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INTERNATIONAL JOURNAL OF BIOMEDICAL LABORATORY SCIENCE

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The journal is intended to disseminate information and knowledge to the international laboratory community by accepting a variety of manuscripts for publication. Those manuscripts should be original research articles, literature or mini-reviews, case studies, brief communications and letters to the editor describing original investigations in all fields of biomedical laboratory sciences.

This journal is the ideal place for all Biomedical Laboratory Scientists, whether recognized experts in the field or starting their career, to publish their findings.

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Editorial

In this Issue: Hot Topics and Clinical Cases in Laboratory Medicine

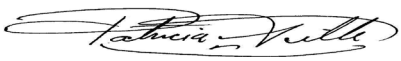
Patricia Tille Ph.D MLS(ASCP) AHI (AMT) FASCs
IJBLS Editor in Chief

This edition of the IJBLS centers on precision, safety, and stewardship in transfusion medicine. A topic that includes a timely review examining how expanding medical and recreational cannabinoid use intersects with blood product safety, especially for transfusion-dependent patients with sickle cell disease and cancer. The authors synthesize emerging evidence that tetrahydrocannabinol (THC) and cannabidiol (CBD) can perturb red cell integrity, platelet function, and coagulation, while noting a striking absence of standardized donor screening for cannabinoids, an actionable gap for laboratories and regulators alike.

Complementing this systems-level lens, a rare and compelling family case series charts three instances of hemolytic disease of the fetus and newborn due to anti-Rh17 alloimmunization, all managed through a rural–tertiary care partnership. The report underscores the power of early antibody identification, serial titers with MCA-PSV surveillance, strategic use of autologous and directed antigen-compatible units, and meticulous perinatal planning, practical lessons for laboratories that may face high-stakes immunohematology with limited resources. It's an instructive blueprint for coordination, inventory foresight, and use of reference laboratories when uncommon phenotypes and high-prevalence antigens collide.

Rounding out the issue, a focused review on machine learning (ML) shows how data-driven models can sharpen perioperative transfusion prediction, reduce unnecessary crossmatching, and align inventory with true patient need. Ensemble methods trained on large, diverse datasets routinely outperform traditional risk scores, yet adoption hinges on transparent models, rigorous external validation, and robust data quality across EHR system, reminding us that technical innovation must travel with implementation science.

Altogether, this demonstrates a need for an integrated future in laboratory medicine: evidence-based policies on novel donor exposures, nimble immunohematology workflows across care settings, and ML-enabled patient blood management that is as thoughtful as it is efficient.



Patricia Tille Ph.D. MLS(ASCP) AHI(AMT) FACSc

Research article

Anti-Malarial Bioactivity of *Garcinia kola* and *Vernonia amygdalina* Ethanolic Extracts in the Treatment of *Plasmodium berghei*-Infected Mice

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Malaria is one of the world's most serious infectious diseases caused by *Plasmodium* parasites. This research was aimed to determine the antimalarial bioactivity of *Gacinia kola* and *Vernonia amygdalina* ethanolic extracts in the treatment of malaria infection using an in vivo mouse model which was infected with *Plasmodium berghi*. The experiment was designed to assess the safety, the curative and prophylactic antimalarial activity of the individual extracts and the combined effect of the two extracts. Mice were evaluated using mean survival time, packed cell volume, rectal temperature and bodyweight. The percentage parasitemia suppression in mice treated with 200mg/kg, 400mg/kg and 600mg/kg of the *G. kola* nut ethanol extract demonstrated suppressive curative test was 40.14%, 45.98% and 61.82%, in four days, respectively. The statistical analysis indicates a significant difference when the mice were treated with different doses of the *G. kola* nut ethanolic extract. In comparison, the percentage parasitemia suppression in mice treated with 200mg/kg, 400mg/kg and 600mg/kg of the *V. amygdalina* leaf ethanolic extract suppressive curative test was 54.62%, 57.49% and 60.78%, in four days. However, there was no significant difference when the mice were treated with the different doses of the *V. amygdalina* leaf ethanolic extract. The study revealed that *G. kola* (nut and leaf), *V. amygdalina* (leaf and stem-bark) ethanolic extracts in the curative and prophylactic test group were effective in the treatment of malaria reducing the percentage parasitemia ≥30% in four days. This study observed that the extracts of *V. amygdalina* and *G. Kola* are potential sources of antimalarial compounds. Further evaluation of the clinical efficacy of these plant extracts in human volunteers is needed.

Keywords: Antimalarial activity, Efficacy, Ethanolic extracts, *Garcinia kola, Vernonia amygdalina*

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Introduction

Malaria remains one of the most serious global health problems, significantly contributing to morbidity and mortality, especially in endemic regions. In many African countries, malaria accounts for over 30% of outpatient visits and hospital admissions.[17] Despite efforts to control the disease, challenges remain due to the high costs of antimalarial drugs, which many Nigerians, particularly those in rural areas, cannot afford. Even for individuals who can afford these medications, safety concerns persist due to the increasing prevalence of drug-resistant strains of Plasmodium species, particularly to frontline antimalarial drugs like artemisinin derivatives.[2]

The management of malaria is further complicated by the absence of a clinically proven vaccine, insecticide resistance in mosquitoes, and the proliferation of fake drugs. In addition, current methods, such as indoor spraying with insecticides, are hampered by limited infrastructure and resources.[2] Moreover, artemisinin-resistant *Plasmodium falciparum* poses a significant threat to malaria control and elimination efforts.[2] Although chloroquine-resistant strains can still be treated with artemisinin derivatives, there is no approved alternative antimalarial drug to replace them if resistance becomes more widespread.

The impact of malaria extends beyond health, affecting the economy through lost working hours, healthcare costs, and reduced national productivity. Malaria is still endemic in over 100 countries, particularly in sub-Saharan Africa, Southeast Asia, and parts of Central and South America. Failure to adequately control malaria will undermine efforts to reduce poverty and childhood mortality in vulnerable communities.[17]

One of the most pressing challenges in malaria control is the emergence of resistance to frontline drugs, including artemisinin, which threatens recent progress in combating the disease.[2] In response, the scientific community is exploring new, affordable, and effective antimalarial agents from medicinal plants.[8] Historically, many conventional antimalarial drugs, such as artemether, chloroquine, and quinine, have been derived from plants or modeled on plant-derived compounds. Over 50% of modern clinical drugs have natural product origins, and medicinal plants play a crucial role in drug development.[7]

Traditional healers have long used plants to treat various infections, and herbal medicine, or phytomedicine, involves the use of seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. An impressive number of modern drugs have been isolated from natural sources, many of which were traditionally used by indigenous people.[3] In regions like Africa, Asia, and Latin America, herbal and traditional medicine is used to meet primary healthcare needs, with up to 80% of the population relying on traditional remedies. Even in industrialized nations, interest in "alternative medicine" has grown in recent years, with medicinal plants continuing to play an important role in health care.[3]

In vivo antimalarial testing typically involves rodent-specific parasites such as *Plasmodium berghei*, *P. yoelii*, and *P. chabaudi* in mice, which assess the development of parasitemia after treatment.[11] While these models may not perfectly replicate human *P. falciparum* infections, they are essential for developing antimalarial drugs. Researching traditionally used medicinal plants like *Garcinia kola* and *Vernonia amygdalina* is crucial for discovering new antimalarial compounds that could contribute to future treatment options.

Methodology

Description of Study Area

The fresh nut and leaf of *Garcinia kola* and *Vernonia amygdalina* plants were collected based on the ethnobotanical description and with the help of a taxonomist and local traditional healers in their natural habitats in Kurmi LGA of Taraba State. Kurmi is located between latitude 6^0 30' and 9^0 36'N and longitude 9^0 10' and 11^0 50'E. Kurmi is bounded in the west by Donga and Takum LGA and on

the east by Gashaka LGA. It is bounded by Bali LGA on the northern part, Ussa LGA on the western part and Sardauna LGA on the southern part. The climatic weather is wet and there is dry and rainy seasons. The soil is generally sandy-loam. Soil color ranges from grayish-brown to brown and it is well drained. It is a high forest region with dense grasses and many tall trees. Most of the residents of Kurmi are Tigun, Ndola and Ichen by tribe and the majority are farmers.

Experimental Design

The fresh nut and leaf of *G. kola and V. amygdalina* plants were collected, washed and air dried then packaged. The *G. kola* and *V. amygdalina* plant samples collected were identified and authenticated with a voucher number 02380 and 02006 respectively by a taxonomist with the Department of Plant Science, Ahmadu Bello University Zaria. The plant screening test was conducted by a laboratory technologist in the chemistry lab of Ahmadu Bello University Zaria, Kaduna State. Male and female (non-pregnant) mice of bodyweight 20g to 35g were purchased at Animal House, National Veterinary Research Institute Vom, Plateau State. The mice were allowed to acclimatize in the Infectious Diseases Research Laboratory, Modibbo Adama University, Yola. The acclimatization was for fourteen days during which they were fed standard rodents' feed (Finisher) and tap water. Then, the mice were equally divided (5 mice/group). And the average weights of the mice in the test group were measured and used to calculate the dosage of plant extract to be administered to the mice.

A total of 140 mice were used used in the curative test. The mice were grouped into seven groups each containing five mice for the treatment group. While for the prophylactic group, the mice were grouped into four groups each containing five mice. In all cases of the plant extract, administration was performed by compulsory oral intubations with the aid of cannula and syringe. The caring and experimental use of the mice during this experiment was performed according to the guidelines recommended by the Center for Drug Evaluation and Research.[5]Parasitemia for both curative and prophylactic tests, synergism potentials of the plant extract, body weight, temperature and packed cell volume (PCV) of the experimental animals were observed and recorded.

Collection and Preparation of Plant Materials

The fresh leaf and stem-bark of *G. kola and V. amygdalina* plants were collected based on the ethnobotanical description and with the help of a taxonomist. The plant samples were cleaned from extraneous materials by carefully washing with clean water, air-dried under a shade at room temperature then cut and reduced to appropriate size. Following cutting the samples were manually ground to powder with a mortar and pestle. The powdered preparations were stored a sterile plastic dish for further use.

Ethanol Extraction of plants

Powdered (100g) of the *G. kola and V. amygdalina* plant samples were macerated with 100 ml of 80% ethanol for 72 hours with intermittent agitation. The supernatant from the agitated material was filtered with 15 cm Whatman grade1 filter paper two times. The filtrate of *G. kola* plant samples were then concentrated using a rotary evaporator (BUCHI R- 250, Switzerland) at 40^{o}C to remove the ethanol. The dried extract were stored at −20°C until used.

Source of Experimental Parasite

The *Plasmodium berghei* clones used in this study were obtained from the National Institute for Pharmaceutical Research and Development, Abuja. The parasites were chloroquine-sensitive ANKA clone phenotypes and were maintained by serial passage in mice intraperitoneally.

Qualitative phytochemical screening Tests

Ethanol extracts of *G. kola* were screened for the presence of secondary metabolites. Thus, tests for alkaloids, flavonoids, terpenoids,

phenols, tannins, saponins, anthraquinones and cardiac glycosides was performed in the chemistry laboratory, Ahmadu Bello University Zaria; using standard test procedures.[9]

Quantitative phytochemical Analysis

Quantitative phytochemical analysis was carried out to determine the quantity of alkaloids, tannins, saponin, flavonoids, phenols and terpenoids

Antimalarial Activity Testing

Inoculation of Mice with Parasites and Extract administration

P. berghei was obtained from a donor mouse. The parasitemia of the donor mice was first determined. A blood sample was collected and diluted with 0.8% normal saline based on the level of parasitemia of the donor mice and the red blood cell count of normal mice, standardizing he volume of 1ml blood containing approximately 5 $\times 10^7$ infected red blood cells of *P. berghei*.. Hence every 0.2 ml of the aliquot should contain about $1x10^7$ *P. berghei* infected red blood cells. Each mouse that was used in the experiment was inoculated with 0.2ml of the infected blood sample containing about $1x10^7$ *P. berghei* ANKA strain intraperitoneally by using a hypodermal needle.

All the extracts were administered using a standard intragastric tube to ensure safe ingestion of the extracts and the drug.[5] Treatment started on day 4 and continued daily for an additional four days (i.e. from day 4 to day 8) and blood samples were collected at day 9, and examined for parasitemia. PCV, body weight, and rectal temperature were examined at pre-infection (PI) day, day 4 and day 9 for all the groups.

Blood samples were collected from all mice in the different groups at day 9, and examined for parasitemia. PCV, body weight, and rectal temperature were examined at pre-infection (PI) day, day 4 and day 9 for all the groups.

Microscopic Examination of the Parasite

On the 9th day (Day-9), 24 hours after the final dose of extract, a blood sample was collected from a tail snip of each mouse.[1] The smears were applied on microscope slides, fixed with absolute methanol for 15 minutes and stained with 15% Giemsa stain at pH 7.2 for 15 minutes. The stained slides were then washed gently using distilled water and air-dried at room temperature. Each stained slide was examined under a microscope with the oil immersion objective of 100X to evaluate the percentage suppression of each extract for the treated and control groups. The parasitemia level was determined by counting a minimum of five fields per slide with 100 RBC in a random field of the microscope. Percentage parasitemia and the percentage of suppression were calculated and recorded.

Determination of Body Weight and Rectal Temperature

The body weight of each mouse in all groups was determined before infection on the first day or pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment) using a sensitive weighing balance (METTLER TOLEDO, Switzerland). The rectal temperature of the mice was measured with a digital thermometer on pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment). The % change in body weight and rectal temperature was calculated and recorded.

Determination of Packed Cell Volume (PCV)

Packed cell volume (PCV) was measured to predict the effectiveness of the test extract and fractions in preventing hemolysis resulting from increasing parasitemia associated with malaria. Blood was collected from the tail of each mouse in heparinized micro hematocrit capillary tubes. The capillary tubes were filled with blood up to 3/4th of their volume and sealed. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettich hematocrit, Germany) with the sealed ends outwards and centrifuged for 5 min at 11,000 rpm. PCV is a measure of the proportion of RBCs to plasma on pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment).[15]

Ethical Considerations

Approval for the study was obtained from Modibbo Adama University, Yola Ethics and Research Committee.

Statistical Analysis

Results were analyzed using SPSS version 24. Comparisons were made between the negative control, positive control (chloroquine) and treatment groups at the various doses. The significance of disparity was determined using a 1-way analysis of variance (ANOVA) and the quantity of the phytochemical quantitative analysis was expressed as Mean ± Standard Error Mean of 3 replicates while the % parasitemia were analyzed and expressed as Mean ± Standard Error mean of 5 replicates, also the mean survival time (MST), the PCV, rectal temperature and bodyweight differences were analyzed and recorded ± Standard Error of the mean of 5 replicates; Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

Results

The phytochemical qualitative analysis of Garcinia kola (leaf and nut) ethanolic extracts revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids in both plant parts (Table 1). Similarly, Vernonia amygdalina (leaf and stem-bark) ethanolic extracts contained alkaloids, phenols, saponins, tannins, and terpenoids, but flavonoids were absent in the stem-bark (Table 2). The quantitative phytochemical analysis of G. kola revealed that the leaf extract contained higher concentrations of alkaloids (2.87 ± 0.03 mg/100g), flavonoids (1.38 ± 0.01 mg/100g), phenols (3.01 ± 0.04 mg/100g), saponins (3.90 ± 0.02 mg/100g), and terpenoids (2.64 ± 0.03 mg/100g) compared to the nut extract, while tannins were slightly higher in the nut extract (0.72 ± 0.01 mg/100g) (Table 3). For *V. amygdalina*, the leaf extract had a higher concentration of alkaloids (6.51 ± 0.03 mg/100g), flavonoids (2.87 ± 0.03 mg/100g), phenols (3.24 ± 0.03 mg/100g), saponins (4.32 ± 0.04 mg/100g), while the stem-bark extract contained higher levels of tannins (2.86 ± 0.01 mg/100g) and terpenoids (0.28 ± 0.02 mg/100g) (Table 4).

