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CASE REPORT

Ruxolitinib for secondary hemophagocytic lymphohistiocytosis: First case report

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Abstract
Hemophagocytic lymphohistiocytosis (HLH) is an immune-mediated disorder resulting in hyperactivation of inflammatory cytokines. If left untreated, the uncontrolled inflammatory response can lead to significant tissue injury and potentially life-threatening multi-organ dysfunction. Conventional immunosuppressive agents are available for the management of HLH, including dexamethasone, cyclosporine, and etoposide; however, patients may not respond to these therapies. Clinicians may turn toward alternative pharmacologic agents that likely have less clinical evidence. We describe a case of secondary HLH that did not respond favorably to conventional treatments. Serum inflammatory markers continued to rise significantly with clinical deterioration and worsening pancytopenia. The severe thrombocytopenia and neutropenia were deemed to have contributed to a spontaneous subdural hematoma and candidemia, respectively. Ruxolitinib, a Janus kinase (JAK) 1/2 inhibitor, was then utilized as a novel salvage therapy based on available *in vivo* murine data at the time. Following initiation, there was improvement seen in several disease markers, including serum ferritin, lactate dehydrogenase, fibrinogen, and liver function tests. However, the pancytopenia did not show signs of recovery. The patient ultimately expired after 7 days of ruxolitinib treatment. It is unclear if the improvement in disease markers was attributed to JAK inhibition alone. However, this experience combined with the positive *in vivo* murine data suggests that ruxolitinib may serve as a potential treatment option for HLH, pending the release of more robust data. To our knowledge, this is the first human case report describing the use of ruxolitinib for HLH. Future studies are warranted to determine the role of ruxolitinib in this setting.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare immune-mediated disorder characterized by hyperactivation of antigen-presenting cells and T-cells, an uncontrolled response of pro-inflammatory cytokines, and impaired natural killer and cytotoxic T-cell function. The disorder can be classified as primary HLH, which results from genetic mutations, or secondary HLH, which is caused by underlying conditions such as viral infections, cancer, or autoimmune diseases [1,2].

The initial presentation may consist of non-specific inflammatory symptoms. Notable clinical features include, but are not limited to, persistent pyrexia, hepatosplenomegaly, pancytopenia, and elevated inflammatory markers. If left untreated, HLH can result in significant tissue injury and potentially life-threatening systemic organ damage [1,3].

Conventional pharmacologic therapies for HLH consist of immunomodulatory agents, including corticosteroids, etoposide, cyclosporine, and intravenous immunoglobulin (IVIG). Treatment recommendations are based on the HLH-94 protocol [4], which has been studied prospectively in pediatric patients [5] and led to the development of the revised HLH-2004 protocol [6]. However, there are no prospective efficacy studies investigating these protocols in adults. Thus, treatment recommendations in adult patients with HLH are mainly extrapolated from the available pediatric data.

Unfortunately, up to 30% of patients do not respond to first-line therapies and may require alternative regimens consisting of other immunosuppressive chemotherapy and/or biologic agents [5]. Currently, there is a lack of robust randomized clinical trials investigating alternative pharmacologic treatments for HLH. Thus, treatment plans are often based upon a compilation of small heterogeneous retrospective studies, case reports, expert opinion, and clinician experience [7,8].

We report the case of a patient with secondary HLH who received dexamethasone, IVIG, etoposide, and rituximab therapy without clinical improvement. We describe our experience utilizing ruxolitinib, a Janus kinase (JAK) 1/2 inhibitor, as a novel salvage treatment for secondary HLH.

