Medical and Research Publications
International Open Access

**Case Report** **Journal of MAR Pathology (Volume 2 Issue 2)**

# Warm-Reacting Anti-Lewis A Antibody: A Case Report

Giancarlo **Tabaro Marrero** [1], William Nicholas **Rose** *[2]

1. University of Wisconsin School of Medicine and Public Health, University of Wisconsin Hospital, 600 Highland Ave, Madison, WI 53792.

2. Department of Pathology and Laboratory Medicine, University of Wisconsin Hospital, 600 Highland Ave, Madison, WI 53792.

**Corresponding Author: William Nicholas Rose,** Department of Pathology and Laboratory Medicine, University of Wisconsin Hospital, 600 Highland Ave, Madison, WI 53792.



**Received Date: May 17, 2023**

**Published Date: June 01, 2023**

***Abstract***

***Background:*** *Clinically significant non-ABO alloantibodies are usually acquired IgG antibodies that react at 37°C, thus potentially causing delayed hemolytic transfusion reactions under physiologic conditions. In contrast, anti-Lewis antibodies are usually low-titer IgM antibodies that react below 30°C and are rarely considered clinically significant. However, warm-reacting anti-$Le^a$ (Lewis A) and anti-$Le^b$ (Lewis B) antibodies have been reported.*

***Case Report:*** *A middle-aged black female G3P3 without previous blood transfusions was diagnosed with a rhabdomyosarcoma after presenting to the ED with orthostatic symptoms due to irregular vaginal bleeding. Two red blood cell units were pre-ordered by the primary team for her scheduled total abdominal hysterectomy and possible bilateral oophorectomy. The antibody screen test identified clinically significant anti-$Le^a$ antibody reacting at room temperature and at 37°C using the reagent red blood cell tube test method. Multiple antibody panels with selected reagent red blood cells confirmed the specificity of the anti-$Le^a$ antibody. A cold agglutinin autoantibody was identified but autocontrol was negative at 37 ºC with anti-human globulin (AHG). Also, a clinically insignificant anti-$Le^b$ antibody was not ruled out. Two units of $Le^a$ and $Le^b$ antigen-negative type O positive red blood cell units were selected that were crossmatched at the 37°C AHG phase with pre-washed donor red blood cells.*

***Conclusion****: We share our experience with a patient with a rare anti-$Le^a$ antibody that reacts at 37°C. It may be favorable to provide $Le^a$ and $Le^b$ antigen-negative red blood cell units crossmatched at 37°C with pre-washed donor red blood cells and AHG anytime clinically significant anti-Lewis antibodies are detected during a patient's lifetime; especially among Le(a-b-) phenotype recipients undergoing chronic red blood cell transfusions. Further investigation is needed to avoid post-transfusion alloimmunizations and determine the clinical significance of kidney allograft rejections among Le(a-b-) phenotype recipients.*

## Introduction

Unlike most non-ABO blood group systems which originate from erythroid precursors, Lewis antigens originate from embryonic endoderm and are commonly found in blood plasma, saliva, breast milk, and urine. [1] Interestingly, the Lewis blood group system is related to the ABO blood group system, and it mainly involves the secretion or synthesis of Lea, Leb, and ABH antigens. Overall, the presence and interaction between the autosomal dominant fructosyltransferases FUT2 and FUT3 determine the Lewis phenotypes in secretions. [1] The Lewis allele expresses the FUT3 enzyme, and the Secretor allele expresses the FUT2 enzyme. The main Lewis phenotypes include Le(a+b-), Le(a-b+), and Le(a-b-). The Le(a-b+) phenotype synthesizes small quantities of Lea antigen as an intermediate product.

Anti-Lewis antibodies are not usually clinically significant for various reasons. [2] First, the prevalence of anti-Lewis antibodies that react at 37°C is low. Second, Lewis antigens easily shed off cells because they are soluble glycolipids that loosely and passively integrate into the cell membrane of red blood cells, lymphocytes, and platelets. [1] This allows the recipient's anti-Lewis antibodies to bind with free donor Lewis antigen in the recipient's blood plasma instead of directly binding to red blood cells. This interaction is thought to reduce the hemolytic effect on red blood cells thereby preventing serious hemolytic transfusion reactions. Third, Lewis antigen concentration is dependent on cell turnover and blood plasma volume. [1][2][3] For example, in pregnancy, the increased blood plasma volume decreases the concentration of Lewis antigens that can transiently promote anti-Lewis antibody formation up to 6 weeks postpartum. Fourth, IgM is less likely to cross the placenta and cause hemolytic disease of the newborn and fetus. In addition, Lewis antigens are poorly expressed in the red blood cells of neonates and may take up to 6 years after birth to fully express their Lewis phenotype in red blood cells and other tissues. [1]

