

Sequential Use of Prednisolone and Cyclosporine Is Effective in the Management of Immunotherapy-Related Hemolytic Anemia

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Abstract

Immune checkpoint inhibitors (CPIs) can cause immune-related organ dysfunctions, including nephritis, pneumonitis, thyroiditis, hepatitis, colitis and more rarely hematological toxicities like immune-related autoimmune hemolytic anemia (irAIHA). Very few cases of irAIHA associated with immunotherapy have been reported, and treatment protocols remain unclear. This is partly because not all irAIHA cases are Coomb's test positive. Causes of anemia in cancer patients undergoing treatment with chemotherapy with or without immunotherapeutic agents can also be multiple. This makes it difficult to initially diagnose irAIHA, especially when CPIs are used concurrently with chemotherapy. Once alternate causes have been ruled out, a treatment plan for irAIHA is initiated based on grade of the anemia. Grade 1-2 irAIHA cases are managed with supportive interventions. However, cessation of therapy is recommended for life-threatening (grade 4) toxicity, severe (grade 3) toxicity that is recurring, or moderate (grade 2) toxicity that does not resolve with appropriate treatment for 3 months. Management of irAIHA usually involves methylprednisolone for 2 - 4 weeks with a slow taper after hemoglobin has normalized. But some cases do not respond to steroids alone and require cessation of immunotherapy or selecting alternate immunosuppressive agents. We report a protocol for treatment of grade 4 irAIHA secondary to programmed death protein 1 (PD-1) blocker pembrolizumab.

Keywords: Immune checkpoint inhibitors; Autoimmune hemolytic anemia; Management

Introduction

Immune checkpoints including cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and programmed death receptor 1 (PD-1) on T-lymphocytes and programmed death receptor ligand 1 (PD-L1) on tumor cells are involved in evasion of cancer cells from immune surveillance. The binding of PD-1 and PD-L1 reduces T-cell activation. Immune checkpoint inhibitors (CPIs) bind to PD-1 or PD-L1 receptors allowing T-cell expansion and cancer cell destruction [1, 2]. However, these activated T-cells are not antigen-specific, leading to off-target effects that cause side effects unique to CPIs. These immune-related adverse effects (irAEs) include nephritis, pneumonitis, thyroiditis, hepatitis, colitis, autoimmune hemolytic anemia (AIHA), immune thrombocytopenia, pure red cell aplasia, as well as hemophagocytic lymphohistiocytosis [3-5].

Very few cases of AIHA associated with immunotherapy have been reported, and so protocols for appropriate management are unclear. Of all irAEs reported to the Food and Drug Administration (FDA) until 2018, the percentages of AIHA cases attributable to pembrolizumab, nivolumab and atezolizumab were 0.146%, 0.211%, and 0.245%, respectively [6]. These small numbers are further complicated by the fact that not all AIHA cases secondary to CPIs are Coomb's test positive. In a series of four cases with cytopenia reported from MD Anderson, three cases of hemolytic anemia were in fact Coomb's-negative [7]. At the same time, other case series and reports show presence of the Coomb's-positive AIHA in all patients studied [8, 9]. Regardless, all these patients have features consistent with hemolysis, including elevated lactate dehydrogenase (LDH), low haptoglobin levels, high indirect bilirubin levels, as well as lack of bone marrow infiltration by cancer cells.

The causes of anemia in cancer patients undergoing treatment with chemotherapy with or without immunotherapeutic agents can be multiple and include blood loss from the tumor itself, nutritional causes, marrow infiltration by tumor cells, as well as marrow suppression from cytotoxic chemotherapy. This makes it difficult to initially diagnose, especially when

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CPIs are being used concurrently with cytotoxic chemotherapy. A complete investigation may include endoscopic evaluation for blood loss and a bone marrow biopsy. Once alternate causes have been ruled out, a treatment plan for immune-related autoimmune hemolytic anemia (irAIHA) is initiated based on grade of the irAE. Grade 1-2 irAEs may be managed symptomatically with supportive therapies. However, cessation of therapy is recommended for life-threatening (grade 4) toxicities, severe (grade 3) toxicity that is recurring, or moderate (grade 2) toxicities that do not resolve with appropriate treatment for 3 months.

