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ORIGINAL ARTICLE

Alloimmunization Rates and Associated Factors in Transfusion-Dependent Patients: a Regional Study from Saudi Arabia

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SUMMARY

Background: **Blood transfusion is effective in treating and managing numerous medical conditions. However, repeated transfusions increase the risk of alloimmunization. This study aimed to determine the prevalence and risk factors associated with alloimmunization in transfusion-dependent patients in the Aseer province.**
Methods: **This study included 149 patients. Data were obtained retrospectively from medical records and include age, gender, diagnosis, blood group, Rh phenotype, presence of alloantibodies, and transfusion history.**
Results: **Out of the 149 patients, 78 (52.3%) were male and 71 (48.6%) were female, with a mean age of 24.58 ± 23.21 years (range 1 - 88 years). Alloimmunization was detected in 15 (10.2%) patients, predominantly in those with blood group O (8, 53%) and Rh-D positive status (12, 80%). A significant proportion (12, 80%) had received > 15 transfusion units per year. The most frequently identified alloantibodies were anti-E (4, 26%) and anti K (4, 26%). Alloimmunization was significantly associated with age, Rh-D blood group, and the number of transfusion units received ($p \leq 0.05$).**
Conclusions: **Transfusion-dependent patients are at increased risk of alloimmunization, particularly those with Rh-D positivity, older age, and a high transfusion burden. The risk of alloimmunization could be mitigated by implementing extended red cell phenotyping and matched transfusion strategies. These findings underscore the need for optimized transfusion policies to minimize alloimmunization-related complications in this vulnerable patient population.**
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INTRODUCTION

Transfusion of blood components is a crucial aspect of patient care in contemporary medicine, with transfusions being essential to treatment of many diseases, including hemoglobinopathies, hematologic malignancies, chronic renal failure, and solid tumors [1,2]. However, the therapeutic use of frequent transfusions may be accompanied by greater risk of complications related to alloimmunization to red blood cells (RBCs) [3].
Alloimmunization poses a significant challenge for those undergoing chronic transfusion therapy, as allo-

antibodies complicate the search for compatible blood types [4]. Previous studies have documented alloantibody presence in patients who have received multiple transfusions, a population that encompasses various conditions across diverse demographics, including sickle cell disease, thalassemia, chronic renal disease, and cancer. Besides red blood cell antigens, a number of risk factors contribute to alloantibody development, with key factors including gender, age, date of the initial blood transfusion, number of transfusion units received, race, genetics, and recipient inflammation status [5-7]. Among transfusion-dependent patient populations, the proportion of those who develop alloantibodies varies significantly depending on several factors, including ancestry, underlying conditions, and the specific protocols used for transfusions [1,2,8,9]. For instance, two studies conducted in India reported widely differing rates, with 3.2% and 7% of patients developing alloantibodies, respectively [1,9]. In contrast, a Greek study found a much lower rate of 1.16%, while a French study reported a remarkably high rate of 29.8% [6,8]. Within the Arab region, the alloimmunization rate among transfusion-dependent patients varies across countries, with Yemen reporting 6% [10], Sudan 21.8% [2], Egypt 9.1% [11], Oman 20% [12], and Kuwait 31.6% [13].

Several studies have documented region-specific alloimmunization rates among transfusion-dependent patients in Saudi Arabia, including Jeddah, Alahsa, and Jazan [14-17]. However, limited information is available about alloimmunization in the Aseer province. The aim of the present study was to complement previously published studies by identifying the rate of and risk factors associated with alloantibody development in patients receiving blood products in the Aseer province. The primary objective was to identify opportunities for enhancing blood transfusion practices to prevent formation of alloantibodies in non-immunized patients and further development of new alloantibodies in immunized patients. These enhancements will improve future treatment efficacy for blood-transfusion-dependent individuals.

MATERIALS AND METHODS

Data collection

We conducted a retrospective study by analyzing blood transfusion records for adult and pediatric patients who received transfusions between January and December 2023. Data for adult patients aged 16 years and older were collected from Aseer Central Hospital (ACH), while data for pediatric patients under 16 years old were obtained from Abha Maternity and Children Hospital (AMCH). Both hospitals are located in Abha City, within the Aseer province of Saudi Arabia.

