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**Remission of cold-agglutinin autoimmune hemolytic anemia and hypereosinophilic syndrome
with rituximab therapy**

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Clinical Implications

Hypereosinophilic syndrome (HES) can be reactive, and several cases have been reported in conjunction with autoimmune hemolytic anemia (AIHA). Treating the underlying autoimmune-driven anemia with targeted therapies such as rituximab has the potential to induce remission of the associated HES.

Hypereosinophilic syndromes (HES) are disorders characterized by absolute eosinophil count (AEC) $\geq 1500/\mu\text{L}$ with evidence of eosinophil-mediated tissue damage.¹ Cold-agglutinin autoimmune hemolytic anemia (AIHA) causes destruction of red blood cells (RBCs) through binding of autoantibodies to RBC surface antigens at temperatures below core body temperature.² We report a case of HES and concurrent AIHA with response to rituximab, an anti-CD20 monoclonal antibody.

A 59-year-old woman on an IRB-approved protocol with a past history of chronic hepatitis B, allergic rhinitis, thyroid nodules, and leiomyomas was evaluated. Thirteen years previously, she developed episodes of periorbital, facial, and neck angioedema with dyspnea, which improved with glucocorticoids and intramuscular epinephrine prescribed for presumed anaphylaxis during several emergency room visits. Peripheral eosinophilia was noted but attributed to allergic rhinitis. Although food reactions were suspected, no reproducible trigger was identified, and skin and serum specific IgE testing was unrevealing. CBC with differential in 2012 showed hemoglobin 9.0 g/dL, hematocrit 31.0%, MCV 70 fL, platelets 412 K/ μL , and AEC 1,408/ μL . Angioedema progressed to involve the hands and feet, occurring daily with pruritus, urticaria, myalgia and arthralgia. Evaluation for an autoimmune etiology demonstrated normal

C3/C4 and absence of anti-U1 RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1, and centromere B antibodies. C1 esterase inhibitor (350 mg/L [>220]) was normal, and she denied a family history of angioedema. Intermittent glucocorticoids provided symptomatic benefit. When a continuous 3-month course of prednisone 10mg was discontinued, symptoms recurred.

Upon evaluation at our institution in April 2013 off therapy, angioedema remained her primary complaint. Laboratory testing demonstrated WBC 11.49 K/ μ L with hypereosinophilia (AEC 3,680/ μ L) and a severe normocytic anemia (hemoglobin nadir 6.5 g/dL, hematocrit nadir 18.7%) with low haptoglobin (<8 mg/dL) reticulocytosis (16.2%, absolute 223.6 K/ μ L [0.5 – 1.7%, 16.4 – 77.6]), peak LDH 1207 U/L (113 – 226), and peak indirect bilirubin 1.3 mg/dL. Serum tryptase was elevated (16.5 ng/mL [<11.5]), as were serum vitamin B12 (1,273 pg/mL [193 – 982]) and ESR (72 mm/hr [0 – 25]). Serum IgE (266 IU/mL [0 – 90]) was high, while IgM was normal (258 mg/dL [34 – 342]). C1 inhibitor function was $>90\%$ (normal) and C1q was not decreased (44mg/dL). Rheumatoid factor (RF) (1300 IU/mL [<15]), ANA (1.3 EU [<0.9]), and anti-CCP (>125 units [<20]) were elevated, while anti-ENA, anti-dsDNA, and anti-MPO were negative. EBV and CMV were negative by PCR. Peripheral smear revealed agglutinated red blood cells and large platelet clumps. Drawing blood resulted in immediate agglutination with cooling in the tube. Following a positive direct antiglobulin (Coombs) test, a transfusion medicine consult was obtained. Workup revealed agglutination with C3, but not IgG after treatment with dithiothreitol to disrupt IgM bonds, consistent with diagnostic criteria of cold agglutinin disease (Figure 1). SPEP was normal. A search for causes of hypereosinophilia was unrevealing, including polyclonal T and B cell receptor rearrangement studies by PCR, normal bone marrow biopsy, negative molecular testing for *FIP1L1-PDGFR*, *D816V KIT*, *V617F JAK2*

and *BCR-ABL* and infectious workup for secondary causes of eosinophilia. CT neck, chest, abdomen, and pelvis revealed no significant lymphadenopathy, hepatosplenomegaly, or other masses concerning for malignancy. FNA of a thyroid nodule was paucicellular but showed cystic fluid with acute inflammatory cells and eosinophils. Salivary gland biopsy revealed chronic sialadenitis with eosinophils (50/hpf) but otherwise lacked characteristic features of IgG4-related disease. Total IgG (1410 mg/dL [767 – 1590]), was normal, with elevated IgG3 (275 mg/dL [18.4 – 106]) and IgG4 (256 mg/dL [2.4 – 121]). *TPSAB1* copy numbers were unremarkable.