Management of irAIHA usually involves initiation of steroids like methylprednisolone for 2 - 4 weeks. A slow taper is recommended after the hemoglobin level has improved or normalized. But not all cases respond to steroids alone and require either cessation of immunotherapy or selecting alternate immunosuppressive agents. We herein report an immunosuppression protocol used in the successful treatment of a grade 4 irAIHA that developed in a patient with cervical squamous cell carcinoma, who was receiving the programmed death protein 1 (PD-1) blocker pembrolizumab.

Studies related to diagnosis and management of irAIHA from immunotherapeutic agents were compiled from PubMed and the Cochrane Database. Sequential laboratory data related to the treatment of acute anemia in the concerned patient were collected using CERNER EMR data extraction protocols. Clinical outcomes were compared with published material on management of hemolytic anemia secondary to CPIs, covering a period from 2011 to 2023. Microsoft Office 365 software was used to prepare data tables. No statistical data analysis was performed in writing this manuscript.

Case Report

A 36-year-old female with stage IVB cervical cancer was admitted to the hospital with acute symptomatic anemia. The patient had been diagnosed with cervical squamous cell carcinoma in January of 2023. Computed tomography (CT) imaging on January 13, 2023, showed a complex cervical mass measuring 8 × 6 × 6.3 cm, along with a uterine mass measuring 7.8 × 6 × 6.2 cm, and multiple cysts in both ovaries. There were also multiple enlarged inguinal, iliac and retroperitoneal lymph nodes. Biopsy from the cervix showed malignant squamous epithelial cells with extensive necrosis.

Pelvic magnetic resonance imaging (MRI) revealed a large, heterogeneous enhancing mass involving the uterine cervix. It extended inferiorly into the upper third of the vagina and proximally into the lower uterine body along with parametrial extension. The mass was inseparable from the pelvic floor, the posterior wall of the urinary bladder, and anterior wall of the rectum. Positron emission tomography (PET) scan confirmed a fluorodeoxyglucose (FDG)-avid complex necrotic cervical mass extending into the lower uterine segment along with numerous FDG-avid left supraclavicular, mesenteric, retroperitoneal, and bilateral external iliac lymph nodes. The biopsy of left iliac and supraclavicular lymph nodes showed metastatic squamous cell carcinoma. PD-L1

Table 1. Baseline Laboratory Values

	March 23, 2023	April 13, 2023
Complete blood count		
Hemoglobin	11	11.8
White blood cells	3.30	7.08
Platelets	275	194
Metabolic profile		
Creatinine	0.51	0.55
Total bilirubin	0.17	0.17

staining confirmed > 1% positivity.

The patient was initially started on a platinum-based triplet regimen using carboplatin, paclitaxel and bevacizumab with the first cycle beginning on March 2, 2023. Pembrolizumab was added after the first two cycles of systemic chemotherapy on April 13, 2023. Baseline hemoglobin prior to starting chemotherapy was 11.0 and 11.8 after two cycles, when pembrolizumab was added. Following the third cycle of pembrolizumab on May 25, 2023, hemoglobin level decreased to 6.3 g/dL. The patient was subsequently hospitalized, and the workup was initiated for the anemia. Baseline laboratory values are summarized in Table 1. Two units of packed red blood cells were given on May 25, 2023, and another two units on June 1, 2023. The workup of anemia showed a negative Coomb's test along with negative titers for human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and parvovirus antigens. The paroxysmal nocturnal hemoglobinuria (PNH) testing yielded negative results and cryoglobulin titer was < 2. Tick-borne panel was also done due to her risk profile, and this was negative as well. The stool occult blood tests were negative. Bone marrow biopsy was then completed due to the recent exposure to systemic chemotherapy and also to rule out marrow involvement with malignancy. It did not show any evidence of aplasia or infiltration with tumor cells. Subsequent labs showed a low haptoglobin level along with an elevated LDH, consistent with a Coomb's test negative hemolytic anemia. With the presumptive diagnosis of irAIHA secondary to pembrolizumab, patient was hospitalized on June 7, 2023 and started on oral prednisolone 100 mg daily. This was continued for 3 weeks, and then cyclosporine was initiated since hemoglobin levels were not improving. A total of 18 units of packed red blood cells had to be given during the hospitalization before the hemoglobin level stabilized. Oral cyclosporine 200 mg/day was started after 3 weeks, which finally led to an improvement of hemoglobin levels. Oral prednisolone was tapered off over the next 2 months (Table 2).

