Immune Checkpoint Inhibitors Induced Autoimmune Haemolytic Anemia: Case Series and Literature Review

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Research Article

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Abstract

Immune checkpoint inhibitors (ICIs) have brought a revolution to the anti-cancer treatment, however, they also trigger a unique spectrum of immune-related adverse events (irAEs). Among irAEs, haemopoietic AEs are rarely reported and mostly severe or even life-threatening, especially autoimmune haemolytic anemia (AIHA). AIHA is presumed to relate to the abnormal formation of circulating autoantibodies against red cell membrane antigens. It usually cannot be discovered timely because of atypical symptoms. It is diagnosed according to presence of hemolysis evidences such as decrease of haemoglobin, increase of indirect bilirubin and lactate dehydrogenase (LDH), urobilinogen, and positive direct antiglobulin test (DAT). Treatments of AIHA are according to clinical experience and consensus, which have not been verified by prospective trial. Here we investigate previous reported ICIs induced AIHA cases including thirty detailedly documented patients. On the other hand, we report three patients who developed AIHA after three different anti-PD-1 antibodies. Most of them were aged patients with melanoma or NSCLC, developed AIHA by anti-PD-1 antibodies and relived with glucocorticoid. 43.3% of previous cases and all of our observed cases had anemia before ICIs treatment, which reminds us of anemia as a risk factor for ICIs induced AIHA. By screening parameters like complete blood examination, reticulocyte, liver function test or DAT test prior to immunotherapy, doctors could exclude pretreatment haemolytic anemia or be aware of post ICIs AIHA. Thus, it is possible to avoid the potentially life-threatening AIHA, or improve the level of pre-alarm and treatment ability of AIHA.

Introduction

In recent decades, the anti-tumor immunotherapy has been rapidly discovered and widely developed due to its favourable anti-tumor effect and well tolerance. Undoubtedly, immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancer mainly by reactivating T cells and upregulating T cell anti-tumor responses.(Zhuang et al., 2020) Among ICIs, anti-programmed cell death 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) antibodies attract most attention due to their satisfactory anti-cancer effect. Although ICIs have passed the safety evaluation and act safer than traditional chemotherapy, they can inevitably trigger a unique spectrum of immune-related adverse events (irAEs),(Kennedy and Salama, 2020) which mainly include the lung, endocrine glands, gastrointestinal system, immune system, and skin, etc.(Zhuang et al., 2020) The haemopoietic system barely gets involved.(Davis et al., 2019) ICIs mediated hematological adverse effects are as rare as 3.69% (35 out of 948 patients) in a descriptive observational study.(Delanoy et al., 2019) The incidence of anemia was 9.8% for all-grade and 5% for grade 3 to grade 5 in a meta-analysis included forty-seven studies of PD-(L)1 inhibitors of 9324 evaluable patients.(Petrelli et al., 2018) In a clinical trial, the incidence of all-grade anemia was 3.84% and grade 3 or higher anemia was 0.78% for patients treated with a single-agent PD-1 or PD-L1 inhibitor.(Wang et al., 2019) Autoimmune haemolytic anemia (AIHA) is an uncommon type of anemia and ICIs induced AIHA in extraordinary rare.

