Know your calculus: What to do with no U + S

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Who am I

- Medical school and residency at the University of Nebraska Medical Center in Omaha
- Fellowship in Transfusion Medicine at the University of Minnesota in Minneapolis
- General private practice pathologist employed by KC Pathology
- Stationed at St. Luke's hospital on the Plaza

MNS blood group

- Discovered in 1927 by Landsteiner and Levine and named after the first three antigens identified in the system: M, N, and S.
- Located on chromosome 4q31.21
- The MNS group consists of 49 antigens on two glycoproteins
- (M and N) and (S and s) are paired antithetical antigens
- The M and N antigens are located on glycophorin A (GPA)
- S and s antigens are located on glycophorin B (GPB)

MNS blood group

- GPA is prevalent in much higher concentrations on the cell membrane than GPB, possibly accounting for lower sensitization to S and s.
- Both GPA and GPB are exploited by *Plasmodium falciparum* as receptors for binding to red cells and may be critical to the invasion process.

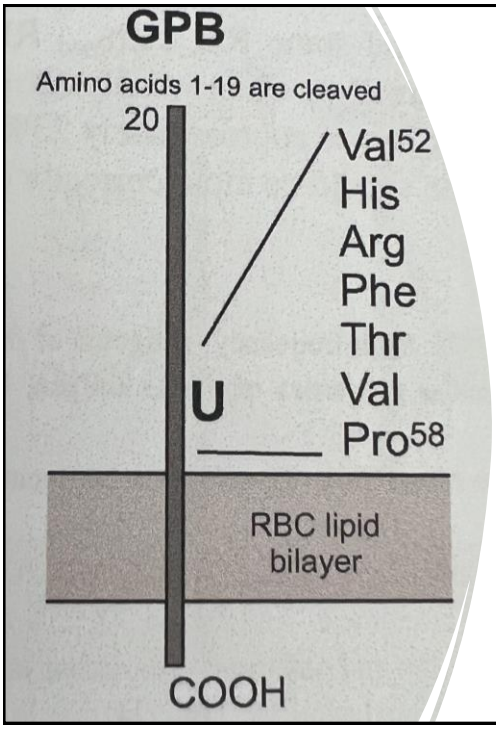
	Prevalence (%)	
Phenotype	**Whites**	**African Americans**
M+ N–	30	25
M+ N+	49	49
M– N+	21	26
S+ s–	10	6
S+ s+	42	24
S– s+	48	68
S– s–	0	2

Antibodies to M and N

- Anti-M is a relatively common antibody, whereas anti-N is less common
- Most anti-M and –N antibodies are of the IgM class and are not active at 37 C. The are usually not clinically significant and can often be ignored in clinical practice.
- Many examples of anti-M are naturally occurring.
- Very rarely, anti-M has been implicated as the cause of acute and delayed hemolytic transfusion reactions, and very rarely anti-M has been associated with severe hemolytic disease of the fetus and newborn.
- Anti-N is not generally associated with either HTRs or HDFN.

Antibodies to S and s

- Antibodies to the S antigen are more commonly of the IgG class.
- Anti-S can be naturally occurring,
- Antibodies to S may result in no to moderate (rare) HTRs and no to severe (rare) HDFN.
- Antibodies to the s antigen are more commonly of the IgG class.
- Antibodies to s may result in no to mild (rare) HTRs and no to severe (rare) HDFN.