The study included 149 patients, comprising 72 children and 77 adults. All patients were either diagnosed at or referred to ACH and AMCH, with diagnoses spanning a variety of conditions, including sickle cell disease, major β-thalassemia, hematological malignancies, solid tumors, hemolytic anemia, bleeding disorders, and bone marrow failure. These patients receive regular transfusions every 2 - 3 weeks in a daycare unit within the outpatient clinic of the respective hospital. To be included in the study, patients must have undergone at least two blood transfusions.

Exclusion criteria included pregnant women, patients not registered as permanent follow-up cases at the hospital, those without available clinical histories, and individuals with any autoimmune disease. Data extracted from medical records encompassed patient demographics (age and gender), diagnosis, and detailed hematological information such as blood group, Rh phenotype, presence of antibodies, and transfusion history.

Laboratory assays

Blood group testing was performed using column agglutination methods with a gel card system known as ID-Cards (Bio-Rad Laboratories, Inc., Hercules, CA, USA). ABO and Rh D blood grouping were performed using the ABO-Rh/Reverse Grouping ID-Card (BIO-RAD, Switzerland). Rh-negative patients were further typed for weak D using ID-Diaclon Anti-D (Bio-Rad). D, C, c, E, e, and K phenotyping were performed with full automation on an ORTHO AutoVue® Innova.

Antibody screening and identification were conducted using the indirect Coombs test, which involved hemagglutination in gel/filtration on BIO-RAD cards utilizing commercial cell panels with known antigen constituents. These included three cell panels for screening (Diacell, Bio-Rad) and an extended panel of 11 cells (ID-DiaPanel, Bio-RAD) for identification, which can be supplemented when necessary with an 11-cell enzyme-treated panel (papain and/or trypsin).

Transfusion policy

According to the policies of the Ministry of Health and American Association of Blood Banks [18], prior to a transfusion, plasma must be analyzed for the presence of new antibodies to RBC antigens. After crossmatching, all patients received ABO/RhD-compatible, phenotypically matched, and leucoreduced units. If a patient was found to have alloantibodies, a negative antigen unit was transfused.

Statistical analysis

GraphPad Prism (version 9.5.1) and Microsoft Excel 19 were utilized for statistical analysis and graph preparation. The chi-squared test and Fisher's exact test were employed to determine the significance of associations between variables, with a p-value ≤ 0.05 considered significant.

RESULTS

Characteristics of the study population

The study included 149 patients whose treatment plans required frequent blood transfusions. Patient ages ranged from 1 to 88 years, with a mean age of 24.58 ± 23.21. In total, 72 (48.3%) were children under 16 years old, while 77 (51.7%) were adults aged 16 years and older (Figure 1A). In terms of gender distribution, 78 patients (52.3%) were male and 71 (47.7%) were female (Figure 1B). Patients were diagnosed with a variety of conditions, including sickle cell disease (70 patients, 46.9%), hematological malignancy (23 patients, 15.4%), major beta-thalassemia (21 patients, 14%), solid tumor (16 patients, 10.7%), hemolytic anemia (9 patients, 6%), bleeding disorder (5 patients, 3.3%), and bone marrow failure (5 patients, 3.3%) (Figure 1C). Categorization of patients based on the number of units received per year revealed that 34 patients (22.8%) received fewer than 10 units annually, 41 patients (27.5%) received between 10 and 15 units, and the greatest proportion (74 patients, 49.6%) received more than 15 units per year (Figure 1D).

Distribution of ABO, Rh antigen, and Rh phenotypes in the study population

Next, we evaluated the ABO blood group distribution within the study population. The most prevalent was group O with 83 patients (55%), followed by group A with 42 patients (28%), group B with 17 patients (11.4%), and group AB with 7 patients (4.6%) (Figure 2A). An overwhelming majority of patients were Rh-positive (140, 94%), while just 9 (6%) were Rh-negative (Figure 2B).

With regard to Rh phenotypes in our study population, the most common was Ccee, found in 49 individuals (32%), followed by ccee and CCee, each present in 29 individuals (19.5%). The CcEe phenotype was observed in 16 individuals (10%); while ccEe was identified in 7 individuals (4.6%). Other phenotypes included CCEE in 4 individuals (2.6%), cceE and cCee in 3 individuals (2% each), and CCEe, cCEE, and ccEE in 2 individuals (1.3% each). The last three phenotypes, cCEe, CceE, and CcEE, were detected in just 1 individual (0.6% each) (Figure 2C).

