Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2021 February 01; 72(2): 194–201. doi:10.1097/MPG.0000000000002940.

Outcomes of Severe Seronegative Hepatitis-Associated Aplastic Anemia: A Pediatric Case Series

Sarah Kemme, MD [Fellow in Pediatric Gastroenterology, Hepatology and Nutrition],
Section of Gastroenterology, Hepatology and Nutrition, and the Digestive Health Institute,
Children's Hospital Colorado, University of Colorado Denver School of Medicine

Marisa Stahl, MD MSCS [Assistant Professor of Pediatrics],
Section of Gastroenterology, Hepatology and Nutrition, and the Digestive Health Institute,
Children's Hospital Colorado, University of Colorado Denver School of Medicine

Dania Brigham, MD [Assistant Professor of Pediatrics],
Section of Gastroenterology, Hepatology and Nutrition, and the Digestive Health Institute,
Children's Hospital Colorado, University of Colorado Denver School of Medicine

Corresponding Author/Reprint Request Author: Sarah Kemme, 13123 East 16th Avenue, B290, Aurora, CO 80045, Telephone: 720-777-3819 Fax: 720-777-7277 sarah.kemme@childrenscolorado.org.

Authorship Roles:

Sarah Kemme, MD

1. Substantial contributions to the study included: conceptualization and design, methodology, investigation, funding acquisition, data curation, and formal analysis
2. Drafted the initial manuscript and reviewed/edited subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Marisa Stahl, MD MSCS

1. Substantial contributions to the study included: conceptualization and design, methodology, investigation, and funding acquisition
2. Reviewed/edited initial and subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Dania Brigham, MD

1. Substantial contributions to the study included: investigation, data curation, and formal analysis
2. Drafted the initial manuscript and reviewed/edited subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Mark A. Lovell, MD

1. Substantial contributions to the study included: investigation, data curation, and formal analysis
2. Reviewed/edited initial and subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Taizo Nakano, MD

1. Substantial contributions to the study included: conceptualization and design, supervision and oversight, data curation, and formal analysis
2. Drafted the initial manuscript and reviewed/edited subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Amy G. Feldman, MD MSCS

1. Substantial contributions to the study included: methodology, investigation, supervision and oversight, data curation, and formal analysis
2. Drafted the initial manuscript and reviewed/edited subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Cara Mack, MD

1. Substantial contributions to the study included: conceptualization and design, methodology, investigation, supervision and oversight, funding acquisition, data curation, and formal analysis
2. Drafted the initial manuscript and reviewed/edited subsequent versions of the manuscript
3. Approves final version to be published
4. Agrees to be held accountable for all aspects of work

Mark A. Lovell, MD [Professor of Pathology],

Section of Pathology and Lab Services, Children's Hospital Colorado, University of Colorado Denver School of Medicine

Taizo Nakano, MD [Assistant Professor of Pediatrics]

Center for Cancer and Blood Disorders, Children's Hospital Colorado, University of Colorado Denver School of Medicine

Amy G. Feldman, MD MSCS [Assistant Professor of Pediatrics], Cara Mack, MD [Professor of Pediatrics]

Section of Gastroenterology, Hepatology and Nutrition, and the Digestive Health Institute, Children's Hospital Colorado, University of Colorado Denver School of Medicine

Abstract

Objectives: Hepatitis-associated aplastic anemia (HAAA) is a potentially life-threatening diagnosis without clear treatment guidelines. The goal of the study was to characterize the presentation, evaluation, histopathology, and outcomes of therapy in children with HAAA to guide future research and to develop standardized care guidelines for this rare disease.

Methods: Retrospective chart review of four patients with HAAA who presented to Children's Hospital Colorado between 2016 and 2019 was conducted. Patient presentation, evaluation, bone marrow and liver pathology, interventions, and clinical course were collected. Immunohistochemistry of liver biopsies was performed.

Results: We treated four patients with HAAA without liver failure. All had evidence of systemic hyperinflammation and CD8⁺ T cell predominant liver tissue infiltration. One had a genetic mutation predisposing him to immune-mediated disease, but all other genetic testing was negative. In three of the four patients, hepatitis was poorly responsive to standard therapy with steroids, azathioprine, or tacrolimus; however, sustained biochemical remission of hepatitis was induced after more aggressive immunosuppressive therapies including Anti-Thymocyte Globulin (ATG) at standard immunosuppressive therapy (IST) dosing for severe Aplastic Anemia (sAA). Two patients underwent hematopoietic stem cell transplant (HSCT); one as first line therapy and one for refractory sAA.

Conclusions: We found that ATG based IST induced remission of hepatitis in patients with steroid-refractory HAAA. This is also an appropriate initial treatment for severe Aplastic Anemia, though may not prevent the need for HSCT. We propose that equine ATG based IST at standard dosing regimen for sAA is a therapy that in select cases can be considered early on in the treatment course and could lead to a sustained remission of both hepatitis and sAA. This should be considered in collaboration with a pediatric hematologist.

Keywords

Anti-Thymocyte Globulin; Hematopoietic Stem Cell Transplant; Immunosuppressive Therapy
Severe Aplastic Anemia; T Cell-Mediated Immunity

Introduction:

Hepatitis-associated aplastic anemia (HAAA) is a well-described clinical syndrome in which an acute episode of hepatitis is identified concurrent to or followed by severe marrow failure and transfusion dependent pancytopenia^{1–5}. HAAA accounts for 2%–5% of cases of severe aplastic anemia (sAA)^{6–8}, is most common among adolescent and young adult males, and is associated with a worse clinical prognosis. The episode of hepatitis can vary from acute to chronic², mild to fulminant⁹, self-limiting to necessitating liver transplant².

sAA, which can occur within 2–3 months following the start of the hepatitis episode^{2,5,10}, can be fatal if not treated promptly^{2,11}. Although the exact mechanism of bone marrow failure is not completely understood, one contributing factor is systemic immune dysregulation that results in immune-mediated destruction of hematopoietic stem cells leading to marrow aplasia². As a result, a variety of immunomodulating therapies and/or hematopoietic stem cell transplant (HSCT) have been trialed with varying degrees of success in inducing remission of the hepatitis and recovery of the marrow. Currently, there are no clear treatment guidelines for children with HAAA. The aim of this study was to perform a detailed characterization of the clinical presentation, evaluation, histopathology, and outcomes of immunosuppressive therapy (IST) in children with HAAA in order to identify areas in need of future study towards the development of standardized care guidelines for this rare disease.