Table 1. Phytochemical qualitative analysis of *G. kola* (nut and leaf) ethanolic extracts

Phytochemical	*Garcinia kola*	
	Leaf	Nut
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+

+ (Present) and - (Absent)

Table 2. Phytochemical qualitative analysis of *V. amygdalina* (leaf and stem-bark) ethanolic extracts

Phytochemical	*V. amygdalina*	
	Leaf	Stem-bark
Alkaloids	+	+
Flavonoids	+	-
Phenols	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+

+ (Present) and - (Absent)

Table 3. Phytochemical quantitative analysis of *G. kola* (leaf and nut) ethanolic extracts expressed as (mg/100g)

Phytochemical	*Garcinia kola*	
	Leaf	Nut
Alkaloids	2.87 ± 0.03[b]	0.63 ± 0.03[a]
Flavonoids	1.38 ± 0.01[b]	0.47 ± 0.01[a]
Phenols	3.01 ± 0.04[b]	0.13 ± 0.02[a]
Saponins	3.90 ± 0.02[b]	2.70 ± 0.03[a]
Tannins	0.43 ± 0.01[a]	0.72 ± 0.01[a]
Terpenoids	2.64 ± 0.03[b]	0.74 ± 0.03[a]

Values were expressed as Mean ± Standard Error mean of 3 replicates. Mean values with different supercripts in the same row differ significantly at $p<0.05$.

Values were expressed as Mean ± Standard Error mean of 3 replicates. Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

Table 4. Phytochemical quantitative analysis of *V. amygdalina* (leaf and stem-bark) ethanolic extracts expressed as (mg/100g)

Phytochemical	*V. amygdalina*	
	Leaf	Stem-bark
Alkaloids	6.51 ± 0.03[a]	3.04 ± 0.03[b]
Flavoniods	2.87 ± 0.03[a]	2.43 ± 0.04[a]
Phenols	3.24 ± 0.03[a]	3.02 ± 0.04[a]
Saponins	4.32 ± 0.04[a]	2.67 ± 0.02[b]
Tannins	1.37 ± 0.04[a]	2.86 ± 0.01[b]
Terpenoids	0.46 ± 0.05[a]	0.28 ± 0.02[b]

Values were expressed as Mean ± Standard Error mean of 3 replicates. Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

The curative effect of *G. kola* nut ethanolic extract on parasitized mice showed a decrease in packed cell volume (PCV) by day 4 post-infection, followed by a partial recovery by day 9. Mice treated with 600 mg/kg of the extract experienced a 20.30% improvement in PCV, whereas the untreated infected group (Group F) had a drastic reduction of 41.50% (Table 5). Similarly, body weight changes revealed a decline by day 4, with partial recovery by day 9 across treated groups, except for the untreated infected group, which exhibited a significant weight loss of 14.96% (Table 6). The rectal temperature of infected mice showed fluctuations, with some treatment groups exhibiting reductions, while others remained stable (Table 7). For *G. kola* leaf ethanolic extract, PCV also dropped by day 4 but showed recovery by day 9, with the highest improvement of 17.92% observed in the group receiving 200 mg/kg of the extract (Table 8). The weight of treated mice slightly declined by day 4, but by day 9, there was a moderate recovery in most groups, with the untreated infected group experiencing the highest weight loss of 12.17% (Table 9). The rectal temperature followed a similar trend, with some treated groups showing a slight decline and others stabilizing (Table 10).

Table.5. Effect of parasite and *G. kola* nut ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	42.66±1.28[a]	32.78±1.41[b]	36.63±1.63[b]	14.14
Group B	400	41.42±2.26[a]	33.16 ±0.89[b]	35.76±1.35[b]	13.66
Group C	600	42.66 ±0.70[a]	30.35 ±1.14[b]	34.00±0.90[b]	20.30
Group D	200	42.12±1.28[a]	25.62 ±0.73[d]	35.88±0.84[c]	14.15
Group E	200	41.72±0.84[a]	41.84±0.52[a]	41.92±0.55[a]	-0.48
Group F	0	42.46 ±0.65[a]	30.20 ±0.50[b]	24.84±0.62[d]	41.50
Group G	0	41.50±0.75[a]	41.76 ±0.81[a]	41.80±0.80[a]	-0.72

Note: Values were expressed as Mean ± Standard Error mean of 5 replicates. Mean values with different superscripts in the same column differ significantly at $p<0.05$. PI stand for (Pre-infection; 24 hours before infection); D4 (Day 4; Post-infection) and D9 (Day 9; 24 hours after treatment).

Table 6. Effect of parasite and *G. kola* nut ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in
		PI	D4	D9	Body weight
Group A	200	30.78±1.34[a]	27.90±1.08[a]	29.43±1.30[a]	4.39
Group B	400	31.78±1.25[a]	29.60 ±1.29[a]	30.80±1.12[a]	3.08
Group C	600	31.70±1.75[a]	29.80±1.59[a]	31.00±1.75[a]	2.21
Group D	200	30.00±0.60[a]	27.74±0.79[a]	28.92±0.53[a]	3.60
Group E	200	30.96±0.48[a]	31.00±0.18[a]	31.30±0.58[a]	-1.10
Group F	0	30.22±1.18[a]	27.10 ±1.49[a]	25.70±1.29[b]	14.96
Group G	0	30.80± 0.84[a]	29.48±0.43[a]	29.82±0.40[a]	3.18

Table 7. Effect of parasite and *G. kola* nut ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	35.10±0.60[a]	36.50 ±0.35[a]	35.17±0.41[a]	-0.19
Group B	400	34.90 ±0.48[a]	36.56 ±0.79[a]	34.96±0.39[a]	-0.17
Group C	600	34.70±0.65[a]	38.40±0.49[a]	36.60±0.84[a]	-5.48
Group D	200	35.80±0.60[a]	7.84±0.49[a]	6.54±0.53[a]	-2.07
Group E	200	35.42±0.62[a]	37.30±0.76[a]	37.32±0.46[a]	-5.36
Group F	0	38.06±0.75[a]	38.98±0.21[a]	38.72±0.30[a]	-1.94
Group G	0	35.92±0.53[a]	36.50 ±0.58[a]	36.28±0.58[a]	-1.00

Table 8. Effect of parasite and *G. kola* leaf ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	40.60±0.48[a]	31.20±0.84[b]	33.78±0.37[b]	17.92
Group B	400	38.04±0.29[a]	28.44±0.95[b]	32.06±0.54[b]	15.72
Group C	600	42.12±1.00[a]	33.26±2.35[b]	37.80±1.20[b]	10.26
Group D	200	41.90±0.07[a]	31.38±1.77[b]	36.46±0.39[c]	12.98
Group E	200	42.86 ±0.79[a]	42.92±1.15[a]	41.88±0.94[a]	2.29
Group F	0	42.28±1.02[a]	31.26±1.55[b]	24.78±0.86[c]	41.39
Group G	0	42.56±0.61[a]	41.62±0.58[a]	41.50±0.40[a]	2.49

Table 9. Effect of parasite and *G. kola* leaf ethanolic extract on Body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	31.06±1.32[a]	28.08±0.90[a]	30.32±0.45[a]	2.38
Group B	400	29.36±1.26[a]	27.36±1.17[a]	28.70±1.18[a]	2.25
Group C	600	32.36±1.61[a]	30.16±2.80[a]	31.20±2.00[a]	3.58
Group D	200	30.46±1.44[a]	27.88±1.43[a]	29.18±1.27[a]	4.30
Group E	200	30.04±1.21[a]	30.16±1.64[a]	30.44±1.26[a]	-1.33
Group F	0	30.74±1.48[a]	28.54±1.39[a]	27.00±1.30[a]	12.17
Group G	0	29.62±0.73[a]	28.72±0.63[a]	28.04±0.51[a]	5.33

Table 10. Effect of parasite and *G. kola* leaf ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	36.04±0.50[a]	38.04±0.06[a]	37.36±0.26[a]	-3.66
Group B	400	35.22±0.41[a]	37.14±0.18[a]	35.52±0.56[a]	-0.85
Group C	600	35.70±0.36[a]	8.50±0.19[a]	36.08±1.89[a]	-1.06
Group D	200	36.12±1.06[a]	38.78±0.44[a]	36.96±0.83[a]	-2.33
Group E	200	35.52±0.54[a]	7.24±0.43[a]	36.88±0.52[a]	-3.83
Group F	0	35.54±0.99[a]	39.94±0.28[a]	38.76±0.35[a]	-9.06
Group G	0	36.24±0.38[a]	36.44±0.14[a]	36.72±0.22[a]	-1.32

The curative potential of *V. amygdalina* leaf ethanolic extract was also assessed, where PCV decreased post-infection and improved after treatment, with the highest recovery of 15.60% observed at a 600 mg/kg dose. Meanwhile, the untreated infected group experienced a significant PCV reduction of 48.77% (Table 11). The body weight of treated mice showed minor fluctuations, while the untreated infected group lost about 11.92% of its body weight (Table 12). The rectal temperature of treated groups remained relatively stable, whereas the untreated infected group exhibited a notable decline (Table 13). Also, PCV levels declined significantly in infected untreated mice (Group F), with a 49.53% reduction, while

treatment with 200–600 mg/kg of *V. amygdalina* stem-bark extract resulted in a lower percentage decrease (Table 14). Similarly, body weight reductions were observed in all treated groups, but the untreated infected group (Group F) showed the highest weight loss of 9.02% (Table 15). Rectal temperature fluctuations followed a similar trend, where infected untreated mice exhibited the most significant drop in temperature (-8.47%), while treated groups maintained relatively stable temperature levels (Table 16).

Table 11. Effect of parasite and *V. amygdalina* leaf ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	43.72±0.65^{a}	33.30±0.48^{b}	37.06±0.54^{b}	15.23
Group B	400	42.48±0.74^{a}	31.96±0.53^{b}	35.90±0.56^{b}	15.49
Group C	600	42.70±0.57^{a}	32.86±0.70^{b}	36.04±0.72^{b}	15.60
Group D	200	43.08±0.61^{a}	31.22±0.75^{b}	37.12±0.75^{c}	13.83
Group E	200	42.00±0.61^{a}	42.14±0.54^{a}	43.18±0.38^{a}	-2.81
Group F	0	42.12±0.41^{a}	31.78±0.89^{b}	21.58±0.60^{c}	48.77
Group G	0	42.96±0.96^{a}	42.62±0.88^{a}	43.08±0.54^{a}	-0.28

Table 12. Effect of parasite and *V. amygdalina* leaf ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	29.79±0.89^{a}	28.18±0.74^{a}	29.44±0.90^{a}	1.31
Group B	400	31.47±1.12^{a}	29.76±1.17^{a}	30.47±1.05^{a}	3.18
Group C	600	30.14±1.44^{a}	28.39±1.47^{a}	29.67±1.25^{a}	1.56
Group D	200	29.62±1.42^{a}	27.47±1.41^{a}	28.47±1.21^{a}	3.88
Group E	200	29.80±0.83^{a}	30.33±1.26^{a}	30.87±1.03^{a}	-3.59
Group F	0	29.44±1.03^{a}	27.00±0.99^{a}	25.93±0.85^{a}	11.92
Group G	0	30.61±0.80^{a}	29.27±0.66^{a}	29.53±0.49^{a}	3.53

Table 13. Effect of parasite and *V. amygdalina* leaf ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (^{0}C)			% change in Temperature
		PI	D4	D9	
Group A	200	35.70±0.56^{a}	36.49±0.40^{a}	36.27±0.57^{a}	-1.59
Group B	400	35.17±0.31^{a}	36.74±0.24^{a}	35.27±0.41^{a}	-0.28
Group C	600	35.69±0.97^{a}	35.37±0.55^{a}	34.09±0.65^{a}	4.48
Group D	200	36.01±0.93^{a}	37.99±0.35^{a}	36.81±0.68^{a}	-2.22
Group E	200	35.46±0.48^{a}	37.89±0.68^{a}	36.81±0.23^{a}	-3.81
Group F	0	37.22±0.90^{a}	38.50±0.43^{a}	38.61±0.32^{a}	-3.73
Group G	0	36.32±0.34^{a}	36.84±0.16^{a}	36.63±0.23^{a}	-0.85

Table 14. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	41.70±0.64^{a}	32.64±1.01^{b}	36.84±0.53^{b}	11.65
Group B	400	41.40±0.48^{a}	30.78±0.82^{b}	35.68±1.25^{b}	13.82
Group C	600	42.16±0.68^{a}	34.24±1.41^{b}	37.62±0.92^{b}	10.77
Group D	200	42.10±1.05^{a}	31.38±0.87^{b}	36.22±0.89^{c}	13.97
Group E	200	42.44±0.83^{a}	42.22±0.56^{a}	41.14±0.53^{a}	3.06
Group F	0	42.28±0.65^{a}	31.26±0.51^{a}	21.34±0.60^{d}	49.53
Group G	0	40.64±0.23^{a}	40.84±0.34^{a}	40.52±0.63^{a}	0.30

Table 15. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	30.93±0.12[a]	27.78±0.95[a]	29.40±1.33[a]	4.95
Group B	400	28.77±1.18[a]	26.61±1.14[a]	27.89±1.21[a]	3.06
Group C	600	32.00±1.39[a]	29.76±1.26[a]	30.83±1.41[a]	3.66
Group D	200	29.86±0.63[a]	27.12±0.78[a]	28.53±0.59[a]	4.45
Group E	200	29.14±0.47[a]	29.22±0.55[a]	29.50±0.47[a]	-1.22
Group F	0	30.38±1.06[a]	28.22±1.35[a]	27.64±1.85[a]	9.02
Group G	0	28.84±0.72[a]	28.38±0.41[a]	28.02±0.35[a]	2.84

Table 16. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	36.00±0.53[a]	37.89±0.25[a]	37.24±0.36[a]	-3.44
Group B	400	35.71±0.42[a]	37.54±0.59[a]	35.93±0.36[a]	-0.62
Group C	600	35.93±0.56[a]	38.60±0.42[a]	36.24±0.46[a]	-0.86
Group D	200	36.16±0.52[a]	38.69±0.50[a]	36.98±0.55[a]	-2.27
Group E	200	35.78±0.38[a]	37.46±0.12[a]	37.23±0.26[a]	-4.05
Group F	0	35.42±0.47[a]	38.90±0.22[a]	38.42±0.30[a]	-8.47
Group G	0	35.91±0.48[a]	36.39±0.52[a]	36.52±0.54[a]	-1.70

The parasitemia suppression in infected mice treated with *G. kola* ethanolic extracts and standard drugs varied across different concentrations. Treatment with 400 mg/kg of *G. kola* nut ethanolic extract resulted in the highest parasitemia suppression of 45.98%, which was significantly higher than the combination of 200 mg/kg of *G. kola* nut extract with 200 mg/kg of *G. kola* leaf extract (40.69%). Meanwhile, treatment with 400 mg/kg of *G. kola* leaf extract alone yielded a lower suppression rate of 36.44% (Figure 1). Similarly, the parasitemia suppression in mice treated with *V. amygdalina* ethanolic extracts