ICIs induced AIHA is one of the most severe and progressive or even life-threatening irAEs with fatality as high as 15%,(Davis et al., 2019) which requires extra awareness and appropriate treatment. AIHA induced by anti PD-1 or PD-L1 antibody is exceptionally rare as reported frequency of 0.95% (9 in 948 patients) in Delanoy's observational study.(Delanoy et al., 2019) It results from red blood cell (RBC) destruction due to an abnormal activation of the immune system, (Jaime-Perez et al., 2013) of which the mechanism remains unconfirmed. It is likely relate to the formation of circulating autoantibodies against RBC membrane antigens, activation of T-cell clones, and suppression of regulatory T cells. Patients who previously accepted ICIs treatment and subsequently developed AIHA may have few atypical symptoms, such as anemic appearance, dizziness, fatigue and dark brown urine. The laboratory tests can show hemolysis related evidences such as a decrease of haemoglobin, an increase of serum indirect bilirubin and lactate dehydrogenase (LDH), urobilinogen, reticulocytosis, low serum haptoglobin and spherocytosis.(Leaf et al., 2019) Diagnosis is based on the direct antiglobulin test (DAT, Coombs test), which is usually positive for anti-immunoglobulin G and/or anti-C3d antisera.(Leaf et al., 2019) Recommendations for treating ICIs induced AIHA come from treatment of primary AIHA,(Jaime-Perez et al., 2013; Zanella and Barcellini, 2014) clinical experiences(Leaf et al., 2019; Tanios et al., 2019) and consensus(Brahmer et al., 2018; Haanen et al., 2018; Thompson et al., 2019) because no prospective trial has been conducted.(Davis et al., 2019) The information shortage of ICIs induced AIHA is because of its uncommon nature or doctors' poor recognition. As such, reporting more cases and retrospectively summarizing previous cases are helpful for enriching the understanding on ICIs induced AIHA.

Case Analysis

Documentally reported ICIs induced AIHA is rare, including 68 cases from the World Health Organization's pharmacovigilance database of individual-case-safety -reports of adverse drug reactions, VigiBase,(Davis et al., 2019) 68 cases from the database of the Food and Drug Administration,(Tanios et al., 2019) and 29 cases from other previous sporadic reports. But only 30 of them were detailedly documented for us to characterize (Table 1, Table 2). Among these 30 cases, 56.7% were men aged from 18 to 85 years old and 43.3% were women aged from 33 to 85 years old. The average age is 65.1 years old, including 65.2 years old for female patients while 64.9 years old for male patients, which indicates a possible prevalence of AIHA in aged patients. The tumor types in order of prevalence were melanoma (56.7%), non-small-cell lung cancer (NSCLC, 30.0%), and other malignancies, which is consistent with the cancer prevalence in Tanios's analysis.(Tanios et al., 2019) 90.0% of the patients' AIHA were caused by anti-PD-1 antibodies, including 40.0% by pembrolizumab, 33.3% by nivolumab, and 16.7% by nivolumab together with ipilimumab. It matches the previous discovery that anti-PD-1 antibodies cause more AEs than anti-PD-L1 antibodies or anti-CTLA-4 antibodies.(Davis et al., 2019) According to the decreasing severity of hemoglobin, all patients' AIHA were equal to or worse than grade 2, except that 2 patients' hemoglobin level was not mentioned.

63.3% patients (19 out of 30) gained AIHA within less than 5 cycles of ICIs. The cycle from starting the ICIs until developing AIHA varied between 1 and 39 cycles with a median of 4 cycles, which is later than 50 days from the data analysis in VigiBase(Davis et al., 2019) and 10 weeks from the cases in Food and Drug Administration database.(Tanios et al., 2019) Except for some cases are not reported, most patients (63.3%) showed positive direct antiglobulin test either mediated by IgG or C3 or their combination. As guidelines suggested,(Brahmer et al., 2018; Haanen et al., 2018; Hill et al., 2017) all patients were treated with glucocorticoids including dexamethasone, prednisone, and methylprednisolone. For patients who did not respond satisfactorily to glucocorticoids, intravenous immunoglobulin (IVIG), anti-CD20 antibody rituximab, and immunosuppressive drug azathioprine, were administered by 10.0%, 13.3%, and 3.3%

respectively. With these treatments, 28/30 patients completely recovered from AIHA while 2/30 expired. Although 76.7% patients suffered grade 3 or higher AIHA, one third of patients re-challenged ICIs with well tolerance.

Table 1. Cases with ICIs induced AIHA detailedly reported in the literature.

Abbreviations: ys: years; ICIs, immune checkpoint inhibitors; AIHA, autoimmune haemolytic anemia; LDH: lactate dehydrogenase; Ret%: percentage of reticulocyte; DAT, direct antiglobulin test; Ref.: reference; F: female; M: male; NSCLC: non-small cell lung cancer; CRC: colorectal cancer; SCC, squamous cell carcinoma; AML: acute myelocytic leukemia; CHOL, cholangiocarcinoma; Pembro: pembrolizumab; Nivo: nivolumab; Ipi: ipilimumab; NR: not reported; GCs: glucocorticoids; Pred: prednisone; MP: methylprednisolone; Dex, dexamethasone; IVIG: intravenous immunoglobulin.