U antigen structure and genetics

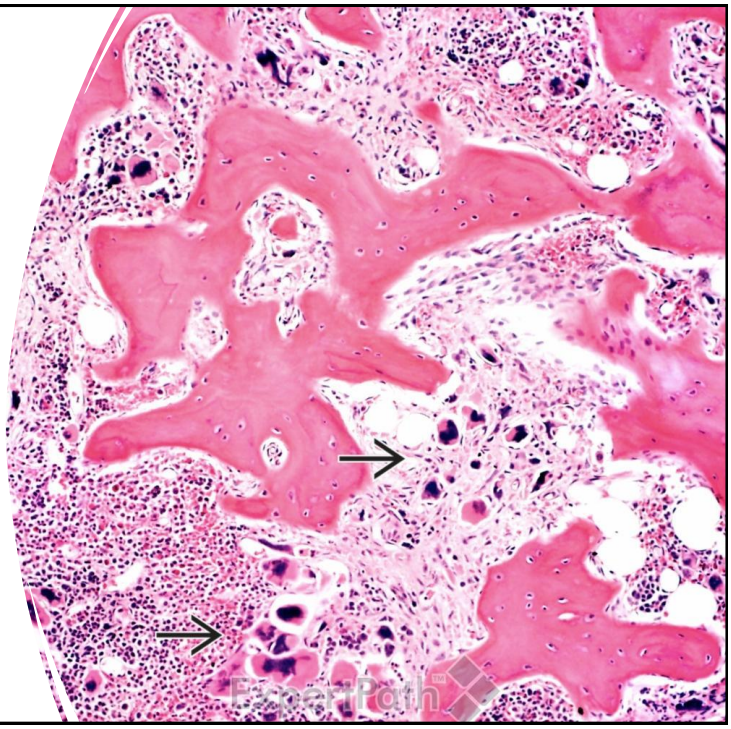
- The red cells of about 1-2% of Americans of African ancestry are S-s- and lack the high-prevalence antigen U.
- 99.9% of Caucasians express the U antigen
- This absence may be due to either a deletion of the coding region of GPB, or a variation of the U antigen (U^{var})
- Anti-U is of the IgG class and has been implicated in autoimmune hemolytic anemia, and may be responsible for severe HTR and HDFN.

Patient presentation intro

- A 78 year old female inpatient with primary myelofibrosis who was hospitalized for chest pain, palpitations, exertional dyspnea
- She is transfusion dependent with a long history of transfusion.
- She has a known cold auto-antibody.
- Her hemoglobin was 5.4 g/dL on admission.
- Type and screen ordered

What is primary myelofibrosis

- Myeloproliferative neoplasm
- Primary driver is *JAK2* V617F, *CALR*, or *MPL* mutations
- The abnormal clonal cells are megakaryocytes
- Fibroblasts secrete collagen and reticulin proteins that cause fibrosis, resulting in decreased hematopoiesis
- Treatment is largely supportive, especially transfusion



Screen results (Immucor Echo)

Batch ID: **Device:** M21120 **Device Order:** Screen

Order Code: Screen **Status:** Partial **Date Time:**

Test Code	Test Type	Index	Lot ID	Result	User Approved	Date Time Approved	User Transmitted	Date Time Transmitted
Screen	Final	0	R421	Positive				

#	Device Test	Well	Image	Original Value	Edited Value	Measured Value	Operator	Date Time
1	Screen 1	A1		4+	4+	100		
2	Screen 2	B1		3+	3+	90		
3	Screen 3	C1		4+	4+	100		
4	Pos Ctrl	D1		4+	4+	100		
5	Screen Interp				Positive			

CBC interpretation

Immunohematology Testing

Red Blood Cell			Direct Antiglobulin Test		
ABO Group	Rh Type	Other Antigen Types	Polyspecific	IgG Specific	Complement
A	Pos		Positive	Positive	Positive

Antibody Identification

Previous Serologic Findings			Current Serologic Findings		
Source	Antibody	Clinical Significance	Source	Antibody	Clinical Significance
Plasma & Eluate	Anti-C	Significant	Eluate	Probable warm autoantibody	See Below
Plasma & Eluate	Anti-U	Significant	Plasma	Anti-U	Significant
Plasma	Cold autoantibody	See Below	Plasma	Anti-C	Significant
			Plasma	Cold autoantibody	See Below

Transfusion Recommendation	Units Provided
C- U- K- See Below	**A segment was provided by the submitting facility from a deglyced C-negative, K-negative, Fy(a-), U-negative unit sent prior to this workup. This unit was tested and found to be nonreactive with the patient's prewarmed plasma: W036216760628.**

CBC interpretation

Additional Comments

An eluate prepared from the patient's cells was reactive with all cells in PEG by the indirect antiglobulin test. These results are consistent with probable warm autoantibody, but this was not confirmed as a valid autologous control was not available. Transfusion of patients with autoantibodies carries a greater than normal risk.

