



# Immunohematology Case Studies 2017 - 10

**Thierry Peyrard**, PharmD, PhD, EurSpLM  
National Institute of Blood Transfusion  
National Immunohematology Reference Laboratory  
Paris, France  
tpeyrard@ints.fr

# Clinical History



- 38 year old pregnant patient, 30<sup>th</sup> week of gestation
- Third pregnancy
- No transfusion history

# Immunohematology Laboratory History



- Blood group O, D+C+E-c-e+, K-
- Positive RBC antibody screen => Antibody identification performed

# Immunohematology Laboratory History



|                     | RH |   |   |   |   |   | KEL |   |   |   | FY |   | JK |   | LE |   | MNS |   |   |   | P | LU |   |   | DO |   | YT |   | CO   |      | XG  | IAT | IAT-papain |
|---------------------|----|---|---|---|---|---|-----|---|---|---|----|---|----|---|----|---|-----|---|---|---|---|----|---|---|----|---|----|---|------|------|-----|-----|------------|
|                     | 1  | 2 | 3 | 4 | 5 | 8 | 1   | 2 | 3 | 4 | 1  | 2 | 1  | 2 | 1  | 2 | 1   | 2 | 3 | 4 | 1 | 1  | 2 | 1 | 2  | 1 | 2  | 1 | 2    | 1    | IAT |     |            |
| 1                   | 0  | + | 0 | 0 | + | 0 | 0   | + | 0 | + | +  | + | +  | 0 | 0  | + | 0   | + | 0 | + | 0 | +  | + | + | +  | 0 | +  | 0 | +    | ++++ | -   |     |            |
| 2                   | 0  | 0 | + | + | + | 0 | 0   | + | 0 | + | +  | + | 0  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | + | +  | 0 | +  | 0 | +    | -    | -   |     |            |
| 3                   | 0  | 0 | 0 | + | + | 0 | +   | + | 0 | + | +  | 0 | 0  | + | 0  | + | +   | 0 | + | + | + | +  | + | + | +  | + | +  | 0 | +    | ++++ | -   |     |            |
| 4                   | 0  | 0 | 0 | + | + | 0 | 0   | + | 0 | + | +  | + | +  | 0 | 0  | + | +   | + | + | + | + | +  | + | 0 | +  | 0 | +  | 0 | +    | ++   | -   |     |            |
| 5                   | 0  | 0 | 0 | + | + | 0 | 0   | + | 0 | + | +  | + | 0  | 0 | +  | 0 | +   | 0 | + | 0 | + | 0  | + | 0 | +  | 0 | +  | 0 | +    | ++++ | -   |     |            |
| 6                   | 0  | 0 | 0 | + | + | 0 | 0   | + | 0 | + | +  | 0 | 0  | + | +  | + | 0   | + | 0 | + | + | +  | + | + | 0  | + | 0  | + | 0    | ++   | -   |     |            |
| 7                   | +  | 0 | + | + | 0 | 0 | 0   | + | 0 | + | 0  | + | 0  | + | 0  | + | 0   | + | 0 | + | + | 0  | + | 0 | +  | 0 | +  | 0 | +    | -    | -   |     |            |
| 8                   | +  | + | 0 | 0 | + | + | 0   | + | 0 | + | +  | 0 | 0  | + | 0  | + | +   | + | + | 0 | + | +  | + | 0 | +  | 0 | +  | 0 | +    | ++   | -   |     |            |
| 9                   | +  | + | 0 | 0 | + | 0 | +   | + | 0 | + | +  | + | 0  | 0 | +  | 0 | +   | + | + | 0 | 0 | +  | 0 | + | 0  | + | 0  | 0 | -    | -    |     |     |            |
| 10                  | +  | + | 0 | 0 | + | 0 | 0   | + | 0 | + | +  | 0 | +  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | 0 | +  | 0 | +  | 0 | +    | -    | -   |     |            |
| 11                  | +  | 0 | 0 | + | + | 0 | 0   | + | 0 | + | +  | + | 0  | 0 | +  | 0 | +   | 0 | + | 0 | 0 | 0  | + | 0 | +  | 0 | +  | 0 | +    | ++++ | -   |     |            |
| 12                  | +  | + | + | 0 | + | 0 | 0   | + | 0 | + | +  | + | 0  | 0 | +  | + | +   | 0 | + | + | 0 | +  | + | 0 | +  | 0 | +  | 0 | +    | ++   | -   |     |            |
| 13                  | +  | + | 0 | 0 | + | 0 | 0   | + | + | + | +  | + | 0  | 0 | +  | + | +   | 0 | + | 0 | 0 | +  | 0 | + | 0  | + | 0  | 0 | ++   | -    |     |     |            |
| 14                  | +  | + | 0 | 0 | + | 0 | +   | + | 0 | + | +  | + | +  | 0 | +  | + | 0   | + | 0 | + | 0 | +  | 0 | + | 0  | + | 0  | 0 | ++++ | -    |     |     |            |
| 15                  | 0  | 0 | 0 | + | + | 0 | 0   | + | 0 | + | +  | 0 | +  | 0 | 0  | + | 0   | + | + | 0 | + | +  | + | + | 0  | + | 0  | 0 | -    | -    |     |     |            |
| <b>Autocontrols</b> |    |   |   |   |   |   |     |   |   |   |    |   |    |   |    |   |     |   |   |   |   |    |   |   |    |   | -  | - |      |      |     |     |            |