Association of alloantibody production with patient demographic and hematological profile factors

All patients were tested for the presence of antibodies during each follow-up visit. Among the 149 patients, alloimmunization was detected in 15 (10.2%). Out of these, 3 cases (20%) were children, while 12 (80%) were adults. Alloimmunization prevalence was significantly higher in adults compared to children ($p \leq 0.05$), whereas the gender distribution was roughly equal, with 9 (60%) alloimmunized males compared to 6 (40%) alloimmunized females. Statistical analysis revealed no significant association of patient gender with development of alloantibodies ($p > 0.05$). With regard to the quantity of packed red blood cells transfused over a year, none of the patients who received fewer than ten transfusions developed alloantibodies. In contrast, among alloimmunized patients, only 3 (20%) received between 10 and 15 transfusions annually, while the majority (12, 80%) received more than 15 transfusions per year. Overall, patients who received more than ten units of packed red blood cells annually exhibited a significantly higher rate of alloimmunization ($p \leq 0.05$).

Among the 15 patients who developed alloantibodies, the ABO blood group distribution was as follows: group O, 8 patients (53%); group A, 5 patients (33%); group AB, 1 patient (7%); and group B, 1 patient (7%). No significant association was found between ABO blood group and development of alloantibodies; however, a significant difference was observed in relation to Rh status. Rh-positive patients accounted for the majority of alloimmunized cases, at 12 patients (80%), compared to only 3 Rh-negative patients (20%) ($p \leq 0.05$). The most common Rh phenotype among alloimmunized patients was Ccee, observed in 6 cases (40%), followed by ccee in 5 cases (33.3%), CCee in 2 cases (13.3%), and CcEe and ccEe in 1 case each (6.7%). However, no specific Rh phenotype was found to have a significant association with the rate of alloimmunization. The most prevalent condition among patients who developed alloantibodies was sickle cell disease, affecting 7 patients (46.7%), followed by hematological malignancies in 4 patients (26.7%), β-thalassemia major in 3 patients (20%), and solid tumors in 1 patient (6.6%) (Table 1).

Frequency of alloantibodies in transfusion-dependent patients

Among blood group systems, the Rh system accounted for the greatest proportion of alloantibodies, detected in 8 patients (53.3%). Antibodies to the Kell system came second, accounting for 4 patients (26.7%). Antibodies to Duffy, Kidd, and MNSs systems were each detected in 1 patient (6.7% per system). Regarding specific antibodies, anti-E and anti-K were the most frequently identified, each observed in 4 patients (26.7%). Anti-C and anti-c antibodies were detected in 2 patients each (13.3%), while anti-Fya, anti-Jka, and anti-s antibodies were each found in 1 patient (6.7%) (Table 2).

DISCUSSION

One serious complication of therapeutic blood transfusions is the development of alloantibodies [7,19]. In recent years, there has been considerable discussion about alloimmunization rates and the factors that influence them, with variation in alloimmunization statistics having been observed across regions and countries [1]. Therefore, this study aimed to assess the rate of alloimmunization and identify contributing factors among patients undergoing multiple transfusions in Asser province, Saudi Arabia. The study involved 149 transfusion-dependent patients, among whom 15 (10.2%) developed

Table 1. Association of demographic and hematological profile factors with alloimmunization.

