had a bone marrow aspirate assessed or a reticuloendothelial organ biopsy performed. Our results emphasize the fact that bone marrow hemophagocytosis is not essential for the diagnosis of sHLH/MAS patients since it might be absent, particularly early on in the disease course (47). Moreover, sHLH/MAS patients are usually critically ill and might not be stable enough to undergo bone marrow biopsy, which might lead to a delay in an urgently needed diagnosis.

Although a late potential diagnostic tool, HLH genetics were also explored as prognostic factors. Of the 5 HLH genes analyzed, 34% of those tested were found to possess at least one heterozygous mutation (Table 2). This is very much in keeping with what has been reported for sHLH/MAS cohorts where genetics have been explored (4, 5). Specifically, in the current cohort, *STXBP2* mutations were found in 5 (13.2%) of 38 patients who were tested. The highest mortality among those with HLH gene mutations was associated with *STXBP2* (p=0.012), as 4 out of 5 patients with an *STXBP2* mutation died. These results are similar to those of Cetica *et al*. who reported 4 (14%) patients with a positive *STXBP2* mutation among HLH patients that lacked mutations in the *PRF1*, *UNC13D*, and *STX11* genes. Of these four patients, two (50%) died (48). Together, these findings suggest that possessing a heterozygous mutation in *STXBP2* represents a significant risk for mortality in the setting of sHLH/MAS. Indeed, we previously showed that one of these fatality-associated *STXBP2* mutations (c.1298 C>T, p.A433V) (Supplemental Table 3) acts in a partial dominant-negative fashion to inhibit NK cell cytolytic activity (5). *STXBP2* is also important for apical trafficking in epithelial cells as it is necessary for fusion of vesicles with the plasma membrane, and lack of *STXBP2* function results in villous atrophy and intestinal atresia critical for gut barrier protection (49-51). Perhaps, the association of *STXBP2* mutations and enteropathy also puts these patients at greater risk of intestinal border bacterial translocation and sepsis.

CONCLUSION

Anakinra (recombinant human IL-1 receptor antagonist) appears to be effective in the treatment of non-malignancy related sHLH/MAS in children, notably those with sJIA, but also in patients with underlying SLE, MCTD, other autoimmune diseases, and infections. Anakinra was less effective in treating those with hematologic malignancies and those with *STXBP2* mtuations. Based on these results, we recommend the use of anakinra as a safe and promising treatment for non-malignancy related sHLH/MAS patients, especially when given early in the disease course. Anakinra is currently being studied in a randomized, double-blinded, placebo-controlled trial to treat sHLH/MAS in children and adults [ClinicalTrials.gov Identifier: NCT02780583].

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Table 1. Clinical and laboratory presentation, and differences between patients who survived and died§

	Total (n=44)	**Survived (n=32)**	**Died (n=12)**	**P value**
Male	19 (43%)	13 (41%)	6 (50%)	1$^{\delta}$
Mean age (years)	10.3 (SD=5.7)	9.6 (SD=5.3)	12.3 (SD=6.5)	0.165$^{\#\delta}$
Presentation				
Persistent fever	43 (98%)	31 (97%)	12 (100%)	1$^{\delta}$
Hepatomegaly	13 (30%)	10 (31%)	3 (25%)	1$^{\delta}$
Splenomegaly	15 (34%)	12 (38%)	3 (25%)	0.5$^{\delta}$
Leucopenia <1,000/μL	24 (55%)	15 (47%)	9 (75%)	0.173$^{\delta}$
Hemoglobin <9gm/L	30 (68%)	20 (63%)	10 (83%)	0.282*$^{\delta}$
Platelets <100,000/μL	31 (71%)	19 (59%)	12 (100%)	**0.008$^{\delta}$**
Trigylcerides >3mmol/L	30 (73%) of 41	20 (69%)	10 (83%)	0.456$^{\delta}$
Fibrinogen <1.5gm/L	16 (44%) of 43	9 (28%)	7 (64%)	0.092*
Hemophagocytosis	14 (40%) of 35	11 (42%)	3 (33%)	1$^{\delta}$
Low NK cell function	13 (54%) of 24	10 (56%)	3 (50%)	1$^{\delta}$
Elevated sCD25	31 (89%) of 35	22 (92%)	9 (82%)	1$^{\delta}$
Hepatitis	43 (98%)	31 (94%)	12 (100%)	1$^{\delta}$
Baseline ferritin (ng/mL)	33,316 (SD=56,514)	28,480.8 (SD=48,582)	48,803.8 (SD=79,565)	1$^{\delta}$ 0.309$^{\#}$