Her AEC peaked at 15,820/ μ L and she was started on prednisone (1 mg/kg) in May 2013 with good clinical and hematologic response, including normalization of her AEC, serum tryptase and hemoglobin. She was unable to taper below 30mg of prednisone daily without recurrence of angioedema and eosinophilia and interferon- α was initiated as a steroid-sparing agent in July 2013, with hydroxyurea added in August 2013. Her AEC and symptoms remained suppressed on this regimen, allowing tapering of prednisone, but when interferon- α was discontinued after 2 months due to neutropenia and arthralgia, her eosinophilia recurred. Bilateral hand/wrist radiographs showed normal bones and joint spaces without evidence of rheumatoid arthritis.

Based on studies demonstrating efficacy of rituximab (an anti-CD20 monoclonal antibody) in cold agglutinin disease³ and the patient's elevated RF, ANA, and anti-CCP suggestive of an underlying rheumatologic disorder, a trial of rituximab was planned. Tenofovir was prescribed to prevent hepatitis B reactivation, and induction therapy commenced with two 375mg/m² IV infusions of rituximab two weeks apart in October 2013, followed by infusions at

6-month intervals. Hydroxyurea and prednisone were discontinued 5 and 10 months after rituximab initiation. A total of 7 rituximab infusions were given. Throughout rituximab therapy, AEC and anti-CCP antibodies remained mildly elevated but gradually declined and normalized. CD20 was normal prior to rituximab, declined to 0/ μ L and recovered post-therapy. Her hemoglobin and haptoglobin remained within normal range without further hemolysis. LDH (Table E1) and IgG remain within normal range several years off rituximab and angioedema episodes also improved.

AIHA in conjunction with HES is rare and, with the exception of this patient, has not been identified in 550 consecutively enrolled hypereosinophilic subjects at our institution. Seven cases of HES and AIHA have been reported in the literature and are summarized in Table E2. Three of the 7 cases of HES were idiopathic, 3 were myeloid, and one patient was diagnosed with eosinophilic granulomatosis with polyangiitis. Glucocorticoids were used as first-line therapy in each, with rapid clinical and hematologic response in all but two cases. In four cases, cyclophosphamide or hydroxyurea were initiated either as glucocorticoid-sparing agents or due to glucocorticoid lack of efficacy. Four patients were in hematologic and clinical remission at the time of report, though follow up duration varied. One patient continued to have relapses of cold-agglutinin AIHA, and 2 died. To our knowledge, our patient is the first with AIHA and presumed reactive HES to respond to treatment with rituximab. Although rituximab is considered first-line therapy for AIHA, relapse has been reported in up to 50%. After receiving rituximab for 2.5 years, it was discontinued and the patient remains in remission on no HES therapy for 4.5 years.

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Figure 1 Legend

Blood smear shows agglutination at room temperature (RT) (top, right) from preexisting IgM (not IgG) bound to sensitized RBCs from fixation/activation of complement (bottom, right) in cold agglutinin disease. The terminal membrane attack complex creates pores causing intravascular hemolysis. After warming, blood smear lacks agglutination (top, left) and RBCs do not bind IgM binding or activate complement (bottom, left).

Medical history	Journal Pre-proof			Outcome	Reference
• AIHA	• 69-year old man with dyspnea on exertion and	• Methylprednisolone 40mg IV BID	• Patient stable at last report		Arquati et

Table E1

Date	Absolute eosinophil count (/mm ³)	Hemoglobin (g/dL)	Lactose Dehydrogenase (U/L)	Serum tryptase (ng/mL)	Notes
29-Apr-2013	3680	8.2	695	16.5	At presentation
6-May-2013	15820	6.6	997	-	Peak symptoms
16-Jan-2014	2480	12	228	4.9	On prednisone
22-Aug-2013	260	13	620	-	On prednisone, hydroxyurea, alpha-interferon
3-Oct-2013	1950	13	304	-	Rituximab initiated
21-Oct-2014	1530	13.8	192	-	Off hydroxyurea and prednisone
16-Apr-2015	570	13.6	180	5.4	On rituximab
10-May-2016	400	15.1	180	5.6	Post-rituximab
13-Oct-2020	350	14.9	222	5.8	4.5 years off rituximab