However, the hemolytic potential of anti-Lea, and less frequently anti-Leb, antibodies is seen when IgM, rarely IgG, react at 37°C. [1][2][3] Anti-Lewis antibodies are thought to occur after non-specific environmental exposure, although pregnancy and transfusion-induced alloimmunization are also thought to play a role. [2] The anti-Lea antibody has been found to be the most common clinically significant anti-Lewis antibody. Approximately 22% of the African American population has the Le(a-b-) phenotype compared to 6% of the White population. Thus, African American patients are more likely to develop clinically significant antibodies since individuals develop antibodies against the Lewis antigens that they lack. [1][2] Current guidelines state that recipients with anti-Lewis antibodies should be crossmatch compatible at 37ºC with AHG. [3] Donor red blood cells are recommended to

be washed before the crossmatch to avoid false negative results due to the Lewis antigen-antibody neutralization in the plasma. **[**4**]**

## Case Presentation

A middle-aged black female G3P3 without previous blood transfusions had a history of a possible stroke, pseudotumor cerebri complicated by bilateral vision loss, and an HPV positive pap smear. The patient presented to the ED after feeling a painful mass protrude from her vagina while urinating. She did not have constitutional symptoms and was in her usual state of health before symptom onset. Although, she endorsed gradually worsening irregular vaginal bleeding and dyspareunia. Past surgical history includes bilateral salpingectomy and tonsillectomy. The patient was not taking any chronic medications and was recently started on Oxycodone for pain control.

Vital signs were within normal limits. The physical exam revealed a friable cystic cluster protruding from her vagina. Exam under anesthesia revealed that the mass covered the entire cervix. Labs were significant for anemia with a hemoglobin of 9 g/dL, normal white blood cell count of 10,000, high platelet count of 510,000, and a negative pregnancy test. Pelvic ultrasound showed mild endometrial stripe thickening. CT scan of her pelvis showed uterine/vaginal prolapse. She subsequently underwent an excisional biopsy that was diagnosed as a rare cervical botryoid rhabdomyosarcoma. CT chest and MRI of pelvis did not identify metastatic disease. The patient was scheduled for a total abdominal hysterectomy and possible bilateral oophorectomy with future adjuvant chemotherapy and possible pelvic radiotherapy after surgery with intent for cure.

Two red blood cell units were pre-ordered by the primary team for her scheduled surgery. The patient did not have a history of previous blood transfusions per chart review. The antibody screen test was positive for a cold agglutinin autoantibody for the first time 3 years after her first pregnancy. Then 5 years after her first pregnancy, the antibody screen revealed a clinically insignificant anti-Lea antibody. The patient developed the newly identified clinically significant anti-Lea antibody 14 years after first testing positive for non-specific cold agglutinin autoantibodies. Interestingly, the patient's antibody screen remained positive between pregnancies but was negative on the days she delivered. This is contrary to what is stated in the literature. **[**2**]**

The patient's blood was typed as B Rh positive. Low ionic strength saline (LISS) solution, saline solution, and modified Alsever's solution were used to suspend the reagent red blood cells at room temperature and at 37ºC. The antibody screen test was performed using the reagent red blood cell 0.8%

Surgiscreen adherence method and it was positive for clinically significant anti-Lea antibody reacting at room temperature and at 37ºC with polyspecific anti-human globulin (AHG).

Non-specific cold agglutinin autoantibodies were also identified exclusively reacting at room temperature. In addition, a cold-reacting anti-Leb antibody was most likely present but was not confirmed since it was clinically insignificant. Autocontrol was negative at 37 ºC with AHG. Identification was confirmed with multiple antibody panels with selected type O reagent red blood cells. The 30-minute test without enhancements but with AHG, the Polyethylene glycol (PEG) & AHG test, and the pre-warm technique with AHG each demonstrated significant agglutination.

Overall, 2+ to 3+ red blood cell agglutination was consistently shown at room temperature and at 37ºC for all the reagent red blood cells that expressed the Lea antigen. These findings support the identification of clinically significant warm-reacting anti-Lea antibody which can potentially cause hemolytic transfusion reactions. We recommended that the patient receives Lewis antigen-negative red blood cell units crossmatched at 37°C with pre-washed donor red blood cells and AHG for all future transfusions. Therefore, two units of Lea and Leb antigen-negative type O positive red blood cell units were selected.

