# Immunohematology Case Studies 2019 - #9

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# Clinical History

- 39-year-old pregnant woman diagnosed with bone marrow hypoplasia at 24 years and without transfusion requirements so far

- Four years before, she had a normal first pregnancy and delivery. At that time, the antibody screen was negative

- In the sixth month of her second pregnancy a presumed antibody to a high frequency antigen was detected in her plasma and a sample was sent to the Immunohematology Reference Laboratory of the Blood and Tissue Bank in Barcelona, Spain

# Serologic History

## Hospital Transfusion Laboratory results

The following serological information was provided:

ABO/Rh: Group A, D+

DAT: Negative

Antibody Screen Results: Positive with the 3 cells (3+)

Antibody Identification Preliminary Results: Positive with all cells (3+) in IAT with untreated and enzyme treated cells (4+)

# Serologic History

## Results in our Reference Laboratory

The results obtained in the hospital were confirmed.

- Group A, D+
- DAT: Negative
- Antibody Screen Method: IAT using Column Agglutination Technology (CAT) and also LISS tube IAT
- Antibody Screen Results: Positive with all cells
- Antibody Identification Methods: LISS tube IAT and IAT using CAT with untreated and enzyme (papain) treated cells
- Antibody Identification Results: Positive with all cells using tube and column in IAT with untreated (3+) and enzyme treated cells (4+)
- In addition we also confirmed that the autocontrol was negative

# Panel Identification

| | | Rh | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNS | | | | Luterano | | Xg$^a$ | | Results | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | C | E | c | e | CW | K | k | Kp$^a$ | Kp$^b$ | Js$^a$ | Js$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Le$^a$ | Le$^b$ | P$_1$ | M | N | S | s | Lu$^a$ | Lu$^b$ | Xg$^a$ | | IAT | Enz | | |
| 1 | $R_1{}^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | 0 | + | 0 | 1 | 3+ | 4+ | | |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | + | 2 | 3+ | 4+ | | |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | 0 | 3 | 3+ | 4+ | | |
| 4 | $R_1r$ | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | + | + | + | + | + | + | + | + | + | 4 | 3+ | 4+ | | |
| 5 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | 5 | 3+ | 4+ | | |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 6 | 3+ | 4+ | | |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | + | + | + | + | + | + | 0 | + | 0 | 7 | 3+ | 4+ | | |
| 8 | $R_0r$ | + | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | + | + | + | 0 | + | + | 8 | 3+ | 4+ | | |
| 9 | r′ r | 0 | + | 0 | + | + | 0 | 0 | + | + | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 9 | 3+ | 4+ | | |
| 10 | rr′′ | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | + | 10 | 3+ | 4+ | | |
| 11 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 11 | 3+ | 4+ | | |
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| Patient | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | | |

The strength and consistency of reactivity with all cells, the negative DAT and autocontrol supported the diagnosis of an alloantibody to a high frequency antigen

# Further Work

- There was no transfusion history
- **Patient extended phenotype** was studied:

1. The extended phenotype for common red blood cell antigens was:

   D+C+c+E-e+ ($R_1$r) K- k+ Kp(a-b+) Jk(a-b+) Fy(a+b+) Lu(a-b+) M+ N+ S-s+

2. Other rare high frequency antigens were investigated with the following results:

   Jr(a+) Vel+ Di(b+) Ge2+ PP1P$^k$+ Kn(a+) Sl(a+) Cs(a+) Cr(a+) P+ Yt(a+) Er(a+) Co(a+) Lan-

3. The Lan phenotype was repeated with a second antiserum obtaining the same result: Lan- phenotype

# Further Serological Work

- **Allogeneic adsorptions** were performed in order to exclude the presence of additional common alloantibodies hidden by the alloantibody to a high frequency antigen

- **Two cells carrying a complementary phenotype** for the most common red cell antigens were employed:

1. $R_1R_1$, K-, Jk(a+b-), Fy(a-b+), SS
2. rr, K-, Jk(a-b+), Fy(a+b-), ss
3. The $R_2R_2$ cell was not employed in this case because the woman's Rh phenotype was e positive and unequivocally a Spanish-Caucasian woman with Caucasian ancestors

- **Allogeneic adsorptions.** After the third adsorption procedure the sample was still reactive against two of the three screening cells. Both adsorbed sera were examined in the Panel

# Further Serological Work

## Results of the allogeneic adsorption procedure

| | | Rh | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNS | | | | Luterano | | Xg$^a$ | | Results | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | C | E | c | e | CW | K | k | Kp$^a$ | Kp$^b$ | Js$^a$ | Js$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Le$^a$ | Le$^b$ | P$_1$ | M | N | S | s | Lu$^a$ | Lu$^b$ | Xg$^a$ | | IAT | Enz | ADS 1 | ADS 2 |
| 1 | $R_1{}^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | 0 | + | 0 | 1 | 4+ | 4+ | 0 | 0 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | + | 2 | 4+ | 4+ | 0 | 1+ |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | 0 | 3 | 4+ | 4+ | 0 | 1+ |
| 4 | $R_1r$ | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | + | + | + | + | + | + | + | + | + | 4 | 4+ | 4+ | 0 | (+) |
| 5 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | 5 | 4+ | 4+ | 0 | 1+ |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 6 | 4+ | 4+ | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | + | + | + | + | + | + | 0 | + | 0 | 7 | 4+ | 4+ | 0 | (+) |
| 8 | $R_0r$ | + | 0 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | + | + | + | 0 | + | + | 8 | 4+ | 4+ | 0 | 1+ |
| 9 | r′ r | 0 | + | 0 | + | + | 0 | 0 | + | + | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 9 | 4+ | 4+ | 0 | 1+ |
| 10 | rr″ | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | + | 10 | 4+ | 4+ | 0 | 0 |
| 11 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | 11 | 4+ | 4+ | 0 | 1+ |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Patient | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 | 0 |