Case report

A 38-year-old woman presented to the hospital with a 10-day history of intermittent fevers and a diffuse rash covering her trunk, back, and upper extremities. Laboratory values were notable for pancytopenia and mildly elevated transaminases. An Epstein—Barr virus (EBV) polymerase chain reaction (PCR) resulted in 14,900 copies/mL, and the patient was diagnosed with an EBV infection with associated pancytopenia and hepatitis. Serum ferritin, lactate dehydrogenase (LDH), and triglyceride concentrations were noted to be 4404 mcg/L, 911 units/L, and 308 mg/dL, respectively; however, further work-up was deferred since the patient's symptoms were resolving. The patient was discharged with supportive care measures and outpatient follow-up. After 8 days, the patient was re-admitted following a syncopal episode with laboratory results showing a progressive pancytopenia and worsening liver function tests (LFTs). Secondary HLH triggered by EBV was high on the differential diagnosis, given the elevated inflammatory markers from the previous admission. After pursuing a full diagnostic work-up, the patient met seven of eight clinical criteria for HLH [6]: fever >38.5 °C, splenomegaly, pancytopenia affecting $\geq$2 cell lineages (hemoglobin, 6.2 g/dL; platelets, 81 $\times$ $10^3/\mu L$; neutrophils, 0.86 $\times$ $10^3/\mu L$), hypertriglyceridemia (467 mg/dL) and hypofibrinogenemia (172 mg/dL), hyperferritinemia (34,162 mcg/L), elevated soluble interleukin-2 (IL-2) receptor concentration (16,339 units/mL), and hemophagocytosis observed on the bone marrow biopsy specimen.

After the diagnosis was confirmed, treatment was initiated on hospital Day 2 with dexamethasone 10 mg/m^2/day and IVIG 1 g/kg/day (see Table 1). After two doses of IVIG, the serum ferritin concentration decreased to 8939 mcg/L and dexamethasone monotherapy was continued. On hospital Day 6, the serum ferritin increased significantly, along with worsening pancytopenia, rising LFTs, and ongoing fevers and malaise. On hospital Day 7, the patient was initiated on etoposide therapy twice weekly, which was dose-reduced to 37.5 mg/m^2 due to poor renal function and a bilirubin concentration >3 mg/dL. A repeat EBV PCR resulted in 108,000 copies/mL, prompting the initiation of rituximab 375 mg/m^2 on hospital Day 11 with the goal of B-cell depletion and viral load reduction [9]. Despite increasing the third and fourth doses of etoposide to 75 mg/m^2 and a repeat 1 g/kg IVIG dose, the patient failed to improve clinically, and ferritin concentrations continued to rise above 100,000 mcg/L. Another dose of rituximab 375 mg/m^2 was administered on hospital Day 21 after a repeat EBV PCR returned at 657,000 copies/mL. A soluble IL-2 receptor concentration remained elevated at 16,986 units/mL. Platelet counts dropped below 10 $\times$ $10^3/\mu L$ on hospital Day 22 despite active transfusions. On hospital Day 25, a subdural hematoma (SDH) measuring 8 mm in diameter was discovered, which was attributed to severe thrombocytopenia secondary to HLH and myelosuppressive therapy. The patient also developed neutropenia and *Candida krusei* candidemia.

Due to the lack of any sustained response to the aforementioned HLH therapies and worsening multi-organ dysfunction, the decision was made to pursue an alternative treatment. On the evening of hospital Day 26, ruxolitinib 20 mg twice daily was initiated (see Table 2). Following the first five doses of ruxolitinib, the ferritin concentration decreased from a peak of 155,726 mcg/L to 89,855 mcg/L. The LDH concentrations were also slowly but steadily downtrending, suggesting a decrease in cell destruction. Additionally, LFTs were also improving. On hospital Day 29, ruxolitinib was held due to renal failure, with plans to resume the following day at a reduced dose of 10 mg daily. During the next 4 days of ruxolitinib treatment, ferritin concentrations decreased rapidly to 24,098 mcg/L, fibrinogen concentrations increased to 374 mg/dL without any cryoprecipitate transfusions, and LFTs continued to downtrend. Despite improving disease markers, the pancytopenia persisted: the platelet count never reached above 50 $\times$ $10^3/\mu L$ and the absolute neutrophil count never

Table 1 Treatment course prior to ruxolitinib.