## Discussion

We share our experience with a middle-aged black female G3P3 without previous blood transfusions found to have clinically significant anti-Lea antibody. This is unusual because nearly all anti-Lewis antibodies are clinically insignificant. **[**1**][**2**][**3**][**6**]** However, the patient's anti-Lea antibody was found to be reactive at room temperature and at 37°C, suggesting an IgM antibody with a wide thermal amplitude, which could potentially cause acute intravascular hemolysis under physiologic conditions. **[**7**][**8**][**9**][**10**]** We did not specifically test our patient to determine the Lewis phenotype or whether the reactive antibody was IgG or IgM. Although, the patient likely has the Le(a-b-) phenotype since the antibody panel was positive for a clinically insignificant anti-Leb antibody. This was not confirmed in the presence of the cold agglutinin autoantibody due to its clinical insignificance.

Current guidelines require crossmatch compatibility at 37 ºC, but do not necessarily require antigen-negative blood transfusions for recipients who have developed anti-Lewis antibodies. **[**6**]** Anti-Lea and anti-Leb antibodies commonly develop in individuals with the Le(a-b-) phenotype without a history of blood transfusions. Nonetheless, anamnestic acute and delayed hemolytic transfusion reactions have been reported from Lewis antigen incompatible red blood cell transfusions. **[**7**][**8**][**9**][**10**][**11**]** Approximately 78% of the population does not express the Lea antigen, thus finding Lea antigen-

negative blood donors would likely not be difficult. [1] However, the frequency of Le(a-b-) phenotype recipients developing a clinically significant anti-Leb antibody after a blood transfusion from a Le (a-b+) phenotype donor has been reported. [9] The incidence of Le(a-b-) phenotype in the African American population is approximately 22% and in the White population it is about 6%. [1][2][3] Therefore, finding Lewis antigen-negative red blood cell units may be a challenge, since most blood donors are White and likely express Lewis antigens.

Interestingly, acute hemolytic transfusion reactions have been implied in patients with anti-Lewis antibodies despite negative pre-transfusion sample testing. [7][8][9][10] Thus, the strategy of solely relying on the crossmatch for the identification of clinically significant anti-Lewis antibodies may be problematic for two reasons. First, Lewis antigens that are soluble in the donor's blood plasma may prevent the detection of clinically significant anti-Lewis antibodies during a crossmatch. [4] Thus, washing donor red blood cells before a crossmatch at 37°C with AHG likely prevents false negative results and acute hemolytic transfusion reactions. [11] However, it is not known how much this increases the detection of low-titer clinically significant anti-Lewis antibodies.

Second, the concentration of anti-Lewis antibodies and Lewis antigens physiologically fluctuates due to changes in plasma volume (e.g. pregnancy), hematocrit (e.g. hemolysis), and lipid levels (e.g. liver disease). This may also cause clinically significant anti-Lewis antibody titers to become undetectable during pre-transfusion testing at any given time. [7] For example, our patient's anti-Lea antibody became undetectable the closer she was to giving birth but reappeared in-between pregnancies despite pre-washing the donor's red blood cell unit. This is the opposite to the typical increase in anti-Lewis antibody titer when the pregnancy is further developed. [1][2][3] In addition, each pregnancy appeared to increase the immunogenicity of the anti-Lea antibody in our patient. This is likely secondary to Lewis antigen exposure during birth, but the mechanism is unclear given that neonates have not been found to significantly express Lewis antigens in their red blood cells or tissues. [1]

Overall, the clinical implications of anti-Lewis antibodies are difficult to study because of their low prevalence and their ability to avoid detection. Therefore, it may be favorable to provide Lea and Leb antigen-negative red blood cell units crossmatched at 37°C with pre-washed donor red blood cells and AHG anytime clinically significant anti-Lewis antibodies are detected during a patient's lifetime. This would reduce false negative results during the crossmatch and avoid the potential of developing clinically significant anti-Lea and anti-Leb antibodies among individuals with the Le(a-b-) phenotype, especially for those undergoing chronic red blood cell transfusions.

Future studies could investigate potential alternatives to Lewis antigen-negative red blood cell units. It may be interesting to see if washing donor red blood cell units with saline before a transfusion could decrease the amount of Lewis antigens and prevent hemolytic transfusion reactions. In addition, the concentration of Lewis antigen within red blood cell units has been noted to decrease the longer a red blood cell unit is stored on the shelf. [12] There may be a specific point in time when red blood cell units become negative for Lewis antigens and could be safely transfused into patients with clinically significant anti-Lewis antibodies. Another solution may be to neutralize all the donor's Lewis antigens with antigen-specific antibodies that are already used for Lewis antigen phenotyping. [13][14][15] It should also be noted that individuals that are blood type O express more Lewis antigen than other ABO blood groups. [8] Therefore, avoiding type O Lewis antigen-positive red blood cell transfusions in the setting of clinically significant anti-Lewis antibodies may be beneficial when Lewis antigen-negative red blood cell units are not available.