With the adsorbed serum 1 the result was negative indicating the complete adsorption of the high frequency antibody. However, the adsorbed serum 2 showed the presence of **an additional antibody of anti-Jk$^a$ specificity.**

# Further Serological Work

- To confirm the presence of anti-Lan in this woman, her unabsorbed plasma was crossmatched with a selection of three cells with different Lan and $Jk^a$ phenotypes

| | Lan+ Jk(a-) | Lan- Jk(a+) | Lan- Jk(a-) |
|---|---|---|---|
| Woman's plasma | +++ | + | 0 |

Positive results were obtained when Lan and/or $Jk^a$ antigens were present, but negative results were obtained when both antigens were absent

Serological results were compatible with the presence of anti-Lan plus anti-$Jk^a$ in a woman carrying a Lan- and Jk(a-) phenotype

# Further Serological Work

## To predict the fetal risk in this case

1. The treatment of the mother's plasma with 2-mercaptoethanol confirmed that the anti-Lan and the anti-$Jk^a$ were of IgG class
2. Antibody's titration showed a titer of 128 for anti-Lan and a titer of 1 for anti-$Jk^a$
3. Crossmatching between the mother's plasma and red cells from the father was very positive (3+) confirming as expected the presence of the Lan antigen in her husband who was later typed as Lan+ and Jk(a+)

# Further Work

## Follow up and searching for compatible blood

1. In the 37th week of gestation the serological results were identical: anti-Lan, IgG, titer 128 plus anti-$Jk^a$, IgG, titer 1
2. Obstetric controls performed until delivery indicated that the fetus was in good condition
3. There were no relatives available for the investigation of the Lan and $Jk^a$ phenotypes
4. The search of compatible donors in the Spanish registry of rare blood donors showed two Lan- Jk(a-) donors (1 A+ and 1 O+) in case a blood transfusion was necessary to the mother or the newborn

# The LAN blood group system
## History

- In 1962, van der Hart and collaborators described an antibody to a high-prevalence red blood cell (RBC) antigen responsible for a severe and acute hemolytic transfusion reaction. The corresponding antigen, Lan, was named for the index case, Mr. Langereis (Dutch origin)

- In 1990, the Working Party on Terminology for Red Cell Surface Antigens of the ISBT decided to place Lan in the 901 series of RBC antigens (901.002)

- As a result of the discovery of its molecular basis in 2012, Lan was officially moved from the 901 series to the novel 33$^{rd}$ blood group system, LAN. This system contains one single antigen to date, LAN1

- Lan is a high prevalence antigen in most populations (>99.9%), and Lan- is considered a rare blood type worldwide (1 in 20.000 in Caucasians, 1 in 50.000 in Japanese, and 1 in 1.500 in black people from South Africa)

# The LAN blood group system
# Molecular basis

- Using the OSK43 monoclonal antibody, Helias and col., were able to elucidate the molecular basis of Lan. A biochemical approach, combining immunoprecipitation test and mass spectrometry analysis, showed that the ABCB6 transporter carries the Lan antigen.

- The *ABCB6* gene is located on chromosome 2q36. People with the rare Lan- phenotype are actually Lan null or ABCB6-/- (two nonfunctional *ABCB6* alleles)

- Multiple null alleles of *ABCB6* have been reported to date. In addition, a few altered alleles of *ABCB6* were recently shown to encode a weak expression of Lan

# The LAN blood group system
## Anti-Lan antibodies: clinical significance

- Anti-Lan has been described as having variable clinical significance. It may cause hemolytic transfusion reactions, ranging from none to severe, and hemolytic disease of the fetus and newborn (HDFN), ranging from none to mild
- Lan- blood should ideally be given to patients with anti-Lan
- The first case of HDFN due to anti-Lan was reported in 1969 and the baby had mild hemolytic disease but fortunately no treatment was required
- In 1983 a second case was reported in which the only treatment required was phototherapy
- Finally, a third case was reported in 1987 in which the newborn also required phototherapy, even though anti-c and anti-$Jk^a$ were also associated

# Clinical and Serological data from the Newborn

The baby was delivered at term without clinical involvement and with normal hemoglobin and bilirubin levels

- The child's blood group was A+
- The direct antiglobulin-test (DAT) was Positive: 2+
- The eluate was also positive (3+) showing an anti-Lan plus anti-$Jk^a$ specificity. The control of the last wash was negative.

| Red cells | Lan+ Jk(a-) | Lan- Jk(a+) | Lan- Jk(a-) |
|---|---|---|---|
| Eluate | +++ | ++ | 0 |

# Conclusions

- This case represents a rare example of anti-Lan (IgG, titer 128) detected in a pregnant woman with potential risk of HDFN
- Fortunately, the baby was not affected before or after the birth
- The simultaneous presence of anti-$Jk^a$ (titer 1) also had no clinical impact on the fetus or newborn
- This observation, together with the 3 cases previously reported, confirms that anti-Lan does not usually cause HDFN and when it does the clinical impact is usually mild

# Lessons Learned by the Case

- The serological findings of this case were compatible with the presence of an antibody against a high prevalence antigen as confirmed

- The homogeneous reaction pattern did not suggest the presence of an additional alloantibody. However, the allogeneic adsorption procedure allowed to identify the presence of an anti-$Jk^a$ hidden by the anti-Lan antibody.

- The clinical significance of anti-Lan prompted the search of Lan- units at the time of delivery to have them available, in case a blood transfusion was required by the fetus or the mother

# References


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# Immunohematology Reference Laboratory

Blood and Tissue Bank.

Barcelona,Spain