Immunohematology Case Studies 2019 – IgM Warm Autoimune Haemolytic anemia

Mirela Raos
Head of Transfusion Medicine Division
Clinical Department of Transfusion Medicine and Transplantation Biology
University Clinical Hospital Zagreb, Croatia
mraos@kbc-zagreb.hr

Clinical History

Medical history

- 57-year-old Caucasian male admitted to our hospital because of severe anemia (Hb=52 g/l)
- Diagnosed with chronic lymphocytic leukemia, (CLL) classified as Rai stage 4 and Binet stage C, failed to respond to fludarabine + cyclophosphamide + rituximab (FCR), was receiving bendamustine + rituximab and is recently included in a clinical study
- Two red cell units are immediately ordered

Clinical History

Transfusion history

- The day before he was admitted to the hospital abroad, where he was transfused with a total of two red cell units (Hb=60 g/l and Hb=51 g/l)
- Previous transfusion history is unknown

Serologic History

Results from the hospital abroad the day before

The following serological information was provided:

ABO/Rh: AB D+

Indirect Antiglobulin Test (IAT): positive

Direct Antiglobulin Test (DAT): positive

Crossmatch in IAT: positive

Current Sample Presentation Data

ABO/Rh: AB, R_1r (D+C+c+E-e+), K neg, Jk (a-b+)

DAT: positive (IgG, C3d, IgM)

Antibody Screen Method: Indirect Antiglobulin Test (IAT) using Column Agglutination Technology (CAT) polyspecific (Biovue, Ortho Clinical Diagnostics)

Antibody Screen Results: Positive

Antibody Identification Method: IAT using CAT-Polyspecific and Neutral (Biovue, Ortho Clinical Diagnostics)

Antibody Identification Preliminary Results: all cells positive in IAT with untreated (3+) and enzyme-treated cells (4+), the autocontrol is positive

Antibody identification panel

	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IAT	Enz
1	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	3+	4+
2	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	3+	4+
3	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	3+	4+
4	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	3+	4+
5	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	3+	4+
6	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	0	3+	4+
7	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	3+	4+
8	0	0	+	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	3+	4+
9	0	0	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	3+	4+
10	0	0	+	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	3+	4+
11	0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	3+	4+
AC																				3+	NT

AC (autocontrol): positive
NT=Not Tested

Direct antiglobulin test

	Initial results	With washed RBCs
IgG	3+	2+
IgA	1+	0
IgM	3+	3+
C3c	1+	0
C3d	3+	3+
Control	1+	0

Agglutination of the patient's RBCs by autoantibodies was observed.
Reaction with the control reagent was positive.
Therefore, the patient's RBC were washed 6 times with 37°C saline.

Challenge with the Current Presentation

- There is an antibody reactive with all panel cells and the autocontrol is positive
- Autoagglutination is observed in the patient's anticoagulated tube of blood
- DAT is positive with IgG, C3d and IgM, but prior to testing the patient's RBCs were washed 6 times in 37°C saline, because a positive reaction with control reagent was obtained at the initial testing
- An initial review of the results would suggest that a cold IgM autoantibody, or more likely, a cold IgM mixed with warm IgG autoantibodies are coating the patient's RBCs and are present in the patient's plasma

Interim Antibody Identification
Possible Answers and Next Steps

- Reactivity appears to be a cold IgM autoantibody or a cold IgM mixed with a warm IgG autoantibody, because autoagglutination is observed, the strength and consistency of reactivity with all cells in the panel was shown (and the reactions with enzyme-treated cells were enhanced), autocontrol and DAT (IgG, IgM, C3d) were positive
- Further testing, particularly elution, titration and thermal amplitude analysis, is needed for a conclusion
- A warm IgM autoantibody is possible, but very rare

Further Work

Elution

- Eluates were prepared from the patient's RBCs using an acid elution kit (DiaCidel, Bio-Rad) and 56°C heat elution
- Neither of the eluates directly agglutinated the test RBCs, but they both reacted in the antiglobulin test