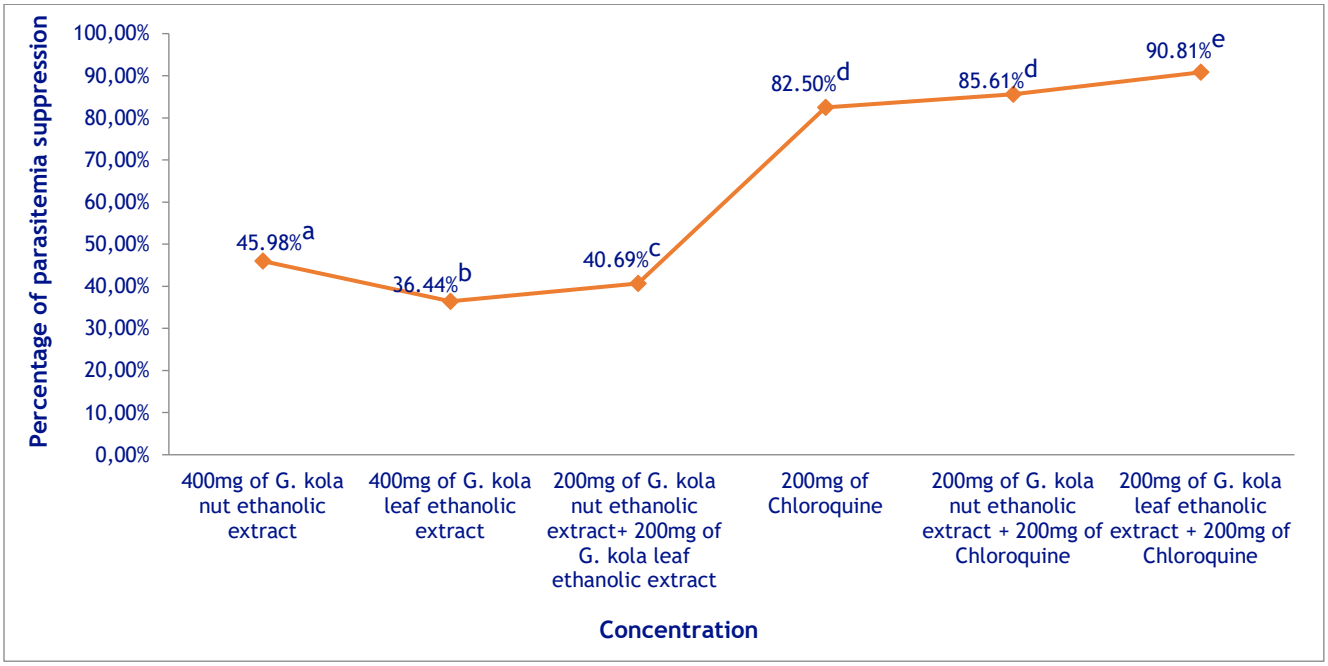

Figure 1. Percentage parasitemia suppression in infected mice treated with single and combined doses of *G. kola* ethanolic extracts and standard drug

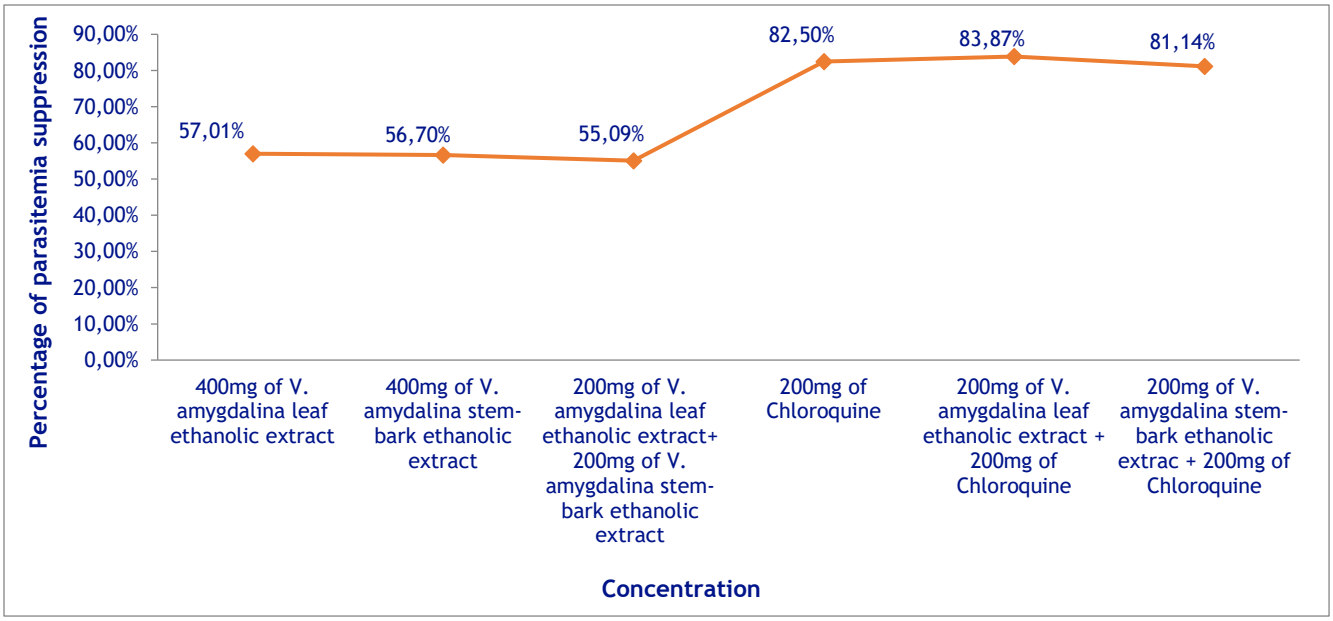

Figure 2. Percentage parasitemia suppression in infected mice treated with single and combined doses of *V. amygdalina* ethanolic extracts and standard drug

showed a slight variation in effectiveness. Administration of 400 mg/kg of *V. amygdalina* leaf extract resulted in 57.01% suppression, which was insignificantly higher than the combination of 200 mg/kg of *V. amygdalina* leaf extract with 200 mg/kg of *V. amygdalina* stem-bark extract (55.09%). Additionally, treatment with 400 mg/kg of *V. amygdalina* stem-bark extract produced a suppression rate of 56.09%, which was also insignificantly higher than the combined 200 mg/kg leaf and stem-bark extract treatment (55.09%) (Figure 2).

Discussion

The present study examined various major classes of phytochemical compounds; it was determined that alkaloid, flavonoids, phenols, saponins, tannins, terpenoids and steroids were present in *G. kola* (nut and leaf), and *V. amygdalina* (leaf and stem-bark) ethanolic extract. These bioactive substances may be responsible for antimalarial activity; the therapeutic and prophylactic efficacy of the two plants evaluated in this research.[1] The two plants possess different classes of phytochemicals such as alkaloids, terpenoids, saponins, and flavonoids and demonstrated anti-plasmodial activity through various mechanisms as previouslyreported.[18] The choice of using organic (ethanol) solvent in this study was based on reports that organic solvents yield more bioactive substances than the aqueous extraction due to an increase in solubility in organic solvents.[6] This implies that organic solvents are good alternatives in evaluating antimalarial plant properties as they can extract a wide spectrum of chemical constituents.

The result of the study showed a significant reduction on the PCV of the infected experimental mice in the curative test groups when observed at day four after infection. After infecting mice with malaria, the host (mouse) demonstrates a reduction in PCV. The underlying cause of this PCV reduction could be loss of infected erythrocytes through parasite maturation, destruction of uninfected red cells in the spleen and liver by macrophage activation and enhanced phagocytosis, reduced erythropoiesis and dyserythropoiesis. However, there was an increase in PCV when extracts at varying concentrations of the four different extracts was administered to the curative test group for four days after infection. This implies that the extract significantly prevented PCV reduction when compared to the negative control, as seen in previous studies.[4,15]

Furthermore, it was observed that there were no reasonable changes in the level of PCV in experimental mice in the prophylactic test group when the mice were not infected with parasites but were administered an extract for four days at a varying concentration of the different extracts used for this study. Unlike the curative group, on the other hand, the prophylactic model did not prevent a reduction in PCV, but the extract-treated group exhibited an improvement in prevention against PCV reduction. This agrees with the findings that ethanolic extracts do not prevent reduction PCV values.[15] However, the finding of this study differed from the research which indicated that the mice treated with the highest dose of the extract (600mg/kg) and the group administered the standard drug (Chloroquine) showed a high increase in PCV on the fifth day.[12] The difference in this finding could be attributed to the differences in the administered concentration of the extracts.

Body weight is another feature used to assess malaria infection in experimental mice. The result of the study showed a slight decrease in the body weight of the infected experimental mice in the curative test groups when observed for four days after infection. Body weight loss in extract-treated mice might be due to a depressing effect as a result of the increment of parasitemia. However, based on the result of the present study, there was a slight increase in body weight when extracts at varying concentrations of the four different extracts were administered to the curative test group for four days following infection with parasites. Even though, the bodyweight gained in the curative test animals for all doses, this could be due to the effect of the ethanolic extract which decreases the parasitemia on established infection since the inoculum was given three days prior to treatment. The finding of this study is similar to previously reported reported that mice treated with crude extracts showed a lower body weight reduction as compared with the non-treated.[12]

Rectal temperature is also an important feature used to assess malaria infection in experimental mice. The result of the study showed a slight increase in the rectal temperature of the infected experimental mice in the curative test groups when observed four days after infection. The finding in this study is related to the finding reported that the extract-treated mice demonstrate an increase in the prevention on rectal temperature reduction than normal control animals even though it was not statistically significant.[1] Active compounds should be able to prevent the rapid dropping of rectal temperature because decrease in internal body temperature will reduce the metabolic activity of the laboratory mice.

Furthermore, the result of this study revealed that there were no reasonable changes in rectal temperature of the experimental mice in the prophylactic test group when the mice were not infected with parasite but were administered with the four different extracts. This implies that the different doses (200mg/kg, 400mg/kg and 600mg/kg) of the two plant extracts (*G. kola* and *V. amygdalina*) significantly protected the decrease in rectal temperature associated with *P. berghei* infection in mice. This indicates that the extracts prevent some pathological processes that can cause the reduction in internal body temperature. Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice. An ideal antimalarial agent obtained from a plant is expected to prevent anemia, body weight loss and regulate temperature in infected mice.

The result of this study revealed that 200mg/kg of *G. kola* nut + 200mg/kg of *G. kola* leaf ethanolic extracts demonstrated a higher percentage of parasitemia suppression than when 400mg/kg of *G. kola* leaf extract was administered alone. Chloroquine administered at 200mg/kg demonstrated a higher percentage parasitemia suppression when in combination with 200mg/kg *G. kola* leaf extract compared to when chloroquine was administered at 200mg/kg alone. The finding of this

study differs from the report that indicated chloroquine is more effective in combination with extract than independently.[10] The possible reason for the difference may be, the use of an aqueous extract of the plant used as an adjuvant with chloroquine. In the present study, the solvent used for the plant extraction was ethanol. Combination therapies are a vital strategy to prevent or delay resistance of parasites and have been approved for other multidrug-resistant infections.

The finding of this study revealed that there were no reasonable changes in the level of percentage parasitemia suppression when 400mg/kg of *V. amygdalin* leaf extract, 400mg/kg of *V. amygdalina* stem-bark extract and 200mg/kg of *V. amygdalina* leaf extract in combination with 200mg/kg of *V. amygdalina* stem-bark extract was administered to the infected mice. This study differs from previous studies that indicated very high parasite growth inhibition at two different doses (200mg/kg and 200mg/kg) of the combined extract of *C. citratus* and *V. amygdalina*. The reason for the difference may be due to the individual or synergistic effect of the two different plants.[14] The result of this study revealed that combining two or more plant parts did not connote higher efficacy compare to when only a single part was used. However, in some situations combining two or more plant parts demonstrated higher efficacy compared to when only a single part was used. These two scenarios indicate that combination therapy of the plants extract provided a synergistic reactions and antagonistic reactions in different scenarios.

Conclusion

This study indicates that the extracts of *V. amygdalina and G. kola* are potential sources of antimalarial compounds. It was observed that the plant extracts used in these studies had a curative antimalarial effect attributed to the presence of bioactive compounds present in the plants. It was also demonstrated that the plant extracts prevent anemia, body weight loss, regulate temperature and improve the mean survival time of the infected mice. Hence this implies that the extract has an important ingredient that is needed for the treatment of malaria. Anemia, body weight loss and body temperature reduction are the general features of malaria. The results of this study could help encourage more identification and validation of natural products, thus facilitating the development of a new generation of antimalarial drugs. Collaboration between the natural product scientists and the traditional healers could be of immense help and assist in the administration of the right doses of antimalarial compounds to avoid the risk of toxicity that may result from these herbal remedies.

Recommendations:

i. There is a need to promote the agricultural production of *G.kola* and *V. amygdalina* plants.
ii. Improve collaboration between traditional medicine and modern medicine.
iii. There is a need to repeat similar studies in a complex immune-compromised mouse model that has been developed to sustain *P. falciparum*-parasitized human erythrocytes *in vivo*.
iv. Evaluation of the *G.kola* and *V. amygdalina* plant extracts on different *Plasmodium* species and animal models are needed to better identify potential antimalarial activity.
v. Further pharmacological screening with bioassay-guided chemical fractionations of the crude extracts should be conducted to isolate the specific active ingredients responsible in the plant.
vi. Elucidating the structure and mechanism of action of the active ingredients of *G.kola and V. amygdalina* plant extracts is also recommended.
vii. There is a need to determine the clinical efficacy of *G.kola* and *V. amygdalina* plant extracts in human volunteers.

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Review article

Impact of Cannabinoids on Blood Product Safety: Risks and Challenges in Transfusion Medicine

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As cannabis use continues to rise, particularly involving tetrahydrocannabinol (THC) and cannabidiol (CBD), new concerns are emerging in transfusion medicine. Legalization in many U.S. states has significantly increased both recreational and medical cannabinoid use. Research suggests that cannabinoids may alter blood function, posing potential risks for transfusion-dependent patients with sickle cell disease (SCD) and cancer. These patients are frequently prescribed cannabinoids for pain and often receive blood transfusions, raising concerns about additional risk from exposure to cannabinoids present in transfused products. Despite improvements in donor screening for infectious diseases, no protocols exist to detect cannabinoid use in blood donors. This represents a critical gap in transfusion safety.

THC may contribute to red cell hemolysis, enhance platelet aggregation, and increase thrombotic risk, while CBD may inhibit platelet function and disrupt coagulation. These pharmacologic effects may compromise transfusion safety in high-risk groups. Although the long-term impact of cannabinoid exposure in transfusion medicine remains unknown, evidence supports the need for immediate investigation. With no existing guidance from the Food and Drug Administration (FDA), interdisciplinary collaboration is essential to assess risks and develop appropriate screening measures to ensure blood product safety.

