

Mixed-field ABO front typing as an early sign of disease recurrence in ABO-matched stem cell transplantation

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ABO group testing is critical for allogeneic stem cell transplantation because mismatches can cause both transfusion and engraftment challenges. Even with ABO-matched donor-recipient pairs, ABO group determination may provide valuable insight into allograft status. Herein, we report a case of a 76-year-old female patient with myeloid neoplasm who underwent ABO-matched stem cell transplantation and in whom mixed-field ABO antigen expression during routine follow-up testing post-transplantation was the first sign of a change in transplant graft status; the mixed-field findings pre-dated changes in formal chimerism testing. This case underscores the potential of mixed-field ABO typing as an early indicator of disease recurrence in ABO-matched stem cell transplants and suggests that, in such cases, more sensitive forms of chimerism testing and/or closer monitoring for disease recurrence, particularly in the clinical setting of myeloid neoplasms, may be warranted. *Immunohematology* 2024;40:89–92. DOI: 10.2478/immunohematology-2024-013.

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ABO group testing in allogeneic stem cell transplantation is essential, since ABO mismatches can lead to challenges from both a transfusion support and cell engraftment standpoint. Notably, even in cases where there is no ABO mismatch between donor and recipient, determining the ABO group can potentially provide unique insight into the status of an allograft. Transplant recipients who are engrafting with donor cells (or losing their donor grafts) may demonstrate subtle but distinct changes in ABO expression through front and back typing results.

Herein, we report a case of a 76-year-old female patient who underwent an ABO-matched stem cell transplantation from a male donor for myeloid neoplasm. The most notable feature of this case is that mixed-field expression in ABO front typing during routine pre-transfusion compatibility testing several months after the transplantation was the first clinical sign of a change in transplant status. Formal chimerism testing

was performed on the patient using a somewhat insensitive assay examining only the presence or absence of X and Y chromosomes in the recipient.

Our group showed, through sensitive neoplastic molecular disease testing performed on current and retained specimens, that the appearance of mixed-field phenomena in ABO front typing clearly correlated with disease recurrence at the molecular level. The findings in this case also have broader implications, suggesting that the pursuit of more sensitive chimerism testing and/or evaluation for disease recurrence may be warranted when mixed-field reactivity or other ABO abnormalities are identified, even in the context of ABO-matched transplants, particularly for individuals with myeloid neoplasms.

Case Report

A 76-year-old woman with a medical history notable for cardiomyopathy, hypertension, and non-sustained ventricular tachycardia was admitted to the hospital with abdominal pain. She was diagnosed with diverticulitis and received treatment. During the admission, she was also investigated for anemia and leukopenia, which ultimately led to a diagnosis of myeloid neoplasm—specifically, myeloproliferative neoplasm, unclassifiable (MDS/MPN-U). She underwent chemotherapy followed by assessment for hematopoietic progenitor cell (HPC) transplantation.

The pre-transplantation bone marrow evaluation using next-generation sequencing (NGS) showed a *DNMT3A* nonsense variant (C861*) at 11 percent variant allele frequency (VAF). Karyotype analysis assay reported 6 of 15 cells with 11q deletion and a derivative chromosome 2.

After the myeloablation, she underwent allogeneic HPC transplantation from a male donor, and both the patient

and the donor had group A, D+ blood types. Although our center routinely gives blood that is compatible with both the recipient and the donor, in this case, no change in ABO group was expected, since the donor and recipient were ABO identical. The chimerism of the sex-mismatched transplant was followed using fluorescence in situ hybridization (FISH) for sex chromosomes (XY-FISH), since the donor was sex-mismatched. The initial FISH performed within 1 month of the transplantation showed that 98 percent of the 200 interphase cells scored had a male (i.e., donor) signal pattern, indicating successful engraftment. FISH, using dual color probes for the *CDKN2C* gene and the *KMT2A* gene, had the normal pattern of two fusion signals. Repeated FISH testing 4 months after the transplantation showed similar results with 100 percent donor myeloid cells. ABO and D typing and an antibody detection test were again performed, and the patient was typed as group A, D+ as expected.

Six months after the transplantation (Fig. 1), the patient's XY-FISH revealed a drop in her chimerism to 78 percent in male donor cells, although the myeloid cells were still 100 percent donor. Additionally, 21 percent of the cells had an abnormal pattern of one fusion signal with the *KMT2A* probe consistent with deletion of 11q. The NGS done concurrently had *DNMT3A* C861* present at 20 percent VAF.

Interestingly, new ABO typing results from a sample sent 1 week after the FISH showed mixed-field agglutination for A

of records and discussion with other team members led to the conclusion that mixed-field reactivity was likely an early sign of disease recurrence.

