EDITORIAL

Asia-type DEL in Serologic RhD-negative Blood Donors.

RHD and *RHCE* genes of the Rhesus system control the production of D antigen, and C, c, E, and e antigens, respectively. [1] Various genetic mechanisms: deleted *RHD* gene, commonly seen in Caucasians; silent mutations, prevalent in Asians; or a pseudogene that inactivates the *RHD* gene, observed among the Africans, resulting in the non-expression of D antigen and hence, being typed as RhD-negative [2]. Caucasians have approximately 15%RhD-negative individuals, while among Asians, only 0.1%–0.5% are RhD-negative. [3]. Similarly, Musa et al. (2023), identified 99% of blood donors in Malaysia are RhD-positive, with only 1% being RhD-negative. [4] Due to the diversity of *RHD*, alleles are categorized as D positive, weak D, partial D, and DEL based on their phenotypic behaviour and molecular structure. While weak D and partial D are identified utilizing the indirect antiglobulin test (IAT), DEL, being the weakliest expressed D, is detected by adsorption/elution techniques. [5] The DEL phenotype typically has 30 or fewer D antigens on the red cell membranes, compared to ~ 7000 for weak D and ~30,000 for normal D [6]. To date, 45 DEL alleles have been discovered and listed on https://www.rhesusbase.info/. Among Asians, 90% of the DEL is of *RHD*01EL.01 (c.1227G>A substitution*) allele, also referred to as Asia-type DEL. [7]. Serologic reagents capable of detecting DEL red cells are unavailable, leading to these cells being overlooked, and typed as RhD-negative. This mislabelling of red cells exhibiting DEL phenotype becomes problematic when anti-D antibody occurs in RhD-negative recipients receiving transfusions from DEL phenotype donors. Ramli S et al, (2018) identified 6.5% and 0.6% of the RhD-negative blood donors from the National Blood Centre, Malaysia, as having DEL and weak D phenotypes, respectively.[8]

Reported cases of anti-D alloimmunization:

a) Thongbut et al, (2023) [9] reported a 17-year-old male Thai patient, with a B RhD-negative blood type. His pretransfusion tests revealed no alloantibodies. He received 17 serologically RhD-negative blood units over seven months, and anti-D was detected before the 12th transfusion. Molecular analysis identified that 5 of the blood donors had the *RHD*01EL.01 (c.1227G>A)* or Asia-type DEL allele.
b) Suksard K, et al (2023) [10] reported the appearance of allo anti-D in 2 cases in Thailand. One case involved an elderly man, in his 70's, developing anti-D and anti-Mi^{a} alloantibodies six weeks after receiving RhD-negative red cells for a total of 10 units. One donor was identified to have Asia-type DEL. The next case involved a 38-year-old woman, undergoing surgery and transfused with 4 units of red cells and later developed anti-D alloantibody. Her antibody screening was negative before the transfusion. Two months later, she required a second surgery, and anti-D was detected during the pretransfusion testing. Investigations revealed that 1 out of the 4 RhD-negative units transfused had a positive adsorption/elution test and verified with molecular genotyping, to identify Asia-type DEL allele.
c) Kim K-H et al. (2009) [11] reported anti-D alloantibody identified in elderly, Korean man after receiving red cell unit transfusions from 4 serologically tested RhD-negative donors. Molecular techniques identified Asia-type DEL allele

(previously denoted as *RHD K409K*) in one of the donors. This case was the first among the Asian population where Asia-type DEL red cells induced primary alloanti-D immunization.

Given the potential for anti-D alloimmunization from DEL phenotype blood donors, should the Blood Transfusion Service perform screening or identification of the DEL phenotype?

Several issues must be considered when deciding on the implementation of additional tests:

a) **Enhanced Screening**: Should RhD screening tests include adsorption and elution tests as mandatory for every negative IAT test to detect the DEL phenotype? This test must be standardised and optimised because it may generate inaccurate findings. [9] In 2022, Malaysia's Health Director-General reported 737,554 blood donations. An estimated 7,375 (1%) donations were RhD-negative, representing the approximate number of blood donors who would need to be screened, thereby increasing healthcare costs for transfusion services.
b) **Inventory Management**: Should blood banks exclude DEL units from their RhD-negative inventory? Yes, due to their immunogenic potential to induce anti-D alloantibody.
c) **Feasibility Studies**: Additional research is necessary to determine the practicality of using adsorption/elution tests for detecting DEL phenotype, as this technique is very challenging.
d) **Molecular Methods:** More research is required on molecular methods, such as polymerase chain reaction-single specific primer (PCR-SSP), to detect the most frequent DEL allele in Asia. Should the strategy focus on molecular detection methods, or are adsorption/elution tests adequate?
e) **Rate of alloimmunization**: Further research is needed in determining the rate of anti-D development in recipients of DEL red cell transfusions.

Keywords: *Adsorption / Acid Elution method; DEL phenotype; RhD negative; RHD 1227 allele.*

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