Keywords: Cannabinoid exposure; Transfusion Medicine; Hematologic Complications

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Introduction

Blood transfusion is fundamental to modern healthcare, supporting the treatment of anemia, hematologic disorders, trauma, and malignancies. The effectiveness of transfusion medicine relies on rigorous donor screening, comprehensive infectious disease testing, and meticulous blood product handling.[1] These measures have significantly enhanced patient outcomes; however, emerging challenges, particularly the increasing prevalence of cannabinoid use, are raising concerns about blood product safety at both national and state levels.[2,3,4] Historically, cannabis was restricted due to its classification as a Schedule I substance;[5] however, legislative shifts have led to increasing medical prescriptions and recreational legalization in multiple states, necessitating an evaluation of its implications for transfusion medicine.[6,7,8]

For transfusion-dependent patients, including those with sickle cell disease (SCD) and cancer, minor coagulation imbalances can have serious clinical consequences.[7,9] In patients with SCD, tetrahydrocannabinol (THC)-induced red blood cell (RBC) membrane instability may impair oxygen transport, potentially worsening vaso-occlusive crises (VOCs), a painful complication common in SCD.[7,10,11] Similarly, in cancer patients receiving anticoagulant therapy, cannabinoid-induced changes in platelet function and clotting factor activity may elevate thrombotic or hemorrhagic risks.[9,12] Recent clinical findings associate cannabis use in SCD patients with increased VOC-related hospitalizations, suggesting worsening disease severity rather than symptom relief.[11,13] Furthermore, cannabis users in transfusion-dependent populations exhibit higher polypharmacy rates compounding transfusion safety concerns due to drug interactions affecting platelet function and coagulation.[9,12]

The legalization of cannabis in multiple states has led to a rise in both medical prescriptions and recreational use, influencing various sectors of healthcare, including transfusion medicine.[14,15] Given the increasing medical and recreational use of cannabis, its presence in the blood donor pool raises critical concerns regarding transfusion safety.[15] Cannabinoids such as THC and cannabidiol (CBD) have gained prominence due to evolving legal frameworks and shifting public perceptions.[4] As more individuals use cannabis-based therapies or recreational products, an increasing number of blood donors present with detectable levels of these compounds. Recent data suggest that up to 13.8% of blood donors may have used cannabis within 72 hours before donation, revealing a critical gap in screening protocols that primarily focus on infectious agents and immunologic compatibility rather than drug-induced hematologic alterations.[3,4]

Unlike rapidly metabolized compounds, cannabinoids accumulate in adipose tissue, allowing THC and CBD to persist in circulation for days or even weeks after use.[16] This prolonged release into the bloodstream raises concerns that transfused blood products may contain residual cannabinoid metabolites, which could contribute to hematologic instability in recipients, particularly those with preexisting coagulation disorders.[16] This prolonged exposure raises concerns that residual cannabinoid metabolites could persist in stored blood products and, upon transfusion, interact with recipient physiology, particularly in individuals with preexisting hematologic disorders.[16,17]

Recent research highlights growing evidence that cannabinoids influence blood components.[3,7,13] THC disrupts RBC membrane integrity, making cells more fragile and prone to hemolysis.[3,13] Additionally, THC induces platelet hyperreactivity, increasing the risk of thrombosis.[3,17] In contrast, CBD inhibits platelet aggregation, which may elevate bleeding risks.[18,19]

Beyond cellular effects, cannabinoids influence coagulation pathways.[17,18] THC exposure has been associated with increased thrombin generation, whereas CBD inhibits fibrin clot formation by altering clotting factor synthesis and activity.[18,19] These disruptions to

coagulation homeostasis raise concerns about transfusion recipients, particularly those using cannabinoids for symptom management, facing increased clotting or bleeding risks.[18,19]

Despite growing evidence of these risks, transfusion protocols lack standardized screening measures for cannabinoid exposure.[15] Additionally, limited longitudinal data on cannabinoid persistence in stored blood products create gaps in understanding post-transfusion effects.[20] Mechanistic studies indicate that cannabinoids induce oxidative stress, alter membrane integrity, and impact coagulation, yet few clinical trials have systematically assessed these findings in transfusion-dependent populations.[21] Implementing mass spectrometry-based detection methods could enhance screening precision by quantifying THC and CBD metabolites in donor blood, ensuring transfusion safety.[20,21,22]

The implications of cannabinoid exposure extend beyond donor eligibility, affecting blood product integrity and recipient safety.[2,13] As medically prescribed THC and CBD use continues to rise, transfusion-dependent patients—particularly those relying on cannabinoids for symptom management —may face increased transfusion-related risks.[2,11,17] The persistence of cannabinoid metabolites in donor blood introduces potential hematologic alterations, which could compromise transfusion efficacy, particularly in vulnerable populations with coagulation imbalances and oxygen transport deficiencies.[10,11,13]

Addressing the risks of cannabinoid exposure in transfusion medicine requires immediate updates to donor screening protocols and transfusion safety policies.[15,23] As the use of cannabinoids increases due to shifting legal and medical landscapes, the potential impact on blood products must be critically evaluated. Future research must prioritize the development of standardized cannabinoid screening measures and risk mitigation strategies to prevent transfusion-related complications and ensure the safety of blood products for all recipients. Cannabinoids alter hematological parameters, necessitating transfusion protocol updates to ensure blood product safety.[3,13,15,23]

Background

The medical practice of blood transfusion provides essential support for treating patients who face blood loss conditions alongside anemia and malignancies and other forms of hematological disorders.[24,25,26] Transfusion therapy has three main purposes: it advances oxygen delivery while providing immune system support along with maintaining blood clotting function primarily for surgeries, traumatic injuries and people who live with SCD or have cancer that requires long-term transfusions.[26] The safety of transfusions remains constant through extensive donor screening combined with blood collection practices alongside laboratory work and donor-receiver compatibility testing under regulatory institutions which minimize both infection transmission during transfusion and adverse reactions.[27]

The advancement of transfusion medicine occurred through both the discovery of scientific knowledge and solutions to newly emerging public health threats. Research into ABO/RH blood group identification methods eliminate life-threatening immune reactions against transfused blood.[1,24] The combination of plastic blood storage bags with anticoagulants and refrigeration technology permits prolonged blood product storage time.[24,28] Pathogen reduction technologies and nucleic acid testing became necessary after human immunodeficiency virus and acquired immunedeficiency syndrome (HIV/AIDS) appeared in the 1980s and the hepatitis C epidemic appeared in the 1990s.[29] Safety failures from the past demonstrates the requirement for strong safety measures and constant oversight of blood collection techniques as well as transfusion administration procedures.[28,30]

Donor screening operates as the core element in transfusion safety because it ensures that only individuals who meet eligibility criteria are permitted to donate blood.[23] The eligibility assessment for becoming a donor

includes standards for age within 18–65 years and body weight greater than 50 kg (110 lb.) and either female or male donor requirements for hemoglobin amounts set at 12.5 g/dL for females and 13.0 g/dL for males.[23] Physical assessments together with health questionnaires evaluate individual medical conditions that would be detrimental to the donor and prevent disease transmission to a recipient during transfusion processes. A review of patients' travel activities helps detect relevant infections like malaria or Zika virus postponing the donation process temporarily.[28,31] Modern nucleic acid testing (NAT) has become the standard procedure for discovering infectious agents including HIV, hepatitis B and C, syphilis, Zika virus, and West Nile virus.[36] Testing and handling protocols have evolved to such an extent that developed countries estimate HIV and hepatitis transmission occurs in only one case out of multiple million transfused products.[24,28] Donating blood is not possible for pregnant individuals and the exclusion extends to six weeks after childbirth.[28]

Blood donation policies receive regular updates through new scientific findings and epidemiological pattern changes.[32] The blood donation policies include permanent exclusion criteria for those with chronic health issues and transfusion-transmissible infections including HIV along with hepatitis B and C, syphilis, and human T-cell lymphotropic virus (HTLV).[33] More recently, the focus has expanded to include pharmacological contaminants, particularly cannabinoids, due to concerns about the potential impact on transfusion safety.[3,13] Unlike alcohol and opioids, which are routinely screened for because of the known effects on coagulation and RBC viability, cannabinoids are not yet addressed through standardized detection protocols in donated blood, despite similar concerns about the physiological effects.[3,13,34]

Blood donation happens through whole blood collection processes or the separation methods of apheresis for different blood supply requirements.[24,28] Whole blood donations are fractionated into red blood cells, platelets, and plasma through separation processes, whereas apheresis selectively collects a specific component such as platelets or plasma while returning the remaining elements to the donor.[24,28] An established set of sterility and safety guidelines for blood donation operates under the oversight of the World Health Organization (WHO) and U.S. Food and Drug Administration (FDA) policies and American Association of Blood Banks (AABB) protocols which comply with national and international standards.[1,24,28,35]

Transfusion-related adverse reactions happen occasionally even after applying strict screening methods alongside safety standards.[24] Three major adverse effects of transfusion are febrile non-hemolytic reactions along with allergic responses and immune-mediated hemolysis that destroys donor red cells. Serious transfusion complications such as transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO) can be fatal to patients.[1,24,28] To control these, standard transfusion practice includes pre-transfusion compatibility testing and the use of irradiated leukoreduction blood products for immunocompromised and close post-transfusion monitoring.[1,24,28]

The integrity of blood transfusion is maintained and must continue to be evaluated in donor screening protocols, laboratory test methods, and storage technologies. While widespread use of screening has greatly decreased the risk of transfusion transmitted infection, usage of cannabinoids poses a new potential safety concern.[21] THC and CBD are both lipophilic compounds that remain in circulation in the blood and may be retained in blood components.[7,9,10,37] For that matter, their presence in donor blood remains uncertain in terms of their potential impact on coagulation and oxygen transport, especially in patients with preexisting hematologic disease.[7,10] The prevalence of individuals using cannabinoids for medical and recreational purposes has increased significantly, which

demands transfusion medicine to develop procedures and policies to implement new cannabinoid-specific screening protocols and mitigation strategies to safeguard the blood products.[4,14,15]

Cannabinoid metabolism

The metabolism of both THC and CBD starts in the liver where it is handled by complex enzymatic processes that determine absorption and systemic circulation.[38] In oxidative metabolism, the cytochrome P450 (CYP450) enzyme system is particularly important with isoforms CYP2C9 and CYP3A4 being of prime significance in the conversion of THC to active metabolites 11-hydroxy-THC (11-OH-THC) and THC-COOH.[16,38] These are lipophilic (fat soluble) metabolites that remain in the circulation because they bind to adipose tissue where they accumulate and slowly are released over time.[38]

CBD metabolizes more slowly than alcohol and opioids and therefore has a longer half-life in the human body.[20,38,39] This persistence is because of the interaction with the cytochrome P450 enzymes.[38] Cannabinoids are present longer in circulation and this raises concern for the accumulation in stored blood products.[20,38] Because cannabinoids are lipid soluble, there may be potential for binding to cellular components in blood and possibly alter structural or functional properties in transfused products.[3,13,20] In addition, temperature fluctuations during storage can affect cannabinoid stability and lead to quality issues in blood product transfusion medicine.[20]

Cannabinoids have pleiotropic effects on blood cell components and coagulation factors beyond pharmacokinetics.[3,12,13,16] The functional integrity of blood elements is essential for achieving favorable transfusion results. The flexible membranes along with the stable structure of red blood cells (RBCs) enable the cells to move throughout microvascular systems for the purpose of oxygen delivery and tissue perfusion.[40] Disruption of RBC membrane lipids by THC reduces cellular deformability and promotes early disaggregateion.[3,10,13] The contribution of this membrane disruption to eryptosis, a form of early programed RBC death, may limit the post transfusion lifespan of donor RBCs. Oxidative stress induced by CBD may cause abnormal RBC morphologies, echinocytosis (spiked cells) and acanthocytosis (irregularly shaped cells), and may interfere with microcirculatory flow and decrease oxygen carrying capacity in transfusion dependent patients.[3,10,13,41]

Expanding the discussion from RBC integrity to platelet function, the hemostatic process begins with platelets that initiates a sequence of adhesion and activation followed by aggregation which results in clot stability.[17,18,19,42] When THC activates the cannabinoid receptor type 1 (CB1), it leads to increased production of thromboxane A_2 (TXA_2), which functions as an aggressive platelet aggregator while causing vasoconstriction.[18,19,42] The drug interaction results in incorrectly aggregated platelets that raise patients' susceptibility to blood clot formation.[9,18,43] The anticoagulant properties of CBD stem from its ability to block the triggering of glycoprotein IIb/IIIa receptors that keep platelets from binding to fibrinogen and forming stable clots.[9,18,19,43] The anticoagulant effects of THC enhance bleeding risks especially among thrombocytopenic patients receiving platelet transfusions.[9,12,17,43]

Blood coagulation proceeds from two pathways intrinsic and extrinsic through thrombin generation and fibrin clot formation until it achieves stable hemostasis.[17] The administration of THC causes elevated levels of clotting factors VII and X in transfusion recipients which leads to increased thrombotic activity.[17,35,38] CBD works to reduce fibrinogen synthesis levels thereby extending coagulation parameters including international normalized ratio (INR), prothrombin time (PT), and partial thromboplastin time (PTT).[12,17,35] Transfusion management becomes more complex for patients with cannabinoid-associated coagulopathies, as these alterations may affect the

efficacy and safety of transfused blood products.[17] This includes patients with thrombocytopenia due to cancer and those with inherited bleeding issues.[35,38]

As a result of these interactions, cannabinoids change blood elements while controlling immune responses resulting in potential negative effects on blood product safety and blood cross-matching. Alloimmunization formation of antibodies against non-self-antigens on transfused cells remains a crucial safety concern in transfusion medicine by activating T-cells while releasing pro-inflammatory cytokines.[44] CBD administration leads to decreased T-cell activation, which might make individuals more prone to graft-versus-host disease (GVHD), mainly affecting immune-compromised patients.[39,44,45] The pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) are affected by THC, which may impair the recipient's ability to respond appropriately to transfused blood products.[45,46] The immunomodulatory characteristics of cannabinoids create issues that might compromise transfusion results in patients with weakened immune systems.[44,47]

The immunomodulatory capabilities of cannabinoids present distinct challenges for transfusion-dependent populations, particularly individuals with SCD and malignant disorders.[44] These patient populations, due to the chronic need for frequent blood product transfusion, will guarantee the development of alloantibodies that will further complicate the challenge of finding compatible blood products and heighten the likelihood of hemolytic transfusion reactions.[11,44,45] Transfusion-transmitted infections combined with reduced treatment efficacy become more likely for immunocompromised individuals who receive chemotherapy or organ transplantation.[39,47] Further research is necessary to analyze cannabinoid impacts on immune regulation, alloimmune reactions, and transfusion safety in high-risk populations, particularly as the use of prescribed and recreational cannabinoids continues to rise within these vulnerable groups.[35,39,44]

Emerging evidence also suggests that cannabinoids have the capacity to affect RBC integrity, platelet function, coagulation pathways, and immune responses, all of which can significantly affect transfusion outcomes.[3,13,44,48] These findings highlight the need to evaluate the risks associated with cannabinoid use in transfusion medicine with well-designed clinical research. To improve transfusion safety, it is essential to implement standardized donor screening protocols and conduct long-term studies that assess the effects of cannabinoids on blood components and recipient responses.[21,23] Supporting the development of safe, effective, and evidence-based transfusion strategies for high-risk patient populations requires a comprehensive understanding of cannabinoid metabolism, blood product hematological alterations, and recipient physiology.[3,13,23]

Clinical implications

Blood transfusions act as a crucial therapeutic approach to manage complications affecting patients who have SCD, cancer, or different chronic hematologic conditions.[28] Patient health stabilization depends on improved oxygen delivery through transfusion along with immune and hemostatic system support.[26] As the use of medical and recreational cannabis increases, clinicians are encouraged to assess the reduction of benefits from using blood products that are contaminated with cannabinoids. Blood product safety is compromised when collected from individuals who use cannabis. THC and CBD, the two primary cannabinoids in medical cannabis, have demonstrated effects on RBC morphology, platelet aggregation, and coagulation pathways.[3,9,13] The unknown health risks that blood transfusion-dependent patients, who are highly reliant on safe and functional blood components, are a concern that needs further investigation.

Regular blood transfusions form a critical therapeutic approach for SCD management for

the treatment of chronic anemia as well as lowering the risk of stroke while decreasing the frequency of VOCs.[49] SCD patients have naturally high RBC turnover rates as well as fragile membranes which means any additional harm to the cells becomes a major clinical concern.[50] Exposure to THC has been shown to compromise erythrocyte membrane stability, promoting hemolysis, and decreasing the ability of transfused red blood cells capacity to carry oxygen.[3,10,13] These adverse effects may exacerbate anemia and hinder the overall efficacy of transfusion therapy in SCD patients, further complicating clinical outcomes.

