Case Report

Management of Mixed Warm/Cold Autoimmune Hemolytic Anemia: A Case Report and Review of Current Literature

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Background. Mixed warm/cold autoimmune hemolytic anemia (AIHA) is a rare diagnostic entity with limited therapeutic options. Previous literature has described the diagnostic difficulty in this pathology and the limited response rates to corticosteroids. Furthermore, there is limited evidence regarding the use of rituximab in this condition.

Methods. Alongside our case report, we conducted a scoping review of case reports/case series describing mixed AIHA, their treatment, and clinical outcomes since 2000. Inclusion criteria included a confirmed diagnosis of mixed AIHA (confirmed warm antibodies and cold agglutinins based on DAT).

Case Summary/Results. We present a case of mixed AIHA in an 83-year-old female presenting with extensive, bilateral pulmonary embolisms and left renal vein thrombosis. The patient underwent extensive workup with no identifiable provoking etiology. Initial treatment involved prednisone therapy was transitioned to rituximab upon diagnosis of mixed AIHA. The patient demonstrated a mixed response with stable hemoglobin and transfusion independence; however, with persistently elevated hemolytic indices following completion of rituximab treatment. Our literature review identified 16 articles; two were excluded for unavailable clinical details. The most commonly associated conditions included autoimmune conditions (n = 5, 26%) and lymphoproliferative disorders (n = 3, 12%). The most common treatment involved corticosteroids; seven studies involved the use of rituximab.

Conclusion. Mixed AIHA represents a complex diagnosis and optimal management is not well established. Consistent with our case, recent literature suggests a promising response to rituximab and a limited response to steroid treatment. Given the limited literature, additional studies are required to elucidate optimal management of this unique pathology.

1. Introduction

Mixed warm/cold autoimmune hemolytic anemia (AIHA) is a rare diagnostic entity with limited therapeutic options. Previous literature has described the diagnostic difficulty in this pathology and a limited response to steroid therapy. Furthermore, there is limited evidence regarding the use of rituximab in this condition. In this case report, we describe a case of severe mixed warm and cold autoimmune hemolytic anemia with a complicated diagnosis which was treated with both steroids and rituximab with a clinical response evidenced by stability in hemoglobin and improvement in hemolytic indices. Further, we conducted a review of recent literature involving case reports and series of mixed warm/cold AIHA and their clinical courses.

2. Research Aim

(i) To highlight the diagnostic difficulties within mixed warm/cold autoimmune hemolytic anemia and use of rituximab as a management strategy within this rare pathology

3. Case Report

Our case involved an 83-year-old female with a past medical history significant for provoked deep vein thrombosis (DVT) following an ankle fracture, hypertension, and osteoporosis endorsing a period of worsening fatigue and exertional dyspnea over the preceding several weeks. Upon admission, the patient was found to have elevated liver
enzymes as well as D-Dimer greater than 4000 μg/L (<500 μg/L) which prompted further workup for thrombembolic disease. Computed tomography angiography identified extensive bilateral pulmonary emboli and a large left renal vein thrombus. Doppler venous US of the legs identified a left-sided proximal DVT. In addition, the complete blood count (CBC) showed a polychromic and normocytic anemia with a hemoglobin of 59 g/L requiring red cell transfusion, leukocytes of 13.7 × 10^9/L, platelets of 277 × 10^9/L, and reticulocytes of 57 × 10^9/L (23–90 × 10^9/L). Further investigations demonstrated evidence of hemolysis including elevated lactate dehydrogenase (LDH) of 1347 U/L (<140 U/L), total and direct bilirubin of 37 μmol/L and 15 μmol/L, respectively (<20 μmol/L and <5 μmol/L), and decreased haptoglobin of less than 0.08 g/L (0.30–2.00 g/L). On the peripheral smear, there were marked spherocytes and mild Howell–Jolly bodies with significant agglutination.