In light of these new findings, the 4-month FISH sample was analyzed with a second look, and the *DNMT3A* C861* variant was present below the reportable level of the assay. The variant was present in 9 of 420 sequencing reads (2% VAF) but was not officially reported because of the low read count and strand bias.

Chimerism testing was repeated in 3 months, which still revealed 100 percent male myeloid cells. The NGS showed *DNMT3A* C861* variant at 35 percent VAF, new *NRAS* G13V variant at 15 percent, new *PTPN11* S502A variant at 12 percent, and new *KRAS* Q61P variant at 3 percent. ABO front typing results remained consistently as mixed field. The patient died a few weeks later because of multiple complications of MDS aggressively turning into acute myeloid leukemia (AML).

Discussion

Chimerism testing for HPC transplantation shows variability across multiple platforms including methods such as short tandem repeat testing, FISH, NGS, and quantitative polymerase chain reaction.¹ NGS is one of the most sensitive testing modalities used by our institution in the post-transplantation period. In our case, the *DNMT3A* C861* mutation was not detectable at a reportable level until 6 months after the transplantation, which correlated with the timing of the ABO discrepancy. More importantly, the mixed-field reactivity in ABO front typing preceded FISH chimerism analysis in predicting disease recurrence, pointing out a disadvantage of FISH testing over its advantages, such as short turnaround time.

The differential diagnosis of mixed-field reactivity and front/back ABO typing discrepancies includes recent out-of-group transfusion, stem cell transplantation, A subgroup, fetomaternal hemorrhage, chimerism, hematologic malignancies, solid tumors, or immune disorders.^{2,3} In cases of myeloid malignancy, it is not uncommon to see mixed-field reactivity due to disappearing antigens.^{4–17} More specifically, disappearance of A with preservation or loss of H via different mechanisms has been documented in the literature.^{18–22} If A- and B-transferase enzymes are affected, H remains intact, but if fucosyl transferase is affected, then H production will decrease as well.^{20,23} In fact, loss of ABO antigens has been suggested to prompt a search for hematologic malignancy in the presence of underlying myelodysplasia.²² In contrast, RBCs

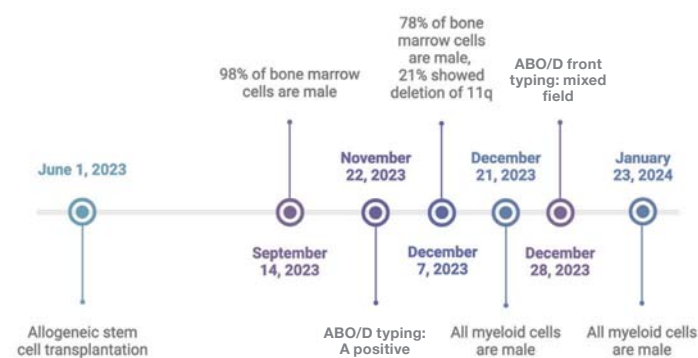


Fig. 1 Timeline of fluorescence in situ hybridization (FISH) and ABO/D typing results

on front typing. There were no recent red blood cell (RBC) or platelet transfusions, and both the recipient and the donor were originally group A, D+, as stated earlier. The antibody detection test was negative, and the last transfusion was 6 months previously. The case was brought to the medical director's attention to assess and resolve the discrepancy. Examination

in patients with solid tumors express normal amounts of ABO antigens, and testing reactivity is lost because of neutralization of testing reagents by the soluble A and/or B antigens secreted by the tumor.²⁰ Other mechanisms involved in antigen loss include loss of heterozygosity or DNA methylation.^{24–28}

Bianco et al.⁵ demonstrated by flow cytometry that, of 29 patients with AML and weakened A expression, 5 patients had lost H, 8 patients had intact H, and 3 patients had lost both H and A. Yoshida et al.²³ describe a case in which a patient had decreased expression of B with normal levels of H, suggesting that the mechanism of this patient's mixed-field reactivity was due to the blockage of conversion from H to B.²³ While there is a patient reported with *RUNX1* mutation possibly causing weakening of A expression,²⁹ a case series of six patients with AML and *RUNX1* and/or *GATA2* mutations did not seem to be associated with antigen expression.⁴ Case studies mention that ABO discrepancies often resolve when the underlying disease is in remission.^{10,11,17}

Some of the confounding factors in the investigation of the mixed-field reactivity in our patient included ABO subgroups and chimerism. A case study described a 70-year-old patient with no transfusion history whose RBCs typed as mixed field with reagent anti-A in her front typing while her back typing showed the presence of anti-B.³⁰ The patient was shown to have a genotype with *ABO*A2.01/O.01.0*. The majority of her RBCs were group O, while some RBCs showed chimerism with some A₂ component that likely belonged to a twin brother who died after birth. A similar case of a 24-year-old male individual also revealed chimerism in his RBCs with 52 percent group B RBCs and 48 percent group AB RBCs.³¹ Subgroups and chimerism are possible causes for mixed-field reactivity that are not associated with any specific diseases, and these cases illustrate possible confounding factors in our patient. However, there is no evidence of A subgroup or ABO chimerism in our patient, supporting the association between the mixed-field reactivity and disease recurrence.