Cannabinoid compounds that influence RBC deformability characteristics, as well as viscosity levels, can potentially worsen blood vessel blockages and lead to elevated frequency of VOCs among SCD patients.[7,11,41] Sickled RBCs that obstruct small blood vessels trigger both tissue damage from reduced blood flow and local tissue inflammation which define these crises.[7,11,41] When THC impairs the membrane flexibility of transfused erythrocytes, it increases cellular rigidity and the likelihood of vascular occlusion.[7,11,41] This risk is further compounded in patients who are exposed to cannabinoids from two routes: their own personal cannabis usage and transfusions involving blood products from cannabinoid-positive donors.[3,11,13]

Alloimmunization, which leads to the development of antibodies against non-self-antigens on transfused red blood cells, creates a major difficulty in managing SCD patients who are frequently in need of blood transfusion therapy.[11,44] The presence of alloantibodies complicates the process of finding compatible blood units while making patients susceptible to delayed hemolytic transfusion reactions (DHTRs).[28] Cannabinoids exert immunomodulatory effects that may alter global immune function by suppressing T-cell activity and disrupting cytokine signaling pathways.[39,44] The immunomodulatory properties have dual effects on alloantibody production by preventing the formation or simultaneously impairing the immune system's ability to recognize or respond to hemolytic reactions following transfusion.[44]

Patients who have cancer share the same high-risk status for transfusion-related complications that SCD patients experience.[8] Thrombocytopenia caused by chemotherapy creates a high-risk state for patients because it leads to spontaneous bleeding and inadequate clot formation.[3,8,13,19,26] Physicians order platelet transfusions for cancer patients to help them maintain adequate platelet count levels to support hemostasis.[28] The biological effects of cannabinoids complicate transfusion safety and efficacy in this population.[3,13,19] THC has been associated with platelet hyperactivity, potentially increasing the risk of thrombosis, while CBD shows anticoagulant properties that inhibit platelet aggregation and elevate bleeding risk.[9,13,17,19,43] Platelet transfusions administered to patients under cannabinoid exposure may display unpredictable efficacy, complicating the clinical management of both hemorrhagic and thrombotic disorders.[9,13,19]

SCDs and cancer patients are not the only transfusion-dependent groups who face potential risks from cannabinoid exposure, individuals with hemophilia represent another patient population.[17,35] Hemophilia requires frequent administration of clotting factor replacement therapy, often delivered through transfusions, to prevent or control bleeding episodes.[12,17,51] THC and CBD may influence coagulation factor activity, but the impact on bleeding duration depends on the direct involvement in blood clotting processes.[17,44] Furthermore, cannabis may enhance the sensitivity of immune systems toward transfused blood components, particularly in immunocompromised individuals.[52] Cannabinoids immunosuppressive properties, especially the inhibition of T-cell function, may elevate the risk of graft-versus-host disease (GVHD) and impair immune responses following transfusion in vulnerable groups, including transplant recipients and patients with autoimmune disorders.[39,44]

Cannabinoid physiology affects clinical outcomes of transfusion-dependent patients

who suffer from SCD as well as those diagnosed with cancer or hemophilia or autoimmune diseases and individuals undergoing immunosuppressive therapy following organ transplantation.[39,44] Blood transfused products alter safety and effective performance because THC and CBD make an impact on the dynamics of coagulation along with red cell integrity and immune system response .[3,13,44] The effects of cannabinoids result in prolonged bleeding time, increased thrombotic potential, and degraded immunological product compatibility between donor and recipient.[17,43] The health complications from THC and CBD therapy can work against treatment objectives and boost transfusion-associated medical issues for patients with health problems. The development of safe blood product transfusion practice mandates the identification of cannabinoid-related risks. There is an urgent need to update blood donor questionnaires to include cannabinoid consumption, as well as to promote temporary cessation of prescribed cannabinoid therapy in transfusion-dependent patients.

Discussion

Cannabinoids present multiple safety risks to transfusion medicine; therefore, health organizations should establish specific guidelines to mitigate these risks. Both THC and CBD degrade blood product quality through hematological alterations, affecting the stability of RBCs, and interfering with platelet aggregation and coagulation mechanisms.[3,10,13,17,19] The changes in the composition and function of blood that result from cannabinoid consumption introduce substantial, measurable changes in health to transfusion-dependent patients, particularly those with SCD and cancer.[3,11,13,53] SCD patients using cannabinoids face increased hospital admissions due to worsening VOCs, demonstrating an immediate need to address transfusion risks from cannabinoids.[11,53]

Despite this growing concern, research evaluating the transfusion-specific consequences of cannabinoid exposure remains limited. While some *in vitro* research on cannabinoid exposure has produced reputable data on impaired blood products, there is not enough comprehensive research evaluating the potential risks associated with transfusing cannabinoid-exposed blood products.[13,20,35] As a result, there is no compelling data to support the transfusion management team in requesting special unique blood products that are cannabinoid-free to prevent adverse reactions for the most vulnerable patients. Research data lacks sufficient information about the prolonged preservation of cannabinoids and the metabolites in blood products and the ability to worsen blood system conditions through cumulative exposure.[54] Cannabinoid potential risks are primarily dependent on research that relies on *in vitro* and animal experiments, which often fail to replicate the outcomes that occur during human transfusion procedures.[13,16,19]

These limitations significantly hinder the ability to develop evidence-based transfusion guidelines. Developing clinically useful guidelines becomes more challenging because of the different routes used to consume cannabinoids, varying periods of exposure, and the absence of standardized dosage information.[43,54] The persistent presence of cannabinoids in blood products donated by cannabinoid users must be measured explicitly through clinical research to determine the impact on blood product safety.

The research gaps are mirrored by regulatory shortcomings in donor screening protocols. The lack of standard cannabinoid-specific donor screening impedes the creation of extensive transfusion safety regulations. Donor screening developed by the FDA along with the WHO focuses primarily on detecting infectious disease markers and does not address cannabinoid use.[23,32] The present gap is exacerbated by federal restrictions on medically prescribed THC and CBD, which create issues related to standardization and difficulties in clinical oversight.[55] Furthermore, the legalization of prescribed medical and recreational cannabis in 24 states in the US complicates legal procedures related to donor privacy and

informed consent.[56] The policies must align with regional laws, making it difficult to establish consistent national standards.[56,57] These factors contribute to inconsistent donor eligibility evaluations and may ultimately reduce the pool of acceptable donors.

To address the regulatory and clinical gaps, a multifaceted approach must be implemented. The medical community must implement evidence-based solutions to resolve problems from cannabinoid exposure in transfusion services. Implementing screening protocols designed to monitor both the recreational and medically prescribed cannabinoid consumption frequencies among donors and immunocompromised patients will assist in policy development by providing crucial data regarding prevalence.[15,22,58] Incorporating cannabinoid-specific questions into donor screening questionnaires will enable more accurate risk assessments of donors without reducing the available donor pool. Collaboration among healthcare professionals, laboratory scientists, bioethicists, and policymakers are essential for creating transfusion safety guidelines that address donor consent, cannabinoid use disclosure, and the associated legal, ethical, and privacy concerns.[55,56,57]

To further support these efforts, clinical practices must reflect the differential risks faced by vulnerable patient populations. Healthcare institutions need to modify transfusion protocols according to the unique risks within a specific population such as SCD, cancer or immunocompromised patients. The use of cannabinoids intensifies RBC deformity and increases RBC viscosity and elasticity, potentially affecting the ability to flow properly, leading to worsening anemia and more frequent VOCs in SCD patients.[3,11,13,35,53] However, transfusion guidelines continue to overlook this fact. Among patients with cancer undergoing chemotherapy treatments, cannabinoids cause changes to blood clotting factors, thereby increasing the risk for thrombocytopenia, and consequently raising the likelihood of potential bleeding.[12,13,17,19,26] The outcomes of blood transfusions for immune-compromised patients, as well as those at risk for allergic reactions and GVHD, become more complex when cannabinoid metabolites are present in donor blood, potentially influencing the immune response in the recipient and complicating the risk of allergic reactions or GVHD in susceptible individuals.[44,45,46,47,52] Cannabinoids' negative impacts on blood product safety support the need for medical facilities to perform pre-transfusion risk assessments, which include cannabinoid exposure among other risk factors.[8,13,17] Integrating cannabinoid screening into pre-transfusion protocols may improve transfusion outcomes and patient safety across high-risk populations, ensuring that evolving patterns of drug use do not compromise the integrity or efficacy of life-saving blood products.

However, translating clinical recommendations into standardized practice remains difficult without conclusive scientific data. Despite the urgency to revise transfusion protocols, significant limitations persist. The existing knowledge about how cannabinoids affect transfusion safety remains imperfect due to various inconsistencies and availability of unreliable, reproducible data.[16,35] The lack of comprehensive research is hindered by the scarcity of human clinical trials, the use of unstandardized dosing methods in studies, and limited knowledge regarding the persistence of cannabinoids in blood components.[20,22] Challenges in experimental design make it difficult for researchers to establish conclusive findings regarding the safety of blood products, especially concerning the persistence of cannabinoids and their effects.

Future investigations must conduct extensive clinical research to determine how ongoing cannabinoid consumption, whether for recreational or medical purposes, can exacerbate hematological alterations in the blood system, thereby compromising the safety of blood products.[38,44] Research in this area requires standardized procedures for cannabinoid measurements combined with reliable analytical approaches to detect the presence of

these compounds and metabolites in stored blood products.[58,59] The development of evidence-based cannabinoid safety thresholds for blood components requires support from regulatory agencies through the facilitation of pilot screening programs. A multidisciplinary team, including transfusion specialists, clinical researchers, toxicologists, and bioethicists, should lead this effort to incorporate cannabinoid detection into donor evaluation protocols while ensuring that ethical considerations are upheld.[55,56]

Conclusion

The emerging threat of cannabinoid exposure in transfusion medicine lacks proper attention, even though it significantly affects the membrane integrity of RBCs, reduces platelet aggregation, and modulates blood coagulation.[3,9,10,13] The increasing number of cannabinoid users in society demands immediate changes to donor eligibility criteria and the creation of validated, reliable testing procedures for cannabinoid detection.[14] The evolution of transfusion medicine plays an integral role in healthcare; ensuring blood product safety is essential and requires multimethod longitudinal research to determine the complete risks associated with cannabinoids, combined with practical cost-effectiveness assessments and a mechanistic understanding.[16] A documented tenfold increase in cannabinoid consumption necessitates collaboration among healthcare professionals, laboratory personnel, and government agencies to establish a national healthcare policy impacting patient blood management, ensuring safety and informed decision-making.[55,60] Cannabinoid exposure threatens transfusion safety, demanding urgent action to prevent unpredictable hematologic risks to vulnerable patients.

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Review article

Management of Three Cases of Hemolytic Disease of the Fetus and Newborn Due to Anti-Rh17 Alloimmunization Within the Same Family by a Rural Hospital Blood Bank

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Hemolytic disease of the fetus and newborn (HDFN) due to anti-Rh17 alloimmunization is rare, with very few cases reported among siblings in the same family. This case series describes three biological sisters, all with blood type O and RhCE-null phenotype (genotype D--/D--), who were evaluated over a three-year period. Two of the sisters developed anti-Rh17 antibodies due to pregnancy, resulting in three separate cases of HDFN in their newborns. The pregnancies were managed collaboratively by a rural hospital blood bank, a tertiary care hospital, and several reference laboratories. Management strategies included prenatal antibody screening, identification and titration, intrauterine interventions, and postnatal blood transfusions using antigen-compatible red blood cells to treat fetal anemia and hyperbilirubinemia. Despite limited resources in the rural setting, coordinated multidisciplinary care enabled the successful management of these complex cases. This report highlights the critical role of early detection, the availability of rare blood units, and the value of collaboration across healthcare systems. It also emphasizes the importance of specialized knowledge in transfusion medicine for managing rare maternal antibodies like anti-Rh17 to improve neonatal outcomes.

Abbreviations: AC, autocontrol; ARC, American Red Cross; DAT, direct antiglobulin test; HDFN, hemolytic disease of the fetus and newborn; IRL, Immunohematology Reference Laboratory; IUFD, intrauterine fetal demise; IUT intrauterine transfusion; IVIG, intravenous immunoglobin; MCA-PSV, middle cerebral artery peak systolic velocity; pRBCs, packed red blood cells; TPE therapeutic plasma exchange.

Keywords: Hemolytic disease of the fetus and newborn (HDFN), anti-Rh17, RhCE-null phenotype, D--/D--

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Introduction

Rh17 (Hr_0) is a high-prevalence Rh antigen found in approximately 99.9% of the population.[1] It comprises a group of epitopes located on all common RhCE proteins, except in individuals with the RhCE-null (genotype D--/D--) or Rh-null phenotypes.[1-4] Anti-Rh17 (anti-Hr_0) antibody is a single antibody with broad and complex specificity, reacting with C/c and E/e antigens.[1] Although rare, anti-Rh17 has been implicated in mild to severe hemolytic disease of the fetus and newborn (HDFN).[1, 5-8] This review describes three cases of HDFN caused by anti- Rh17 in siblings from an American Hispanic family. The family includes three biological sisters, each spaced approximately three years apart in age. Over a three-year follow-up period, their ages ranged as follows: Sister 1 was 25–28 years old, Sister 2 was 22–25 years old, and Sister 3 was 19–22 years old.

Patient Profile

Sister 1

At 24 years of age, Sister 1 presented to the emergency department of a rural regional hospital with complaints of abdominal pain, nausea, and vomiting. Upon admission, laboratory tests and imaging studies indicated acute cholecystitis, requiring emergent cholecystectomy. During the pretransfusion workup, serologic testing revealed Sister 1's blood type as O, Rh(D) positive. Her antibody screen was positive with all red cell panels. Antibody identification showed pan-reactivity with all red cells on the antibody identification panels, while the autocontrol (AC) and direct antiglobulin test (DAT) were both negative. Crossmatching attempts failed to find any compatible units with the patient's plasma. Due to the urgency of the surgery and the complex serologic findings, Sister 1's specimen was sent to the regional American Red Cross (ARC) Immunohematology Reference Laboratory (IRL) for further antibody identification.

While awaiting results from the IRL, Rh extended phenotyping was performed for Sister 1 Her Rh phenotype was determined to be D+, C-, c-, E-, e-, consistent with the rare D--/D-- genotype. Subsequent IRL testing identified the presence of anti-Rh17 antibody in her plasma and ruled out any additional underlying alloantibodies. Red blood cell antigen typing results further confirmed the RhCE-null phenotype.

Family members

Due to the rarity of the RhCE-null phenotype and the presence of anti-Rh17 antibody, an immunohematological workup was performed for Sister 1's immediate family members, including her husband, first child, father, mother, Sister 2, and Sister 3. The results are summarized in Table 1. Molecular genotyping was not performed due to health insurance limitations, which restricted access to advanced diagnostic testing. As a result, Rh genotype determination was based on serologic phenotyping and family inheritance patterns.

Table 1. Blood Typing, Rh Phenotype, and Presumed Genotype of Sister 1's Family

	Blood typing ABO	Rh(D)	Rh phenotyping	Presumed Rh genotype
Sister 1	O	POS	D+ C- c- E- e-	*D--/D--*
Husband	O	POS	D+ C+ c+ E+ e+	*R1/R2*
First child	O	POS	D+ C+ c+ E- e+	*R1/D--*
Parents and siblings				
Father	O	POS	D+ C+ c- E- e+	*R1/D--*
Mother	O	POS	D+ C+ c- E- e+	*R1/D--*
Sister 2	O	POS	D+ C- c- E- e-	*D--/D--*
Sister 3	O	POS	D+ C- c- E- e-	*D--/D--*

ABO, blood type; POS, positive; Rh(D), RhD antigen.