# Immunohematology Laboratory History



|              | RH |   |   |   |   |   |   | KEL |   |   |   | FY |   | JK |   | LE |   | MNS |   |   |   | P | LU |   |   | DO |   | YT |   | CO |   | XG | IAT  | IAT-papain |      |   |
|--------------|----|---|---|---|---|---|---|-----|---|---|---|----|---|----|---|----|---|-----|---|---|---|---|----|---|---|----|---|----|---|----|---|----|------|------------|------|---|
|              | 1  | 2 | 3 | 4 | 5 | 8 | 1 | 2   | 3 | 4 | 1 | 2  | 1 | 2  | 1 | 2  | 1 | 2   | 3 | 4 | 1 | 1 | 2  | 1 | 2 | 1  | 2 | 1  | 2 | 1  |   |    |      |            |      |   |
| 1            | 0  | + | 0 | 0 | + | 0 | 0 | +   | 0 | + | + | +  | + | 0  | 0 | +  | 0 | +   | 0 | + | + | + | +  | 0 | + | 0  | + | +  | + | +  | + | +  | +    | +          | ++++ | - |
| 2            | 0  | 0 | + | + | + | 0 | 0 | +   | 0 | + | + | +  | + | 0  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | + | +  | 0 | +  | 0 | +  | + | +  | +    | -          | -    |   |
| 3            | 0  | 0 | 0 | + | + | 0 | + | +   | 0 | + | + | 0  | 0 | +  | 0 | +  | + | 0   | + | + | + | + | +  | + | + | +  | + | +  | 0 | +  | + | +  | +    | ++++       | -    |   |
| 4            | 0  | 0 | 0 | + | + | 0 | 0 | +   | 0 | + | + | +  | + | 0  | 0 | +  | + | +   | + | + | + | + | +  | 0 | + | 0  | + | 0  | + | +  | + | +  | +    | ++         | -    |   |
| 5            | 0  | 0 | 0 | + | + | 0 | 0 | +   | 0 | + | 0 | +  | + | 0  | 0 | +  | + | 0   | + | 0 | + | + | 0  | + | + | 0  | + | 0  | + | +  | + | +  | +    | ++++       | -    |   |
| 6            | 0  | 0 | 0 | + | + | 0 | 0 | +   | 0 | + | 0 | +  | + | 0  | 0 | +  | + | 0   | + | 0 | + | + | +  | + | + | 0  | + | 0  | + | 0  | + | +  | +    | ++         | -    |   |
| 7            | +  | 0 | + | + | 0 | 0 | 0 | +   | 0 | + | 0 | +  | 0 | +  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | 0 | +  | 0 | +  | 0 | +  | 0 | +  | 0    | -          | -    |   |
| 8            | +  | + | 0 | 0 | + | + | 0 | +   | 0 | + | + | 0  | 0 | +  | 0 | +  | + | +   | 0 | + | + | + | 0  | + | + | 0  | + | 0  | + | 0  | + | +  | +    | ++         | -    |   |
| 9            | +  | + | 0 | 0 | + | 0 | + | +   | 0 | + | + | +  | + | 0  | 0 | +  | 0 | +   | + | + | 0 | 0 | +  | 0 | + | 0  | + | 0  | 0 | 0  | 0 | 0  | -    | -          |      |   |
| 10           | +  | + | 0 | 0 | + | 0 | 0 | +   | 0 | + | + | 0  | + | +  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | 0 | +  | 0 | +  | 0 | +  | 0 | +  | -    | -          |      |   |
| 11           | +  | 0 | 0 | + | + | 0 | 0 | +   | 0 | + | + | +  | + | 0  | 0 | +  | + | 0   | 0 | 0 | 0 | + | 0  | + | + | 0  | + | 0  | + | 0  | + | +  | ++++ | -          |      |   |
| 12           | +  | + | + | 0 | + | 0 | 0 | +   | 0 | + | + | +  | + | 0  | 0 | +  | + | +   | 0 | + | + | 0 | +  | + | 0 | +  | 0 | +  | 0 | +  | 0 | +  | +    | ++         | -    |   |
| 13           | +  | + | 0 | 0 | + | 0 | 0 | +   | + | + | + | +  | + | 0  | 0 | +  | + | +   | 0 | + | 0 | 0 | +  | 0 | + | 0  | + | 0  | 0 | 0  | 0 | 0  | ++   | -          |      |   |
| 14           | +  | + | 0 | 0 | + | 0 | + | +   | 0 | + | + | +  | + | +  | 0 | +  | + | 0   | + | 0 | + | 0 | +  | 0 | + | 0  | + | 0  | 0 | 0  | 0 | 0  | ++++ | -          |      |   |
| 15           | 0  | 0 | 0 | + | + | 0 | 0 | +   | 0 | + | + | 0  | + | 0  | 0 | +  | 0 | +   | 0 | + | + | 0 | +  | + | + | +  | 0 | +  | 0 | 0  | 0 | 0  | -    | -          |      |   |
| Autocontrols |    |   |   |   |   |   |   |     |   |   |   |    |   |    |   |    |   |     |   |   |   |   |    |   |   |    |   | -  | - |    |   |    |      |            |      |   |