Initial hemolysis workup included a Direct Antibody Test (DAT) which was invalid twice due to positive controls. Investigations revealed positive cold autoantibody at 4 degrees Celsius (°C) and negative ones at 37°C. Due to clinical suspicion of cold autoimmune hemolysis, the patient was subsequently started on folic acid supplementation, and cold avoidance was recommended. Secondary etiologies including infection were investigated including HIV, EBV, and hepatitis B serologies, all of which were negative as well as SARS-CoV-2 PCR. Malignancy workup including CT chest, abdomen, and pelvis did not reveal evidence of malignancy. A bone marrow biopsy was also performed, demonstrating erythropagocytosis without evidence of a lymphoproliferative disorder, and paroxysmal nocturnal hemoglobinuria markers were also sent and found to be negative.

Due to ongoing clinically significant hemolysis, the patient was started on a trial of prednisone at a dose of 1 mg/kg for four days. Unfortunately, the patient continued to have significant hemolysis demonstrated by ongoing transfusion requirements as well as elevated hemolytic markers, and additional investigations were undertaken. Due to ABO type discrepancies, samples were sent to our reference lab at Canadian Blood Services for further analysis. The patient’s red cells were found to be reactive by immediate spin with different monoclonal ABO antisera (anti-A, anti-B, and anti-A, B) due to the presence of a cold autoantibody. The determination of the cold antibody titre was unable to be performed and therefore remained unknown in this case. The interference was not eliminated by the washing patient’s red blood cell suspension three times with prewarmed saline and retesting. The valid ABO typing was obtained by using the prewarmed technique and incubation at 37°C for an hour. A valid DAT result was obtained from testing the patient’s dithiothreitol (DTT)-treated cells. The patient’s plasma was reactive with all papain-treated cells tested by the indirect antiglobulin testing including autocontrol. An acid eluate prepared from the patient’s red cells was reactive with all cells tested by the gel method indicating the presence of a warm autoantibody. Cold autoabsorption with rabbit erythrocytes stroma was also performed to remove the cold autoantibody, and the adsorbed plasma was still reactive, but the strength of the reaction was reduced by gel. A diagnosis of both cold and warm autoantibodies was confirmed, while all major clinically significant alloantibodies were excluded.

Based on these findings, prednisone was discontinued, and the patient was treated with rituximab 375 mg/m^2 for a total of four doses. The prednisone course was limited to four days due to the discovery of cold antibodies as well as ongoing significant anemia. The patient began demonstrating signs of response after 2 doses of rituximab evidenced by decreasing transfusion requirements, improved hemoglobin stability, and improvement in hemolytic indices. Follow-up at 5 months after rituximab completion demonstrated ongoing hemoglobin stability with evidence of compensated hemolysis (Table 1).

4. Discussion

Mixed warm/cold autoimmune hemolytic anemia is a relatively rare entity and current evidence regarding optimal management of these cases is limited. Furthermore, there is considerable variability between sources in diagnostic criteria which further complicates the diagnosis and management of this pathology [1].

Previous studies have found that mixed warm/cold AIHA account for 6.5–8.3% of AIHA cases and approximately 50% of which are idiopathic without an identifiable secondary etiology [2, 3]. Interestingly, an association between mixed AIHA and SLE has been described; however, this was not observed in our case [4, 5]. Mayer et al. investigated 2194 patients with detected warm autoantibodies and found only 2 patients, less than 0.1%, had features consistent with mixed warm/cold AIHA which makes the true prevalence of this pathology difficult to ascertain [1].

The use of steroids in the management of this condition has been previously described with a mixed response, and in several cases, patients required splenectomy due to ongoing hemolysis (Table 2) [1, 4–18]. We conducted a literature review of case reports published and available on PubMed since the year 2000 with confirmed mixed AIHA (confirmed warm antibodies and cold agglutinins based on DAT) and their clinical course (Table 2). Additional case reports were excluded for unavailable clinical details [19, 20]. Rituximab, a monoclonal CD20 antibody, is another treatment that has recently been employed as an efficacious therapeutic strategy amongst warm and cold AIHA [21–24]. Rituximab has been described for the management of mixed AIHA, and in a case report, a patient responded with resolution of hemolysis after two courses of rituximab following initial management with prednisone [8]. Our study similarly demonstrated an initial response following one course of rituximab with stabilization in hemoglobin, and will continue to be observed for long-term remission.