Outcomes	30 (SD=40)	18 (SD=16)	62 (SD=62)	**0.0005*#**
Hospitalization (days)	14,435	1,651	51,511	0.075δ
Ferritin within 15 days of anakinra	(SD=79,842)	(SD=2,967)	(SD=157,590)	
	19,256	-26,830	+2,707	0.201#
Ferritin change (15 days)	(SD=66,334)	(SD=47,078)	(SD=104,703)	
	72% (SD=62%)	85% (SD=17%)	35% (SD=115%)	**0.0202*#**
% ferritin drop 15 days	1.7 (SD= 1.1)	1.6 (SD=1)	2 (SD=1.4)	0.297*#
Day of defervescence after anakinra				

t-test (between two means), δ Fisher exact text, * p value is statistically significant.

§ BM = bone marrow; NK cell = natural killer cell; sJIA = systemic juvenile idiopathic arthritis; SLE = systemic lupus erythematosus; MCTD = mixed connective tissue disease; APLA = antiphospholipid antibody syndrome; SpA = spondyloarthritis.

Table 2. Hemophagocytic lymphohistiocytosis (HLH) genetic testing results§

Test Result	Total (38) tested	Survived (28)	Died (10)	P value
No HLH gene mutation	25 (66%)	20 (80%)	5 (20%)	0.263
Any HLH heterozygous gene mutation	13 (34%)	8 (62%)	5 (38%)	0.263
UNC13D	6 (16%)	5 (83%)	1 (17%)	N/A
STXBP2	5 (13%)	1 (20%)	4 (80%)	**0.012***
PRF1	1 (3%)	1 (100%)	0	---
RAB27A	1 (3%)	1 (100%)	0	---

*Significant P value <0.05

§ HLH = Hemophagocytic lymphohistiocytosis; STXBP2 = syntaxin binding protein-2 gene; PRF1 = perforin-1 gene

Table 3. Causes of Death§

Pt #	Diagnosis	Cause of death	Concurrent Immunosuppression
16	SLE	septic shock	IVMPx3, CYC
19	SLE/MCTD	MOSF	none
22	EBV	septic shock	prednisolone, rituximab
24	EBV	MOSF	IVMPx3, dexamethasone, CsA, etoposide
27	HHV6	fungemia, MOSF	prednisolone, CsA, dexamethasone, etoposide
32	Sjogren	MOSF, shock	IVMPx3, IVIG, plasmapheresis
34	Crohn disese	C. difficile colitis, septic shock	IVMPx3
35	pre-B cell ALL/E. coli	shock, pancreatitis, MOSF	CsA, dexamethasone, induction chemotherapy
36	T cell ALL	disseminated Fusarium fungal infection (BMT)	Dexamethasone, etoposide
37	pre-B cell ALL	disseminated mucormycosis	dexamethasone
38	Gastroparesis-TPN dependent/CMV	Aspergillus central line infection, MOSF	IVMPx3, CsA, abatacept, tocilizumab
41	Unknown	GVHD4, fungemia (after BMT)	dexamethasone, etoposide, BMT

§ ALL = Acute lymphoblastic leukemia; MOSF = multiorgan system failure; EBV = Ebstein Barr virus; HHV6 = Human Herpes virus 6; GVHD = graft versus host disease; BMT = bone marrow transplantation; SLE = systemic lupus erythematosus; MCTD = mixed connective tissue disease; IVMP = intravenous methylprednisolone

Table 4. Predictive factors correlated to outcomes§

Outcome	Predictive factors	P value
Mortality	**Increased with:**	
	Thrombocytopenia	**0.025**
	HScore cell lineage	**0.033**
	STXBP2 mutation	**0.004**
	Decreased with:	
	Earlier start of anakinra	**0.046**
	sJIA	**0.006**
Ferritin improvement	**Increased with:** Earlier start of anakinra	**0.001**

§ STXBP2 = syntaxin binding protein-2 gene; sJIA = systemic juvenile idiopathic arthritis

Table 5. Survival based on diagnosis

Diagnosis	Number	Survival rate	P value
Systemic JIA	13	13 (100%)	0.006
Malignancy (leukemia)	3	0 (0%)	
Lupus and related#	10	7 (70%)	
Infection alone	6	3 (50%)	
Other/unknown*	12	9 (75%)	

- systemic lupus erythematosus (n=5), mixed connective tissue disesase (n=3), Sjoren disease (n=1), anti-phospholipid antibody syndrome (n=1)

* - gastoparesis (n=2), spondyloarthritis with uveitis (n=2), sarcoidosis (n=1), Crohn disease (n=1), mitochondrial disorder (n=1), undefined vasculitis (n=1), unknown (n=4)