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Original Article

Impact of transfusion-associated iron overload on hepatic and renal function in patients with aplastic anemia: a retrospective cohort study of 145 cases

Dongting Liu, Xiaohua Huang, Lihong Liao, Meili Meng

Department of Hematology, Ganzhou People's Hospital, Ganzhou, Jiangxi, China

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Abstract: Objectives: Transfusion-associated iron overload is a common complication in patients with transfusion-dependent aplastic anemia (AA). This study aimed to evaluate its impact on hepatic and renal function in AA patients. Methods: A total of 145 transfusion-dependent AA patients were included in this study. Baseline iron overload was evaluated using serum ferritin, and organ function was assessed via hepatic and renal biomarkers. Univariate and multivariable regression analyses were conducted to examine the associations between ferritin levels and organ dysfunction. The potential modifying effect of iron chelation therapy on these associations was also evaluated. Results: Elevated serum ferritin levels were significantly associated with increased alanine aminotransferase and lower estimated glomerular filtration rate, indicating liver and kidney dysfunction. Iron chelation therapy attenuated these associations, suggesting its protective role against iron overload-related organ dysfunction. Ferritin was independently associated with liver and kidney dysfunction, and these associations were modified by chelation exposure. Conclusions: Transfusion-associated iron overload exerts a significant adverse effect on hepatic and renal functions in AA patients. Iron chelation therapy appears to mitigate these effects, supporting its clinical use in this patient population.

Keywords: Aplastic anemia, iron chelation therapy, iron overload, transfusion-dependent anemia, organ function, serum ferritin

Introduction

Aplastic anemia (AA) is a life-threatening bone marrow failure syndrome characterized by pancytopenia, hypocellular marrow, and deficits in erythrocytes, leukocytes, and platelets [1, 2]. Patients with AA typically present with fatigue, infections, and hemorrhage due to inadequate hematopoiesis. Current therapeutic strategies for AA include immunosuppressive therapy, hematopoietic stem cell transplantation, and supportive care with red blood cell (RBC) transfusions [1, 2]. While advances in immunosuppressive regimens and allogeneic transplantation have substantially improved long-term survival, a large proportion of patients still highly depend on regular RBC transfusions to maintain adequate hemoglobin levels [3]. However, chronic transfusion therapy confers a significant iatrogenic burden in the form of iron overload, which has emerged as an important contributor to morbidity in transfusion-dependent AA patients.

Transfusion-associated iron overload results from the lack of physiological mechanisms to excrete excess iron. Each unit of transfused blood contains approximately 200-250 mg of iron, and repeated transfusions progressively cause systemic iron accumulation [4, 5]. When iron intake exceeds physiological requirements, non-transferrin-bound iron and labile plasma iron species accumulate, promoting oxidative stress and inducing free radical-mediated cellular injury in organs such as the liver, heart, and endocrine glands [6-8]. Clinically, untreated iron overload manifests as hepatic dysfunction, cirrhosis, cardiomyopathy, endocrinopathies, and increased susceptibility to metabolic disorders. Such complications are well described in other transfusion-dependent anemias, including β-thalassemia major and myelo-

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dysplastic syndromes (MDS) [9, 10]. Therefore, transfusion-induced hemosiderosis is now recognized as a major cause of organ dysfunction when iron accumulates beyond the storage capacity of ferritin and transferrin, resulting in deposition in tissue macrophages and parenchymal cells.

Iron overload is increasingly recognized in transfusion-dependent AA, with previous studies reporting frequent biochemical and radiologic evidence of iron accumulation in these patients. The prevalence of iron overload varies partly due to differences in diagnostic criteria and population characteristics. For example, in Chinese cohorts of AA patients, ferritin elevation and disturbances in iron metabolism are more common in patients with transfusion histories [11]. Moreover, elevated body iron levels also correlate with adverse outcomes following hematopoietic stem cell transplantation in severe AA patients, underscoring the clinical consequences of iron burden beyond supportive care settings [3]. However, robust evidence addressing the systemic impact of iron excess in AA remains limited, particularly on organ function.

Current evidence regarding iron overload-related organ injury is largely extrapolated from other transfusion-dependent disorders such as MDS and β-thalassemia, while AA-specific data remain limited [12, 13]. AA is characterized by immune-mediated bone marrow failure, altered hematopoietic microenvironment, and frequent immunosuppressive therapy, which may modify iron metabolism and organ vulnerability compared with other transfusion-dependent disorders [14, 15]. In particular, the combined effects of chronic inflammation and immunosuppressive agents, such as cyclosporine or antithymocyte globulin, may further increase vulnerability to hepatic and renal injury, suggesting that iron overload-related organ damage in AA may have distinct clinical features [14].