In summary, mixed warm/cold AIHA represents a complex diagnosis, and the optimal management of these cases has not been well elucidated. Case series report largely limited early response following steroid therapy, and recent case reports suggest a promising response to rituximab. However, given the limited literature surrounding this topic, additional investigations would be required to further elucidate the optimal management of this pathology.
<table>
<thead>
<tr>
<th>Value</th>
<th>At presentation</th>
<th>Preprednisone*</th>
<th>Postprednisone</th>
<th>Prerituximab**</th>
<th>Postrituximab</th>
<th>5 months postrituximab</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>59</td>
<td>44</td>
<td>74</td>
<td>69</td>
<td>95</td>
<td>99</td>
<td>4.0–11.0 × 10⁹/L</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>37</td>
<td>47</td>
<td>41</td>
<td>46</td>
<td>57</td>
<td>21</td>
<td>3–17 μmol/L</td>
</tr>
<tr>
<td>LDH</td>
<td>1347</td>
<td>1300</td>
<td>1467</td>
<td>1489</td>
<td>1584</td>
<td>692</td>
<td>84–246 U/L</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt;0.08</td>
<td>&lt;0.08</td>
<td>Not tested</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.30–2.00 g/L</td>
</tr>
</tbody>
</table>

*Prednisone 1 mg/kg for four doses. **Rituximab 375 mg/m² weekly for four doses.
Table 2: Case reports of mixed autoimmune hemolytic anemia and their clinical course.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of publication [ref]</th>
<th>Number of patients</th>
<th>Associated condition</th>
<th>Treatment employed</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer et al</td>
<td>2008 [1]</td>
<td>2</td>
<td>SLE (2)</td>
<td>(1) No treatment (2) Prednisone (30 mg/day) and azathioprine (100 mg/day)</td>
<td>(1) Did not require treatment (2) Response after uncertain duration</td>
</tr>
<tr>
<td>Wondergem et al</td>
<td>2006 [4]</td>
<td>1</td>
<td>SLE</td>
<td>Prednisone (1 mg/kg/day uncertain duration), IVIG</td>
<td>Persistent hemolysis despite prednisone, response to IVIG and subsequent tapering of steroids with resolution of hemolysis</td>
</tr>
<tr>
<td>Sudha Reddy et al</td>
<td>2011 [5]</td>
<td>1</td>
<td>Unknown</td>
<td>Prednisolone (2 mg/kg/day for 4 weeks), tapered to alternating day steroids</td>
<td>Response, tapered to corticosteroids on alternating days</td>
</tr>
<tr>
<td>Tanaka et al</td>
<td>2006 [6]</td>
<td>1</td>
<td>Following chicken pox infection</td>
<td>Methylprednisone (1000 mg/day uncertain duration), prednisolone (60 mg/day for 4 weeks tapered to 10 mg/daily)</td>
<td>Initial response to methylprednisolone, worsening thrombocytopenia following taper treated with reintroduction of methylprednisolone and IVIG (400 mg/day for 5 days) with stabilization, recurrent hemolysis requiring reintroduction of prednisone</td>
</tr>
<tr>
<td>Win et al</td>
<td>2007 [7]</td>
<td>2</td>
<td>(1) Unknown (2) Splenic T-cell angioimmunoblastic non-Hodgkin's lymphoma</td>
<td>(1) Prednisone (40 mg/day uncertain duration) and IVIG (0.4 mg/kg for 5 days) (2) prednisone (60 mg/kg, increased to 100 mg/kg uncertain duration) and IVIG (2 mg/kg for 2 days); VCP (cyclophosphamide 750 mg/m², vincristine 2 mg and prednisone 50 mg uncertain number of cycles); rituximab (325 mg/m² for 2 weeks); splenectomy</td>
<td>(1) Response with stabilization in hemoglobin (2) No response to steroids/IVIG, chemotherapy or rituximab; response to splenectomy with stabilization in hemoglobin</td>
</tr>
<tr>
<td>Morselli et al</td>
<td>2002 [8]</td>
<td>1</td>
<td>Unknown</td>
<td>Prednisone (1 mg/kg/day) with taper; over three weeks to 25 mg/daily, rituximab (325 mg/m² for 2 weekly courses)</td>