- **Anti-M**, with dosage effect (stronger reactivity on M+N- RBCs, with a *MM* genotype)
- M antigen destroyed by papain treatment of RBCs
- Negative autocontrols => **probable allo-anti-M** but M/N typing required to conclude

# Immunohematology Laboratory History



## M/N typing

**M**-N+ => Anti-M concluded to be an alloantibody

New blood samples investigated a few weeks later  
in a **second laboratory**: anti-M confirmed

**New M/N typing** in this second laboratory: **M+N+**!

Strong reaction for M (4+), equivalent to the M+N-  
control RBCs



# Results



## Blood samples referred to the IRL

- Confirmation of anti-M reactivity
- M-N+ type confirmed with two different sources of anti-M reagents
- Control with the anti-M used by laboratory #2 who found a 4+ reactivity for M => confirmation of the M+ type
- Genotyping test =>  $MNS^*2/MNS^*2$  => predicted M-N+ type

**=> Patient M+ or M- ? Auto- or allo-anti-M ?**



# Discussion



- Typical issue of **cross-reactivity** with blood typing reagents
- **Cross-reactivity between antigens occurs when an antibody directed against one specific antigen is successful in binding with another, different antigen. The two antigens in question have similar three-dimensional structural regions, known as epitopes, which allow the antibody for one antigen to recognize a second antigen as being structurally the same antigen**
- **Totally different context from a contamination of a polyclonal reagent by an unexpected antibody to a low-prevalence antigen**



# Discussion



**Some widely used monoclonal anti-M clones (e.g. 2514E6 and M-11H2) strongly cross-react with the low-prevalence He antigen (Henshaw, MNS6)**

Example of warning in the manufacturer's instruction manual:

## PERFORMANCE DATA

A performance assessment of the reagents was conducted on a random samples panel of known common phenotypes including clinical and neonatal samples. The samples were drawn in the recommended anticoagulants (E.D.T.A., heparin, citrate). The expert assessment demonstrated 100 % specificity for each of the reagents with respect to the expected results.

ANTI-M (MNS1) can recognize unspecifically some HENSCHAW red blood cells (M -, He + is an extremely rare phenotype).

**~7-10% of people of African descent are He+  
=> not "extremely rare" in some countries!**



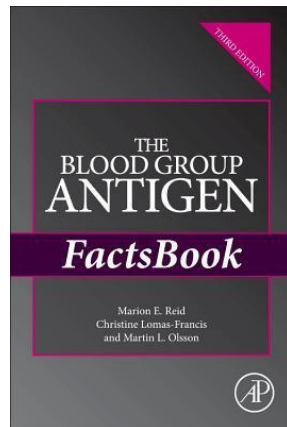
**Of note, in our experience, 90% of S-s-U+<sup>var</sup> type P2 and 100% of S-s-U+<sup>var</sup> type NY are He+**

# Discussion



## He Antigen

### Terminology



ISBT symbol (number)

MNS6 (002006 or 2.6)

Obsolete name

Henshaw

History

Named for the first He+ proband, Mr. Henshaw; the original anti-He, present in a rabbit anti-M serum, was identified in 1951.