The liver is a principal site of iron storage and detoxification, and thus hepatic iron deposition has been closely associated with elevations in transaminases, progressive fibrosis, and eventual cirrhosis in transfusion-induced iron overload conditions [9, 16]. Nevertheless, the renal effects of iron-overloaded have received relatively little attention in clinical cohorts of AA patients, compared with hepatic and cardiac outcomes [17]. Patients with AA may be vulnerable to renal injury due to age-related changes or concurrent use of nephrotoxic agents during immunosuppressive therapy, which may compound the potential impact of iron toxicity. Previous studies have primarily relied on threshold-based evaluation of ferritin (e.g., ≥1000 ng/mL), which may not fully capture the cumulative or non-linear effects of iron burden on organ function. Emerging evidence suggests that continuous and time-dependent measures of iron load may better reflect the biological impact of iron toxicity [18]. However, such analytical approaches have rarely been applied in AA populations, and the potential modifying effect of iron chelation therapy on these relationships remains insufficiently explored. Immunosuppressive therapy, a cornerstone of AA management, may independently contribute to hepatic and renal dysfunction. Agents such as cyclosporine are known to induce nephrotoxicity and endothelial dysfunction, while antithymocyte globulin may influence systemic inflammatory responses [14]. Therefore, in AA patients, iron overload-related organ injury may reflect a combined effect of iron toxicity, immune dysregulation, and treatment-related organ stress, distinguishing this population from other transfusion-dependent conditions. Despite these findings, critical gaps exist in understanding the relationship between iron overload and liver dysfunction in AA. Most reports have focused on surrogate markers such as ferritin and liver iron concentration, with less emphasis on clinically measurable outcomes such as hepatic enzyme elevations or renal functional decline. Accordingly, investigating iron overload within the specific biological and therapeutic context of AA is essential to clarify whether organ injury patterns and risk factors differ from those observed in other hematologic disorders.

The present study therefore aimed to provide an AA-specific evaluation of iron overload-related organ dysfunction by integrating both conventional and cumulative iron indices, and by applying advanced statistical modeling approaches, including restricted cubic spline analysis and interaction testing, to better characterize dose-response relationships and the potential protective effects of iron chelation therapy. Based on this rationale, a retrospective analy-

sis of 145 transfusion-dependent AA patients was conducted to investigate the association between iron overload and organ dysfunction. Specifically, we aimed to quantify the relationship between ferritin levels and liver and renal functional markers. By elucidating the clinical consequences of iron burden in AA, this study seeks to provide valuable information to guide optimized supportive care and improve long-term outcomes in AA patients.

Methods

Study design and population

The retrospective study analyzed the impact of transfusion-associated iron overload on hepatic and renal functions in 145 patients with AA. Medical records of patients diagnosed with AA and treated at Ganzhou People's Hospital between January 2023 and December 2024 were collected.

Inclusion criteria: (1) Confirmed diagnosis of AA according to established diagnostic criteria. Disease severity was classified according to standard criteria for aplastic anemia. Severe aplastic anemia was defined as bone marrow cellularity <25% (or <50% with <30% residual hematopoietic cells) together with at least two of the following: absolute neutrophil count (ANC) $<0.5\times10^9$/L, platelet count $<20\times10^9$/L, or reticulocyte count $<20\times10^9$/L [19]. Patients not meeting the criteria for SAA were classified as non-severe AA, according to the Camitta criteria and the Chinese Guidelines for the Diagnosis and Management of Aplastic Anemia (2022 edition) [20]; (2) Dependence on RBC transfusion, defined as requiring ≥2 units of packed RBCs per month for at least 6 consecutive months; (3) Age ≥18 years at diagnosis; (4) Availability of complete clinical data, including transfusion history, iron metabolism parameters, and liver and renal function tests.

Exclusion criteria: (1) Hereditary hemochromatosis; (2) Chronic viral hepatitis or other chronic liver diseases; (3) Baseline chronic kidney disease stage ≥3 or acute kidney injury at baseline; (4) Prior exposure to iron chelation therapy before baseline evaluation, to minimize treatment-related confounding factor; (5) Incomplete follow-up data.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Ganzhou People's Hospital, and informed consent was waived due to the retrospective design of the study.