<td>Response to initial steroid treatment with following taper, recurrence in hemolysis with response and stabilization in hemoglobin with 2 cycles of rituximab</td>
</tr>
<tr>
<td>Qiao et al</td>
<td>2016 [9]</td>
<td>1</td>
<td>Primary Sjören syndrome</td>
<td>Methylprednisone (40 mg/kg/day uncertain duration) transitioned to pulse methylprednisone (1000 mg/day for 3 days) followed by prednisone 30 mg/day (unknown duration), patient received G-CSF and cyclophosphamide 0.2 mg every other day to treat concomitant agranulocytosis</td>
<td>Complete response with resolution of hemolysis</td>
</tr>
<tr>
<td>Imataki et al</td>
<td>2020 [10]</td>
<td>1</td>
<td>Idiopathic, prior autologous stem cell transplant for DLBCL</td>
<td>Prednisolone (1 mg/kg for unknown duration)</td>
<td>Progressive hemolysis without response</td>
</tr>
<tr>
<td>Hirano et al</td>
<td>2016 [11]</td>
<td>1</td>
<td>SLE</td>
<td>Prednisolone (2 mg/kg for 2 months and subsequent taper), 2 courses of methylprednisolone pulse (unknown dosage/duration) MMF (1000 mg/day tapered 500 mg/day for unknown duration)</td>
<td>Response with stabilization in hemoglobin</td>
</tr>
<tr>
<td>Study</td>
<td>Year of publication [ref]</td>
<td>Number of patients</td>
<td>Associated condition</td>
<td>Treatment employed</td>
<td>Response</td>
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</tr>
<tr>
<td>Scaramucci et al</td>
<td>2005 [12]</td>
<td>1</td>
<td>Idiopathic</td>
<td>Prednisone (1 mg/kg/day unknown duration with taper), rituximab (375 mg/m² four weekly courses)</td>
<td>Response with prednisone with relapse following taper, complete response to rituximab</td>
</tr>
<tr>
<td>Haller et al</td>
<td>2009 [14]</td>
<td>1</td>
<td>Post liver transplant without evidence of alloantibodies, EBV and CMV positive</td>
<td>IV methylprednisone (1 mg/kg QID for 7 days) transitioned to prednisolone (1 mg/kg daily), rituximab (325 mg/m² for 4 weekly courses), IVIG (0.5 g/kg, dose for 3 weekly doses), prednisolone tapered to 2.5 mg daily over 6 months and subsequently continued as GVHD prophylaxis</td>
<td>No response to initial methylprednisone, response to rituximab with stabilization in hemoglobin and resolution of transfusion requirements</td>
</tr>
<tr>
<td>Zhang et al</td>
<td>2012 [15]</td>
<td>1</td>
<td>Hodgkin lymphoma</td>
<td>IV methylprednisone (unclear dose and duration), 1 cycle of rituximab (unclear dose)</td>
<td>No response to initial methylprednisone, response to rituximab with hemoglobin stabilization</td>
</tr>
<tr>
<td>Rai et al</td>
<td>2017 [16]</td>
<td>2</td>
<td>Idiopathic</td>
<td>Both cases received corticosteroids (unknown medication, dose and duration)</td>
<td>Both had response to corticosteroid therapy with improvement in hemolysis however long term follow up unknown</td>
</tr>
<tr>
<td>Gupta et al</td>
<td>2011 [17]</td>
<td>1</td>
<td>Idiopathic</td>
<td>IV methylprednisone (unknown dose and duration), plasmapheresis for 7 daily doses, rituximab (375 mg/m² for 4 weekly doses)</td>
<td>No response to initial corticosteroid or plasmapheresis, stabilization in hemoglobin following initiation of rituximab and transfusion independence</td>
</tr>
<tr>
<td>Webster et al</td>
<td>2004 [18]</td>
<td>1</td>
<td>Idiopathic</td>
<td>Cyclophosphamide 50 mg daily and prednisone 10 mg alternating days for 1 month; rituximab 700 mg IV for 4 weekly doses</td>
<td>Discontinued cyclophosphamide/prednisone due to side effects and progressive hemolysis, response to rituximab with resolution of transfusion requirements and hemoglobin stabilization</td>
</tr>
</tbody>
</table>

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

The manuscript was already published as an Abstract at the Canadian Transfusion Medicine Society conference [25].

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References