### Occurrence

African Americans

3%

Blacks in Natal

Up to 7%

Caucasians

Not found

### Antithetical antigen

“N” (MNS30)

# Discussion



- **If an anti-M is found in a M+ patient with negative autocontrols, do not conclude there is a "partial M". This has not been reported to exist.**
- **Polyclonal anti-M are not concerned by this cross-reactivity issue**
- **The anti-M clones that cross-react with He are, however, considered the most performant anti-M**
- **Anti-M clone BS57 does not cross-react with He**
- **Some anti-M clones (E3, E6, 425/2B) also cross-react with the low-prevalence M<sup>g</sup> antigen (MNS11) => less problematic because this antigen is very rare in all populations (except in Switzerland but prevalence <1%)**

## **Anti-M Monoclonal Antibodies Cross-Reacting With Variant M<sup>s</sup> Antigen: An Example of Modulation of Antigenic Properties of Peptide by Its Glycosylation**

By Ewa Jaśkiewicz, Marcin Czerwinski, Danuta Syper, and Elwira Lisowska

Some monoclonal antibodies (MoAbs) directed against blood group M-related epitope of glycophorin A (GPA) were found to agglutinate rare variant erythrocytes carrying GPA of M<sup>s</sup> type. In contradistinction to normal GPA-M or -N, the N-terminal portion of GPA-M<sup>s</sup> is not glycosylated. Therefore, the multipin peptide synthesis was used for testing the specificity of the cross-reacting MoAbs. Among several anti-M and anti-N MoAbs tested, only three anti-M (E3, E6, 425/2B) agglutinated M<sup>s</sup> erythrocytes and showed binding to the synthetic octapeptides corresponding to N-terminal sequences of GPA-M (SSTTGVAM), GPA-N (LSTTEVAM), and GPA-M<sup>s</sup> (LSTNEVAM). Testing multiple peptide analogs (window and replacement analysis) showed that these MoAbs were specific for peptidic epitope in which Met8 and Val6 were the most essential amino acid residues. The amino acid replacements Ser1 ↔ Leu1 or Gly5 ↔ Glu5 (M v N) and Thr4 ↔ Asn4 (M and N v M<sup>s</sup>) had no or negligible effect on the reaction of synthetic peptides with the MoAbs. However, when Ser2, Thr3, and Thr4 carry O-linked sialooli-

gosaccharides (normal GPA-M or -N), the MoAbs recognize Gly5- and sialic acid-dependent blood group M-related epitope. An interesting finding concerning anti-M/M<sup>s</sup> MoAbs described here is the fact that glycosylation of amino acid residues adjacent to the most important part of peptidic epitope not only differentially modulates the proper exposure of peptidic epitope, but also alters the requirement for some amino acid residues present within the epitope. Pathologic conditions, including hematologic disorders, are often accompanied by alterations in protein glycosylation, resulting not only from differences in the structure of antigen polypeptide chain, but also from changes in specificity or expression of enzymes involved in glycosylation. Our present findings draw attention to possibility of the bidirectional modulation of protein antigenicity by glycosylation and may be helpful in interpretation of some results obtained with MoAb used for diagnostic or other purposes.

© 1994 by *The American Society of Hematology.*

*Blood*, Vol 84, No 7 (October 1), 1994: pp 2340-2345

# Summary and Conclusions



- **Risk to falsely conclude to an auto-anti-M (that does exist) in a M-He+ patient if the anti-M reagent cross-reacts with He+ RBCs**
- **Beware of possible cross-reactions with some monoclonal antibodies, potentially responsible for false-positive results**
- **Always carefully read the package insert of the manufacturer and limitations of the reagent**
- **Choose the reagent the most adapted to the profiles of patients of your laboratory => an anti-M that cross-reacts with He+ RBCs is certainly not the best option in a laboratory that deals with many patients/donors of African descent**

# Summary and Conclusions



- **Cross-reactivity sometimes explains discrepancies between phenotype and genotype**
- **Cross-reactivity may also explain apparent parentage exclusion: example of a child typed as M+N+ with a cross-reacting reagent with He+ RBCs, with mother and father previously typed as M-N+ with an anti-M that does not cross-react with anti-He**