In cases of stem cell transplantation like our patient, most ABO discrepancies are due to ABO-mismatched transplants. For example, a post-transplantation juvenile patient with myelomonocytic leukemia that showed 100 percent engraftment with conversion from group O to group AB later developed anti-A₂.³² This case did not have a longer follow-up, but the authors argued that this ABO grouping mismatch could represent disease recurrence, similar to previous literature describing ABO-mismatched stem cell transplantation cases. To our knowledge, our case is the first to describe new mixed-

field reactivity in ABO-identical stem cell transplantation that represented a sign of disease recurrence.

In light of the literature and cases discussed earlier, one could argue that failure of engraftment in our patient resulted in deactivation of ABO-related enzymes and loss of A, likely in RBCs of the donor. It is important to note that this is a report of a single specific stem cell transplantation case, and more comprehensive data are needed to draw generalizable conclusions. However, the clear correlation between the mixed-field A front typing and the timing of disease occurrence in our case suggests strongly that ABO discrepancies can be an early sign of recurrence, even in the ABO-identical stem cell transplantation setting.

Conclusion

Mixed-field ABO front typing can be an early indicator of recurrent disease in ABO-matched stem cell transplants for patients with MDS.

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References

1. Blouin AG, Askar M. Chimerism analysis for clinicians: a review of the literature and worldwide practices. *Bone Marrow Transplant* 2022;57:347–59. doi: 10.1038/s41409-022-01579-9.
2. Meny GM. Recognizing and resolving ABO discrepancies. *Immunohematology* 2017;33:76–81.
3. Qiu H, Wang X, Shao Y. Forward and reverse typing discrepancy and crossmatch incompatibility of ABO blood groups: cause analysis and treatment. *Hematology* 2023; 28:2240146. doi: 10.1080/16078454.2023.2240146.
4. Jeong IH, Seo JY, Choi S, Kim HY, Cho D. ABO blood group antigen changes in acute myeloid leukemia and no significant association with *RUNX1* and *GATA2* somatic variants. *Ann Lab Med* 2023;43:635–7. doi: 10.3343/alm.2023.43.6.635.
5. Bianco T, Farmer BJ, Sage RE, Dobrovic A. Loss of red cell A, B, and H antigens is frequent in myeloid malignancies. *Blood* 2001;97:3633–9. doi: 10.1182/blood.v97.11.3633.
6. Fathima S, Killeen RB. ABO typing discrepancies. Treasure Island, FL: StatPearls Publishing. Updated 17 December 2023. Available from <https://www.ncbi.nlm.nih.gov/books/NBK585061/>. Accessed 6 March 2024.