The patient was discharged home after 4 weeks, on oral cyclosporine with a tapering protocol for prednisolone. Hemolysis had significantly improved during the first 2 weeks of induction treatment (Table 3). On the follow-up visit, hemoglobin was stable at 13 from week 9. Subsequently, starting week 10 cyclosporine was tapered gradually over 6 weeks and finally stopped on October 13, 2023, with hemoglobin level at week 16 remaining stable at 12.7. Patients eventually com-

Table 2. Treatment Schedule

Treatment days	Hb range	Total PRBCs	Prednisolone	Cyclosporine
D1 - 7	3.9 - 5.7	7	None	None
D8 - 14	5.5 - 8.2	7	100 mg	None
D15 - 21	5.3 - 6.5	3	100 mg	None
D22 - 28	5.8 - 6.6	1	Steroid taper	200 mg
Weeks 5 - 8 (month 2)	11.8 - 12.3	0	Steroid taper	200 mg
Weeks 9 - 12 (month 3)	12.5 - 13	0	None	100 mg
Weeks 13 - 16 (month 4)	10.9 - 12.7	0	None	50 mg

Hb: hemoglobin; PRBCs: packed red blood cells.

pleted a total of eight cycles of the triplet regimen before going on to maintenance bevacizumab on November 2, 2023.

Discussion

Immune checkpoint inhibitors have come to be extensively used in the management of multiple solid tumors. But their use has shown that even as they are very effective in improving response and survival rates in many oncological conditions, they also have unique side effects, collectively called irAEs that are attributable to their use. These include hematological side-effects that can range from irAIHA to pure red cell aplasia. The pathogenesis of irAIHA remains unclear considering that Coomb’s test is not always positive. However, it remains unclear as to whether irAIHAs are truly Coomb’s test negative. It may actually be that there is immunoglobulin (Ig)G sensitization that remains below the threshold of detection by currently available testing platforms. It may also be from removal of IgG during the testing process. Another possible reason is that the antibodies may belong to IgA or IgM subtypes [10, 11]. Regardless, their relation to CPIs is very likely, considering the elevated levels of other markers like Th-17 cells and reduced levels of cytokines like interleukin (IL)-10, which are consistently seen in other CPI-related complications [12, 13]. There are other cytokines too, which are elevated in AIHA, including IL-2 and IL-12. These cytokines cause differentiation of CD4⁺ naive T cells into Th 1 cells that further augment the cellular immunity response [14]. Also seen is elevation of transforming growth factor-β, which promotes differentiation of the Th 17 subset, adding to the amplification of the autoimmune cascade [15]. This also explains the response to immunosuppressive agents like intravenous (IV) steroids. The most commonly used