Day	Ferritin (mcg/L)	LDH (units/L)	Dexamethasone (mg/m^2/day)	IVIG (g/kg)	Etoposide (mg/m^2)	Rituximab (mg/m^2)
1	34,162	2301	–	–	–	–
2	25,729	–	10	1	–	–
3	11,812	–	10	1	–	–
4	8939	–	10	–	–	–
5	–	–	10	–	–	–
6	29,293	3408	6.25	–	–	–
7	37,222	2910	12.5	–	37.5	–
8	41,047	2780	12.5	–	–	–
9	42,996	3070	12.5	–	–	–
10	36,437	2369	12.5	–	37.5	–
11	75,558	4660	10	–	–	375
12	51,710	2483	10	–	–	–
13	84,848	3006	10	–	–	–
14	109,899	3108	25	–	–	–
15	75,738	2276	25	–	75	–
16	117,257	5530	25	–	–	–
17	–	4980	25	–	–	–
18	60,266	2684	25	1	–	–
19	118,579	4222	25	–	–	–
20	121,271	3876	25	–	75	–
21	148,432	6270	25	–	–	375
22	109,655	5580	25	–	–	–
23	110,243	5645	25	–	–	–
24	113,032	5280	12.5	–	75	–
25	139,043	7260	–	–	–	–

IVIG = intravenous immunoglobulin; LDH = lactate dehydrogenase.

reached above 0.13 $\times$ 10^3/μL. Repeat abdominal imaging did not show any change in the hepatosplenomegaly. The patient ultimately experienced expansion of the SDH and a new intraparenchymal hemorrhage. With a worsening clinical status and poor prognosis, terminal extubation was performed. After the patient expired, results of a genetic sequence analysis returned and did not show any mutations in 14 genes associated with HLH. Therefore, the patient was deemed to have secondary HLH caused by an EBV infection.

Discussion

HLH is a potentially fatal syndrome, with mortality rates reaching up to 50% in pediatric patients even with protocolized treatment [10]. In adult patients, mortality rates have been reported to exceed 40% [1]. Of note, treatment recommendations from the HLH-94 and HLH-2004 protocols have been adopted in the adult population, but have yet to be validated in a prospective study of adult patients. To date, the only published clinical trial in the adult population investigated the utility of liposomal doxorubicin, etoposide, and methylprednisolone as salvage therapy for refractory HLH [11]. Since patients may not respond favorably to conventional therapies, several targeted treatment strategies affecting specific or numerous cytokine signaling pathways have been under investigation [7]. Herein, we have described our single patient experience using the JAK1/2 inhibitor ruxolitinib as a novel salvage therapy for secondary HLH.

Table 2 Ruxolitinib treatment course.

Day	Ruxolitinib (mg/day)	Ferritin (mcg/L)	LDH (units/L)	AST/ALT (units/L)	Bilirubin (mg/dL)	Fibrinogen (mg/dL)	Triglyceride (mg/dL)
26	20	155,726	13,456	1978/477	4.4	218	–
27	40	60,325	12,507	1347/358	3.9	204	–
28	40	90,368	10,720	1048/289	2.9	143	875
29	–	89,855	–	846/224	2.3	144	–
30	10	67,298	8389	566/176	2	142	662
31	10	45,105	–	462/168	1.9	245	–
32	10	32,234	–	379/133	1.7	259	–
33	10	24,098	–	–	–	374	–

AST/ALT = aspartate transaminase/alanine transaminase; IVIG = intravenous immunoglobulin; LDH = lactate dehydrogenase.

JAKs are crucial components of cytokine receptor subunits and are essential in the transmission process of cytokine-induced signals. Following cytokine binding, tyrosine residues are phosphorylated by JAKs, allowing them to recruit signal transducers and activators of transcription (STATs). STATs translocate to the nucleus and ultimately activate gene transcription [12,13]. JAK inhibition will affect signaling pathways of numerous cytokines including interferon-gamma (IFN-γ) and IL-2.