Methods:

This study was approved by the University of Colorado Institutional Review Board (IRB #17–0854). Four patients with HAAA presented to Children's Hospital Colorado between 2016–2019. Retrospective chart review was conducted to determine patient presentation, diagnostic evaluation including blood tests (see Table 1), bone marrow and liver biopsy pathology, bone marrow aspirate, therapeutic interventions, and subsequent clinical course. Three of the four patients had whole exome sequencing (WES), as well as specific genetic mutation analyses associated with aplastic anemia (Table 2).

Results:

Overview of Case Presentations:

All four patients initially presented with severe hepatitis (defined as ALT and/or AST >1,000 U/L), without acute liver failure¹². Diagnostic work-up for acute and chronic hepatitis (including infectious, genetic, autoimmune, toxin, and anatomic) was negative in all patients (evaluation detailed in Table 2). Liver histology for all patients demonstrated marked portal and pan-lobular T-cell predominant inflammation and sAA was confirmed with analysis of peripheral blood and bone marrow biopsy and aspirate demonstrating severe suppression of two of three peripheral blood cell lines and a severely hypocellular bone marrow of less than 25%. None of the patients had growth delay, dysmorphic or syndromic features, radial-ray anomalies, or midline defects. All are Caucasian with no significant family history pertinent to the presentation. The average time frame of the diagnosis of sAA was 7.0 ± 9.0 weeks from the presentation of the liver disease.

Patient A: A 6-year-old male presented with fatigue, pruritus, abdominal pain, and hepatosplenomegaly on physical exam. At time of presentation, abnormal laboratory values included aminotransferase levels- $>2,000$ U/L, INR- 1.49, conjugated bilirubin- 6.1 mg/dL, WBC- $3.54 \times 10^3/\mu\text{L}$, ANC- $1.48 \times 10^3/\mu\text{L}$, hemoglobin- 13.6 g/dL, platelets- $86 \times 10^3/\mu\text{L}$, and evidence of systemic hyperinflammation and immune dysregulation [ferritin- 792 ng/mL, soluble IL-2 receptor (sIL2R)- 7,987 unit/mL, Natural Killer (NK) cell function- $<2\%$ function] (Figure 1A, Table 1). Perforin and Granzyme B studies demonstrated increased positivity in CD8^+ T cells with borderline-low positivity in NK cells (Table 1). Functional studies to evaluate for inherited bone marrow failure syndrome (IBMFS) were negative, including chromosome fragility and telomere length assays. WES and sAA-associated genetic testing was negative (Table 2). Liver histology demonstrated marked diffuse inflammation with hepatocyte necrosis and cholestasis, but without interface hepatitis or fibrosis (Figure 2: 2A-2E). Two weeks after presentation, the initial bone marrow biopsy demonstrated moderately hypocellular marrow (50%) with megakaryocytic hypoplasia and rare hemophagocytosis; a follow-up bone marrow biopsy approximately two months later demonstrated severe hypocellularity (20%). The hepatitis partially responded to steroids (40 mg/day) and tacrolimus (trough goal 8–10 ng/mL); however, to control refractory and progressive systemic hyperinflammation, he received etoposide ($150 \text{ mg}/\text{m}^2/\text{week} \times 4$ weeks) (Figure 1A). Etoposide led to partial improvement in hepatitis and increased time between platelet transfusions, but disease was still active. Formal sAA IST with equine anti-thymocyte globulin (ATG) ($40 \text{ mg}/\text{kg}/\text{day} \times 4$ days) with tacrolimus maintenance was then given, which resulted in remission of hepatitis, transfusion independence with improved peripheral blood counts in two months, and normalization of bone marrow hypocellularity in twelve months (Figure 1A). He was continued on tacrolimus monotherapy for one year after ATG was given, followed by mycophenolate mofetil (MMF) for one year due to tacrolimus induced renal toxicity, and has now been off all immunosuppression for over one year.

Patient B: A 7-year-old female presented with epigastric abdominal pain and scleral icterus, followed by diffuse jaundice and fatigue. At time of presentation, abnormal laboratory values included aminotransferase levels- >2000 U/L, conjugated bilirubin- 5.9 mg/dL, WBC- $3.00 \times 10^3/\mu\text{L}$, ANC- $0.97 \times 10^3/\mu\text{L}$, hemoglobin- 15 g/dL, and platelets- $154 \times 10^3/\mu\text{L}$ (Figure 1B, Table 1). Liver histology demonstrated marked diffuse hepatitis, including extensive interface hepatitis with occasional plasma cells, prominent cholestasis, and fibrous expansion of portal tracts with Ishak stage 2/6 fibrosis¹³. At that time she was given a diagnosis of seronegative autoimmune hepatitis (AIH). The AIH went into biochemical remission with standard of care therapy (steroids 1 mg/kg/day and azathioprine 1 mg/kg/day). Due to lack of patient follow-up, laboratory testing was limited for the first three months after presentation. Four months after presentation the WBC was $1.04 \times 10^3/\mu\text{L}$ and platelets were $29 \times 10^3/\mu\text{L}$, concerning for the development of sAA (Figure 1B). At that time she had a 6-TG level of 79 pmol/ 8×10^8 RBC, suggestive of etiology other than azathioprine-induced marrow failure. Bone marrow biopsy and aspirate demonstrated a severely hypocellular marrow ($<10\%$) with marked decrease in megakaryocytic and myeloid