Table 2. Characteristics of patients with ICIs induced AIHA detailedly reported in the literature.

Age/ys	Gender	Diagnosis	ICIs	LDH/U/L	Hemoglobin /g/dL			Ret%	DAT	Mediator	Treatment	Outcome	ICIs Re-challeng
					Baseline	Nadir	Δ						
52	F	Melanoma	Pembro×3	1266	12.5	6.3	6.2	0.1	IgG		GSs, IVIG	Recover	No
55	M	Melanoma	Pembro×1	399	10.4	6.3	4.1	0.3	IgG, C3		Pred	Recover	Pembro
67	M	Melanoma	Pembro×8	NR	15.4	6.6	8.8		NR		MP	Recover	No
68	M	Melanoma	Pembro×12	530	13.6	8.5	5.1	4.7	IgG		Pred	Recover	Nivo
79	F	Melanoma	Pembro×1	235	-	5.0	-	-	IgG, C3		GCs	Recover	No
81	F	Melanoma	Pembro×2ys	1176	9.1	3.9	5.2	8.9	IgG, C3		MP, Pred	Recover	No
85	M	Melanoma	Pembro×2	885	12.8	8.2	4.6	3.3	Negative		Pred, Rituximab	Recover	No
85	F	Melanoma	Pembro×10	1151	12.9	6.4	6.5	6.4	IgG		Pred, IVIG, azathioprine	Recover	No
71	F	NSCLC	Pembro×4	623	10.1	5.5	4.6	0.3	Negative		Dex	Recover	Pembro
73	M	NSCLC	Pembro×13	1615	11.0	7.4	3.6	NR	NR		Pred	Recover	No
78	M	NSCLC	Pembro×1	492	11.7	6.0	5.7	7.8	NR		Pred	Recover	NR
69	F	CRC	Pembro×1	716	11.7	9.4	2.3	4.3	NR		Pred	Recover	No
52	M	NSCLC	Nivo×24	383	13.4	5.9	7.5	18.9	IgG		MP	Expired	No
59	F	NSCLC	Nivo×12	505	11.9	6	5.9	16.8	IgG, C3		Pred, IVIG, Rituximab	Recover	No
63	F	NSCLC	Nivo×4	976	11.7	4	7.7	14.8	IgG		MP, Pred	Recover	No
60	M	NSCLC	Nivo×21	335	8-9	4.3	-	17	IgG		MP, Pred	Recover	Nivo
70	M	NSCLC	Nivo×2	330	10.5	5.5	5.0	NR	C3		Pred	Expired	No
78	M	NSCLC	Nivo×39	648	11–13	8.6	-	17.0	IgG		Pred, rituximab	Recover	No
75	F	Hodgkin's lymphoma	Nivo×2	409	12.3	6.4	5.9	NR	IgG		Pred	Recover	Nivo
82	M	Cutaneous SCC	Nivo×8	NR	NR	NR	-	NR	IgG, C3		Pred	Recover	NR
85	M	Melanoma	Nivo×5	682	≥10	6.4	-	7.9	IgG		Pred	Recover	No
33	F	AML	Nivo×1	791	9.3	7.2	2.1	4.9	Negative		MP, Pred	Recover	No