- HES (idiopathic) - Heavy cigarette, alcohol use - Thyroid adenoma s/p resection, hypothyroidism - COPD	- fatigue - Hgb nadir: 8.9 g/dL - Peak AEC 4,000/μL - Exam: icteric sclerae, reduced breath sounds, + wheezing - Imaging: bilateral peribronchitis, hepatosplenomegaly and steatosis - PFTs: severe airway obstruction.	- with rapid improvement - Steroids continued at 80mg daily x 1 month, then tapered	on prednisone 12.5mg daily	al Am J. Hematol 1995
- HES (idiopathic) x 20 years - AIHA x 16 years, progressive until splenectomy 6 years post diagnosis - Chronic active hepatitis (CAH) x 18 years - DVT, PE (14 years prior) - Small bowel infarct s/p surgery (4 years prior)	48-year old man with dyspnea, pleuritic chest pain, abdominal pain, diarrhea - Hgb nadir: 6.1 g/dL - Peak AEC: 101,178/μL - Exam: febrile, tachycardic, tachypneic, scleral icterus, grade II systolic ejection murmur at LSB, diffusely tender abdomen - Acute kidney injury - Exploratory laparotomy: small retroperitoneal hematoma	- Methylprednisolone 80mg IV daily, empiric antibiotics - Methylprednisolone 1g IV once, 60mg IV q8h, daily hemodialysis, blood transfusions, with no response - Cyclophosphamide 100mg IV daily, immunoglobulin 30mg daily - Pressors started for hypotension - Death on hospital day 12	Autopsy: hematomas in pancreas, hepatic necrosis, bronchopneumonia, pericardial effusion, no eosinophilic infiltrate in any organ, but Charcot-Leyden crystals (CLC) found in renal tubular lumina. Bone marrow without eosinophils	Hirszel P et al. Am J Kidney Dis. 1988
- HES/EGPA (chronic sinusitis, asthma, nasal polyposis) x 1.5 yrs - AIHA	44-year old man with asthma, neuropathic pain, lower extremity weakness, purpuric papules - Hgb nadir g/dL - Peak AEC 21,500/μL - Exam: diffuse expiratory wheezes, mild hepatomegaly, livedo reticularis, diminished ankle jerks - Biopsies: Sural nerve: axonal loss and Schwann cell proliferation. Purpuric papules: vascular occlusion of small arteries and venules, mononuclear infiltration, extravascular granuloma	- Prednisolone 1mg/kg/day with abrupt drop in AEC, asthma improvement by day 3, resolution of skin lesions by 2 weeks - Prednisolone tapered to 10mg/day with exacerbation of asthma and mononeuritis multiplex - Cyclophosphamide 50mg/day added	Remission maintained with combination therapy of cyclophosphamide and prednisolone, duration unknown	Kojima K et al. Int J Hematol. 1996
- NHL in remission (stage IV follicular s/p 6 cycles of CHOP) - AIHA - HES (myeloid)	72-year old man with new onset anemia - Hgb nadir: 8.7 g/dL - Peak AEC: 16,000/μL - Bone marrow biopsy: dysplastic granulopoiesis, increased dysplastic eosinophils, CLCs, and erythrophagocytosis by eosinophils	1st episode of AIHA responded to prednisone 1mg/kg/d x 18 weeks, then dose reduced. Relapse did not respond to glucocorticoids - Hydroxurea 30mg/kg/d started with excellent response	Sudden sepsis 12 weeks later resulted in death	Kuk JS et al. Am J Hematol. 2006
- CAH - Hypereosinophilia vs HES - AIHA	14-year old boy with malaise, fevers, headaches - Hgb nadir: 8.1 g/dL	Prednisone 60mg daily resulted in clinical and serological improvement	Prednisone tapered to 7.5mg daily, but CAH recurred twice without hemolysis or	Panush RS et al. Gastroente

	• Peak AEC 7,437/μL • Exam: oral T 100 F, pallor, scleral icterus, RUQ tenderness, smooth liver edge palpable at costal margin • Liver biopsy: CAH, inflammatory portal infiltrate, piecemeal necrosis.		eosinophilia, requiring hospitalization, high-dose steroids	rology. 1973
• AIHA • HES (myeloid - translocation (3;5)(p13;q13)0 • Fibroids s/p hysterectomy	• 45-year old woman with abdominal pain, jaundice, weight loss • Hgb nadir: 4.3 g/dL • Peak AEC 29,550/μL • Exam: scleral icterus, pallor, tender hepatosplenomegaly	• 1st presentation responded to pRBCs and glucocorticoids • 2nd presentation responded to prednisone and hydroxyurea • Hydroxyurea continued at 1-2g/day • Bone marrow biopsy at 4 years: myeloid predominance, eosinophilia, translocation (3;5)(p13;q13)	• Patient was asymptomatic on hydroxyurea at the time of report	Shanske AL et al. Br J Haematol 1996
• AIHA • HES (myeloid) • h/o musculoskeletal pain, used ibuprofen frequently	• 25-year old male with fever and malaise • Hgb nadir: 5.4 g/dL • AEC peak: 49, 900/μL • Exam: hypotension, tachypnea, scleral icterus • Abdominal ultrasound: hepatosplenomegaly	• pRBCs, methylprednisolone 120mg IV q6h x 1 day, then prednisone 60mg daily with good response	• Discharged after 5 days with glucocorticoid taper and avoidance of NSAIDs • Lost to follow up	Sweidan AJ et al. J Blood Med. 2015

Table E2

Abbreviations: AEC- absolute eosinophil count; AIHA- autoimmune hemolytic anemia; CAH-chronic active hepatitis; CHOP- cyclophosphamide, doxorubicin, vincristine, prednisone therapy; CLC- Charcot-Leyden crystal; COPD- chronic obstructive pulmonary disease; DVT- deep venous thrombosis

EGPA- eosinophilic granulomatosis with polyangiitis; HES- hypereosinophilic syndrome; Hgb- hemoglobin; NHL- non-Hodgkin lymphoma; PE- pulmonary embolism; PFT- pulmonary function test; pRBCs- packed red blood cells.