Further Work

Titration and thermal amplitude analysis

- Master dilutions of serum samples were prepared for titration and thermal amplitude analysis at 4°C, 20°C, 30°C and 37°C
- Separate sets of test tubes containing the master dilutions of serum were used for testing at each of the four temperatures (4°C, 20°C, 30°C, 37°C) to avoid carryover of agglutination from one temperature to the next
- Serum samples were tested with untreated and papain-treated RBCs
- RBCs that were tested were a pool of adult RBCs, a pool of cord RBCs and autologous RBCs
- All testing was performed in test tubes without presence of LISS additive

Further Work

- Thermal amplitude analysis showed that the autoantibody optimally reacted at 37°C and 30°C with untreated and papain-treated RBCs (see table)
- The titer was 2 at 4°C in the tube test

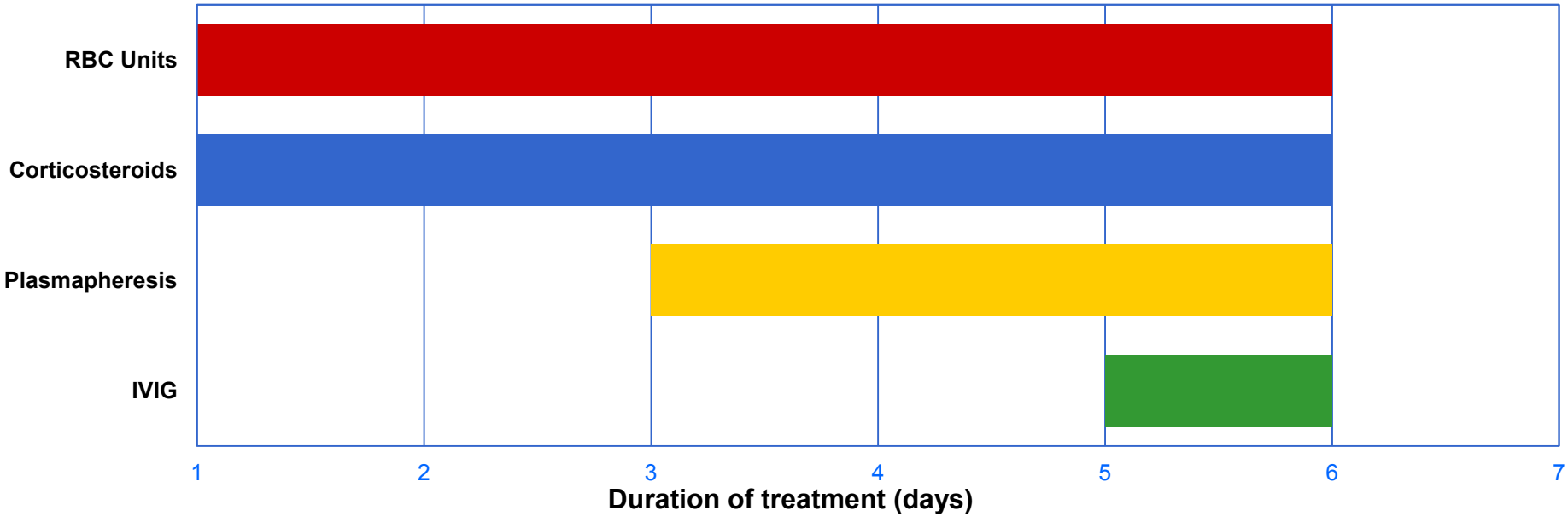
RED BLOOD CELLS TESTED		4 °C	20 °C	30 °C	37 °C
Untreated RBCs	Pool of adult RBCs	1+	1+	1+s	2+
	Pool of cord RBCs	1+	1+	1+s	2+
	Autologous RBCs	neg	neg	w	1+
Papain-treated RBCs	Pool of adult RBCs	1+	2+	3+	4+
	Pool of cord RBCs	1+	2+	3+	4+
	Autologous RBCs	neg	neg	1+	2+

Further Testing Results and Interpretations

- Serum samples with acidification, after addition of a fresh normal serum as a source of complement, and serum samples treated with 0.01 M dithiothreitol (DTT) were also tested with untreated RBCs in the tube test
- Agglutinin also reacted after serum acidification and the addition of fresh normal serum, but the latter did not enhance reactions
- No reactivity was observed after the serum was treated with DTT pointing out that in the patient's serum only IgM antibodies were present