47	M	Melanoma	Ipi×1	522	9.5	6.2	3.3	3.2	NR	Dex	Recover	Ipi
68	F	Melanoma	Ipi×3	580	NR	6.0	-	0.2	NR	MP	Recover	NR
71	M	Melanoma	Ipi×4	NR	NR	NR	-	NR	IgG, C3	Pred	Recover	NR
18	M	Melanoma	Ipi+Nivo×4	784	12.4	6.2	6.2	<0.5	Negative	MP, Pred	Recover	Ipi+Nivo
43	F	Melanoma	(Ipi+Nivo)×2	1406	10	5.6	4.4	6.5	IgG, C3	MP, Rituximab	Recover	Ipi+Nivo
48	M	Melanoma	(Ipi+Nivo)×3	770	7.2	3.5	3.7	11.1	IgG, C3	MP, Pred	Recover	No
67	F	Melanoma	(Ipi+ Nivo)×4	426	13.2	8.7	4.5	8.8	IgG	Pred	Recover	Nivo
67	M	Melanoma	(Ipi+ Nivo)×2	1574	13.7	6.2	7.5	8.5	Negative	Dex, Pred	Recover	Ipi+Nivo

Characteristics	ICIs induced AIHA (n=30)
Sex, n (%)	
Female	13(43.3)
Male	17(56.7)
Age in years, average (range)	65.1(18-85)
Female	65.2(33-85)
Male	64.9(18-85)
Cancer type, n (%)	
Melanoma	17(56.7)
NSCLC	9(30)
Lymphoma	1(3.3)
Colorectal cancer	1(3.3)
Cutaneous squamous cell carcinoma	1(3.3)
Acute myelocytic leukemia	1(3.3)
Immunotherapy agent(s), n (%)	
Pembrolizumab	12(40)
Nivolumab	10(33.3)
Ipilimumab	3(10)
Ipilimumab+ Nivolumab	5(16.7)
ICIs cycles before onset	
Median (range)	4(1-39)
Average (range)	7.7(1-39)
AIHA Grade	
Grade 1	0
Grade 2	5(16.7)
Grade 3	14(46.7)
Grade 4	9(30.0)
Not reported	2(6.7)
DAT Mediator, n (%)	
IgG	10(33.3)
C3	1(3.3)
IgG, C3	8(26.7)
NR	6(20)
Negative	5(16.7)
Treatment, n (%)	
GSs (dexamethasone, prednisone, methylprednisolone)	30(100)
Intravenous immunoglobulin	3(10)
Rituximab	4(13.3)
Azathioprine	1(3.3)
Recovery from ICIs induced AIHA, n (%)	
Yes	28(93.3)
No	2(6.7)
Re-challenge with ICIs, n (%)	
Yes	10(33.3)

No	16(53.3)
NR	4(13.3)

Abbreviations: ICIs, immune checkpoint inhibitors; AIHA, autoimmune haemolytic anemia; DAT, direct antiglobulin test; GCs: glucocorticoids.

Case series

Case 1: Non-small cell lung cancer

A 62-year-old man (Table 3) presented to the department of oncology complaining with chest distress for half a year, occasional chest pain for half a month and vomiting after eating for a week. He also felt mental infirmity, loss of appetite and troubled sleep. During this past half year, he lost two kilograms. In the past fifty years, he suffered from a skin allergy very often but allergen was unknown. Seven years ago he was diagnosed with silicosis due to five years work history in coal mine. His smoking and alcohol history were both going back almost fifty years. His physical examinations were unremarkable except for a mild anemia appearance, while no hemorrhage was found. Blood routine showed a red blood cell count of 2.87×10^{12} /L with hemoglobin level of 87 g/ L. Iron, vitamin B12, and folate deficiency were ruled out. Positron emission tomography-computed tomography (PET-CT) revealed nodules or masses with increased glucose metabolism in both lungs, bilateral hilar and mediastinal lymph nodes, sacrum, left adrenal gland and pleura. After tissue biopsy, pathological diagnosis was made as squamous cell carcinoma of lung with PD-L1 expression over 50%, while molecular diagnosis did not found any valuable mutation. Thus, pembrolizumab was administered as first line treatment. However, one day later the patient's complete blood examination showed an astonishing decrease of RBCs to 1.34×10^{12} /L and hemoglobin to 50 g/L with normal leukocyte and platelet counts. Supplement of iron and vitamin B12 was not helpful. Furthermore, hemoglobin level lowered to 45 g/L with RBCs of 1.30×10^{12} /L in three days. The absolute reticulocyte count was 0.031×10^{12} /L and percentage of reticulocyte was 3.49%. Liver function test revealed an increase in lactate dehydrogenase (LDH, 289.7 U/L), total bilirubin (27.6 umol/L) and indirect bilirubin (13.4 umol/L). Besides, the patient complained with dark brown urine and an increased urobilinogen was found in his urine, while urine and fecal occult blood test were both negative. The DAT test was positive for IgG and C3 antibodies. Additionally, a positive antinuclear antibody was detected (granular pattern) at a titer of 1:80. Anti-U1-nRNP antibody, anti-Ro-52 (52kDa) antibody, anti-histone antibody were also found positive. Echocardiography showed slight pulmonary arterial hypertension with pulmonary arterial systolic pressure of 33mmHg, while bone marrow biopsy was normal and abdominal ultrasonography did not find hepatomegaly or splenomegaly. In accord with the evidences stated above, the patient was diagnosed as AIHA and immediately received intravenous 80mg/day methylprednisolone together with other supportive treatment like gastric protection. RBC transfusion was not performed because of increased risk of additional hemolysis. Luckily, he responded well with rising level of hemoglobin and RBCs. Subsequently, his intravenous steroid was gradually tapered, and replaced with oral steroid. Finally he weaned off oral steroid in a month when his hemoglobin completely recovered.