Ruxolitinib has been investigated for myelofibrosis, corticosteroid-refractory graft-versus-host disease, and alopecia areata, demonstrating clinical benefits and reductions in pro-inflammatory markers [14—19]. In the United States, ruxolitinib currently has labeled indications for the treatment of myelofibrosis and polycythemia vera. It is generally dosed between 5 mg and 25 mg by mouth twice daily based on the indication, hematologic parameters, organ function, and drug interactions [20].

Currently, there is a lack of human data regarding the use of ruxolitinib in patients with HLH. Das et al. [21] published *in vivo* animal data in murine models of induced primary and secondary HLH using ruxolitinib 90 mg/kg twice daily for approximately 5 days. Mice with induced primary HLH who received ruxolitinib exhibited reversal of splenomegaly, anemia, and thrombocytopenia; reduced levels of IFN-γ, transcription necrosis factor-α, and the absolute number of $CD8^+$ T-cells; and improved survival. Mice with induced secondary HLH that received ruxolitinib exhibited reversal of splenomegaly and pancytopenia as well as reversal of IFN-γ, IL-6, and IL-12 levels. Maschalidi et al. [22] also published *in vivo* murine data of ruxolitinib 1 mg/kg twice daily for 14 days in mice with full-blown HLH. When compared to the control group, treatment with ruxolitinib resulted in improved hematologic parameters, decreased serum levels of pro-inflammatory cytokines, reduced macrophage activation and tissue infiltration, and increased survival. These results shed light on ruxolitinib's JAK inhibition as a potential treatment option through its ability to attenuate the hyper-inflammation and disease manifestations associated with HLH.

After the patient failed to respond favorably to conventional therapies, now with severe pancytopenia, the medical team agreed on a trial of ruxolitinib for off-label treatment of HLH based on the *in vivo* murine data. Following initiation, serum ferritin, LDH, and LFTs began to steadily downtrend. It is unclear if the decline in disease markers was due to a rapid response to ruxolitinib or a delayed response to the increased dose of etoposide and/or the repeat dose of rituximab. Pancytopenia and hepatosplenomegaly persisted while receiving ruxolitinib, although the patient only received 7 days of therapy; it is also unclear if longer treatment duration would have resulted in improvement. A repeat tissue or bone marrow biopsy was not performed, which did not allow us to determine the extent of hemophagocytosis during ruxolitinib treatment. Finally, the patient's fever curve while receiving therapy was difficult to interpret in the setting of concomitant neutropenia, septic shock, and intracranial hemorrhage.

To our knowledge, this is the first human case report describing the use of ruxolitinib for HLH. Future studies are warranted to determine the role of ruxolitinib in primary and/or secondary HLH, optimal dosing and duration of therapy, short- and long-term outcomes, and a proactive approach to monitoring for clinical response and toxicities. Currently, there is a pilot study underway investigating the efficacy of ruxolitinib 15 mg twice daily in adult patients with secondary HLH (NCT02400463).

Conclusion

HLH is a serious and potentially fatal disease process. Although numerous pharmacologic agents are available, patients may not respond favorably to conventional options, leading clinicians to turn toward alternative and developing therapies that likely have less clinical evidence. JAK inhibition with ruxolitinib was administered to our patient with secondary HLH after she continued to clinically decline despite conventional therapies. It is unclear if the improvement in several disease markers after initiation of ruxolitinib could be attributed to the agent alone. However, this experience combined with the positive *in vivo* murine data suggests that ruxolitinib may serve as a potential treatment option for HLH, pending the release of more robust data. If treatment with ruxolitinib is being considered, clinicians and patients should discuss the risks and benefits prior to initiating therapy since it is considered an off-label indication for the management of HLH.

Conflict of interest

The authors declare that there is no conflict of interest.

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