precursors consistent with sAA. Functional studies to evaluate for IBMFS, WES, and other sAA-associated genetic testing were negative. With an HLA matched sibling donor available and due to platelet transfusion dependence and severe neutropenia from her sAA, she proceeded directly to HSCT (Figure 1B). HSCT preparative regimen consisted of equine ATG (30 mg/kg/day $\times$ 3 days), cyclophosphamide (50 mg/kg/day $\times$ 4 days), and methylprednisolone (1 mg/kg/day $\times$ 5 days). She received standard immunosuppression with MMF and cyclosporine post HSCT to prevent graft-versus-host disease (GVHD). She is currently doing well three years after presentation and has been off all immunosuppression for almost two years.

Patient C: A 7-year-old male presented with fever, abdominal pain, jaundice, scleral icterus, and hepatomegaly on physical exam. At time of presentation, abnormal laboratory values included aminotransferase levels- $>2,500$ U/L, conjugated bilirubin- 2.6 mg/dL, WBC- $2.53 \times 10^3/\mu\text{L}$, ANC- $1.2 \times 10^3/\mu\text{L}$, hemoglobin- 11.1 g/dL, platelets- $182 \times 10^3/\mu\text{L}$, and evidence of systemic hyperinflammation and immune dysregulation [ferritin- 522 ng/mL, sIL2R- 13,310 units/mL, NK cell function- $<1\%$ function] (Figure 1C, Table 1). Perforin and Granzyme B studies demonstrated increased positivity in CD8⁺ cells (Table 1). WES revealed a heterozygous gene mutation of tumor necrosis factor receptor superfamily member 13B (*TNFRSF13B*, variant c.542C>A), also known as Transmembrane activator and CAML interactor (*TACI*) (Table 2). This mutation has been reported previously in individuals with immunodeficiencies, including in common variable immunodeficiency as homozygous, compound heterozygous, and heterozygous phenotype. It has also been reported frequently in unaffected controls¹⁴. It was felt to have contributed to but not been wholly responsible for the patient's specific presentation. Liver histology demonstrated diffuse portal, interface and lobular hepatitis with ductular proliferation and damage, hepatocyte necrosis, mild cholestasis, and no fibrosis. Five weeks after presentation a bone marrow biopsy and aspirate demonstrated a severely hypocellular marrow (30%) with decreased trilineage hematopoiesis ($<20\%$) and some erythrophagocytosis. The hepatitis partially responded to steroids (1 mg/kg/day) and tacrolimus (trough goal 8–12 ng/mL). With refractory hepatitis and a clinical diagnosis of sAA, he received formal IST with equine ATG (40 mg/kg/day $\times$ 4 days) and tacrolimus maintenance (Figure 1C). Six months after presentation he also started adjuvant intravenous immunoglobulin (IVIG), 1 g/kg for four doses for physiologic serum replacement of decreased IgG levels of <500 mg/dL. Twelve months post IST, his hepatitis was in remission but he failed to achieve even a partial sAA response and thus proceeded to HSCT with a haplo-identical HLA family donor. HSCT preparative regimen consisted of rabbit ATG (2 mg/kg/day $\times$ 3 days), fludarabine (30 mg/m²/day $\times$ 5 days), cyclophosphamide (14.5 mg/kg/day $\times$ 2 days pre and $\times$ 2 days post for prevention of rejection), and total body irradiation. He received standard post HSCT immunosuppression with MMF and tacrolimus to prevent GVHD per institutional protocol (Figure 1C). He remained on tacrolimus (trough goal 5–15 ng/mL) due to rash concerning for GVHD; this was tapered six months after HSCT and discontinued ten months after HSCT (Figure 1C). He is currently doing well nearly three years after presentation and has been off all immunosuppression for one year.

Patient D: A 5-year-old male presented with fatigue, jaundice, scleral icterus, acholic stools, and hepatosplenomegaly on physical exam. At time of presentation, abnormal laboratory values included aminotransferase levels- >2500 U/L, conjugated bilirubin- 6.6 mg/dL, WBC- $4.38 \times 10^3/\mu\text{L}$, ANC- $2.33 \times 10^3/\mu\text{L}$, hemoglobin- 13.6 g/dL, reticulocyte count- 3.7%, platelets- $26 \times 10^3/\mu\text{L}$, and evidence of systemic hyperinflammation and immune dysregulation [ferritin- 456 ng/mL, sIL2R- 20,600 unit/mL, NK cell function- <3% function] (Figure 1D, Table 1). sAA-associated genetic testing was negative (Table 2). Liver histology demonstrated severe lymphoplasmocytic interface hepatitis, marked bile duct inflammation, ductular proliferation, and Ishak stage 1/6 fibrosis¹³. Four days after presentation, a bone marrow biopsy and aspirate demonstrated moderate hypocellular marrow (40%) with decreased trilineage hematopoiesis (< 20%) and megakaryocytic hypoplasia with rare erythrophagocytosis. His hepatitis and moderate to severe marrow aplasia did not respond to steroids (40 mg/day) and tacrolimus (trough goal 10–12 ng/mL). With refractory disease he received formal sAA IST with equine ATG (40 mg/kg/day $\times$ 4 days) and tacrolimus maintenance (Figure 1D). In addition to tacrolimus maintenance therapy, he required MMF for two months due to a flare in his hepatitis (Figure 1D). He is doing well ten months after presentation on tacrolimus monotherapy (trough goal now 8–10 ng/mL), transfusion independent, with normalization of peripheral blood counts.

