RESEARCH LETTER

blood advances

TO THE EDITOR:

Severe autoimmune intravascular hemolytic anemia in patients receiving alemtuzumab for multiple sclerosis

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Alemtuzumab is a recombinant humanized anti-CD52 monoclonal antibody against a glycosylated, glycosylphosphatidylinositol-anchored, cell-surface protein (CD52) which is expressed at high levels on T and B lymphocytes.[1,2] It was approved by the European Medicines Agency in 2013 and by the Food and Drug Administration (FDA) in 2014 for relapsing remitting multiple sclerosis (RRMS) (*Lemtrada*).[2,3] Alemtuzumab is also FDA-approved for B-chronic lymphocytic leukemia (B-CLL) under the brand name *Campath* and used off-label for immune disorders such as graft-versus-host disease and aplastic anemia, and occasionally as a part of the conditioning regimen for solid organ and hematopoietic stem cell transplantations.[4] An interesting paradox to its immunosuppressive effects is its autoimmune effects, which are sometimes serious, warranting an FDA-issued black box warning.[2] Among the autoimmune disorders, thyroiditis (20%-40%) is the most common, followed by immune thrombocytopenia (2.2%) and autoimmune nephropathies (0.3%).[5,6] The secondary autoimmune disorders tend to arise within the first 5 years of follow-up, with the peak incidence occurring in the first 3 years after the last dose.[7]

Rare singular cases of autoimmune hemolytic anemia (AIHA) occurring months after alemtuzumab infusion are emerging. Here, we summarized our case along with review of all the reported cases of post-alemtuzumab AIHA in patients with RRMS.

The case of a patient with RRMS who developed post-alemtuzumab AIHA encountered at our center was described. Three other cases from the randomized controlled trials comparing alemtuzumab vs interferon beta 1a trials (CARE MS-I/II and CARE MS extension) were reviewed and case details were obtained from the research team.[8,9] Simultaneously, a literature search using the terms "autoimmune hemolytic anemia," "multiple sclerosis," and "alemtuzumab" in PubMed, Ovid, Medline, and Cochrane Library from 1 January 2010 to 1 January 2022 revealed 6 additional cases.[10-15] Relevant demographic and clinical data were collected and summarized (Table 1). All the analyses were descriptive and exploratory. Our primary aim was to assess and characterize the clinical landscape of post-alemtuzumab AIHA.

A 36-year-old male presented to the emergency department with symptoms of malaise, dyspnea, and dark urine for 3 days. His past medical history was significant for RRMS for 6 years. He had previously relapsed on interferon-1B and fingolimod and received alemtuzumab 11 months before admission.

He denied recent infections, new medication, or over the counter products. Physical examination revealed tachycardia, pallor, and jaundice. Laboratory results were significant for anemia (Hb, 5.4 g/dL; baseline, 15 g/dL), thrombocytopenia (platelets, 60 × 10^3 per μL), elevated lactate dehydrogenase (LDH, 900 units/liter), and undetectable haptoglobin (<8 mg/dL). His total bilirubin was 4 mg/dL with direct fraction <1 mg/dL. Urine analysis revealed hemoglobinuria with no red blood cells. Pretransfusion peripheral blood smear showed significant spherocytosis and thrombocytopenia. Direct antiglobulin test was immunoglobulin G (IgG)-positive. He was immediately given matched blood transfusion with

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Table 1. Clinical characteristics of cases with alemtuzumab associated AIHA

Features	Case A (present case)	Case B (CARE MS I trial)[8]	Case C (CARE MS II trial)[16]	Case D (CARE MS extension trial)[9]>	Case E (Meunier et al)[10]	Case F (Di Ioia et al)[11]	Case G (Rieckmann et al)[12]	Case H (Tzartos et al)[14]	Case I (Metz et al)[13]	Case J (Alnahdi et al)[15]	Mean ± SD
Age (y)	36	43	43	52	28	34	34	31	33	52	38.6 ± 8.5
Sex	Male	Female	Male	Female	Male	Male	Female	-	Female	Male	
Previous MS treatments	IFNb1b fingolimod	-	IFNb1a	-	IFNb1b mitoxantrone fingolimod	IFNb1a natalizumab fingolimod	IFNb1b natalizumab fingolimod	IFNb1a natalizumab fingolimod	IFNb1b natalizumab fingolimod	IFNb1a natalizumab fingolimod	
No. of ALZ cycles received	1	2	2	3	1	1	1	1	1	1	
Cumulative ALZ dose (mg)	60	96	96	132	60	60	60	-	60	60	76 ± 26
Time of hemolysis to last ALZ dose (mo)	11	15	9	3	11	12	9	12	8	8	10.3 ± 2.3
Lowest haptoglobin (mg/dL)*	<8	-	-	<8	<8	<8	<8	<8	-	<8	
Hb Nadir (g/dL)	4.7	6.7	8.6	2.9	3.5	3.9	2.8	5.7	2.4	5.3	4.7 ± 2
DAT (IgG/C3d)	IgG	IgG/C3d	IgG/C3d	IgG/C3d	IgG/C3d	IgG	IgG/C3d	IgG/C3d	-	-	
Treatment	Transfusion CORT RTX, IVIG, Cy	CORT	Folic acid Vit B12	Transfusion CORT	Transfusion CORT IVIG, RTX	Transfusion CORT, IVIG	Transfusion CORT, PLEX	Transfusion CORT, PLEX, RTX	Transfusion CORT, IVIG, Cy, PLEX	CORT, PLEX	
Time to complete hematological recovery (wk)	6	16	8	8	8	4	-	13	-	16	9.6 ± 4.8
Outcome	Alive	Alive	Alive	Alive	Alive	Alive	Dead	Alive	Dead	Alive	
Other concomitant autoimmune effects	ITP	Grave disease	ITP	-	-	Autoimmune nephropathy	ITP	Relapsed MS	DNL	DAH, nephropathy	

ALZ, Alemtuzumab; CORT, corticosteroids; Cy, cyclophosphamide; DAT, direct antiglobulin test; DNL, disseminated necrotizing leukoencephalopathy; DAH, diffuse alveolar hemorrhage; Hb, hemoglobin; IFN, interferon; IVIG, intravenous immunoglobulin; PLEX, plasma exchange; RTX, rituximab; Vit, vitamin.