Furthermore, another area of medicine where anti-Lewis antibodies have been thought to be clinically insignificant is transplant medicine. However, kidney allograft rejections have been reported among Le(a-b-) phenotype recipients due to donor Lewis antigen incompatibility. [16][17] Liver allograft rejections have not been associated with the Lewis blood group system. [18] This is likely because the kidney contains higher concentrations of Lewis antigens for unclear reasons. Further investigation is needed among Le(a-b-) phenotype recipients to determine the clinical significance of anti-Lewis antibodies causing kidney allograft rejection. [1][2][3].

## References

1. Simon, Toby L., McCullough, Jeffrey, Snyder, Edward L., Solheim, Bjarte G., and Strauss, Ronald G., eds. Rossi's Principles of Transfusion Medicine. Hoboken: John Wiley & Sons, Incorporated; 2016, Pages 165-168.

2. Edited by Jeffrey McCullough. Transfusion Medicine 5th edition. Hoboken, NJ: Wiley-Blackwell; 2021.

3. Barbara J. Bain, Imelda Bates, and Michael A. Laffan. Dacie and Lewis Practical Haematology; Book, Twelfth Edition, 2017.

4. Harmening DM, Taghizadeh M: The Lewis System. In Harmening DM, (ed): Modern Blood Banking and Transfusion Practices. FA Davis, Philadelphia 1994, Pages 133-145.

5. Cooling L. ABO H, and Lewis blood groups and structurally related antigens. In: Roback JD, editor. American Association of Blood Banks Edition. Technical Manual. 17th ed. Bethesda, Maryland: AABB; 2011, Pages 376-378.

6. Rajeswari Subramaniyan. Serological characteristics of Lewis antibodies and their clinical significance – A case series. Hematology, Transfusion and Cell Therapy; 2021.

7. Höglund P, Rosengren-Lindquist R, and Wikman AT. A severe hemolytic transfusion reaction caused by anti-Le(a) active at 37 °C. Blood Transfusion; 2013, Volume 11, Issue 3, Pages 456-459.

8. Thakral, B., Jain, A., Saluja, K., Sharma, R.R., Singh, T.S.K. and Marwaha, N. Acute hemolytic transfusion reaction by Lea alloantibody. American Journal of Hematology; 2006, Volume 81, Pages 807-808.

9. Alexander A Delk, Richard R Gammon, Harold Alvarez, Nancy Benitez, and Frieda Bright. A Hemolytic Transfusion Reaction Caused by an Unexpected Leb Antibody; Laboratory Medicine, May 2021, Volume 52, Issue 3, Pages 303–306.

10. Irani MS, Figueroa D, and Savage G. Acute hemolytic transfusion reaction due to anti-Le(b). Transfusion; October 2015, Volume 55, Issue 10, Pages 2486-2488.

11. Virginia Duncan, Huy P. Pham, and Lance A. Williams III. A possible case of a haemolytic transfusion reaction caused by anti-Lea antibody. Blood Transfusion; 2015, Volume 13, Pages 535-536.

12. Fung, Mark K.; Eder, Anne; Spitalnik, Steven L.; Westhoff, and Connie M. Technical Manual. American Association of Blood Banks, 19th edition, October 3, 2017.

13. Hossaini AA. Neutralization of Lewis antibodies in vivo and transfusion of Lewis incompatible blood. American Journal of Clinical Pathology; April 1972, Volume 57, Issue 4, Pages 489-93.

14. Shulman, I.A. and Nakayama, R. The routine use of soluble Lewis antigens in the identification of Lewis system antibodies. Transfusion journal; 1993, Volume 33, Pages 37-41.

15. Das SS and Chakrabarty R. Optimization of blood safety through essential characterization of naturally occurring Lewis antibody. Global Journal of Transfusion Medicine; 2018, Volume 3, Pages 121-124.

16. Boratyńska M, Banasik M, Hałoń A, Patrzałek D, and Klinger M. Blood group Lewis alloantibodies cause antibody-mediated rejection in renal transplant recipients. Transplant Procedure; 2007, Volume 39, Issue 3, Pages 2711-2714.

17. Spitalnik S, Pfaff W, Cowles J, Ireland J, Scornick J, and Blumberg N. Humoral immunity to Lewis blood group antigens correlates with renal transplant rejection. Transfusion journal; 1983, Volume 23, Page 422.

18. Yevgen Chornenkyy, Alcino Pires Gama, Christopher Felicelli, Nigar Khurram, Adam L Booth, Joseph R Leventhal, Glenn Eugene Ramsey, and Guang-Yu Yang. Alloimmunization Against RBC Antigens Is Not Associated with Decreased Survival in Liver Transplant Recipients; American Journal of Clinical Pathology; March 2023, Volume 159, Issue 3, Pages 255–262.