medication has been IV Solu-Medrol, at a dose of 1 mg/kg with conversion to oral steroids and a taper once hemoglobin levels have recovered and markers of hemolysis show resolution. However, this intervention alone is not always successful due to the heterogeneity of irAIHA. In such situations, the use of rituximab is often considered. However, unlike corticosteroids and immunosuppressants like cyclosporine or mycophenolate, the effect of anti-CD20 therapies takes time to become apparent. Also, anti-CD20 therapies have a limited mechanistic effect, which may not be optimal, considering the widespread inflammatory sequelae of CPIs, involving multiple pathways and many different organs. Corticosteroids on the other hand produce their anti-inflammatory effects through multiple effects including but not limited to reduction in cytokine productions, modulation of the function of cells including T lymphocytes, neutrophils and even macrophages, inhibition of vasodilation, as well as decrease in vascular permeability [16]. This again shows the limits of using medications like rituximab, which have limited ways of ameliorating the pro-inflammatory cascade from CPIs. It also has no effect on pathways involving activation of T lymphocytes. Therefore, in patients with irAIHA who have an inadequate response to IV steroids, it is better to consider alternate immunosuppressive agents than anti-CD20 therapies. Cyclosporine, unlike rituximab, has more extensive immunomodulatory effects, including suppression of T lymphocyte-mediated inflammation and suppression of CD25-mediated regulatory T-cell activation. Cyclosporine, unlike steroids, does not affect neutrophils or macrophages, while having unique effects, including suppressing B-cell-dependent T-cell activation, as well as reduced dephosphorylation of nuclear factor of activated T cells, which in turn reduces IL levels [17]. These immune-modulatory mechanisms and their prevalence in CPI-related irAIHA make cyclosporine a better choice than rituximab in situations where the response to first-line corticosteroids in resolving irAIHA is suboptimal.

Table 3. Induction Phase Laboratory Values

Week	Haptoglobin	LDH	Bilirubin: total/direct
Week 1	< 10	1,368	1.79/0.3
Week 2	< 10	2,334	2.30/0.5
Week 3	< 10	1,578	1.11/0.2
Week 4	< 10	1,048	0.89/0.2

LDH: lactate dehydrogenase.

New findings

Widespread use of CPIs has increased the incidence of irAIHAs. But unlike AIHAs, irAIHAs are not always antibody or complement mediated. Increased levels of Th-17 cells and low IL-10 levels indicate alternate pathophysiological mechanisms. Consequentially, there has been difficulty creat-

ing effective treatment protocols. Use of anti-CD20 antibodies is common in AIHAs, but its effectiveness remains unclear in irAIHAs. Use of these antibodies is also not cost-effective in developing countries. This report explains the utility of sequential use of methylprednisolone followed by cyclosporine if there is insufficient response to steroids. Both agents are more cost-effective than plasma exchange and anti-CD20 antibodies.

Conclusions

Immunotherapeutic agents including pembrolizumab and nivolumab are associated with multiple irAEs including AIHA. The management of irAIHA due to CPIs is very different from that due to marrow suppression, infiltration and/or bleeding, which all need to be ruled out first. Protocols for managing irAIHA are not clearly defined partly from the rarity of this adverse event. However, when they occur, they can be debilitating to the patient. A complete workup should include a bone marrow biopsy besides tests like Coomb's. And even if Coomb's test is negative, in the absence of other causes such anemias should be considered as immune-related. This report provides support for fixed dosing of IV Solu-Medrol for 10 days followed by cyclosporine in the event of inadequate response to IV steroids. Our protocol recommends early cessation of steroids in such situations and use of induction and tapering doses of cyclosporine, which is more likely to be effective in resolving resistant irAIHA. This treatment sequence appears to be cost-effective while also providing durable responses.

Learning points

Check point inhibitors can cause irAIHA. irAIHA is rare, and therefore there are limited guidelines on treatment protocols.

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Conflict of Interest

The authors have no conflict of interest to disclose.

Informed Consent

The patient's informed consent for publication of this manuscript after removal of all patient identifiers was obtained.

Author Contributions

Niravkumar Brahmabhatt wrote the manuscript. Ghulam Ghous prepared the tables. Kapil Meleveedu provided editorial help. Nancy Vander Velde provided editorial help. Mark Hunter provided editorial help. Hari Parameshwaran helped with expert comments. Cherian Verghese wrote the manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author. Study protocol, patient data and clinical study report will be accessible to any individual who wishes to access the data from the time of publication and for 5 years thereafter.

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