Variable	Category	Alloantibodies n (%)		p-value
		positive 15 (10.2%)	negative 134 (89.8%)	
Age	< 16 years	3 (20%)	69 (51.5%)	0.020
	≥ 16 years	12 (80%)	65 (48.5%)	
Gender	male	9 (60%)	69 (51.5%)	0.531
	female	6 (40%)	65 (48.5%)	
Units received per year	< 10 units	0 (0%)	34 (25.3%)	0.026
	10 - 15 units	3 (20%)	38 (28.3%)	
	> 15 units	12 (80%)	62 (46%)	
Blood group	O	8 (53%)	75 (55%)	0.887
	A	5 (33%)	37 (27.5%)	
	B	1 (7%)	16 (11.9%)	
	AB	1 (7%)	6 (4.4%)	
	Rh positive	12 (80%)	128 (95.5%)	0.016
	Rh negative	3 (20%)	6 (4.5%)	
Rh phenotype	Ccee	6 (40%)	43 (32%)	0.726
	ccee	5 (33.3%)	24 (20%)	
	CCee	2 (13.3%)	27 (18%)	
	CcEe	1 (6.7%)	15 (11.2)	
	ccEe	1 (6.7%)	6 (4.5)	
Disease	sickle cell disease	7 (46.7%)	63 (47%)	0.675
	hematological malignancy	4 (26.7%)	19 (14.1%)	
	β-thalassemia major	3 (20%)	18 (13.5%)	
	solid tumor	1 (6.6%)	15 (11%)	

Table 2. Frequency of alloantibodies in transfusion-dependent patients.

System	n (%)	Alloantibody	Frequency n (%)
Rh	8 (53.3)	E	4 (26.7)
		C	2 (13.3)
		c	2 (13.3)
Kell	4 (26.7)	K	4 (26.7)
Duffy	1 (6.7)	Fya	1 (6.7)
Kidd	1 (6.7)	Jka	1 (6.7)
MNSs	1 (6.7)	s	1 (6.7)

RBC alloantibodies. This observed rate of alloantibody formation aligns with findings from multiple prior investigations in Saudi Arabia. For example, studies conducted in Jeddah on patients with sickle cell disease have reported rates of 12.8% [20] and 17.4% [21]. Likewise, a study on patients in Jazan with thalassemia and sickle cell disease revealed respective alloimmunization rates of 12.9% and 13.2% [17]. Among patients with sickle cell disease in the Eastern Province of Saudi Arabia, a prevalence of 13.7% has been reported [22]. Finally, for patients from the Al-Ahsa region with sickle cell disease and thalassemia, alloimmunization rates of 16.7% and 11.97% were observed [15]. One of the key distinctions between our study and previous research is that the majority of studies conducted in Saudi Arabia have focused on sickle cell disease, thalassemia, or

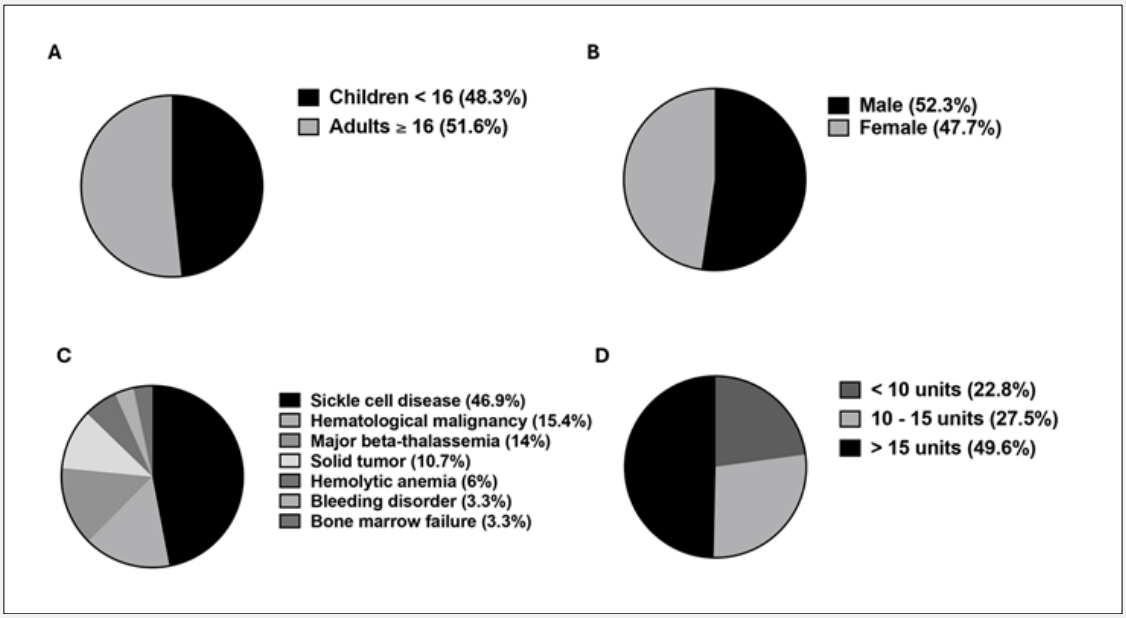

Figure 1. Pie charts showing age, gender, clinical diagnoses, and number of units received per year among the study population (n = 149 transfusion-dependent patients).

