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High thermal amplitude auto-anti-"N" complicating pretransfusion compatibility tests on a patient with malaria and bladder cancer

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Abstract:

BACKGROUND: Antigen "N" is a high-frequency antigen of the MNS blood groups and carried on glycophorin B that is resistant to enzymatic cleavage by trypsin, and provides differential diagnosis of its antibody specificity to N being present of glycophorin A. Naturally occurring IgM antibodies to N are known to be clinically insignificant, as against the IgG counterpart with clinical relevance.

AIM: Auto-anti-"N" association with the bladder cancer was explored for its clinical significance as well as its interference in grouping anomaly.

MATERIALS AND METHODS: A warm environment was created while blood sampling for the laboratory work up as the patient had a high-titer auto-cold agglutinin causing spontaneous hemagglutination. The antibody was tested by standard serological methods with the red cell, antisera, and enzymes prepared in house or obtained commercially.

RESULTS: The case was admitted to hospital with high fever and hematuria; he was diagnosed with malaria and bladder cancer. He required transfusions in the face of severe anemia. His blood sample posed problems in compatibility tests due to autoantibody present. Serological workup revealed its specificity as anti-"N."

CONCLUSION: Auto-anti-"N" as a cause of severe anemia could not be attributed to, for concurrent malarial infection. However, its presence may have some association with the underlying malignant condition.

Keywords:

Auto-anti-N, carcinoma of urinary bladder, high thermal amplitude, malaria infection

Introduction

Antigen "N" is a high-frequency antigen of the MNS (MNS 002) blood group system and assign the numerical term MNS 030 by the International Society of Blood Transfusion.[1] It is present in almost all individuals sparing rare individuals typed as S-s-U+ of African descent.[2] While the M and N antigens are carried on glycophorin A (GPA)[3] and are sensitive to cleavage by proteolytic enzymes such as papain, ficin, bromelain, trypsin, the antigens S, s, U, and "N" are situated on glycophorin B (GPB)[4] and are resistant to cleavage by trypsin,[5,6] the feature provides a differential diagnosis on antibody to N or "N" identification exercise.[6]

Although the most common form of anti-N is naturally occurring IgM antibody that is innocuous in nature,[7] its rare IgG counterpart could cause hemolytic transfusion reaction,[8,9] hemolytic disease of the fetus and newborn,[10] or autoimmune hemolytic anemia (AIHA)[11-14] indicating to its clinical significance. Irrespective of its clinical relevance, auto-anti-N with high thermal

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amplitude, had posed discrepant results in pretransfusion compatibility tests.[15,16] While there was no differentiation made on earlier reports as to whether the antibody involved was specific to N or "N" antigens, we describe here by the first time a case of an auto-anti-"N" causing ABO blood group discrepancy in a patient with malarial infection and bladder cancer.

Materials and Methods

The patient's blood specimens were collected in an anticoagulant ethylenediaminetetraacetic acid (EDTA) and/or without any anticoagulant (plain) for the study. As the patient's blood specimen showed overwhelming autoagglutination, elution, and absorption procedures were employed on the patient's red cells and serum respectively to perform blood grouping. For this, the patient's red cell suspension was kept in warm water bath (45°C) for 5 min with intermittent shaking, followed by washing twice using warm saline. This had helped in removing auto-antibody coat from red cells that had facilitated forward grouping. Autoabsorption of the patient's serum was carried out by incubating serum with autologous warm-saline-washed red cells. Two consecutive rounds of absorption, the first at room temperature (RT) and the second one at 4°C for 1 h each, completely removed auto-antibody that allowed performing reverse group and other serological work up without any hindrance. The same steps were used in absorption-elution experiments carried out on allogenic red cells as well.

Red cell panel used was of in-house source as well as of commercial origin (Ortho-Clinical Diagnostics, USA). Standard serological methods were employed throughout as per Bhatia.[17] The enzyme treatment to red cells was carried out by incubating the red cells with 1% papain solution in normal saline at 37°C, followed by three times washing with and suspended in normal saline. One drop of 10% NaCl solution was added to nine drops of 0.25% trypsin-EDTA reagent (Gibco, India) to make it isotonic. A volume the red cells were mixed with equal volume of trypsin solution, incubated at 37°C, washed by and suspended in normal saline. The immunoglobulin nature of the antibody in serum and eluate was determined by Dithiothreitol as has been described by Mollison *et al*.[18]

Clinical and other laboratory details were made available from the patient's medical record file.

