



Immunohematology Case Studies 2016 - 01

Sofia Lejon Crottet, PhD
Swiss National Immunohematology
Reference Laboratory
Interregional Blood Transfusion SRC Ltd.
sofia.lejon.crottet@itransfusion.ch

Clinical History



- 74 year old male with multiple myeloma undergoing a treatment with Daratumumab (DARA)
- Admitted to the hospital with anemia, three units of blood needed

Immunohematology Laboratory History



- In December of 2015
 - Negative antibody screen
 - DAT negative
 - Extended genotype (KEL, JK, FY, MNS, CO, YT, LU, DO)

Current Sample Presentation Data



ABO/Rh: O, D+ C+ c+ E- e+

DAT: negative

Antibody Screen Method: IAT in Gel Antiglobulin (ID Gel AHG) card with six test cells in LISS, four untreated and two cells treated with papain (Bio-Rad) were used simultaneously

Antibody Screen Results:

all four untreated test cells were weakly reactive, while the papain cells showed a very weak reactivity

Antibody Identification Method:

IAT in ID Gel AHG card with eight untreated test cells (in-house) in LISS (ID/IAT) and IAT in natural card with papain treated cells (in-house) in LISS (papain/IAT)

Antibody Identification Preliminary Results:

all cells reacted weakly positive in ID/IAT, whereas only three out of eight papain treated cells reacted very weakly with the patient plasma

Question



- 1) What could be the cause of the panreactivity seen in ID/IAT?

Challenge with the Current Presentation



The clue to this case is the treatment with DARA. DARA is an IgG1k human monoclonal antibody targeting the cell-surface protein CD38. It is in clinical development for the treatment of multiple myeloma (MM). MM cells have a high expression of CD38 making it an interesting therapeutic target. However, CD38 is also expressed at low levels on red blood cells (RBC). The anti-CD38 (DARA) found in the patients plasma/serum is what is causing the panreactivity as it binds to CD38 on the test cells.

In-house Panel Results



Nr.	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	ID/IAT	papain
1	+	+	0	0	+	0	0	+	0	w	0	+	+	0	+	+	+	+	0	w	0
2	+	0	+	+	0	0	+	+	+	0	+	0	0	+	+	+	+	+	+	w	0
3	+	+	0	0	+	+	0	+	0	+	0	+	0	w	+	0	+	0	+	w	0
4	0	0	+	0	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	w	-/+
5	0	+	+	0	+	0	0	+	+	0	+	+	0	+	+	0	+	0	+	w	0
6	0	0	+	+	+	0	0	+	0	+	+	0	0	+	0	+	+	+	0	w	0
7	0	0	+	0	+	0	0	+	+	+	+	0	0	+	+	+	+	+	+	w	-/+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	0	+	w	-/+

Interim Antibody Identification Possible Answers and Next Steps



Due to the presence of CD38 on the test cells DARA will interfere with the antibody screening, antibody identification as well as the crossmatch. DARA bound to the test cells will thus mask detection of potential allo-antibodies to erythroid antigens.

DARA will however not interfere with the standard identification of ABO/RhD antigens. This is not true for the RhD testing at antiglobulin phase.

Question



2) What would be the next step?

Further Work



In order to eliminate the interference of anti-CD38 test cells can be treated with trypsin or dithiothreitol (DTT).

Reduction or elimination of reactivity due to anti-CD38 is achieved by enzymatic digestion by trypsin or disulfide bond reduction by DTT, with trypsin treatment less efficient than DTT treatment.

Further Testing Results and Interpretations



Nr.	D	C	c	E	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	trypsin	DTT
1	+	+	0	0	+	0	0	+	0	w	0	+	+	0	+	+	+	+	0	0	0
2	+	0	+	+	0	0	+	+	+	0	+	0	0	+	+	+	+	+	+	0	0
3	+	+	0	0	+	+	0	+	0	+	0	+	0	w	+	0	+	0	+	0	0
4	0	0	+	0	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	0	0
5	0	+	+	0	+	0	0	+	+	0	+	+	0	+	+	0	+	0	+	0	0
6	0	0	+	+	+	0	0	+	0	+	+	0	0	+	0	+	+	+	0	0	0
7	0	0	+	0	+	0	0	+	+	+	+	0	0	+	+	+	+	+	+	0	0
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	0	+	0	0

Question



- 3) Which blood group antigens are completely or partially destroyed by trypsin and/or DTT?

Further Testing Results and Interpretations



Trypsin, which is an endopeptidase belonging to the family of serine proteases, cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by a proline.

It is important to remember that blood group antigens Ch, Rg, Yk^a, Kn^a, McC^a, and partially JMH, and antigens in the GE, LU, MN blood group systems are destroyed by trypsin.

Further Testing Results and Interpretations



DTT, which is a reducing agent, acts on CD38 by disrupting the extracellular disulfide bonds.

DTT also destroys blood group antigens, such as Raph and JMH, and antigens in the KEL, KN, LW, LU, YT, DO and IN blood group systems.

Papain, which is a cysteine protease enzyme, will also have a proteolytic activity on CD38.

Antigen typed cord cells have been reported to be useful in ruling out antibodies to high prevalence antigens

Question



- 4) What could be done in order to reduce the risk of an alloimmunization?

Genotyping Results



Since certain blood group antigens are destroyed when the test cells are treated with trypsin or DTT, it is recommended to pheno- or genotype the patient (if possible before treatment with DARA). In that way, the patients can be transfused with phenosimilar blood units without time delay and the risk of alloimmunization is reduced.

Summary and Conclusions



- DARA interferes with the antibody screening, causing an panreactivity that can mask clinically significant allo-antibodies
- The interference can be eliminated by treating the test cells with trypsin or DTT
- Detection of clinically significant antibodies like anti-K and anti-Yt^a is not feasible when using DTT treated test cells
- Transfusion of ABO/RhD and K compatible blood units is recommended
- Knowledge of patient red cell antigen types that are destroyed by DTT or trypsin may also be important in future transfusion therapy

Lessons Learned by the Case



The information that the patient is undergoing a Daratumumab therapy is essential for a correct and rapid pretransfusion workup.

An antibody screen and an extended genotype before the first Daratumumab distribution will contribute to a blood product distribution without time delay.

References



1. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55(6Pt2):1545-1554.
2. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion*. 2015;55(6Pt2):1555-62.