Sister 1's parents were in a consanguineous marriage and were found to be compound heterozygotes for the DCe/D--. Sister 1 experienced an uneventful first pregnancy and delivered a healthy baby at 23 years of age in a non-consanguineous marriage, with no history of transfusion or miscarriage. Sister 1's husband was determined as DCe/DcE. Both Sister 2 and

Sister 3 – also homozygous for the D--/D--, did not have a history of pregnancy or previous transfusion.

Diagnostic Processes

Clinical interventions

Sister 1

During Sister 1's second pregnancy at 25 years of age, prenatal screening and monitoring were initiated early in the first trimester. At 12 weeks' gestation, her baseline anti-Rh17 titer was measured at 4, and the pregnancy progressed normally through the second trimester. However, a significant rise in anti-Rh17 titer was observed at 27 weeks' gestation, reaching 2048, representing an eight-fold increase, as reported by the regional reference laboratory, Quest Diagnostics. At 28 weeks' gestation, the fetus developed hydrops fetalis and experienced intrauterine fetal demise (IUFD).

Due to the limited capacity of the rural regional hospital and Sister 1's complicated obstetric history, she was referred to a tertiary hospital for management of her third pregnancy at the age of 26. Doppler ultrasonography was used to monitor for fetal anemia, specifically measuring middle cerebral artery peak systolic velocity (MCA-PSV). There were no signs of fetal anemia until the early third trimester. In preparation for delivery, Sister 1 completed a 500 mL autologous whole blood donation at gestational age of 30 weeks for potential use during her scheduled cesarean section. Additionally, Sister 2 made a directed donation of 250 mL of antigen-compatible packed red blood cells (pRBCs), which was aliquoted into small pediatric bags, each containing approximately 30 mL of pRBCs. Two aliquots were stored in the blood bank refrigerator to support potential intrauterine transfusions (IUTs) in the event fetal anemia was detected. The remaining six aliquots were cryopreserved to ensure the availability of antigen-compatible pRBCs for the neonate, if needed in the future.

Sister 1's initial anti-Rh17 antibody titer was 8 and increased gradually, though not significantly, throughout early third trimester. However, at 32 4/7 weeks' gestation, the titer rose sharply to 2,048. Concurrently, the middle cerebral artery peak systolic velocity (MCA-PSV) exceeded 1.5 multiples of the median (MoM), indicating a high likelihood of fetal anemia. Given the elevated risk, early delivery by cesarean section was performed at 33 week's gestation as the most favorable option for both maternal and fetal outcomes. The newborn's Rh phenotype was determined to be D+, C+, c–, E–, e+, with a presumed genotype of DCe/D--, as shown in Table 2.

Table 2. Laboratory Testing and HDFN Outcomes for Sister 1 and Siblings Over Three Years

	Antibody screen & identification panel	AC & DAT	Antibody presence	HFDN & outcome
Sister 1 (25y-28y)				
1st pregnancy (23y)	NK	NK	NK	Normal
2nd pregnancy (25y)	All positive	Negative		IUFD
3rd pregnancy (26y) Infant (DCe/D--)	All positive	Negative		Moderate HDFN, alive
Sister 2 (22y-25y)	All negative	Negative	None	
Sister 3 (19y-21y)				
19y-20y	All negative	Negative	None	
1st pregnancy (21y) Infant (DcE/D--)	All positive	Negative	Anti-Rh17	Mild HDFN, alive

AC, autocontrol; Anti-Rh17, anti-Rh17 antibody; DAT, direct antiglobulin test; HDFN, hemolytic disease of the fetus and newborn; IUFD, intrauterine fetal demise; NK, not known.

The infant developed moderate hyperbilirubinemia and anemia, requiring intravenous immunoglobulin (IVIG) infusions, phototherapies, an exchange transfusion and antigen-compatible pRBCs transfusion, directly

donated by Sister 2. One week after discharge from the tertiary hospital, the infant received an additional 50 mL transfusion of rejuvenated, deglycerolized, and irradiated antigen-compatible pRBCs at the local rural hospital. This unit was prepared and shipped from the tertiary hospital via the ARC transport service. Three days later, the baby was discharged with stable condition from the local hospital.

Sister 3

Sister 3 had no history of pregnancy or blood transfusion and consistently tested negative for red cell antibodies during the first two years of the observation period. She made a directed donation of pRBCs and cryopreserved prior to pregnancy for potential future use. Her first pregnancy occurred at age 21, and the antenatal course was unremarkable. She delivered at 38 weeks of gestation. Upon delivery, Sister 3's antibody screen was positive, and the antibody was identified as anti-Rh17 with a titer of 16. No additional alloantibodies were detected, as reported by the ARC IRL. The newborn's cord blood was DAT-positive, and the baby presented with mild anemia and hyperbilirubinemia. Anti-Rh17 was also identified in the eluate from the cord blood. The infant's Rh phenotype was D+, C-, c+, E+, e-, with a presumed DcE/D--, as shown in Table 2. An immediate transfusion of 30 mL of antigen-compatible, rejuvenated, deglycerolized and irradiated pRBCs, previously donated by the mother, was administered, followed by phototherapy. The baby was discharged one week later in stable condition, with no further pRBCs transfusions required.

Sister 2

Sister 2 had no history of pregnancy or transfusion and consistently maintained a negative antibody screen throughout the three-year observation period. As a result, she was considered the most suitable donor for directed blood donation to support the treatment of HDFN within the family.

Patient Follow-up

Over the three-year period, both Sister 1 and Sister 3 had live-born infants, delivered in the same year approximately three months apart. Following treatment for hyperbilirubinemia and anemia, both infants demonstrated normal growth and development, with no evidence of neurological abnormalities during follow-up. All the three sisters were enrolled into rare donor registry. The laboratory testing results over the three-year period are shown in Table 2.

Discussion

The absence of the Rh17 antigen, characteristic of the RhCE-null phenotype, is caused by rare RHCE variant alleles resulting from genetic alterations that inactivate or delete both copies of the RHCE gene.[9-11] Individuals with this genotype (D--/D--) lack expression of C/c and E/e antigens on red blood cell membrane but typically show enhanced expression of the D antigen.[12]

The frequency of the D-- haplotype varies among ethnic groups, with a higher prevalence reported in Japanese populations (0.0032) and American Hispanics (0.005).[1,13] Individuals with this rare RhCE-null phenotype may develop the rare alloantibody anti-Rh17 when exposed to conventional RhCE antigens through pregnancy, transfusion, or transplantation. Once immunized, individuals with anti-Rh17 face significant clinical challenges, as demonstrated by the cases in this report. This antibody targets high-prevalence Rh17 antigens, which can lead to mild to severe HDFN or delayed hemolytic transfusion reactions. [6-8,14]

Although cases of HDFN due to anti-Rh17 alloimmunization have been reported in the literature, this is the first documented report of multiple HDFN cases occurring among siblings within the same family. Notably, all high-risk pregnancies and cases of mild to severe HDFN occurred within a three-year period in a rural hospital setting.[15] The hospital's blood bank team faced considerable challenges, including limited testing capabilities and restricted access to specialized

blood product preparation, while managing the care of this uniquely affected family. In collaboration with a tertiary healthcare facility and reference laboratories, the blood bank of a rural hospital developed a comprehensive management strategy for high-risk pregnancies and HDFN due to anti-Rh17 alloimmunization. As demonstrated in this family's cases, the approach included prenatal monitoring, intrauterine interventions, and postnatal managements. A flowchart outlining this strategy is presented in Figure 1. Notably, this approach is also applicable to high- risk pregnancies and HDFN caused by other maternal red blood cell alloantibodies.

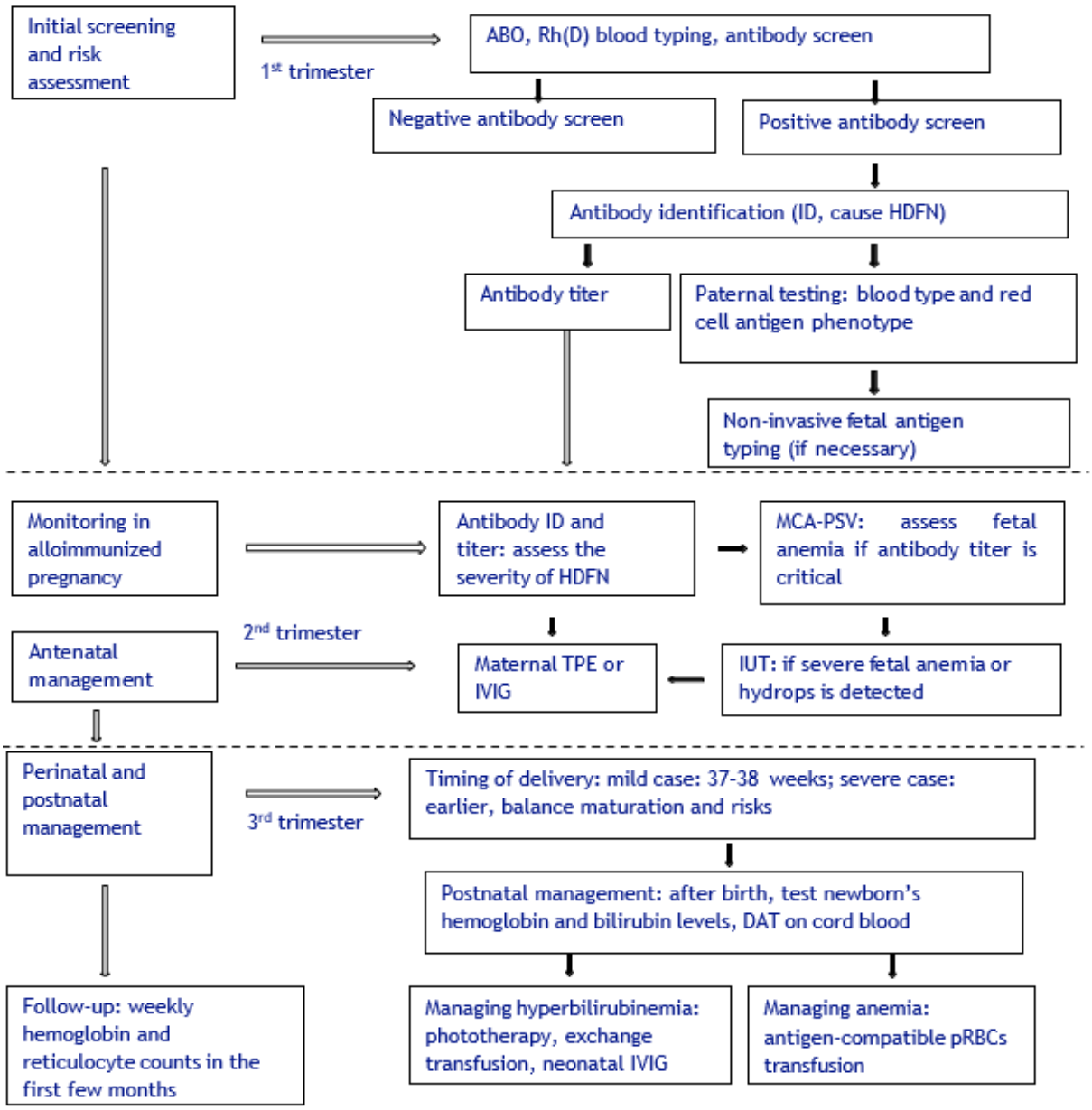

Figure 1. Flowchart for management of HFDN with maternal red blood cell antibody ABO, blood type; antibody ID, antibody identification; DAT, direct antiglobulin test; HDFN, hemolytic disease of the fetus and newborn; IUT intrauterine transfusion; IVIG, intravenous immunoglobin; MCA-PSV, middle cerebral artery peak systolic velocity; pRBCs, packed red blood cells; Rh(D), RhD antigen; TPE, therapeutic plasma exchange.

During the first trimester of pregnancy, prenatal antibody screening, identification, and titration are essential for assessing the risk and potential severity of HDFN. If clinically significant antibodies are detected, paternal testing should be performed to determine the presence of the implicated red cell antigen and assess the likelihood of fetal antigen positivity.16 If the father is heterozygous for the antigen or if paternity is uncertain, non-invasive fetal antigen typing using cell-free fetal DNA from maternal blood should be considered to further evaluate fetal risk.[16]

During the second trimester, regular monitoring of maternal antibody titers is critical for assessing the severity of HDFN. For most clinically significant antibodies, a titer of 16 is considered the critical threshold.[16] If titers reach or exceed this level, serial ultrasounds to measure the MCA-PSV are performed to detect fetal anemia.[16,17] When MCA-PSV indicates severe anemia or if other signs of fetal hydrops are present, IUT is typically indicated. In particularly high-risk cases, such as Sister 1's second pregnancy, she was referred to a tertiary hospital for potential therapeutic plasma exchange (TPE).[16,17] Unfortunately, her anti-Rh17 titer rose sharply within a short period, resulting in intrauterine fetal demise (IUFD) before TPE could be initiated.

In her subsequent pregnancy, a proactive approach was taken. The blood bank team coordinated with a reference laboratory's blood center to arrange directed donations of antigen- compatible red blood cells. These units were processed and cryopreserved in preparation for potential IUT.[17] In addition, intravenous immunoglobulin (IVIG) may be considered to delay the need for IUT in certain cases.[17]

During the third trimester, delivery at a tertiary care center should be considered for high- risk pregnancies. The optimal timing of delivery requires balancing fetal lung maturity with the risks associated with ongoing hemolysis and IUTs. In milder cases, delivery is typically planned around 37–38 weeks of gestation, while earlier delivery may be necessary for more severe cases, as seen in Sister 1's third pregnancy.[15,18] Sister 3 developed anti-Rh17 during the third trimester of her first pregnancy, despite having no prior transfusion history – likely due to an anamnestic immune response. [6,19]

Postnatal management for the newborns of Sister 1 and Sister 3 focused primarily on red blood cell transfusions to treat anemia and phototherapy to manage hyperbilirubinemia. In the event of severe hyperbilirubinemia, exchange transfusions using antigen-compatible pRBCs reconstituted with AB plasma should be prepared as a treatment option. [20,21] In addition, neonatal IVIG may be administered to reduce red blood cell destruction and help delay or avoid the need for exchange transfusion.[17]

Finding a suitable donor for the RhCE-null phenotype is extremely challenging due to the rarity of this antigen profile. Fortunately, all three sisters in this family share the RhCE-null phenotype, making them valuable potential donors for rare donor registries. Sister 2, who has no history of pregnancy or transfusion and consistently negative antibody screens, stands out as the most ideal donor. Even in the presence of anti-Rh17 antibodies, washed maternal red blood cells can serve as an effective and safe source of blood for treating fetal anemia and hyperbilirubinemia, especially in emergency or resource-limited situations.[7,21]

Conclusion

The cases reported here demonstrate that, despite the challenges of managing HDFN due to anti-Rh17 in a resource-limited rural setting, health care providers and laboratory professionals successfully treated the affected infants and managed complicated pregnancies through collaboration with tertiary healthcare facilities. This experience contributes valuable insight into the management of HDFN caused by anti-Rh17 antibodies.

Disclosure

The author declares no conflicts of interest.

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Review article

Machine Learning Algorithms Improve Blood Utilization in Surgical Transfusion Management

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Red blood cell transfusions are essential in perioperative care but are frequently overutilized, increasing costs and exposing patients to unnecessary harm. Traditional transfusion risk scores lack the precision needed for personalized care, often not accounting for the complexity of patient-specific variables. Machine learning (ML) has emerged as a promising tool to improve the accuracy of transfusion risk prediction by analyzing large, complex datasets and identifying non-linear relationships among clinical factors. A comprehensive review of published ML-based transfusion prediction models was conducted, focusing on surgical applications in cardiac, orthopedic, and general procedures. Studies were analyzed based on algorithm type, performance metrics, input variables, and model transparency. Implementation challenges, including data quality, clinical acceptance, and infrastructure limitations, were also examined. ML enables more accurate, individualized prediction of perioperative transfusion needs. ML models outperformed traditional methods in predictive accuracy, particularly those built using large data sets and ensemble techniques such as gradient boosting. Simpler models like logistic regression performed well with smaller datasets. Barriers to implementation included fragmented electronic health records, variability in data standardization, and limited external validation. The "black-box" nature of some ML algorithms poses additional implementation challenges for providers including trust and adoption. For successful clinical integration, models must be transparent, validated across diverse populations, and supported by standardized, high-quality data. ML-based transfusion prediction models improve blood utilization and enhance surgical outcomes.

