Immunohematology Case Studies
2016 - 5

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Clinical History

A 78 yrs old patient had coronary artery bypass surgery.
In the perioperative period, she received 14 red cell units (A Rh pos) within 1 week.
Antibody screen remained negative throughout.

Three weeks later, she had severe pneumonia and was on artificial ventilation.
An antibody screen was requested when the patient had hemoglobin 8 g/dl.
This antibody screen was positive.
Serologic History

Initial presentation:

Blood type A
Rh: D+ C+c+E-e+ (CcDee, R1r)
Kell: K-
Antibody screen (3 cells): negative (IAT + neutral)

All transfused units were A CcDee K-
Current Sample Presentation Data

ABO/Rh: A Rh pos
DAT: negative
Antibody Screen Method: IAT (BioRad ID gel)
Antibody Screen Results: positive (0.5 to 1+)
Antibody Identification Method: IAT (BioRad ID gel)
Antibody Identification Preliminary Results:
Almost all cells weak positive, autocontrol negative
Challenge with the Current Presentation

Based on the initial panel this case is of category “autocontrol negative / almost all cells positive”

This constellation suggests that transfused red blood cells might not be compatible and requires further, often laborious work-up.
Panel

All cells positive independent of the antigen constellation!
Challenge with the Current Presentation

The most frequent causes for the “autocontrol negative / almost all cells positive” pattern are:

- An antibody to an antigen of high prevalence
- A combination of antibodies to “the usual” antigens
- An antibody interfering with the commercial cell preparation
- An autoantibody with a suppressed autoantigen

→ The laboratory did some additional work
In another panel, there were some weak to negative cells.
There was no obvious correlation with the antigen pattern.

Note: antigen typing was considered confounded by recent massive transfusion.

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Influence of ABO type and test conditions

There was no correlation with ABO type

Agglutination at 20° C and 4° C with 4 test cells type A1 and 4 test cells type O (“H“)

Autocontrol („EK“) and reactivity with 3 cell antibody screen in the following techniques:
Neutral (NaCl), Liss, 4° C saline, standard ID, PEG and Albumine

In the cold, there was non-specific reactivity.
Albumin-IAT was positive
Panel

A third panel was done but did not give much additional information
Challenge with the Current Presentation

Based on the three panels, the following explanations are unlikely:

- An antibody to an antigen of “really” high prevalence, like Vel, Lu\textsuperscript{b}, Kp\textsuperscript{b}, Yt\textsuperscript{a}
  - For these antigens, all cells would be expected to be positive
  - In addition, there was no correlation Lu\textsuperscript{a} and Kp\textsuperscript{a} status
- A combination of antibodies to “the usual” antigens
  - In that case, a pattern would be expected
Crossmatches with ABO / RH compatible units (all A R1r) were mostly positive:

Only 2 of 40 units were negative

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<tr>
<th>Konserven-Nr</th>
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<th>Kell</th>
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Crossmatch

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Crossmatches with ABO / RH compatible units (all A R1r) were mostly positive:

Only 2 of 40 units were negative

The positive crossmatch with ABO-identical cells is an argument against
- antibodies to commercial test cells
- antibodies to blood O cells (e.g. anti-IH)
The two compatible units differed in their antigen pattern
- Another argument against a combination of antibodies to "conventional" antigens

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Interim Antibody Identification
Possible Answers and Next Steps

The laboratory suspected an antibody to an antigen of higher to high frequency.

Therefore, two additional tests were done:
(1) Cross-match with rare cells
(2) Neutralization with plasma
Crossmatch with rare cells

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All available rare cells showed a positive cross-match.
## Crossmatch with rare cells

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All available rare cells showed a positive cross-match

The ability to identify an antibody to an antigen of higher frequency heavily depends on the “rare“ cells available in a laboratory.

In the case shown, only Y₄⁺, Y₅⁺, K₅⁻, K₁⁻ and L₅⁻ were tested. Y₅⁺ and K₅⁻ have a frequency of 98% resp. 92% and would have fitted the “all positive with some negative“ pattern.
Neutralization with plasma

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Neutralization with plasma

Some blood group antibodies are directed to proteins that are abundantly present in plasma. The by far most important example is the CH/RG blood group system:

Ch antigens are on complement C4B,
Rg antigens are on complement C4A

These antibodies are inhibited by plasma
Although both specificities would fit the pattern, no inhibition was observed
Further Work

The case was referred to our laboratory
Further Work

The case was referred to our laboratory

Which tests should be repeated?
Is there any test lacking that we would have done?
Did the colleagues miss some special condition?
Further Work

Which tests should be repeated?
Decision 1: Let`s do our basal diagnostics, because we know how to interpret these tests

Tests were done in BioVue technique using AutoVue Innova:
[ABD Conf - ABD confirmation; 8BVSF Poly - 3 cell antibody screen in IAT; Auto Poly - autocontrol in IAT; FicABScr - 3 cell antibody screen in IAT (left) and with ficin-treated cells on neutral column (right)]
Further Work

Which tests should be repeated?
Decision 1: Let`s do our basal diagnostics, because we know how to interpret these tests

Legend: Tests were done in IAT (pAHG), papain-IAT (PAP) and Neutral 4°C; results were independently scored by two persons (“1. Ableser” and “2. Ableser”)
Further Work

Decision 1: Let`s do our basal diagnostics, because we know how to interpret these tests