Case 2: Intrahepatic cholangiocarcinoma

A 63-year-old woman (Table 3) visited the hospital complaining with upper abdominal distension and occasional abdominal pain. CT scan revealed multiple nodules in the liver, peritoneal cavity and pelvic cavity. Tissue biopsies from liver nodule and peritoneal mass both indicated intrahepatic cholangiocarcinoma. After intraperitoneal perfusion chemotherapy of cisplatin and two cycles of intravenous chemotherapy of paclitaxel and carboplatin, the tumor progressed unexpectedly. Anti-angiogenic therapy of anlotinib could not control the disease either. Thus, anti PD-1 antibody camrelizumab (200mg) was applied. Four days after the second cycle, the patient complained with dizziness, fatigue, and soy-colored urine. Immediate blood routine test indicated severe anemia in which RBCs was 1.85×10^{12} /L and hemoglobin level was 51 g/L, while previous RBCs was 4.02×10^{12} /L and hemoglobin was 106 g/L before camrelizumab therapy. Suspended leukocyte-reduced RBCs transfusion was performed, however, the hemoglobin level decreased to 40 g/L instead. Urobilinogen became positive in the urine. Besides, chemical analysis revealed an increase in total bilirubin (40.7 umol/L) and indirect bilirubin (30.79 umol/L). Serum LDH also reached as high as 1395 IU/L. With highly suspicious of AIHA, the patient was started on daily intravenous methylprednisolone at 40mg although the Coombs test was negative. Two days later, even though the bilirubin and LDH half decreased, the anemia worsened with 37 g/L of hemoglobin and 1.01×10^{12} /L of RBCs. Urobilinogen also became strong positive. Considering the dosage of steroid may be insufficient, doctors increased the methylprednisolone dosage to 80 mg twice a day. Gradually, the patient's hemoglobin level improved, and methylprednisolone was tapered as well. In total the patient was treated with steroids for twenty days, and hemoglobin completely recovered in fifty days.