7. Chenna D, Mohan G, Reddy VR, Shastry S. The disappearance of blood group antigens: a clue to the clinical diagnosis of leukemia. *Transfus Apher Sci* 2019;58:48–9. doi: 10.1016/j.transci.2018.11.010.
8. Sarma R, Kuli K, Kalita C, Iqbal A, Gupta S. Loss of blood group antigens in haematolymphoid malignancy: a case series from a cancer institute from North East India. *Asian Pacific J Cancer Care* 2024;9:165–8. doi: 10.31557/APJCC.2024.9.1.165.
9. Elzein HO. Association of leukemia with ABO blood group distribution and discrepancy: a review article. *Cureus* 2024; 16:e56812. doi: 10.7759/cureus.56812.
10. Ting SC, Sainamthip P, Hsiao HH, Liu TC. Discrepancy of ABO typing in acute leukemia patients. *Kaohsiung J Med Sci* 2016;32:595–6. doi: 10.1016/j.kjms.2016.06.004.
11. Nambiar RK, Narayanan G, Prakash NP, Vijayalakshmi K. Blood group change in acute myeloid leukemia. *Proc Bayl Univ Med Cent* 2017;30:74–5. doi: 10.1080/08998280.2017.11929536.
12. Desai P, Naukudkar A, Rajadhyaksha S. ABO blood group discrepancies in blood donor and patient samples at a tertiary care oncology centre: analysis and serological resolution. *Hematol Transfus Cell Ther* 2023;S2531-1379(23)00168-2. doi: 10.1016/j.htct.2023.07.011.
13. Mishra D, Ray GK, Mahapatra S, Parida P. ABO typing error resolution and transfusion support in a case of an acute leukemia patient showing loss of antigen expression. *Cancer Transl Med* 2019;5:80–2. doi: 10.4103/ctm.ctm_28_19.
14. Shafiq M, Karim F. Red cell antigen loss in a patient with chronic myeloid leukemia: a case of ABO discrepancy. *Transfus Apher Sci* 2015;52:103–4. doi: 10.1016/j.transci.2014.11.004.
15. Van Loghem JJ Jr, Dorfmeier H, Van Der Hart M. Two A antigens with abnormal serologic properties. *Vox Sang* 1957;2:16–24. doi: 10.1111/j.1423-0410.1957.tb03429.x.
16. Cho J, Hur M, Moon H, Yun Y, Lee MH, Han S-H. Loss of red cell A antigen in a patient with acute myeloid leukemia: a case report and review of the literature. *Korean J Blood Transfus* 2011;22:277–83.
17. Grujić J, Bujandrić N, Malenković G. Resolving blood group discrepancy in a patient with acute myeloid leukemia: a case report. *Medicinski Pregled* 2022;75:314–6. doi: 10.2298/MPNS2210314G.
18. Miola MP, de Oliveira TC, Guimaraes AAG, Ricci-Junior O, de Mattos LC. ABO discrepancy resolution in two patients with acute myeloid leukemia presenting the transient weak expression of A antigen. *Hematol Transfus Cell Ther* 2024;46:85–8. doi: 10.1016/j.htct.2022.01.015.
19. Reid ME, Bird GW. Associations between human red cell blood group antigens and disease. *Transfus Med Rev* 1990;4:47–55. doi: 10.1016/s0887-7963(90)70247-7.
20. Winters JL, Howard DS. Red blood cell antigen changes in malignancy: case report and review. *Immunohematology* 2001;17:1–9.
21. Kuhns WJ, Oliver RT, Watkins WM, Greenwell P. Leukemia-induced alterations of serum glycosyltransferase enzymes. *Cancer Res* 1980;40:268–75.
22. Lopez M, Bonnet-Gajdos M, Reviron M, Janvier D, Huet M, Salmon C. An acute leukaemia augured before clinical signs by blood group antigen abnormalities and low levels of A and H blood group transferase activities in erythrocytes. *Br J Haematol* 1986;63:535–9. doi: 10.1111/j.1365-2141.1986.tb07531.x.
23. Yoshida A, Kumazaki T, Dave V, Blank J, Dzik WH. Suppressed expression of blood group B antigen and blood group galactosyltransferase in a preleukemic subject. *Blood* 1985;66:990–2.
24. Bianco-Miotto T, Hussey DJ, Day TK, O'Keefe DS, Dobrovic A. DNA methylation of the ABO promoter underlies loss of ABO allelic expression in a significant proportion of leukemic patients. *PLoS One* 2009;4:e4788. doi: 10.1371/journal.pone.0004788.
25. Orlov I, Lacombe L, Pellicer I, et al. Genotypic and phenotypic characterization of the histoblood group ABO(H) in primary bladder tumors. *Int J Cancer* 1998;75:819–24. doi: 10.1002/(sici)1097-0215(19980316)75:6<819::aid-ijc1>3.0.co;2-y.
26. Gao S, Worm J, Guldberg P, et al. Genetic and epigenetic alterations of the blood group ABO gene in oral squamous cell carcinoma. *Int J Cancer* 2004;109:230–7. doi: 10.1002/ijc.11592.
27. Chihara Y, Sugano K, Kobayashi A, et al. Loss of blood group A antigen expression in bladder cancer caused by allelic loss and/or methylation of the ABO gene. *Lab Invest* 2005;85:895–907. doi: 10.1038/labinvest.3700268.
28. Kronstein-Wiedemann R, Nowakowska P, Milanov P, et al. Regulation of ABO blood group antigen expression by miR-331-3p and miR-1908-5p during hematopoietic stem cell differentiation. *Stem Cells* 2020;38:1348–62. doi: 10.1002/stem.3251.
29. Hayakawa A, Sano R, Takahashi Y, et al. RUNX1 mutation in a patient with myelodysplastic syndrome and decreased erythrocyte expression of blood group A antigen. *Transfusion* 2020;60:184–96. doi: 10.1111/trf.15628.
30. Sharpe C, Lane D, Cote J, et al. Mixed field reactions in ABO and Rh typing chimerism likely resulting from twin haematopoiesis. *Blood Transfus* 2014;12:608–10. doi: 10.2450/2014.0261-13.
31. Miola MP, de Araujo C, Junior OR, de Mattos LC. Mixed field resolution in ABO phenotyping in a rare case of a blood donor with hematopoietic mosaicism. *Hematol Transfus Cell Ther* 2022;46:306–10. doi: 10.1016/j.htct.2022.08.006.
32. Hermelin D, Zhang D, Blackall D. Resolution of an unexpected ABO typing discrepancy in a 9-month-old patient with juvenile myelomonocytic leukemia. *Clin Case Rep* 2020;8:2358–60. doi: 10.1002/ccr3.3101.