Updated Clinical Information

- The patient was treated with red cell units on a daily basis (in total 9 units were transfused), steroids (Solu-Medrol 2x125 mg iv), plasmapheresis (3 cycles) and intravenous immunoglobulins (0.5 g/kg), but failed to respond and died due to anemia complications after 6 days (see table)



Updated Clinical Information

- Laboratory parameters during hospitalization are shown in the following table

Parameters/ Days of hospitalization	1ST DAY	2ND DAY	3TH DAY	4TH DAY	5TH DAY	6TH DAY
Hb (g/L)	52	52	44	34	40	35
Rtc ($x10^9$/L)	-	-	8	14.9	5	-
PC ($x10^9$/L)	97	100	118	149	152	159
WBC ($x10^9$/L)	32.7	31.1	29.0	29.0	19.3	12.4
T-Bil (µmol/L)	31	33	30	35	53	43
LDH (U/L)	563	561	497	293	302	226

Differentiating IgM warm AIHA from cold agglutinin syndrome and “mixed” type AIHA

	IgM WAIHA	CAS	Mixed type AIHA
DAT	C3 + IgM (+IgG)	C3	IgG+C3, C3
Ig class	IgM, IgG	IgM	IgG, IgM
Eluate	IgM (+IgG)	Non reactive	IgG
Serum	IgM 30-37°C, Titer at 4°C <64	60% titer ≥1000 at 4°C, IgM 4-37°C	IgG IAT + IgM 30-37°C

Conclusions

- A warm reacting IgM autoantibody was identified in a patient serum sample
- Anti-IgG, -IgM and -C3d were detected on the patient's RBCs
- A warm reacting IgM autoantibody was causing severe warm AIHA (WAIHA)
- The patient failed to respond to steroids, plasmapheresis and intravenous immunoglobulins and rapidly died due to complications from anemia

Summary of Case Challenges

- Autoagglutination was noted in a tube of the patient's anticoagulated blood
- DAT was positive due to anti-IgG, -IgM and -C3d
- Neither of the eluates obtained from acid and heat elution directly agglutinated RBCs, but they both reacted in the antiglobulin test
- The autoantibody optimally reacted at 37°C and 30°C
- Enzyme-treated RBCs enhanced reactions
- The 4°C titer was low (only 2)
- The serum sample treated with DTT showed only IgM antibodies

Lessons Learned in the Case

Warm AIHA associated with IgM warm autoantibodies is very difficult to diagnose. It can be confused with cold agglutinin syndrome, caused by IgM cold autoantibodies reactive up to 30°C, or with “mixed” type AIHA, characterized by both IgG warm autoantibodies and IgM cold autoantibodies reactive up to 30°C to 37°C.

Autoagglutination of the patient's RBCs can hint at the possible presence of an IgM warm autoantibody. Knowing the serologic features of IgM warm autoantibodies, in particular a serum agglutinin optimally reactive or only reactive at 37°C, is important in confirming the diagnosis of IgM WAIHA.

Lessons Learned in the Case

The mechanism of hemolysis in IgM WAIHA is a severe complement-mediated intravascular hemolysis.

There is no special treatment for IgM WAIHA and patients are treated the same way as for other WAIHAs. However, in refractory patients complement inhibition may be an effective therapeutic modality and eculizumab may benefit in warm IgM AIHA as a short-term therapy to improve refractory hemolysis. In addition, rituximab may be an effective long-term therapy for warm IgM AIHA.

Since hemolysis may be life-threatening and difficult to treat, precise serologic diagnostics is of paramount importance for determining prognosis.

Lessons Learned by the Case

Serologic characteristics of IgM WAIHA from this case
Agglutination of the patient's RBCs by autoantibodies is present
RBCs are coated with C3 and IgM (and also IgG)
The serum agglutinin reacts optimally at 37°C and 30°C
The 4°C titer is low
Enzyme-treated RBCs enhances reactions

References

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