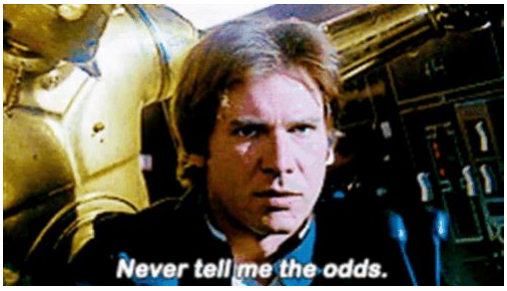
Cold autoantibodies are not usually clinically significant. Prewarmed testing may be required to circumvent the cold autoantibody in pretransfusion testing.

The presence of anti-K could not be excluded at this time. Until the presence of anti-K can be excluded, donor blood selected for transfusion should be negative for the K antigen.

Donor blood lacking the U antigen is extremely rare (1%) and often not readily available. If the patient's health permits, it is strongly recommended that they donate red blood cells for themselves and others.
Siblings of the patient may also be U negative and should be encouraged to be tested for the U antigen. If found to be negative, it is also strongly recommended that they donate for themselves and others.
For questions, please, contact the reference laboratory 816-968-4053.

Compatibility math

- Caucasian
  = (%O + %A)*(1-%C)*(1-%K)*(1-%U)
  = (0.45 + 0.40)*(1 – 0.68) * (1 – 0.09) * (1 – 0.99)
  = 0%
- Black
  = (%O + %A)*(1-%C)*(1-%K)*(1-%U)
  = (0.49 + 0.27)*(1 – 0.27) * (1 – 0.0.2) * (1 – 0.98)
  = 1.1%



Next steps

- Patient started treatment with Retacrit (epoetin alfa-epbx)
- Request for rare donor units made to the New York Blood Center
  - Less than a dozen U negative units available in the country
- After discussion with SLH pathology, the patient's transfusion threshold was moved to 5.0 g/dL and later 4.5 g/dL
- Lab draw frequency decreased

Treatment log

- Transfused one U negative unit on admission day (day 0)
- Transfused one U negative unit on day +7
- Transfused one U negative unit on day +18
- Transfused one U negative unit on day +22
- Transfused one U negative unit on day +23
- Transfused two U negative units on day +43

Something's got to give

- Around day +50, there are no units available in the United States
- No viable family donors
- Monocyte monolayer assay (ordered earlier) is resulted

Monocyte monolayer assay

- The monocyte monolayer assay (MMA) is used to determine the clinical significance of alloantibodies produced by blood transfusion recipients.
- Useful in a situation such as ours where compatible blood products are not available.
- Essentially, this labor-intensive assay uses donor mononuclear cells, donor antigen positive red cells, and patient plasma to assess the antigen generating ability of the patient to attempt to predict their clinical reactivity to an incompatible blood transfusion.

MMA Results

Testing on [redacted] gave the following results:

Antibody Source	RBC Source	AHG (IgG)	AHG (C3b/C3d)	MI Adhered RBCs (%)	MI Ingested RBCs (%)	MI Total (%)
Pos Control 20220321LH	Screen Cell I & II Lot V256422	4+	N/A	0.75	47.75	48.50
[redacted]	[redacted] (auto)	0✓	0	0.50	0.25	0.75
	W036522038979, O+, C-	2+	0	2.25	0.75	3.00
	W036522090802, O+, C-	2+	Mi+	1.25	2.00	3.25
	W036522113755, O+, C-	2+	Mi+	5.75	2.75	8.50
	W036522112965, O+, C-	2+	0	5.25	1.00	6.25
	W036522090744, O-, C-	2+	Mi+	2.75	0.25	3.00
	W036522101389, O-, C-	2+	0	3.75	0.00	3.75

MMA Results

An MI of zero or 0 indicates there were no adhered or phagocytized red cells. Our experience with this testing procedure is similar to others (TRANSFUSION, 2004, 44; 1273-81); in that, MI values of ≤5 have indicated that incompatible blood can be given without the risk of an overt hemolytic transfusion reaction but it does not guarantee normal long-term survival of those RBCs. MI values ranging from 5–20 have a reduced risk of clinical significance, but signs and symptoms of transfusion reaction may occur. Similarly, an MI of >20 indicates the antibody has clinical significance, which may range from abnormal RBC survival to clinically obvious reactions.

Using a routine 60-minute saline incubation, the autocontrol was nonreactive using anti-IgG and anti-C3. The MI for the autocontrol is 0.75, indicating the warm autoantibody is currently considered clinically insignificant. However, if the antibody titers are lower than the detectable limits of the assay, it may cause a false negative result.