Detailed immunohistochemistry of liver tissue for each patient was performed in order to demonstrate the degree and severity of inflammation, as well as to clearly characterize the nature of the immune infiltrates. The hematoxylin and eosin stain of the diseased liver tissue show extensive hepatitis both in portal and lobular areas compared to normal liver tissue (Figure 2: 1A, 2A). CD4⁺ staining is predominantly in a sinusoidal pattern, reflecting the CD4 antigen that is found on sinusoidal endothelial cells and Kupffer cells lining the sinusoids^{15,16}. This sinusoidal pattern is consistent in both normal and diseased liver tissue (Figure 2: 1B, 2B). There are no significant CD4⁺ T cell infiltrates in diseased liver portal tracts or parenchyma compared to normal liver tissue. The majority of the cellular inflammation in the diseased liver tissue was identified to be CD8⁺ T cells. In the normal liver tissue, there are a few CD8⁺ T cells (Figure 2: 1C). However, in the diseased liver tissue, there is a dramatic increase in CD8⁺ T cells in both the portal tracts and parenchyma (Figure 2: 2C). CD20⁺ staining demonstrate a few B-cells in normal liver tissue, without a significant increase in diseased liver tissue (Figure 2: 1D, 2D). CD56⁺ staining for NK cells is noted in the portal tract of normal liver tissue (Figure 2: 1E) and subjectively looks diminished in diseased liver tissue (Figure 2: 2E). Fibrosis based on Ishak staging was absent in two patients and stage 1–2 fibrosis in two patients¹³.

Discussion:

This case series highlights unique aspects of the presentation and therapeutic outcomes of four children with HAAA. In addition to acute hepatitis and marrow aplasia, all patients demonstrated evidence of increased systemic inflammation and immune dysregulation including decreased NK cell function and CD8⁺ T cell activation (sIL2-R, perforin). The systemic hyperinflammation was demonstrated histologically in the liver as excessive CD8⁺

T cell infiltration and in the marrow as increased plasma cell infiltration. These children did not meet clinical criteria for secondary hemophagocytic lymphohistiocytosis (HLH) (i.e. absence of persistent fevers, absence of splenomegaly in two patients, no significant erythrophagocytosis in bone marrow). The phenomenon of CD8⁺ T cell activation in severe hepatitis is similar to that recently described in a cohort of children with indeterminate acute liver failure (iALF), some of whom also had sAA (25% of cohort)¹⁷. Immunohistochemistry of the iALF livers demonstrated dense CD8⁺ T cell infiltrates producing high levels of perforin, reflecting activation. This pattern was unique to iALF cases and not found in control patient livers. Furthermore, immunohistochemistry of the iALF livers showed lack of infiltrates of CD4⁺ T cells, CD20⁺ B cells and CD56⁺ NK cells; a similar immunophenotype to our cohort. A follow-up study from this group revealed that the majority of patients with iALF and sAA also had evidence of increased systemic inflammation and immune dysregulation (elevated sIL-2R, deficient NK cell function)¹⁸. It is notable that the liver and bone marrow, both of which have hematopoietic potential, are susceptible to severe injury in the setting of systemic immune dysregulation. Collectively, these studies and the immune phenomenon seen in HAAA suggest that pediatric HAAA is one of many phenotypic end points caused by severe immune dysregulation. The presentation of immune dysregulation can range from focal end organ disease (AIH, sAA) to multifocal disease (including HAAA) to systemic disease (including Macrophage Activating Syndrome and HLH). Future research should focus on mechanism of acquired immune dysregulation to clearly understand the pathogenesis of these distinct phenotypes, including HAAA.

The diagnostic workup for the liver disease and bone marrow failure included numerous infectious disease studies, all of which were negative. Genetic causes of immune dysregulation were sought in this cohort, including genetic mutations known to be associated with sAA, as well as WES. All patients completed a comprehensive marrow failure evaluation including screening for IBMFS, immunodeficiencies, rheumatologic disorders, and paroxysmal nocturnal hemoglobinuria. Only one patient had a genetic mutation predisposing to immune-mediated disease- a mutation in *TNFRSF13B*, variant c.542C>A (*TACI*), which has been reported to be associated with both common variable immunodeficiency and autoimmunity^{19,20}. Future studies could consider whole exome and/or genome sequencing analyses in order to accurately determine the existence of genetic mutations associated with HAAA.

An important observation in three of the four patients presented here is that the liver disease was minimally responsive to corticosteroid therapy, which induced only transient and incomplete response. In stark contrast, the liver disease responded to equine ATG following traditional sAA IST regimen and led to a sustained biochemical remission of hepatitis in our cohort. This has been previously reported in one patient with iALF and sAA who received ATG (40 mg/kg/day × 4 days) early in disease course and subsequently avoided liver transplantation²¹. In addition to treating the liver disease, equine ATG is an accepted initial treatment for sAA though may not induce remission of marrow suppression^{22,23}. In a recent ten year retrospective review of 314 pediatric patients with sAA, only 71.2% of subjects treated with ATG based IST achieved an objective response²⁴, thus HSCT may still be necessary for refractory marrow suppression regardless of status of hepatitis. Considering the lower-dose rabbit ATG utilized for refractory HAAA (10–20 mg/kg/dose × 5 days²⁵) and

liver transplant rejection ($1.5 \text{ mg/kg/day} \times 7\text{--}14 \text{ days}^{26}$), collaboration with a pediatric hematologist/oncologist familiar with therapy of sAA may be appropriate. This collaboration is imperative when also considering that sAA may evolve and require more aggressive IST or HSCT.

The spectrum of systemic hyperinflammation observed in immune regulatory disorders varies from self-limited inflammation to life-threatening cytokine storm. Accurately assessing the degree of systemic hyperinflammation from laboratory evaluation, physical exam, and degree of end organ injury helps guide appropriate therapy. Whereas a mild, self-limiting degree of inflammation may allow therapy to focus on the underlying cause of disease, a severe cytokine storm may require rapid, aggressive use of non-specific immunosuppression (e.g., etoposide and dexamethasone). Etoposide was utilized in one case in the setting of rapidly progressive systemic hyperinflammation. With better control of systemic inflammation, but persistent AIH and sAA, the patient went on to receive ATG based IST which ultimately led to sustained remission of liver and marrow disease.