Case 3: Gastric adenocarcinoma

A 55-year-old woman (Table 3) presented to the emergency department complaining with dizziness, nausea and haematemesis, which also happened once seven days ago. Emergency blood test showed the RBCs count of 2.5×10^{12} /L with hemoglobin level of 78 g/ L. Fecal occult blood test was positive. The hepatic and renal functions including LDH were normal. Upper gastrointestinal hemorrhage was diagnosed by gastroscop and at the same time a huge elevated mucosa was found at the gastric corpus. Abdominal CT scan revealed local thickening of gastric wall and magnetic resonance imaging (MRI) discovered multiple nodules in the liver. A biopsy of the elevated mucosa led to a gastric adenocarcinoma diagnosis. After hemostatic therapy and blood transfusion, the patient's hemoglobin level increased to 97 g/ L. Concerned that the patient may not be able to tolerate chemotherapy, she accepted immunotherapy and was prescribed with 140mg nivolumab. The next two days, she suffered from low-grade fever, followed by soy-colored urine on the fourth day. Her hemoglobin level lowered to 85 g/L, while the LDH reached at 3288 IU/L, total bilirubin at 39.6 umol/L and indirect bilirubin at 31.2 umol/L. Urine occult blood test was strong positive but urine erythrocyte was negative. In addition, a positive antinuclear antibody in granular pattern was detected at a titer of 1:100. Therefore, the patient was clinically diagnosed with AIHA and accepted intravenous methylprednisolone 40mg per day. She responded well to steroid because her hemoglobin gradually increased and bilirubin decreased. Accordingly, steroid was tapered and changed to oral dexamethasone in a month. Afterwards, oral steroid was weaned off within two weeks when hemoglobin level completely recovered.

Table 3. Cases with ICIs induced AIHA in our report.

Age/ys	Gender	Diagnosis	ICIs	LDH/U/L	Hemoglobin /g/dL			Ret%	DAT Mediator	Treatment	Outcome
					Baseline	Nadir	Δ				
62	M	NSCLC	Pembrolizumab×1	289	8.7	5.0	3.7	3.49	IgG, C3	MP	Recover
63	F	Intrahepatic CHOL	Camrelizumab×2	1395	10.6	5.1	5.5	-	Negative	MP	Recover
55	F	Gastric adenocarcinoma	Nivolumab×1	3288	9.7	8.5	1.2	-	-	MP	Recover

Abbreviations: ys: years; ICIs, immune checkpoint inhibitors; AIHA, autoimmune haemolytic anemia; LDH: lactate dehydrogenase; Ret%: percentage of reticulocyte; DAT, direct antiglobulin test; F: female; M: male; NSCLC: non-small cell lung cancer; CHOL, cholangiocarcinoma; MP: methylprednisolone.

Discussion

Immunotherapy has become a popular therapy for cancer patients in recent years, among which ICIs have been successfully applied in multiple cancers, such as melanoma, Hodgkin's lymphoma, lung cancer, gastric cancer, etc. Although ICIs demonstrated a favorable safety profile compared to traditional chemotherapy,(Davis et al., 2019; Wang et al., 2019) ICIs induced adverse events should not be ignored.(Kennedy and Salama, 2020) As a systemic therapy, ICIs get involved in the whole immune system in addition to the local immune environment of tumor. Thus, ICIs related AEs could highly spread to the whole body or even be life-threatening with fatality as high as 15%.(Davis et al., 2019) AIHA is one of the most severe ICIs induced AEs but is rarely reported.(Delanoy et al., 2019; Kennedy and Salama, 2020; Wang et al., 2019) Beside of 68 cases from the VigiBase,(Davis et al., 2019) 68 cases from the Food and Drug Administration database,(Tanios et al., 2019) and 29 cases from other sporadic reports, we report three patients who developed AIHA after being subjected to treatment of anti-PD-1 antibodies. According to detailed analysis of thirty cases (Table 1, Table 2), and observation on three practical cases (Table 3), we found that ICIs induced AIHA mostly harasses the aged patients with melanoma or NSCLC. But it should be aware of the bias of higher usage rate in melanoma and NSCLC patients. Anti-PD-1 antibodies, particularly pembrolizumab and nivolumab, induce more AIHA than anti-PD-L1 antibodies or other ICIs. It may be explained by their molecular mechanism of interrupting wider signal pathways including PD-L1 and PD-L2.