A) Age distribution. B) Gender distribution. C) Clinical diagnoses. D) Number of units received per year.

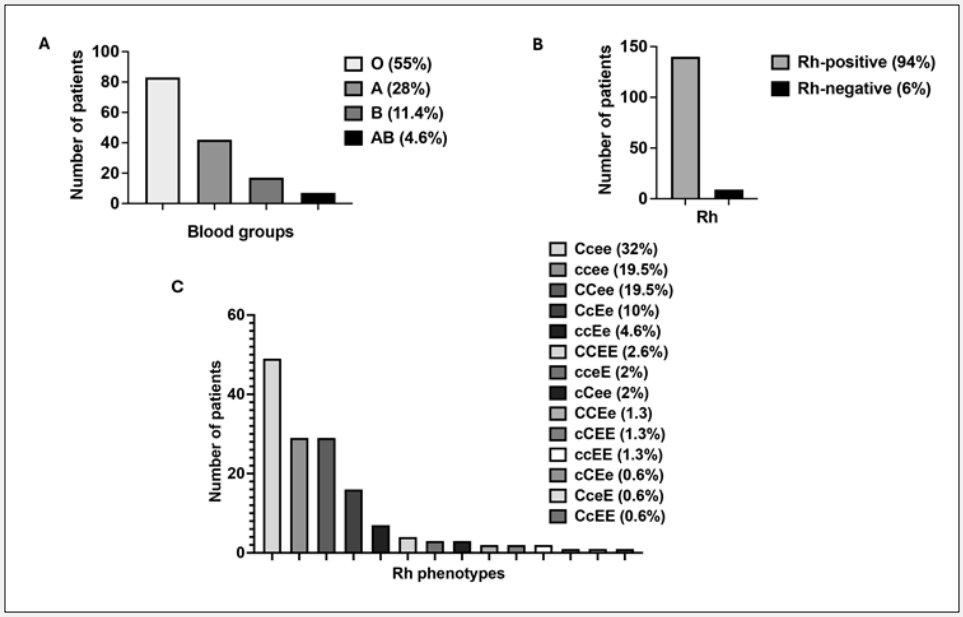

Figure 2. Column graphs showing the distributions of ABO blood group, Rh blood group, and Rh phenotypes in the study population (n = 149 transfusion-dependent patients).

A) ABO blood group. B) Rh-D blood group. C) Rh phenotypes.

both, whereas our study encompassed all patients requiring regular blood transfusions regardless of their underlying diagnosis. Alloimmunization rates have surged in several Arab countries, with reported rates of 31.6% and 20% among sickle cell disease and thalassemia patients in Oman [12], 23.6% among sickle cell disease patients in Kuwait [13], and 21.83% among transfusion-dependent patients in Sudan [2]. In contrast with these prevalence values, studies from India have observed rates ranging from 5.6% to 8.6% [23], with one study reporting a notably lower rate of 2.8% [1]. These disparities highlight the influence of regional and population-specific factors on alloimmunization prevalence. Compared to countries with fewer ethnicities among the population, alloimmunization rates are notably higher in countries with immigrants from differing ethnic backgrounds [2,6]. The relatively lower alloimmunization rate observed in this study may be attributed to a lack of routine post-transfusion screening for transient RBC alloantibodies and the racial similarity between donors and recipients, which likely resulted in greater RBC antigen compatibility. At the same time, many cases are transferred to ACH and AMCH after developing antibodies in other centers.