Initial therapy for younger patients with sAA is a matched sibling HSCT or IST if a matched sibling donor is unavailable. One patient received up front HSCT from their matched sibling donor after AIH had already resolved with standard of care therapy. A second patient went on to require HSCT due to refractory marrow suppression after IST despite resolution of hepatitis.

Based on these observations, in the setting of severe acute immune-mediated hepatitis with concern for sAA, equine ATG IST at standard dosing regimen for sAA is an acceptable therapy that can be considered early on in the treatment course for select patients, as it can lead to a sustained remission of hepatitis and may also lead to remission of sAA. Patients receiving ATG based IST should be counseled on the increased risk of infection and follow local practices for the presentation of fever and severe cytopenias. After T cell targeted induction therapy, similar maintenance therapy should also be considered for this CD8^+ predominant T cell disease. Although cyclosporine remains the most common T cell suppressing maintenance agent utilized in sAA IST, tacrolimus may be a comparable alternative that happens to overlap in its application as a maintenance agent for autoimmune hepatitis²⁷.

Limitations to this study include the retrospective nature of the study design, the missingness of data pertaining to diagnostic workup and the variability as to the type of immunosuppressant and the timing of initiating the immunosuppressant to treat the liver and bone marrow diseases. Based on observations from this retrospective study, a prospective study with an itemized list of diagnostic testing, a plan for frequency of monitoring for development of sAA, monitoring markers of systemic inflammation and immune dysregulation and a proposed algorithm for treatment is necessary in order to clearly define the best practice for managing HAAA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

We thank Andy Veasey, BS for running Whole Exome Sequencing and Naomi Meeks, MD for reviewing and interpreting results of Whole Exome Sequencing.

Conflicts of Interest and Sources of Funding:

No conflicts of interest are declared.

T32 DK067009-15 (Mack CL, Brigham D, Stahl M, Kemme S)

The Judith Sondheimer Pediatric GI Fellow Research Fund (Kemme S, Stahl M)

AHRQ K08 HS026510-01A1 (Feldman, AG)

References:

1. Patel KR, Bertuch A, Sasa GS, Himes RW, Wu H. Features of Hepatitis in Hepatitis-associated Aplastic Anemia: Clinical and Histopathologic Study. *J Pediatr Gastroenterol Nutr.* 2017;64(1):e7–e12. [PubMed: 28030425]
2. Gonzalez-Casas R, Garcia-Buey L, Jones EA, Gisbert JP, Moreno-Otero R. Systematic review: hepatitis-associated aplastic anaemia--a syndrome associated with abnormal immunological function. *Aliment Pharmacol Ther.* 2009;30(5):436–443. [PubMed: 19508613]
3. Osugi Y, Yagasaki H, Sako M, et al. Antithymocyte globulin and cyclosporine for treatment of 44 children with hepatitis associated aplastic anemia. *Haematologica.* 2007;92(12):1687–1690. [PubMed: 18055993]
4. Rauff B, Idrees M, Shah SA, et al. Hepatitis associated aplastic anemia: a review. *Virology.* 2011;8:87. [PubMed: 21352606]
5. Brown KE, Tisdale J, Barrett AJ, Dunbar CE, Young NS. Hepatitis-associated aplastic anemia. *N Engl J Med.* 1997;336(15):1059–1064. [PubMed: 9091802]
6. Locasciulli A, Bacigalupo A, Bruno B, et al. Hepatitis-associated aplastic anaemia: epidemiology and treatment results obtained in Europe. A report of The EBMT aplastic anaemia working party. *Br J Haematol.* 2010;149(6):890–895. [PubMed: 20456352]
7. Wang H, Tu M, Fu R, et al. The clinical and immune characteristics of patients with hepatitis-associated aplastic anemia in China. *PloS one.* 2014;9(5):e98142. [PubMed: 24845454]
8. Savage WJ, DeRusso PA, Resar LM, et al. Treatment of hepatitis-associated aplastic anemia with high-dose cyclophosphamide. *Pediatr Blood Cancer.* 2007;49(7):947–951. [PubMed: 17252566]
9. Hagler L, Pastore RA, Bergin JJ, Wensch MR. Aplastic anemia following viral hepatitis: report of two fatal cases and literature review. *Medicine (Baltimore).* 1975;54(2):139–164. [PubMed: 1117835]
10. Safadi R, Or R, Ilan Y, et al. Lack of known hepatitis virus in hepatitis-associated aplastic anemia and outcome after bone marrow transplantation. *Bone Marrow Transplant.* 2001;27(2):183–190. [PubMed: 11281388]
11. Cengiz C, Turhan N, Yolcu OF, Yilmaz S. Hepatitis associated with aplastic anemia: do CD8(+) kupffer cells have a role in the pathogenesis? *Dig Dis Sci.* 2007;52(9):2438–2443. [PubMed: 17443406]
12. Squires RH Jr., Shneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr.* 2006;148(5):652–658. [PubMed: 16737880]
13. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol.* 1995;22(6):696–699. [PubMed: 7560864]
14. Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 13B; TNFRSF13B. MIM Number:604907;11/11/2011: . World Wide Web URL: <https://omim.org/entry/604907>. Accessed 1/22, 2020.