→ Confirmed:
  The antibody reacts with almost all cells

→ New:
  The antibody does not react in direct agglutination in the cold

→ New:
  The antibody did not react
  - with papain-treated cells in IAT
  - with ficin-treated cells in direct test
Further Work

Is there any test lacking that we would have done? Our scheme for antibodies to high frequency antigens includes the following additional tests:

- Typing by molecular and serologic methods for "usual" antigens
- Neutralization with recombinant proteins
- Use of a commercial "special cell" panel
For molecular typing, we used a „exploratory“ in-house single SNP method. This method suggested that the patient could form an anti-Jk\textsuperscript{b} and anti-S. The serologic typing was in concordance, showing only weak reactivity with anti-Jk\textsuperscript{b} and anti-S (a remnant of the massive transfusion)

In addition, molecular typing suggested that the patient was likely positive for Vel, Lu\textsuperscript{b}, Yt\textsuperscript{a} and Kp\textsuperscript{b}

As the pattern was not suggestive of a mixture of antibodies, no further genotyping was instituted
Neutralization with recombinant proteins

We have some recombinant proteins available for neutralization. The set-up is similar to neutralization with plasma: Patient plasma is first incubated with recombinant protein (or saline control), the mixture is than used for a normal IAT.

For the test, we use cells compatible for DCcEe, K, Jk^a/Jk^b, Fy^a/Fy^b, Ss in order to prevent masking by these antibodies. All tests remained reactive, hence no antibody specificity was identified.
As part of our work-up, we use a special cell panel that includes many cells with rare phenotypes. Two cells were negative:

- one cell marked as Lu:14+ Kn(a+/vw)
  - Anti-Lu8??? (should have been inhibited by LU-protein)
  - Anti-Kn??? (should have been inhibited by KN-protein)
- one cell marked as Rg-
  - Anti-Rg??? (should have been inhibited by C4A and by plasma)
Further Testing Results and Interpretations

What did we miss?
Further Testing Results and Interpretations

What did we miss?

→ The “many positive, some negative pattern” is extremely suspicious for an antibody in the CH/RG or KN blood group system

→ Anti-Kn\textsuperscript{a} (and anti-Lu8) are expected to be reactive in papain-IAT

→ Anti-Rg would fit the pattern perfectly, but should be neutralized by plasma or C4A
Further Testing Results and Interpretations

What did we miss?

→ Anti-Rg would fit the pattern perfectly, but should be neutralized by plasma or C4A

→ Anti-Rg often shows a HTLA pattern ("high titer low avidity")

→ Apparently, a titer was lacking in the work-up.
Further Testing Results and Interpretations

What did we miss?

Antibody titer was 1:1024

Was it “too much antibody” for our neutralization reagents?

Neutralization was repeated with diluted plasma
Further Testing Results and Interpretations

What did we miss?

1:2  1:4  1:8  1:16  1:32  1:64

The typical “high titer low avidity” pattern is seen:
Reaction at 1:2 is only faint (even in gel technique).
The reactions remain unchanged up to a 1:64 dilution
Conclusions

What did we miss?

→ Neutralization was repeated with diluted plasma (1:128)
→ C4A specifically neutralized the diluted antibody
→ The anti-Rg hypothesis was correct!
Conclusions:

Neutralization of diluted plasma

What did we miss?

→ Neutralization was repeated with diluted plasma (1:128)
→ C4A specifically neutralized the diluted antibody
→ The anti-Rg hypothesis was correct!
Conclusions: Neutralization of diluted plasma

What did we miss?

→ Plasma did also work with the diluted patient plasma
Anti-Rg is a “generic” name for antibodies to complement C4B.

There are two specificities, Anti-Rg1 and Anti-Rg2:
- Rg1 depends on Val at position 1188 and Leu at position 1191
- Rg2 in addition needs Asn at position 1157

The discrimination of Anti-Rg1 and Anti-Rg2 is beyond the capacities of our laboratory. Almost all antisera contain both specificities.
Anti-Rg reacts with ~98% of all cells. It does best react in IAT (in columns) and does not react with papain-treated cells. Typically, there are some cells in a panel that react slightly weaker.

The presence of “weak” or even negative cells in a panel with many positive cells may cause diagnostic confusion and long hunts for possible combinations of antibodies present in the serum.
Anti-Rg

Anti-Rg is considered to be clinically insignificant. There are reports of allergic reactions to plasma or plasma-containing platelet units.

We usually recommend transfusion with antigen positive units tested for compatibility after neutralization with plasma. In addition we try to match for clinically relevant antigens in order to avoid a missed weak yet clinically relevant antibody.
Summary of Case Challenges

This case included several challenges and pitfalls:
- The patient had a recent massive transfusion confounding serologic antigen typing
- The patient had rapidly developed an antibody of very high titer that overwhelmed the usual neutralization capacity of neutralization tests
- The case was referred after considerable prior testing. We had to decide which tests were to be repeated and which results were to be considered valid.
Lessons Learned by the Case

- Neutralization tests may fail, if the antibody titer is extremely high.
- The reaction pattern gives important information. In our case, we were almost certain that the antibody was an anti-Rg because the pattern fitted so well. Therefore, we were eager to repeat the neutralization test.
- Even a clinically irrelevant antibody may harm the patient, because he may not receive the blood units he needs in time
  - In our case, no transfusion was needed
References

Reid ME, Lomas-Francis C, The blood group antigen facts book


Rare Cell Panel:
DRK-BSD Identification Red Cell – Special
(produced by Red Cross Blood Service BaWü-He)