AIHA is an autoimmune disorder in which RBCs are destroyed prematurely due to the abnormal production of antibodies directly against the patient's own RBCs.(Jaime-Perez et al., 2013) Yet the pathogenesis of ICIs induced AIHA remains unclear. It is known that PD-1 is also expressed on B-lymphocytes and plays a role in B cell regulation and development. Anti-PD-1 antibodies could affect the proliferation of autoreactive B cell clone and increase the production and secretion of autoantibodies, which lead to RBCs self-attack. On the other hand, it is likely to be related to the activation of T cells and diminishing of the regulatory T cells, which could cause direct damage of RBCs. It is reported that the number of Th17 cells and secretion of IL-17 increased in AIHA patients and changed along with the disease activity. Transferring the Th17 cells into a murine AIHA model could enhance the development of AIHA and blockade of IL-17 could abrogate AIHA in turn. In non-transferred mice, blockade of IL-17 could reduce the incidence of AIHA with less severity of AIHA activity.(Xu et al., 2012) Mqadmi, et al(Mqadmi et al., 2005) found that depletion of CD25⁺ regulatory T cells using anti-CD25 increased the incidence of AIHA in C57/Bl6 mice from 30% to 90%. The adoptive transfer of purified CD4⁺CD25⁺ regulatory T cells from immunized mice prevented the induction of AIHA. Furthermore, serum IL-33 was discovered elevated in patients with AIHA and positively correlated with AIHA disease activity. Enforced IL-33 promoted AIHA incidence and accelerated disease activity, while blockade of IL-33 interfered with AIHA incidence and ameliorated disease activity.(Bu et al., 2015) In our report (Table 3), three patients previously with anemia were treated with three different anti-PD-1 antibodies, which were pembrolizumab, camrelizumab, and nivolumab. Their anemia was all successively exacerbated and developed AIHA. Likewise, 43.3% patients in Table 1 had anemia before ICIs treatment. Thereupon, we suspect that cancer patients with anemia may be a special group either with high erythrocyte fragility, existing special antibody, or with activated immune system of high tendency to produce abnormal antibody. If pre-existing anemia is a risk factor of developing AIHA, the risk assessment has to be necessarily done prior to initiating ICIs treatment. Hemolysis related tests, such as direct and indirect Coombs tests, could be performed to exclude pretreatment hemolytic anemia. Besides, screening pre-existing autoimmune disorders is also important because autoimmune status undoubtedly relates to AIHA when AIHA is an autoimmune disorder.(Ogawa et al., 2018) As a result, it is possible to avoid a potentially life-threatening AE, or improve the level of pre-alarm and treatment ability of AIHA.

ICIs induced AIHA is not hard to diagnose with low hemoglobin and RBCs, reticulocytosis and/or anisocytosis, elevated LDH and indirect bilirubin level, positive Coomb's test and low serum haptoglobin.(Leaf et al., 2019) Nevertheless, the diagnosis is often delayed because the patient usually does not have typical symptoms until anemia becomes severe. The most common symptoms in AIHA are similar to general anemia such as fatigue, dizziness and breathlessness. In our experience, when the first patient complained with fever and dark brown urine, his hemoglobin level has been as low as 50 g/L. Similarly, the hemoglobin level in the second case was as low as 51 g/L when symptoms of dizziness, fatigue, and soy-colored urine were discovered. In the third case, the patient pre-existing hemorrhagic anemia confounded the physician's awareness and judgement of the type of anemia, even though she complained with fever and soy-colored urine. Glucocorticoids were not used until the fifth day after nivolumab. Fortunately her anemia was grade 2 and immediately controlled with initial 0.8mg/kg/d methylprednisolone. As ICIs are more and more widely used, ICIs induced hematologic AEs should be paid more attention in order to obtain timely treatment benefits. Mostly cases either from previous documents (Table 1, Table 2) or our observation (Table 3) showed positive DAT test. Hence, DAT test could be routinely administered for patients with anemia after ICIs treatment to early identify AIHA.