15. Scoazec JY, Feldmann G. Both macrophages and endothelial cells of the human hepatic sinusoid express the CD4 molecule, a receptor for the human immunodeficiency virus. *Hepatology*. 1990;12(3 Pt 1):505–510. [PubMed: 2205556]
16. Nakamura S, Muro H, Suzuki S, et al. Immunohistochemical studies on endothelial cell phenotype in hepatocellular carcinoma. *Hepatology*. 1997;26(2):407–415. [PubMed: 9252152]
17. Chapin CA, Burn T, Meijome T, et al. Indeterminate pediatric acute liver failure is uniquely characterized by a CD103. *Hepatology*. 2018;68(3):1087–1100. [PubMed: 29603342]
18. Chapin CA, Horslen SP, Squires JE, et al. Corticosteroid Therapy for Indeterminate Pediatric Acute Liver Failure and Aplastic Anemia with Acute Hepatitis. *J Pediatr*. 2019;208:23–29. [PubMed: 30770193]
19. Leonardi L, Lorenzetti G, Carsetti R, et al. Rare TACI Mutation in a 3-Year-Old Boy With CVID Phenotype. *Front Pediatr*. 2019;7:418. [PubMed: 31681716]
20. Barroeta Seijas AB, Graziani S, Cancrini C, et al. The impact of TACI mutations: from hypogammaglobulinemia in infancy to autoimmunity in adulthood. *Int J Immunopathol Pharmacol*. 2012;25(2):407–414. [PubMed: 22697072]
21. Molleston JP, Vik TA, Steiner SJ, Kernek KM. Non-ABC acute hepatic failure associated with aplastic anemia can be treated with immunosuppression. *J Pediatr Gastroenterol Nutr*. 2008;46(2):216–219. [PubMed: 18223384]
22. Young NS. Aplastic Anemia. *N Engl J Med*. 2018;379(17):1643–1656. [PubMed: 30354958]
23. Janka GE, Lehmborg K. Hemophagocytic syndromes--an update. *Blood Rev*. 2014;28(4):135–142. [PubMed: 24792320]
24. Rogers ZR, Nakano TA, Olson TS, et al. Immunosuppressive therapy for pediatric aplastic anemia: a North American Pediatric Aplastic Anemia Consortium study. *Haematologica*. 2019;104(10):1974–1983. [PubMed: 30948484]
25. Tung J, Hadzic N, Layton M, et al. Bone marrow failure in children with acute liver failure. *J Pediatr Gastroenterol Nutr*. 2000;31(5):557–561. [PubMed: 11144443]
26. Palmer WC, Taner CB, Keaveny AP, Nakhleh RE, Nguyen JH, Rosser BG Jr. Antithymocyte Globulin Use for Corticosteroid Nonresponsive Rejection After Liver Transplantation. *Transplant Proc*. 2018;50(10):3606–3614. [PubMed: 30577245]
27. Alsultan A, Goldenberg NA, Kaiser N, Graham DK, Hays T. Tacrolimus as an alternative to cyclosporine in the maintenance phase of immunosuppressive therapy for severe aplastic anemia in children. *Pediatr Blood Cancer*. 2009;52(5):626–630. [PubMed: 19148946]

What is Known and What is New:**What is Known:**

- Hepatitis-associated aplastic anemia (HAAA) is an entity in which acute hepatitis is identified concurrent to or followed by bone marrow failure and can be fatal.
- The precise etiology of bone marrow failure in HAAA is unknown, though studies have shown increased systemic inflammation and immune dysregulation.

What is New:

- Anti-Thymocyte Globulin following traditional severe Aplastic Anemia (sAA) immunosuppressive therapy (IST) regimen is associated with a sustained remission of hepatitis in some children with HAAA. In select cases, it may be a considered therapeutic choice early in the course of HAAA. Future clinical data of its use will inform whether ATG should be a clinical recommendation for HAAA.
- There are a broad spectrum of phenotypes within the umbrella term of immune dysregulation, that range from focal end organ disease (including autoimmune hepatitis, sAA) to multifocal disease (including HAAA) to systemic disease (including Macrophage Activating Syndrome and Hemophagocytic Lymphohistiocytosis). The similarities and differences of these different phenotypes and associated immune phenomena warrant further investigation.

Figure 1: Aminotransferase and CBC Changes with Therapy:

Shown are laboratory results over time for Patients A-D. For each patient (A.-D.) the top graph shows aminotransferase levels and the lower graph shows the WBC, absolute lymphocyte count (ALC) (left axis) and platelet count (right axis). The time frame of administration of various therapies is shown with arrows. *Patient B had HSCT preparative regimen of equine ATG, cyclophosphamide, and methylprednisolone. *Patient C had HSCT preparative regimen of rabbit ATG, fludarabine, cyclophosphamide, and total body irradiation. Steroids= oral and IV formulations; Tacro= Tacrolimus; ATG = Anti-Thymocyte Immune Globulin 40 mg/kg; AZA= Azathioprine; HSCT = Hematopoietic Stem Cell Transplant; CsA = Cyclosporine A; ETOP = Etoposide 150 mg/m²; MMF = Mycophenolate Mofetil

Figure 2: Immunohistochemistry of liver tissue at 200x magnification.

1A – 1E represent immunohistochemistry of normal liver tissue. 1A: hematoxylin and eosin stained normal liver tissue. 1B: CD4⁺ immunostain. 1C: CD8⁺ immunostain. 1D: CD20⁺ immunostain. 1E: CD56⁺ immunostain.

2A – 2E represent immunohistochemistry of liver tissue for Patient A. 2A: hematoxylin and eosin stained liver tissue. 2B: CD4⁺ immunostain. 2C: CD8⁺ immunostain. 2D: CD20⁺ immunostain. 2E: CD56⁺ immunostain.