Keywords: Perioperative blood transfusion, machine learning, risk prediction, blood utilization

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Introduction

Transfusion services in a hospital aim to provide an adequate blood product supply to patients. Maintaining a healthy inventory of blood products is a critical responsibility, ensuring their availability and prompt delivery to patients in need.[1] However, despite their life-saving potential, blood products are often used unnecessarily. While generally safe, blood transfusions carry inherent risks to patient health and contribute to increased costs for both patients and healthcare institutions.[1,2] To mitigate unnecessary transfusions, hospitals have implemented various strategies, such as restrictive transfusion guidelines, aiming to optimize blood utilization and ensure that only patients with clinical necessities receive transfusions.

While unexpected bleeding situations can arise, pre-planned surgeries offer an opportunity to anticipate and manage transfusion needs. Accurate prediction of transfusion requirements in surgical patients is essential for effective resource allocation and planning.[3] Traditionally, clinicians rely on preoperative hemoglobin (HgB) levels, surgical risk factors, and clinical judgment to guide transfusion decisions.[4,5] However, these methods are not always dependable, as human error and practice variability can lead to inconsistencies. Additionally, even when transfusion guidelines are unmet, clinicians may still request blood products preemptively as a precaution, resulting in unnecessary reservations and potential waste of resources.[5,6] Addressing these challenges requires more precise and data-driven approaches to improve transfusion practices in the surgical setting.

Machine Learning (ML), a branch of artificial intelligence (AI), offers a promising solution by using electronic health data to find patterns and accurately predict transfusion needs.[4,7,8,9] By analyzing clinical and demographic factors, ML algorithms can assist clinicians in making data-driven, patient-specific decisions regarding blood product utilization. As healthcare continues to embrace digital transformation, integrating ML into perioperative transfusion practices has the potential to enhance decision-making, reduce unnecessary blood use, and improve patient care. ML algorithms accurately predict transfusion needs for surgical procedures, leading to improved blood utilization and patient outcomes.

Background

Blood transfusions are a lifesaving procedure made possible through voluntary blood donations.[1] Maintaining an adequate blood product inventory is a critical responsibility of transfusion services, ensuring prompt availability. However, while transfusions are essential in many clinical scenarios, their use must be evaluated to prevent unnecessary administration and associated risks. Over the past few decades, organizations like the World Health Organization (WHO) and Association for the Advancement of Blood & Biotherapies (AABB), have emphasized the use of patient blood management (PBM) programs to optimize transfusion practices and reduce inappropriate use of blood products.[10,11]

Although they are generally safe, unnecessary transfusions pose significant health risks to patients. Patients receiving unwarranted transfusions are exposed to potential adverse effects, including transfusion reactions, transfusion-associated circulatory overload, and alloimmunization.[10] Additionally, inappropriate transfusions have been associated with prolonged hospital stays, increased healthcare costs, and resource wastage. A study evaluating transfusion appropriateness in 15 hospitals found that nearly 50% of transfusions were deemed unnecessary.[2] Despite national and institutional efforts to implement restrictive transfusion strategies, unexplained variations in transfusion practices remain, particularly in noncardiac surgeries.[12] The persistence of inappropriate transfusions raises questions about the effectiveness of guidelines, clinician adherence, and potential gaps in transfusion education. Addressing these challenges is essential to ensuring both

optimal patient outcomes and the efficient use of a limited resource.

Predicting the need for transfusion in the perioperative setting is essential for effective blood inventory management.[3,12] Hospital transfusion services allocate substantial resources to managing surgical transfusions, including testing, preparation, and distribution of blood products. Each unit of blood transfused during surgery requires approximately 30 minutes of preparation by a medical laboratory professional.[3] Preoperative evaluation of surgical patients enables the identification of transfusion risk factors, allowing clinicians to optimize management strategies before surgery or arrange for blood product availability.

Early identification of patients at substantial risk for transfusion facilitates prompt interventions, such as anemia management, and enables the use of autotransfusion techniques, such as cell salvage, in eligible patients.[3] Furthermore, restricting blood product preparation to those patients with actual need prevents unnecessary sequestration of units from the blood supply inventory. However, despite perioperative transfusion guidelines, studies suggest that risk assessment tools and ordering practices are inefficient in accurately predicting transfusion needs.[6,12,13] This inefficiency underscores the need for improved predictive models that integrate patient-specific variables, surgical factors, and laboratory data to enhance decision-making and reduce unnecessary transfusions.

Guidelines for transfusion

Effective surgical preparation is critical for ensuring patient safety and optimizing clinical outcomes. Before surgery, the surgical team evaluates the patient's clinical status, including laboratory results, to confirm readiness for the procedure. Blood management in the perioperative setting is guided by HgB thresholds and transfusion risk scores, which vary based on the type of surgery.[4,14] Transfusion guidelines recommend a HgB threshold of 8 g/dL for patients undergoing cardiac or orthopedic surgery, as well as those with pre-existing cardiovascular conditions.[15] However, making the right decisions about blood product use is complex, and transfusion should not be solely based on laboratory values.

Transfusion risk scores can help predict perioperative blood requirements for different surgical procedures. One widely used tool is the Maximum Surgical Blood Ordering Schedule (MSBOS).[16] This system estimates the historical RBC transfusion rates for specific procedures based on Current Procedural Terminology (CPT) codes, providing an average transfusion requirement per surgery. Institutions typically set a threshold—often at 5%—to determine whether a type and screen (T&S) test is necessary.[17] If the historical transfusion rate for a procedure exceeds the threshold, a preoperative T&S is recommended. Conversely, if the likelihood of transfusion is below 5%, routine T&S may not be needed unless specific risk factors exist, such as a positive antibody screen or the use of anticoagulants and antiplatelet agents. [17]

Implementing the MSBOS has proven efficient in reducing the amount of blood product orders.[6] However, one limitation is that these calculations are not frequently updated and do not account for variability in provider-specific or patient-specific characteristics.[17,18] Surgical techniques vary among providers, affecting transfusion needs, making it essential to incurporate individual provider transfusion history into preoperative evaluations. Additionally, adherence to MSBOS recommendations remains low, as some clinicians override the system, relying instead on personal judgment to ensure blood availability.[17,19]

The provider's intuition and clinical experience significantly influence perioperative blood product ordering.[19-21] Studies reveal inconsistencies in transfusion practices for adult surgical patients, suggesting that decision-making extends beyond objective clinical data. Factors such as the providers' training, past experiences, and clinical intuition contribute to transfusion decisions.[22] Research

shows that provider judgment can independently predict surgical outcomes, highlighting the complex interplay between standardized guidelines and individualized clinical assessment.

Although transfusion risk scores and clinical guidelines provide a structured framework, they allow room for provider discretion.[15] A key metric for evaluating transfusion ordering practices is the crossmatch-to-transfusion (C/T) ratio.[10] A high C/T ratio — where crossmatched RBC units stay unused — suggests over-ordering of blood products in the perioperative setting. When surveyed, surgeons with high C/T ratios cited concerns about delays in blood availability from transfusion services as a primary reason for ordering blood in advance as a precautionary measure.[13]

Traditional statistical models underpin many of these transfusion decision tools, but they come with limitations. These models require strict assumptions, such as linear relationships between variables, which may not always align with real-world clinical conditions. Traditional regression analyses evaluate predictors independently or within predefined subsets based on prior knowledge, often overlooking interactive effects between variables.[23] This can lead to the omission of significant predictors, reducing the model's accuracy and effectiveness in transfusion decision-making. Advancements in ML and data-driven approaches may offer improved predictive models that better reflect the complexity of perioperative transfusion needs.

Considerations for cardiac surgeries

Cardiac surgeries account for a considerable proportion of RBC use in transfusion services due to their invasive nature, high-dose anticoagulation, and exposure to cardiopulmonary bypass.[24,25] The need for RBC transfusion in cardiac procedures varies widely, with approximately 15% of patients requiring large volumes of blood, while more than half do not require transfusion at all. This high-risk subset represents 80% of all blood products used in cardiac surgery, underscoring the importance of accurately predicting not just transfusion likelihood but also the number of RBC units required.[25]

Transfusion risk assessment in cardiac surgery involves multiple risk actors and patient-specific characteristics. Two widely validated tools are the Transfusion Risk Understanding Scoring Tool (TRUST) and Transfusion Risk and Clinical Knowledge (TRACK). TRACK, developed in Italy, predicts transfusion risk based on 5 preoperative factors: age, weight, sex, surgical complexity, and preoperative hematocrit (HCT) level.[26] TRUST, developed in Canada, incorporates 8 factors: HgB level, body weight, sex, age, emergency status, creatinine level, prior cardiac surgery, and procedural complexity.[27]

A systematic review compared the predictive values of different transfusion risk predictive models using c-statistics, or its parallel, area under the receiver operating characteristic curve (AUC) values, where 0.5 represents random chance and 1.0 represents perfect prediction.[24] Nine different models were found in the literature. However, due to poor reporting and substantial risk of bias, only 2 models, TRUST and TRACK were evaluated. Both TRUST and TRACK showed moderate predictive accuracy, with c-statistics of 0.74 and 0.72, respectively. The models also seemed to slightly overestimate the number of patients needing a transfusion. Additionally, this systematic review found performance variability of the scores among studies, as ordering perioperative transfusion is influenced by differences in adherence to PBM guidelines, population characteristics, and provider-driven decision-making. [24]

Beyond patient-related variation, recent studies highlight increasing provider-related discrepancies in RBC ordering, even when risk scores are implemented in the institution.[21,24] Given the high frequency of transfusions in cardiac surgery, refining predictive tools to incorporate detailed patient data, provider-specific variability, and ML algorithms could enhance the accuracy and clinical utility of transfusion decision-making. Developing a more dynamic and adaptable prediction model

could lead to improved transfusion planning and better resource utilization.

Considerations for orthopedic surgeries

Preoperative anemia is a common concern in orthopedic patients, particularly due to their advanced age, which increases the risk of perioperative transfusions.[28] Additionally, the growing use of oral anticoagulants in the elderly population presents further challenges in managing blood loss and transfusion needs. Given the complexity of care, providers cannot rely solely on HgB thresholds when making transfusion decisions.

Total knee arthroplasty (TKA) and total hip arthroplasty (THA) rank among the most performed orthopedic surgeries worldwide.[29,30] These procedures can lead to significant blood loss, with up to 46% of patients requiring RBC transfusions either during or after surgery.[29] Importantly, postoperative blood transfusions are linked to extended hospital stays and a higher risk of complications related to both transfusion and reduced mobility. Orthopedic surgeons have adopted measures to mitigate transfusion risk, including tranexamic acid administration and autotransfusion devices, though these strategies introduce their own risks and challenges.[30]

Given the multifactorial nature of transfusion risk in orthopedic surgery, rigid guidelines may not always be practical.[28] Key predictors of transfusion include advanced age, low body mass index (BMI), low preoperative HgB levels, and the use of surgical drains.[29] By integrating these factors alongside procedure-specific variables, clinicians can develop personalized transfusion plans that refine blood management, reduce costs, and enhance patient safety. Leveraging ML and advanced predictive modeling could further refine these estimations, enabling more efficient perioperative blood utilization.

Machine learning and surgical transfusion risk prediction

Computational technology and electronic data are transforming healthcare.[4,7,8] Artificial intelligence (AI) is a broad field encompassing machines capable of making decisions and performing complex, human-like tasks. Within AI, pattern recognition from large and complex datasets is being increasingly applied in the healthcare field.

Machine learning (ML), a subset of AI, enables systems to learn from data in a manner similar to human learning, improving performance over time.[4,7,8] There are 3 types of ML algorithms: supervised learning, unsupervised learning, and reinforcement learning. Supervised ML, a statistical method widely used for predictive modeling, employs algorithms to classify data and perform regression analysis. These models are trained using historical electronic health records, finding patterns in patient characteristics and procedural details to make accurate predictions for future cases.

In the context of transfusion risk prediction for surgical patients, ML models analyze years of surgical data, incorporating procedure characteristics, patient demographics, laboratory results, surgeon-specific factors, and historical blood product usage.[4] The type and amount of data is unlimited. In fact, the learning process of ML benefits from vast amounts of data to perform a more accurate prediction, as long as the data is well organized and defined.[4,8,9,31] By leveraging institutional data, ML algorithms can uncover novel patterns and associations, enabling tailored risk assessments.

The most effective ML techniques used for transfusion risk prediction are supervised regression models, including single logistic regression (LR) algorithms, Gaussian processes, and decision trees, or ensemble methods, such as gradient boosting (GB) and random forest.[4,8] These supervised ensemble methods use a combination of different ML algorithms to enhance prediction accuracy when a large amount of data is used. While the models differ in their mathematical approaches and simplicity, they share the fundamental ability to recognize complex patterns within data.

A primary challenge of ML models is their interpretability. Many advanced models function as "black boxes," making it difficult to

understand the reasoning behind their predictions. However, ML's main value relies on its ability to analyze extensive datasets for the identification of transfusion trends linked to individual surgeons, surgical techniques, and institutional blood utilization patterns. Published ML models have shown high predictive accuracy, as measured by AUC, c-statistics, and other performance metrics (Tables 1, 2, and 3). To upset the lack of transparency, ML model developers work on creating programs to explain the ML prediction. Transparency is a valuable asset to help providers trust the ML models.

Most of the published ML models are tailored to orthopedics and cardiac surgeries due to the high incidence of blood transfusions during these procedures and their unique patients' characteristics. Few models have been developed and validated for broader surgical applications (Table 1). Lou et al. created and published S-PATH, a GB ML model trained with data from years of elective surgical encounters from multiple hospitals across the United States.[14] S-PATH innovation incorporates patient and surgery-specific variables in the building of predictive algorithms. By analyzing a large multi-institutional dataset with diverse transfusion practices, S-PATH translates general procedure-based transfusion risks into personalized predictions based on patient comorbidities and preoperative laboratory results. The most significant variables identified are a high MSBOS and low HCT. The model has an outstanding predictive performance and is one of the few models that has been externally validated.[14]

When applied in different hospitals, the S-PATH model adjusts to local transfusion rates while refining risk estimation based on patient-specific factors. A subsequent external validation study further assessed the model's performance in a variety of hospital settings.[3] While incorporating hospital-specific transfusion rates slightly improved accuracy, model effectiveness varies significantly among institutions, underscoring the need for local validation before implementation. Building on this work, other researchers have explored ML approaches tailored to specific surgical populations to enhance predictive performance.[32]

Park et al. focused on developing a ML classifier to predict intraoperative transfusion risk specific for noncardiac surgeries.[32] In this single center study, the main variables identified that influence the need for transfusion are operation time, preoperative HgB level, and open surgery. Longer surgeries are linked to higher transfusion risks due to complexity, complications, and patient factors. The model accounts for different surgery durations and allows surgeons to input expected longer times for better accuracy. Preoperative anemia and open surgery are also strong independent risk factors. Other factors like prothrombin time, sodium levels, and age play a smaller role, but ML enhances predictions by considering multiple variables, even if their clinical significance is not fully understood.[32]

Machine learning models for cardiac surgeries

Several ML models have been developed for transfusion risks during cardiac surgery (Table 2). Wang et al. designed an ML-based model for estimating RBC transfusion needs in cardiothoracic procedures.[33] This model use LR algorithms and demonstrates strong predictive performance for cases involving 0 to 3 RBC units transfused but its accuracy diminishes when predicting transfusions of 4 or more units. Despite this limitation, the model is still valuable for blood inventory management, as it accurately predicts transfusion requirements using only preoperative variables.[33] The use of preoperative variables in the ML models allows for their comparison with traditional risk scores in different populations.