Results

The case

Clinical details

The patient, an 80-year-old male, a smoker, was admitted to a city hospital with malarial fever (101°F) for 6 days, cough with white expectoration, abdominal pain, nausea, and painless hematuria. On examination, he was found to have pallor ++, no icterus, or edema. There was no organomegaly or ascites observed. X-Ray chest, kidneys, ureters, and bladder-intravenous pyelogram, kidney, and ureters-all were normal. However, bladder film showed an irregular filling defect on the left of midline, though the bladder margin was not altered. Ultrasonography showed normal liver, spleen, kidneys, seminal vesicles, and prostate. Full urinary bladder examination showed a presence of irregular papillary growth arising from the bladder neck just to the left of midline (28 mm × 19 mm × 12 mm thickness). An avascular tumor in the bladder was removed completely by transurethral resection (TUR). The histopathological examination showed grade II transitional cell carcinoma with focal inner muscle inversion suggesting to the stage B1.

Hematology parameters

The level of hemoglobin (Hb) was found as 5.6 g/dL. Peripheral blood film showed the abnormal red cell morphology with a presence of hypochromia, anisocytosis/poikilocytosis, ovalocytosis, tear drop cells, and the ring form of *Plasmodium falciparum* malaria parasite. Reticulocyte count was 11%. The differential white blood cell (WBC) percentage showed polymorphs, 80; lymphocyte, 16; eosinophil, 3; and monocyte one among the total WBCs count of 13,000/cu mm. Biochemical parameters such as blood urea, serum creatinine, bleeding time, clotting time, serum bilirubin, serum lactate dehydrogenase, urine albumin, and urine sugar were within the normal limit. The microscopic examination of urine showed a significant presence of pus cells (15–20) and red blood cells (>500) under the high-power resolution. A negative Widal test had ruled out the typhoid infection as a cause of the prevailing high fever. As the patient's Hb level was dropped to 4.7 g/dL in the following 2 days, blood transfusion was indicated. His blood specimen was referred to blood bank for necessary arrangement.

Immunohematological work up

He had no history of transfusion nor did he ever group. Pretransfusion blood specimen showed a presence of strong auto agglutination that had posed problem in grouping. In the forward grouping, strong red cell agglutination was observed with reagent antisera, and in the reverse grouping his serum showed a strong agglutination of groups A, B, and O red cells. Autocontrol test, by incubating his red cells with autologous serum, showed a weak agglutination at RT but strong agglutination at 4°C. A direct antiglobulin test (DAT) on his red cells was positive (+1). However, the results on the autocontrol test and the DAT could not be taken at face value since his red cells were already in

an agglutinated form. Processed in warm environment, his blood specimen was grouped as AB, Rh. D positive by forward and reverse grouping. He was transfused 6 units of homologous crossmatch compatible blood that went uneventfully with an increment of Hb to 9.3 g/dL.

Other serological features

The reaction pattern obtained on testing his serum with cell panel had suggested its specificity as anti-N. Further to this, his serum had agglutinated the red cells of 9 random donors who possessed N antigens but reacted weakly with the red cells of other 5 donors who lacked N, thus confirming its specificity as anti-N. This auto-anti-N eluted from the patient's red cells strongly agglutinated the red cells from the donors with M−N+ and M+N+ but weakly with M+N-phenotypes. Second, the patient's red cell typing, performed on his warmed-washed red cells, revealed his phenotype to be M+N+. The auto-antibody was an IgM immunoglobulin as his serum treated with 2-mercaptoethanol reagent lost its reactivity. The patient's serum titrated with red cells with M−N+ phenotype showed a high-titer value of 1:512 at RT and 1:2048 (at 4°C) by the saline tube test. The reactivity was significantly weakened when papain enzyme treated red cells were used in the test [Table 1].

In a separate experiment, the patient's serum was absorbed at 4°C using a donor's red cells with M+N− phenotype, and that had completely removed the autoantibody from his serum. The eluate prepared from these sensitized red cells did not react with the papain treated red cells from M+N− and M−N+ donors but had reacted with trypsin treated red cells thereby suggesting to its specificity as anti-"N" [Table 2].