Table 1:

Laboratory Values at Time of Hepatitis Presentation

Laboratory Values At Time of Presentation				
Laboratory Test	Patient A	Patient B	Patient C	Patient D
ALT (10–35 U/L)	2,445	2,763	2,763	2,502
AST (15–40 U/L)	3,134	2,287	2,789	3,485
Total Bilirubin (0.2–1.2 mg/dL)	10.7	7.4	6.7	7.5
Direct Bilirubin (0–0.3 mg/dL)	6.1	5.9	2.6	6.6
GGT (11–21 U/L)	163	64	178	146
INR (<1.5)	1.49	1.2	0.98	1.05
WBC (5.7–10.5 × 10 ³ /μL)	3.54	3.00	2.53	4.38
ALC (1.23–2.69 × 10 ³ /μL)	1.43	0.97	0.56	1.31
ANC (1.8–5.4 × 10 ³ /μL)	1.48	0.97	1.2	2.33
Platelets (150–500 × 10 ³ /μL)	86	154	182	26
Hemoglobin (11.1–14.5 g/μL)	13.6	15	11.1	13.6
Reticulocyte Count (0.8–2.2%)				3.7
Ferritin (10–60 ng/mL)	792		522	456
Fibrinogen (150–400 MGS/dL)	140		185	177
Triglycerides (<75 mg/dL)	213		279	316
Soluble IL-2 R (45–1,105 unit/mL)	7,987		13,310	20,600
NK Cell Function (50:1) (20%)	2		1	3
NK Cell Function (25:1) (10%)	2		1	2
NK Cell Function (12:1) (5%)	1		0	1
NK Cell Function (6:1) (1%)				1
NK Cell Lytic Units (2.6%)	0		0	0
NK Cell CD16/56% (4–26%)	2		4	1
Perforin:				
Positive of NK (73–91%)	76		95	
Intensity (98–181 MCF)	112		166	
Positive of CD8 (0–16%)	28		27	
Positive of NKT (0–28%)	NR			
Granzyme B:				
Positive of NK (80–98%)	92		98	
Intensity (152–825 MCF)	539		1,661	
Positive of CD8 (0–61%)	88		87	
Positive of NKT (0–72%)	NR			
CD56 Bright Positive (1.7–13.4%)	1.3		9.3	
Surface Marker (SM) T&B Panel:				
WBC Total (4,000–12,000/μL)	5,810	730	10,000	3,980

Laboratory Values At Time of Presentation				
Laboratory Test	Patient A	Patient B	Patient C	Patient D
Lymph Abs (1,000–5,500/ μ L)	471	530	130	530
CD16/CD56 NK Cells (4–17%)	2	<1	4	1
CD16/CD56 Abs (100–480/ μ L)	8	<5	6	7
CD19 (B4) SM (13–27%)	30	5	12	10
CD19 SM Abs (270–860/ μ L)	142	27	16	53
CD3 (T3) SM (60–76%)	68	94	83	88
CD3 SM Abs (1,200–2,600/ μ L)	322	498	108	468
CD4 (T4) SM (31–47%)	5	11	19	8
T4 SM Abs (650–1,500/ μ L)	21	57	25	43
CD8 (T8) SM (18–35%)	53	81	55	77
T8 SM Abs (370–1,100/ μ L)	248	429	71	409
CD4/CD8 SM (0.98–3.24)	0.09	0.13	0.35	0.11

Table 2:

Diagnostic Evaluation

Diagnostic Evaluation				
Infectious Studies	Patient A	Patient B	Patient C	Patient D
Adenovirus Blood PCR	Neg	Neg		
Blood Bacterial Culture	Neg		Neg	Neg
Cytomegalovirus Blood PCR	Neg	Neg	Neg	Neg
Epstein-Barr Virus Blood PCR	Neg	Neg	Neg	Neg
Enterovirus Blood PCR				Neg
Hepatitis A Antibody		Neg	Neg	Neg
Hepatitis B Surface Antigen	Neg	Neg	Neg	Neg
Hepatitis C Antibody	Neg	Neg	Neg	Neg
Hepatitis E Antibody	Neg		Neg	Neg
Herpes Simplex Virus Blood PCR	Neg	Neg		Neg
Human Herpesvirus 6 Blood PCR	Neg	Neg	Neg	Neg
Human Immunodeficiency Virus	Neg	Neg	Neg	Neg
1/2 Antibody				
Human T-Lymphotropic Virus	Neg	Neg		
1/2 Antibody				
Parvovirus B19 Blood PCR	Neg		Neg	Neg
Respiratory Virus Panel			Neg	Neg
Urine Bacterial Culture	Neg			
Genetic Studies				
Alpha-1 Anti-Trypsin Level	Normal	Normal	Normal	Normal
Autoimmune				Neg
Lymphoproliferative				
Syndrome DN TCR / Alpha				
Beta Pos Cells Testing				
Bone Marrow Failure Panel ¹		Neg		
Ceruloplasmin	Normal	Normal	Normal	Normal
Chromosome Fragility Study	Normal	Insufficient Cells	Normal	Insufficient Cells
Copper (Quantitative, Liver)		Normal		
Dyskeratosis Congenita Panel ²	Neg			
Fanconi Mutagen Sensitivity	Neg	Neg	Neg	
HLH Panel ³	Neg			
Myelodysplastic Syndrome Panel ⁴	Neg			Neg
Paroxysmal Nocturnal			Neg	
Hemoglobinuria				

Diagnostic Evaluation				
Infectious Studies	Patient A	Patient B	Patient C	Patient D
Immunophenotypic Analysis				
Primary Immunodeficiency Panel ⁵				Neg
Telomere Length Measurement	Normal	Normal	Normal	Normal
Whole Exome Sequencing	Neg	Neg	<i>Mutation: TNFRSF13B, variant c.542C>A</i>	
XIAP Protein Expression	Normal			
Autoimmune Studies				
ANA	Neg	Neg	Pos 1:80	Neg
Anti-LKM	Neg	Neg	Neg	Neg
Anti-Smooth Muscle Antibody	Neg	Neg	Neg	Neg
c-ANCA	Neg			
Liver Cytosol Antibody	Neg			Neg
Lymphocyte Stimulation	Low			
p-ANCA	Neg			
Panel Reactive Antibody	Normal			
Soluble Liver Antigen	Neg		Neg	Neg
Toxin Studies				
Serum Acetaminophen Level	Normal		Normal	Normal
Urine Drug Screen	Neg			
Anatomic Evaluation				
Anatomy on Ultrasound [*]	Normal	Normal	Normal	Normal
Anatomy on MRCP [*]	Normal	Normal	Normal	Normal