To compare the prediction ability of TRACK and TRUST cardiac transfusion risk scores and ML algorithms in the Brazilian population, Cunha et al. developed an LR-ML model trained on local patient data, where age and body surface area (BSA) are the top variables influencing transfusion needs.[18] Despite using a smaller dataset than previous studies, the LR-

Table 1. Published Machine Learning Models for Surgery

Study	Characteristics	Number of cases		Type of validation	Prediction accuracy	Significant variables found	Type of ML used	Inter-pretable design
		Training	Validation					
Lou et al,[14] 2022	Multicenter. 722 hospitals in US. Surgical cases.	2,439,694	16,053	External	c-statistic: 0.938	- MSBOS* - Hematocrit* - Platelet count - INR - PTT - Creatinine - Sodium - Albumin - Bilirubin - Patient demograph-ics - Patient comorbid-ities	Gradient boosting machine	Yes
Lou et al,[3] 2024	NA.	NA.	1,000,927	External. Multicenter. 414 hospitals in US.	AUC using hospital-specific priors: 0.9246 AUC using NSQIP-wide priors: 0.9100	NA.	NA.	NA.
Park et al,[32] 2025	Single center. South Korea. Noncardiac surgery patients	4378	1877	Internal	AUC: 0.836	- Operation time* - Preoperative Hgb* - Surgical approach – open surgery* - ASA physical status - Emergency operation - PFT – mild Obstructive panel - Preoperative AST, BUN, Creatinine - Operation type – stomach	LR	No

Abbreviations: MSBOS, Maximum Surgery Blood Ordering Schedule; INR, International Normalized Ratio; PTT, activated partial thrombin time; NA, not applicable; AUC, area under the curve; NSQIP, National Surgical Quality Improvement Program; Hgb, hemoglobin; ASA, American Society of Anesthesiologists; PFT, pulmonary function test; AST, alanine aminotransferase; BUN, blood urea nitrogen; LR, logistic regression.
*Most meaningful variables.

Table 2. Published Machine Learning Models for Cardiac Surgery

Study	Characteristics	Number of cases		Type of validation	Prediction accuracy	Significant variables found	Type of ML used	Interpretable design
		Training	Validation					
Wang et al,33 2022	Single center. USA. CT surgery	2,410	437	Internal	AUC: 0.826	- ECMO initiation - ECMO continuous - ECMO cannulation - Thoracoab Dominal aortic aneurysm repair - Barometric Pressure (blood gas analysis) - Potassium - Ionized calcium - Hgb - Alb - RV >96h	GPR	No
Cunha CBC, et al,18 2024	Single center. Brazil. Cardiac surgery	396	99	Internal	AUC: 0.735	- Age* - BSA* - Hgb* - Gender* - Prior cardiac surgery - Use of CPB	LR	Yes
Hur et al,9 2024	Single center. Republic of Korea. Thoracic surgery	6,200	1,643	Internal	RMSE: 3.203 R2: 0.399	- MSBOS* - Hgb* - PT - Platelet count - Comorbidity (cancer) - PTT - Comorbidity (renal disease) - Comorbidity (myocardial infarction) - Coumarin derivative	XG boosting	Yes

Abbreviations: CT, Cardiothoracic; AUC, area under the curve; ECMO, Extracorporeal Membrane Oxygenation; Hgb, hemoglobin; ALB, albumin; RV, respiratory ventilation; GPR, Gaussian Process Regression; BSA, body surface area; CBP, cardiopulmonary bypass; RMSE, Root Mean Square Error; R^2, root square; MSBOS, Maximum Surgical Blood Ordering Schedule; PT, prothrombin time; INR, International Normalized Ratio; PTT, activated partial thrombin time; XG boosting, extreme gradient boosting.
*Most meaningful variables.

Table 3. Published Machine Learning Models for Orthopedic Surgery

Study	Characteristics	Number of cases		Type of validation	Prediction accuracy	Significant variables found	Type of ML used	Interpretable design
		Training	Validation					
Chen et al,23 2023	Multicenter. 7 hospitals in China. Orthopedic surgery.	47,684	11,921	Internal	AUC: 0.831	- Operation type* - Age* - RBC count - Preoperative erythropoietin - ALB - PTT -BMI	CatBoost	No
Zhou et al,34 2024	Single center. China. Hip fracture surgery.	2,228	99	Internal: 557 External: 122	AUC: 0.887 AUC: 0.834	- Type of surgery* - Duration of surgery* - Hyponatremia* - Preoperative anemia* - Age* - Types of anesthesia - Stroke - Wait time for surgery - ASA physical status - Hypertension - Sex	RF	Yes
Zang et al,35 2024	Single center. China. Hip fracture surgery.	2,431	730	Internal	AUC: 0.85	- Operation time* - Preoperative HgB* -Femoral head Necrosis - ASA physical status - Osteoarthritis - THA - Anemia - Autotransfusion - Fibrinogen - ALB	Ridge classifier	Yes
Zhu et al,30 2024	Multicenter center. 3 hospitals in China. THA for femoral neck fracture.	829	NP	Internal External	c-statistic: 0.98 c-statistic: 0.93	- IBL* - Preoperative HgB* - Operation time* - Preoperative ALB - BMI - Anticoagulant history - TXA use	LR	Yes

Abbreviations: AUC, area under the curve; RBC, red blood cell; ALB, albumin; PTT, activated partial thrombin time; BMI, body mass index; ASA, American Society of Anesthesiologist; RF, random forest; HgB, hemoglobin; THA, total hip arthroplasty; NP, not published; IBL, intraoperative blood loss; TXA, tranexamic acid; LR, logistic regression.
*Most meaningful variables.

ML model outperforms TRACK and TRUST in predicting transfusion risk. While these traditional risk scores incorporate similar variables — age, weight, sex, HgB/HCT levels, and history of prior surgery — they were developed using data from populations with higher baseline HgB levels than those in Brazil. This discrepancy underscores the limitations of generalized risk scores and highlights ML's ability to provide tailored predictions.[18] Other models have been designed to enhance prediction accuracy by integrating clinical decision tools with dynamic patient data.

Hur et al. introduced the pMSBOS-TS model, integrating the MSBOS with patient-specific clinical and laboratory data to enhance transfusion predictions for thoracic surgery.[9] The model shows superior predictive accuracy compared to MSBOS alone, reducing unnecessary T&S orders by one-third. Notably, it predicts transfusion likelihood and estimates the number of RBC units needed. The variables with the highest predictive impact include a high MSBOS score and low preoperative HCT. To improve transparency, a clinical decision support system is incorporated, explaining the rationale behind predictions. However, a significant limitation of the model is its reliance on single-center data, reducing its generalizability.[9]

Machine learning models for orthopedic surgeries

In orthopedic surgery, ML models have identified novel transfusion risk factors. Besides the proven predictors—advanced age, low HgB, anticoagulant use, and low BMI—a multicenter ML model also identified low albumin (ALB) levels and prolonged activated partial thromboplastin time (APTT) as significant risk factors for postoperative RBC transfusion.[23] Preoperative ALB levels reflect nutritional status and liver function, with low values suggesting malnutrition and anemia, both of which increase transfusion risk. Similarly, prolonged APTT indicates impaired coagulation, heightening intraoperative and postoperative bleeding risk and, consequently, transfusion likelihood. [23] These findings have prompted more focused investigations into transfusion risks within specific orthopedic procedures such as hip surgeries.

Two independent research teams developed ML models for transfusion risk prediction in hip surgery. Zhou et al. used single-center data to identify preoperative variables linked to intraoperative RBC transfusion risk in unilateral hip fracture surgery.[34] This model indicates that internal fixation surgery, prolonged operative duration, and hyponatremia significantly increase intraoperative transfusion risk. The model underwent external validation, achieving excellent predictive performance. Zang et al. developed a similar ML model focused on perioperative transfusion prediction for hip surgery patients.[35] This model extends the predictive timeframe to 72 hours postoperatively but lacks external validation, limiting its broader applicability. Building on these efforts, other models have been designed for other types of orthopedic procedures, such as THA, to further refine transfusion risk predictions. [30]

Zhu et al. 2024 developed a ML predictive model for patients undergoing THA following femoral neck fractures.[30] After selecting key features and processing data, researchers identified 7 independent risk factors for blood transfusion: BMI, surgical duration, intraoperative blood loss, anticoagulant history, tranexamic acid usage, preoperative HgB, and preoperative ALB. Although the model performs well in internal validation, its predictive accuracy declines slightly when evaluated with external datasets. [30] The reduced effectiveness highlights the challenges of generalizability in ML models. However, despite these limitations, the model is still a valuable tool for assessing transfusion risk, supporting clinical decision-making, and improving perioperative blood management.

Discussion

The integration of machine learning into transfusion risk prediction represents a significant advancement in perioperative care. While traditional risk scores offer acceptable

accuracy, they often lack adaptability and fail to capture the complexity of individual patient profiles. Recent studies highlight the limitations of these conventional methods and emphasize the need for more precise, personalized tools. ML models stand out for their ability to process large datasets and detect complex, non-linear relationships among variables, something traditional models struggle with. These models have shown accuracy across a range of surgical specialties, offering a more dynamic and data-driven approach to predicting transfusion needs. Their flexibility and scalability make them a valuable asset in enhancing clinical decision-making and improving patient outcomes.

A central advantage of ML-based models is the ability to continuously learn and evolve as new data becomes available. This dynamic learning ability allows models to improve over time, unlike traditional static scoring systems, which often rely on fixed parameters and outdated population data.[18] Furthermore, ML approaches enable personalization and customization of predictions based on patient-specific variables, such as comorbidities, medications, intraoperative variables, and laboratory trends, providing a more tailored approach to transfusion planning. Similarly, including hospital and procedure-specific historical data in the building of the algorithm improve prediction accuracy.[3,9,23,34] Such individualized predictions lead to better resource allocation, reduce blood product wastage, and improve patient outcomes. The customization process is also affected by the chosen ML method, which differs based on the size and complexity of the data.

The types of ML methods used differ. As expected, ensemble methods, such as CatBoost, random forest, and GB demonstrate a higher prediction accuracy in larger data sets.[9,14,23,34] Simpler methods, such as LR, ridge classifier, and Gaussian regression, are valuable with smaller data sets.[18,30,32,33,35] While model selection plays a key role in prediction accuracy, other factors such as study design and dataset origin introduce limitations and potential biases.

There are persistent limitations and biases evident in the use of ML methods and evaluations. Most studies were conducted in single-center environments with retrospective designs, limiting the generalizability of the findings.[18,32-35] External validation is notably lacking, and the models developed in one healthcare setting may not perform equally well in another due to differences in surgical practices, data documentation, and patient demographics. The predictive models demonstrate robust performance on internal validation data; however, those with external validation show a decrease in accuracy when applied to external datasets.[3,30,34] This shows that due to the level of personalization, the algorithms benefit from incorporating local data in the learning process to achieve an excellent prediction. One example of a model that embraces the localized learning approach is the S-PATH model, which has undergone external validation across multiple hospital settings.[3,14] This model allows the input of local hospital and procedure-specific transfusion risks. S-PATH prediction accuracy is higher using local datasets than when using transfusion risks from a national dataset.[3] However, the model's performance still varied among different hospitals, showing that unmeasurable contributors, such as local transfusion culture, continue to affect the rate of perioperative transfusions.

Another notable limitation in ML models is related to the quality and quantity of the data. Small sample sizes, inconsistent definitions of transfusion triggers, and heterogeneous data preprocessing methods further complicate comparative analysis and model reproducibility. A common question in ML is how much training data is needed for the model to work well.[31] This is important because the amount of data can significantly impact the accuracy and reliability of the model. Finding the right balance between data quantity and quality is essential. It is widely accepted that using larger training datasets (typically more than 1,000 instances) tends to result in more

accurate models.[31] With larger datasets, ML algorithms are better equipped to identify complex patterns and relationships, improving overall model performance. Notably, the ML model that demonstrated lower performance was trained with fewer than 400 cases.[18]

Additionally, during model validation, careful consideration must be given to the selection of input variables. Omitting critical clinical data, such as coagulation parameters or medication use, limits the impact of the model's ability to accurately predict transfusion needs.[30,34,35] However, even when data quality and quantity is addressed, translating ML predictive models into routine clinical practice introduces a new set of logistical challenges.

Successful integration of ML into healthcare requires a robust information technology infrastructure to support data integration from various sources and real-time processing, introducing a logistical limitation to many institutions. Furthermore, data used for model training must be accurate, standardized, and well-organized. This is a significant hurdle in many hospitals where electronic health records are fragmented or inconsistently maintained.[8,33] Additionally, the "black-box" nature of some ML algorithms pose challenges for clinical acceptance, as transparency and interpretability are vital in medical decision-making.[23,32,33] When authors lack transparency when publishing ML model selection or performance metrics, they are limiting the interpretability and replicability. Transparency in the ML model helps clinicians trust the prediction, as they can see and make sense of the associated risk. Organizational readiness, including provider's trust and continuous education, is crucial for adoption.

In addition to technical and organizational limitations, potential biases were also observed. Selection bias is a concern in retrospective studies where the dataset may not represent the broader surgical population.[36] Additionally, publication bias may favor studies that report higher model performance, while negative or inconclusive findings are less likely to be published. The exclusion of patient cases due to missing data, electronic unavailability of important variables that influence transfusions risk, and authors not reporting how missing data was handled, introduces the possibility of information bias.

Although the development of innovative technologies, practices, and care models mark significant milestones in healthcare, technical innovation is only part of the equation. Successfully replicating and expanding healthcare innovation from one study or setting to a different context is neither straightforward nor guaranteed. Future research should address these limitations through multicenter, prospective studies with diverse populations and standardized data collection. There is a clear need for external validation of existing models, as well as the development of models that incorporate explainable components to ease clinical adoption. Additionally, the inclusion of blood products beyond RBCs is necessary when evaluating perioperative transfusion risk to support comprehensive product availability. Finally, the integration of ML models within electronic health record systems should be explored, with emphasis on assessing the real-world impact on transfusion practices, cost savings, and patient outcomes.

Conclusion

Effective prediction of transfusion needs is essential for optimizing blood inventory management, ensuring that limited blood products are allocated efficiently, while minimizing the risk of shortages and reducing unnecessary waste. ML offers a transformative approach to predicting perioperative transfusion risk. By leveraging large, diverse datasets and identifying complex, non-linear patterns, ML models can significantly enhance clinical decision-making, improve blood utilization, and lead to better patient outcomes across a wide range of surgical specialties.

However, integrating these models into clinical practice presents several challenges. Performance variability across institutions, limited external validation, retrospective

study designs, and inconsistent data quality all hinder widespread clinical adoption. Operational obstacles such as fragmented electronic health records, lack of standardized input variables, and the "black box" nature of some ML algorithms further complicate implementation. The effectiveness of ML models depends on access to high-quality, structured data and the ability for continuous validation and updates to ensure ongoing accuracy.

While the path to clinical integration of ML in perioperative transfusion prediction is complex, the potential benefits make it a worthwhile endeavor. With thoughtful design, rigorous validation, and interdisciplinary collaboration, ML has the capacity to redefine how health care approaches transfusion planning. ML models outperform perioperative transfusion guidelines by using real-time data, recognizing complex patterns, and personalizing decisions, leading to more accurate and efficient blood utilization in surgical patients.

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