Cells from six group O donor units with varying phenotypes (C-) were selected to challenge the reactivity of the anti-U. All six donor unit cells were reactive 2+ using anti-IgG, and three cells were microscopically reactive with anti-C3. The test cells yielded MI values ranging from 3.0-8.5, indicating the antibody is minimally clinically significant against certain cells.

If transfusion is necessary, the patient should be able to receive U antigen positive red blood cells with minimal risk of an overt transfusion reaction. If antigen positive units are transfused, they should be administered with caution and the patient monitored during and after transfusion for hemolysis and secondary immune response. If the patient needs additional transfusion, the MMA can be repeated before selecting units to transfuse.

The monocyte monolayer assay is mediated by monocytes to predict extravascular hemolysis occurring in the liver, spleen and lymph nodes. Variables such as immunoglobulin class and subclass, and complement activation may affect the clinical significance of the antibody despite MMA predictions. In addition, if antibody titers fall below detectable limits, the assay could yield false negative results.

Prevention of hemolytic transfusion reactions with intravenous immunoglobulin prophylaxis in U– patients with anti-U

Nay Win,[1] Muhsin Almusawy,[2] Loraine Fitzgerald,[3] Guy Hannah,[3] and Tom Bullock[4]

- Case report series of three U- patients transfused U+ pRBCs
- Two received IVIG prophylaxis with the following regimen:
  - 0.5 g/kg IVIG given day before transfusion
  - 1 g/kg given day of transfusion
  - 0.5 g/kg given day after transfusion
- The two patients given U+ units with prophylaxis had no discernable acute or delayed HTR

TRANSFUSION PRACTICE

Transfusions of least-incompatible blood with intravenous immunoglobulin plus steroids cover in two patients with rare antibody

Nay Win,[1] Malcolm Needs,[1] Nicole Thornton,[2] Robert Webster,[3] and Cherry Chang[4]

- Case report series of two patients with rare antibodies (anti-U and anti-Jra)
- Both received IVIG, one as prophylaxis and the other as post-exposure
- Neither patient given IVIG had any discernable acute or delayed HTR

Treatment regimen

- The patient was given 60 mg prednisone the day before transfusion
- Given 50 g IVIG (Privigen) the day of transfusion (0.6 g/kg)
- Transfused one unit of pRBCs that were C-, U+
- Continued on 60 mg of prednisone
- She had a mild uptick in her LDH and serum haptoglobin
  - LDH: 311 U/L from 249 U/L baseline
  - Haptoglobin: 376 mg/dL from 340 mg/dL baseline
- Hgb increased from 5.0 g/dL to 5.8 g/dL, which persisted for the next 7 days, after which she was transfused another unit of C-, U+ blood with repeated steroid/IVIG prophylaxis

MMA follow up results (2 months after U+ transfusion)

Testing on [redacted] gave the following results:

Antibody Source	RBC Source	AHG (IgG)	AHG (C3b/C3d)	MI Adhered RBCs (%)	MI Ingested RBCs (%)	MI Total (%)
Pos Control 20220321LH	Screen Cell I & II Lot V257379	3+	N/A	1.50	43.50	45.00
[redacted]	[redacted] (auto)	mi+	0	4.00	4.50	8.50
	W036523024711, O+,C-	mi+	mi+	2.00	1.50	3.50
	W036523017388, O+, C-	0	0	2.00	1.00	3.00
	W036523024703, O-, C-	0	0	3.50	0.25	3.75
	W03652302378, O+, C-	0	0	2.50	0.50	3.00
	W036523004147, O-, C-	mi+	0	14.00	0.25	14.25
	W036523017357, O-, C-	mi+	0	1.50	0.50	2.00

Patient outcome

- Patient is still with us and remains transfusion dependent, and receives the same IVIG prophylactic regimen

Thanks/questions

- Many thanks to the following individuals
  - The excellent blood bank staff in the Saint Luke's System
  - The individuals at Community Blood Center / NYBCe
  - My colleague Ben Wagenman MD, who was the lead contact on this case
  - My kindergartner and toddler for tolerating me while I put this together

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5. Win N, Needs M, Thornton N, Webster R, Chang C. Transfusions of least-incompatible blood with intravenous immunoglobulin plus steroids cover in two patients with rare antibody. Transfusion. 2018 Jul;58(7):1626-1630