*Normal range, 30 to 200 mg/dL.

relief of symptoms and was started on high dose intravenous corticosteroids and intravenous immunoglobulin with daily transfusions to keep Hb above 5 to 6 g/dL. However, after 10 days of admission, he continued to have active hemolysis, necessitating second- and subsequently third-line treatment with rituximab and cyclophosphamide. Two weeks after cyclophosphamide treatment, his Hb started to improve and haptoglobin, LDH, and bilirubin normalized. He achieved transfusion-independence by 6 weeks since diagnosis. At his last follow-up, 1 year since discharge, he remained hemolysis-free with normal platelet counts, and his RRMS did not relapse.

Among the 10 patients reported with post-alemtuzumab AIHA, 5 were males and 4 were females. Median age was 35 years (range, 31-52 years) (Table 1). Before the initiation of alemtuzumab, 4 patients received interferon beta 1a (IFN-β1a) and 4 received interferon beta 1b (IFN-β1b). Besides IFN, patients also received fingolimod (7/10), natalizumab (5/10), and mitoxantrone (1/10).

Seven patients completed only 1 cycle of alemtuzumab, 2 completed 2 cycles, and 1 received 3 cycles. Median onset of hemolysis was 10 months from last dose (range, 3 to 15 months). Median Hb at presentation was critically low at 4.3 g/dL. Direct antiglobulin test results were available in 8 patients, all were IgG-positive and 6 were C3d-positive, indicating that both IgG and classical complement pathway activation possibly triggered hemolysis. Other concomitant autoimmune disorders were present in 8 patients, including 3 with immune thrombocytopenia (Evan syndrome), 1 with Grave disease, 2 with autoimmune nephropathy. One patient had alveolar hemorrhage which was unclear whether it was autoimmune-related. No specific autoantibodies were reported in these patients.

Apart from supportive transfusions, 9 patients received corticosteroids. Additional immunomodulatory treatments included intravenous immunoglobulin (4), plasma exchange (4), cyclophosphamide (2), and rituximab (2). Eight patients recovered from AIHA; however 2 succumbed to death because of complications of severe AIHA. Median time from diagnosis of AIHA to normalization of hematological parameters was 8 weeks (range, 4 to 16 weeks).

Because of the risk of autoimmunity, urinalysis for periodic monitoring of blood count and renal function is recommended by the FDA in patients with RRMS who have received alemtuzumab till 48 months after the last dose.[2] By targeting CD52, alemtuzumab primarily depletes circulating T and B lymphocytes via antibody dependent cellular toxicity and complement-dependent cytolysis.[17] After depleting dysregulated lymphocytes, the immune system starts to reconstitute around 4 to 6 months and reaches a healthy homeostasis, leading to the remission of autoimmune disease.[18] However, the new, reconstituted immune system may develop autoimmunity, a so-called "immune-rebound" effect contributed by multiple factors such as proliferation of residual T cells, failure of thymic reconstitution, increased production of interleukin-21 which causes increased T-cell turnover, and hyperpopulation of immature and naïve B cells. B-cell reconstitution often precedes T-cell reconstitution because of the lower B-cell depleting efficacy of alemtuzumab, leading to hyperpopulation of autoreactive B cells in the absence of regulatory T cells.[19] B-cell proliferation and function capacity remain increased for at least 2 years after treatment.[20] The mechanism by which alemtuzumab is associated with AIHA is likely because of unbalanced immune reconstitution after generalized immune downregulation by knockout of CD52 harboring immune cells, a novel mechanism of drug independent yet drug-induced hemolysis with specific high-risk period after drug administration.[21] Circumstantial proof of this mechanism lies in the fact that alemtuzumab is paradoxically, albeit rarely, used in conjunction with rituximab to treat refractory AIHA.[22]

Patients with MS are predisposed to other autoimmune disease such as thyroiditis, psoriasis, and inflammatory bowel disease; however no clear association between MS and AIHA has been established.[23] AIHA, after other disease modifying therapy such as IFN and fingolimod, has been reported.[24,25] On the other hand, the occurrence of post-alemtuzumab AIHA is not limited to MS but also seen in alemtuzumab-conditioned allogeneic stem cell transplantation.[26,27]

Our concise review showed the probable association between alemtuzumab and severe AIHA in patients with RRMS. Our literature review is limited by an inherent reporting bias of only severe cases. The overall incidence of post-alemtuzumab AIHA may be higher. Despite estimates from clinical trials, pharmacovigilance data are around 0.21%.[9] Nonetheless, heightened awareness of secondary autoimmune adverse events of alemtuzumab is important for providing timely and proper treatment to patients. At present, the FDA recommends monitoring complete blood count at baseline and at monthly intervals until 48 months after the last treatment in patients receiving alemtuzumab for RRMS. Our review highlighted the need to perform additional work up once a patient develops anemia during monitoring, in order to diagnose AIHA in a timely fashion.

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