^{*} All imaging had abnormalities consistent with acute hepatitis, but no anatomic abnormalities]

Genes Tested in Panels:

¹ ACD, AK2, ANKRD26, ATR, BRCA1, BRCA2, BRIP1, CEBPA, CECR1, CSF3R, CTC1, DDX41, DKC1, ELANE, ERCC4, ETV6, FANCA, FANB, FANCC, FANCD2, FANCE, FANCF, FANGC, FANCI, FANCL, FANCM, G6PC3, GATA1, GATA2, GF11, GF11B, HAX1, JAGN1, LIG4, MPL, NBN, NHP2, NOP10, PALB2, PARN, PAX5, RAB27A, RAD51, RAD51C, RMRP, RPL11, RPL15, RPL19, RPL23, RPL26, RPL27, RPL31, RPL35A, RPL36, RPL5, RPS10, RPS15, RPS17, RPS19, RPS24, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS7, RTEL1, RUNX1, SAMD9, SAMD9L, SBDS, SLX4, SRP72, TAZ, TCIRG1, TERC, TERT, THPO, TINF2, TP53, TSR2, UBE2T, USB1, VPS13B, VPS45, WAS, WRAP532

² DKC1, NHP2, NOP10, TERC, TERT, TINF2, and WRAP53

³ AP3B1, BLOC1S6 (PLDN), TNFRSF7 (CD27), ITK, LYST, MAGT1, PRF1, RAB27A, SH2D1A, SLC7A7, STX11, STXBP2, UNC13D, XIAP (BIRC4), 253 Kb inversion, UNC13D (MUNC13–4)

⁴ Chromosome enumeration assay for interphase cells using chromosome 5, 7, 8, and 20q12 DNA probes; myeloid/lymphoid leukemia (MLL) gene at 11q23

⁵ ACD, ACP5, ACTB, ADA, ADAM17, ADAR, AICDA, AIRE, AK2, AP3B1, ARPC1B, ATM, BACH2, BCL10, BCL11B, BLM, BLNK, BTK, C1QA, C1QB, C1QC, C1S, C2, C3, CARD11, CARD14, CARD9, CASP10, CASP8, CD19, CD247, CD27, CD3D, CD3E, CD3G, CD40, CD40LG, CD46, CD55, CD59, CD70, CD79A, CD79B, CD81, CD8A, CDCA7, CEBPE, CECR1, CFB, CFD, CFH, CFI, CFP, CFTR, CHD7, CIITA, CLCN7, CLPB, COLEC11, COPA, CORO1A, CR2, CSF2RA, CSF2RB, CSF3R, CTC1, CTLA4, CTPS1, CTSC, CXCR4, CYBA, CYBB, DCLRE1C, DDX58, DGKE, DKC1, DNAJC21, DNMT3B, DOCK2, DOCK8, ELANE, EPG5, ERCC6L2, EXTL3, FADD, FAS, FASLG, FERMT3, FOXP3, G6PC3, G6PD, GATA2, GF11, GINS1, HAX1, HELLS, HYOU1, ICOS, IFIH1, IFNAR2, IFNGR1, IFNGR2, IGLL1, IKKB, IKZF1#, IL10, IL10RA, IL10RB, IL12B, IL12RB1, IL17RA, IL17RC, IL1RN, IL21, IL21R, IL2RA, IL2RG, IL36RN, IL7R, IRAK4,

IRF2BP2, IRF8, ISG15, ITGB2, ITK, JAGN1, JAK1, JAK3, KRAS, LAMTOR2, LAT, LCK, LIG4, LPIN2, LRBA, LYST, MAGT1, MALT1, MAP3K14, MASP1, MEFV, MKL1, MOGS, MRE11A, MSN, MTHFD1, MVK, MYD88, MYO5A, NBN, NCF1, NCF2, NCF4, NCSTN, NFKB1, NFKB2, NFKBIA, NHEJ1, NHP2, NLRC4, NLRP1, NLRP12, NLRP3, NOD2, NOP10, NRAS, NSMCE3, OFD1, ORAI1, OTULIN, PARN, PEPD, PGM3, PIGA, PIK3CD, PIK3R1, PLCG2, PMS2, PNP, POLE, POLE2, PRF1, PRKCD, PRKDC, PSENEN, PSMB8, PSTPIP1, PTPRC, RAB27A, RAC2, RAG1, RAG2, RASGRP1, RBCK1, RECQL4, RFX5, RFXANK, RFXAP, RHOH, RLTPR, RMRP, RNASEH2A, RNASEH2B, RNASEH2C, RNF168, RNF31, RNU4ATAC, RORC, RPSA, RTEL1, SAMD9, SAMD9L, SAMHD1, SBDS, SERPING1, SH2D1A, SLC29A3, SLC35C1, SLC37A4, SLC46A1, SLC7A7, SMARCA1, SMARCD2, SP110, SPINK5, SRP72, STAT1, STAT2, STAT3, STAT5B, STIM1, STK4, STX11, STXBP2, TAP1, TAP2, TAPBP, TBX1, TCF3, TCN2, TERC, TERT, TFRC, THBD, TNF2, TMC6, TMC8, TMEM173, TNFAIP3, TNFRSF13B, TNFRSF1A, TNFRSF4, TRAF3IP2, TREX1, TRNT1, TTC7A, TYK2, UNC119, UNC13D, UNC93B1, UNG, USB1, USP18, VPS13B, VPS45, WAS, WDR1, WIPF1, WRAP53, XIAP, ZAP70, ZBTB24 and ZNF341.