According to general clinical experience and consensus on AIHA so far,(Brahmer et al., 2018; Haanen et al., 2018; Jaime-Perez et al., 2013; Leaf et al., 2019; Tanios et al., 2019; Zanella and Barcellini, 2014) implementation of the appropriate treatment should depend on the severity of AIHA.(Brahmer et al., 2018) Glucocorticoids are the mainstay treatment for autoimmune side effects caused by immunotherapy,(Davis et al., 2019) which also fit for ICIs related AIHA.(Leaf et al., 2019; Tanios et al., 2019) For patients suffering from above grade 2 AIHA, corticosteroid is suggested as the first-line therapy beside of elimination of ICIs.(Brahmer et al., 2018) After initial remission, the dosage should be tapered down slowly with caution, while maintenance dose is frequently

required. If no sign of improvement or worsening symptoms while on corticosteroids, doctors should consider initiating conventional immunosuppressive drugs, such as IVIG, cyclosporin A, and mycophenolate mofetil.(Brahmer et al., 2018; Zanella and Barcellini, 2014) AIHA is presumed to be mediated via autoantibodies produced by B-lymphocytes and plasma cells.(Jaime-Perez et al., 2013) The intervention of drugs that are specific for the B-cell compartment may reduce the secretion of abnormal antibodies. B-cell specific blocking does not interrupt T-cell mediated anti-cancer immune function, so that to preserve the therapeutic effects of ICIs. Thus, B-cell depletion with rituximab is an alternative method for relapsed or refractory AIHA.(Reynaud et al., 2015) Otherwise, new anti-CD20 monoclonal antibody ofatumumab and anti-CD52 monoclonal antibody alemtuzumab are optional therapeutic approaches.(Zanella and Barcellini, 2014) RBCs transfusion is beneficial in increasing oxygen-carrying capacity provided by the transfused RBCs as a supportive treatment, however, it may increase the risk of further hemolysis and its application require extra cautiousness. In our second case, the patient's anemia did not improve after RBC transfusion, instead, her hemoglobin deteriorate to 40 g/ L. It is methylprednisolone that brought her hemoglobin back to normal gradually and saved her from severe anemia. Our other two cases, the same as the previously reported 28 cases shown in Table 1, responded well to glucocorticoids and recover from AIHA. Whereas two men(Palla et al., 2016; Tanios et al., 2019) were not successfully rescued by corticosteroids, instead they expired in a short time. For patients not benefiting from glucocorticoids, second-line therapy of other immune-suppressive or immuno- modulating drugs should be considered.

Recent guidelines suggest that patients with less than grade 3 AIHA should temporary discontinue ICIs and patients with equal to or worse than grade 3 AIHA should permanently discontinue ICIs.(Brahmer et al., 2018; Haanen et al., 2018; Thompson et al., 2019) Our three patients followed the guideline by not re-challenging ICIs and were administered different therapy. Nevertheless, ten(Algaze et al., 2018; Khan et al., 2017; Leaf et al., 2019; Tardy et al., 2017) out of thirty patients (Table 1) reused ICIs and did not subsequently report uncontrolled AIHA. The safety of re-challenging ICIs is uncertain based on mere experience from limited clinical cases. Further prospective trials are required to test whether or not it is worthy to take such big a risk.

Conclusion

ICIs induced AIHA requires more consciousness due to its severity and high fatality. Early recognition and prompt treatment are important in mitigating its severity and improving the recovery of AIHA. Previously reported cases and our three cases remind us that risk assessments, especially hemolysis related tests, are necessary and needed to be done prior to initiating ICIs treatment for patients previously with anemia. Because of the rarity of AIHA, guidelines for treatment, monitoring and re-challenging are all immature, which demands further prospective trials to optimize public understanding on AIHA.

Abbreviations

ICIs, immune checkpoint inhibitors; PD-1, programmed cell death 1; PD-L1, programmed death ligand-1; AEs, adverse events; irAEs, immune-related adverse events; AIHA, autoimmune haemolytic anemia; NSCLC: non-small cell lung cancer; CHOL, cholangiocarcinoma; RBC, red blood cell; LDH, lactate dehydrogenase; DAT, Coombs test, direct antiglobulin test; PET-CT, Positron Emission Tomography-Computed Tomography; CT, computed tomography; MRI, magnetic resonance imaging; IVIG: intravenous immunoglobulin.

Declarations

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Conflict of interest statement

The authors declare that there is no conflict of interest.

Consent

Written informed consent was obtained from the patients or their legally authorized representative for publication of patient related clinical data.

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