The patient was treated with anti-malarial drug as well as TUR to remove the bladder tumor.

The follow-up studies were carried out after 2 weeks. His laboratory findings showed Hb 9 g/dL, with red cell morphology continued to show a presence of anisocytosis, hypochromia, poikilocytosis, ovalocytosis, tear drop cells, a weak positive DAT, presence of anti-N but with a negative autocontrol test and an absence of malaria parasites.

Discussion

MNS (ISBT MNS 002) system is a unique mix of two distinct categories of antibodies, namely, the "red cell immune" warm reacting clinically significant antibodies to the S, s, U antigens and the naturally occurring cold temperature reacting clinically insignificant antibodies to the M, N, and M^g antigens.[7] Naturally occurring anti-N in the presence of N antigen has also been reported among healthy individuals.[19] So also an innocuous high titer IgM auto-anti-N was found in an octogenarian with bladder cancer.[20] Clinically, significant auto-anti-N as a cause for AIHA has also been reported.[11-14]

Severe hemolytic anemia in the present case might not solely be attributed to autoimmune anti-"N" as the patient had concurrent malarial infection that could be one of the causes for clinical hemolysis.

The antigens M and N are carried on GPA[3] and are sensitive to cleavage by proteolytic enzymes including trypsin, while the antigens S, s, U, "N" are present on GPB[4] and are resistant to enzymatic cleavage by trypsin,[5,6] though they are sensitive to other enzymes mentioned. Auto-anti-N in the present case did not react with papain treated red cells but reacted strongly with the trypsin-treated red cells indicating to its specificity toward cryptic antigen "N" (ISBT 030).[5,6]

Innumerable cases of anti-N-like antibody, termed as anti-N^f, were reported among the patients undergoing hemodialysis.[16] Formaldehyde, used as sterilant to sanitize the dialysis equipment, was responsible to bring about antigenic change in the red cells present to stimulate an immune response to produce anti-N^f.[21] Our patient was not on hemodialysis but

Table 1: Titer values of autoantibody in the patient's serum against red cells with group O, NN

Test temperature	Test tech	Serum dilutions (starting from undiluted to serial double dilutions)											
		1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	4096	Titer
RT	Saline	+4	+4	+4	+4	+3	+2	+1	+w	0	0	0	512
	Pap	w	0	0	0	0	0	0	0	0	0	0	4w
4°C	Saline	+4	+4	+4	+4	+4	+3	+3	+2	+2	+1	0	2048
	Pap	+2	+w	0	0	0	0	0	0	0	0	0	8w

RT: Room temperature

Table 2: Results on eluate prepared from M−N+ red cells exposed to the patient's serum at 4°C (antibody eluted at 45°C)

Test cells phenotype M+N-			Test cells phenotype M−N+		
Untreated	Papain-treated	Trypsin treated	Untreated	Papain-treated	Trypsin treated
w	0	+2-1	+4	0	+4

Inference: anti-"N" specificity

had a habitual smoking. It has been reported that the tobacco smoke consists a variety of carcinogens including formaldehyde,[22] that is known to cause cancer in different organs including urinary bladder.[23] Furthermore, the patients with bladder cancer have auto-antibodies to tumor-associated antigens.[24] In this context, it is conceivable that our case with a smoking habit and a presence of auto-anti-"N" may have some bearing towards underlying pathogenesis of the bladder cancer. It is worth looking into the presence of anti-"N" among the people who are habitual smoker in general and those involved in formaldehyde industry. Increased risk of cancer among those who work in formaldehyde environment has been established.[22]

Conclusion

The patient's blood specimen showed a presence of strong auto agglutination that has posed problems in the forward and the reverse blood grouping. Auto-absorption of serum removed reactivity against A, B, and O red cells allowing interpretation of the reverse grouping. A positive autocontrol test and the DAT could not be validated in this case as the patient's red cells were already in an agglutinated form. Besides, hemolytic anemia could have been set because of the concurrent malarial infection and not necessarily due to AIHA caused by auto-anti-"N." However, the presence of auto-anti-"N" may have some relevance with underlying malignant condition.

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Conflicts of interest
There are no conflicts of interest.

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