The majority of alloantibodies identified in our patient population were specifically directed against antigens within the Rh and Kell blood group systems. The high rate of Rh system alloimmunization is due to its strong immunogenicity compared to other blood group systems [24]. The most prevalent antibodies in our study were anti-E and anti-K, each detected in 26% of alloimmunized patients, with no cases of multiple alloantibodies observed. These findings are consistent with previous studies conducted across Saudi Arabia. For instance, a study from Jazan reported anti-E antibodies in 27% of participants and anti-K antibodies in 14% [17]. Similarly, anti-E and anti-K have twice been identified as the most common alloantibodies among patients in Jeddah, respectively observed in 32.4% and 21.6% [16] and 23.7% and 17% [21] of patients. In Al-Ahsa, anti-K and anti-E are also the predominant alloantibodies, detected in 35.7% and 30% of alloimmunized patients [15].

Our study also revealed a significantly lower alloimmunization rate in children (20%) compared to adults (80%), which aligns with several previous studies [12,25,26]. However, other reports have found no significant link between age and antibody formation [27-29]. Our interpretation is that whereas children in this study receive their complete treatment plan, from diagnosis to blood transfusions, at a single center, adults are often referred to ACH only after they have developed antibodies while being treated at other hospitals. Additionally, in our observations, alloimmunized children received an average of 14.6 units of transfused blood each year, while alloimmunized adults received an average of 23 units. This notable difference in transfusion volume may also explain the different rates of alloimmunization between children and adults.

In our study sample, alloantibody development was nominally higher in males (60%) than in females (40%), but alloimmunization rate did not exhibit a statistically significant difference with gender ($p > 0.05$). These findings are similar to a study conducted in Sudanese patients; [2] however, research from Greece [8] and Palestine [30] has also reported higher alloimmunization rates in females than in males. The authors attributed these higher rates to females having greater susceptibility for biological reasons, specifically exposure to foreign antigens during pregnancy.

We did observe a significant association between the quantity of packed red blood cells transfused and alloimmunization ($p \leq 0.05$), which aligns with previous reports [1,4,31,32]. These findings suggest that risk of alloantibody formation escalates with the number of blood units transfused to the patient.

With regard to the ABO blood group distribution in our study population, the proportions align with findings from another study conducted in the Aseer province [33]. Additionally, we found no statistically significant association between ABO blood group and alloimmunization, which is consistent with other reports [16,28]. However, a significant association was observed between Rh blood group and rate of alloimmunization ($p \leq 0.05$), which contrasts with previously published studies [21,28]. At the same time, we found no significant association between Rh phenotype and alloimmunization, similar to previously published data [34].

Regarding disease diagnosis, we found patients with sickle cell disease to have the highest rate of alloimmunization, with 46% affected. However, we did not find a statistically significant association between disease and alloantibody production. This elevated rate of alloimmunization among sickle cell patients likely reflects the significant prevalence of sickle cell diagnoses within our study population. It has previously been suggested that the increased alloimmunization rate in patients with sickle cell disease is primarily due to their frequent need for blood transfusions, as it is well established that alloimmunization rate rises with the frequency of blood transfusions [2]. Additionally, the short lifespan of red blood cells in patients with sickle cell anemia leads to an abnormal inflammatory condition, which can increase the immune response involved in production of alloantibodies [35].

Limitations

Although this study is limited by its relatively small sample size, the findings provide important insights into the factors driving alloimmunization in transfusion-dependent patients in the Aseer province. The use of retrospective data in this study resulted in some patients having missing or incomplete information; more particularly, there were gaps regarding dates of diagnosis and initial transfusion. Additionally, in some cases, patients received blood transfusions at facilities outside our centers, leading to a lack of information on the blood units transfused and the outcomes of post-transfusion tests.

CONCLUSION

This study examined the rate of alloimmunization in patients who depend on blood transfusions in the Aseer province. The results showed that several factors contribute to alloimmunization, particularly age, frequency of transfusions, and Rh blood group. To enhance quality of life for transfusion-dependent patients, it is essential to conduct antibody screening and identification each time they receive blood products. Notably, our findings revealed that the most common alloantibodies targeted the Rh and K antigens. This indicates that extended phenotyping for the Rh system (D, C, c, E, e) and Kell factor can help reduce the rate of future alloimmunization, prevent complications, and improve responses to blood transfusions. In addition, we recommend implementing a special protocol that considers the ethnic homogeneity between donors and recipients.

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Informed Consent Statement:
Patient consent was waived as the study was conducted retrospectively.

Data Availability Statement:
The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Declaration of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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