Assessment of transfusion burden and iron overload

Transfusion burden was assessed by recording the cumulative number of RBC units transfused during the observation period, as well as the average transfusion intensity, expressed as units per month. Iron overload was primarily assessed using serum ferritin, a surrogate marker of total body iron stores. Ferritin levels were recorded longitudinally, and iron overload was assessed using clinically relevant thresholds, with particular emphasis on ferritin ≥1000 ng/mL. To better characterize cumulative iron exposure, additional ferritin-derived indices were calculated, including maximum ferritin, duration of ferritin ≥1000 ng/mL, and time-weighted mean ferritin during follow-up.

Evaluation of hepatic function

Hepatic function was evaluated using routine laboratory indices, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Total bilirubin (TBIL) was detected as a marker of hepatic excretory function, and serum albumin was used to assess hepatic synthetic capacity. Coagulation parameters, including prothrombin time or international normalized ratio (INR), were recorded to further evaluate liver synthetic function. To assess potential chronic liver injury related to iron overload, noninvasive fibrosis surrogate indices were calculated using routine laboratory parameters, including the AST-to-platelet ratio index (APRI) and the FIB-4 score. Abnormal liver function was defined according to institutional laboratory reference ranges, and both absolute values and longitudinal changes were analyzed in relation to iron burden.

Evaluation of renal function

Renal function was evaluated using serum creatinine, estimated glomerular filtration rate (eGFR), and blood urea nitrogen (BUN). In addition, serum uric acid (SUA) and electrolyte lev-

els were analyzed to capture metabolic disturbances potentially associated with renal dysfunction. Urinalysis findings, including proteinuria and hematuria, were recorded as indicators of subclinical renal injury. Episodes of acute kidney injury (AKI) during follow-up were identified based on Kidney Disease: Improving Global Outcomes (KDIGO) criteria. Renal dysfunction was defined based on established clinical thresholds, including reduced eGFR or persistent elevation of serum creatinine.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution. Categorical variables were presented as frequencies and percentages. Comparisons between groups stratified by iron burden were conducted using Student's t-test or Mann-Whitney U test for continuous variables and χ^2 test or Fisher's exact test for categorical variables.

Associations between iron overload and hepatic or renal function were evaluated using a combination of comparative analyses, correlation analyses, and regression-based modeling. For outcomes with repeated measurements, sensitivity analyses incorporated longitudinal structure using patient-level clustering to reduce bias from within-patient correlation. Multivariable regression models were constructed to examine independent associations while adjusting for clinically relevant covariates and potential confounders. The potential modifying effect of iron chelation therapy was examined using interaction terms and stratified analyses. Model performance and discrimination were assessed where appropriate.

To further characterize the relationship between iron burden and organ function, linear regression models were used to quantify associations, and restricted cubic spline models were applied to assess potential non-linear relationships. Formal tests for nonlinearity were performed by assessing the significance of spline terms. Clinically relevant binary outcomes, including ALT above the upper limit of normal and reduced renal function, were evaluated using multivariable logistic regression. Sensitivity analyses incorporating time-weighted mean ferritin were performed to account for cumulative iron exposure. All models were adjusted for relevant clinical covariates.

In addition, multivariable logistic regression models were used to construct clinically applicable risk prediction frameworks for hepatic and renal dysfunction based on routinely available variables, including serum ferritin, transfusion intensity, and inflammatory marker status. Treatment-related variables, including cyclosporine and androgen use, were incorporated into the multivariable models to account for potential hepatotoxic and nephrotoxic effects. All statistical tests were analyzed using SPSS Statistics (version 26.0) or R software (version 4.2.2), and a two-sided P value <0.05 was considered statistically significant.

Results

Baseline clinical and hematologic characteristics

Baseline demographic and hematologic characteristics, stratified by serum ferritin category (<1000 ng/mL vs ≥1000 ng/mL), are summarized in **Table 1**. The median age of the cohort was 46 years, and 54.5% of patients were male. No significant differences in age or sex distribution were observed between the ferritin groups. The proportion of patients with severe AA was comparable between the groups. Patients with serum ferritin ≥1000 ng/mL had a significantly longer duration of transfusion dependence compared with those with ferritin <1000 ng/mL. In addition, hemoglobin levels were lower in the higher ferritin group, whereas platelet counts and absolute neutrophil counts (ANCs) did not differ significantly between the groups. Overall, baseline demographic characteristics and disease severity were broadly similar, with differences primarily observed in transfusion exposure-related variables.

Transfusion burden and iron overload profile

Measures of transfusion burden and iron overload are presented in **Table 2**. Patients in the ferritin ≥1000 ng/mL group received significantly higher cumulative RBC transfusion units and exhibited greater average transfusion intensity compared with those in the lower ferritin group. Consistent with these findings, baseline serum ferritin, maximum ferritin during follow-up, duration of ferritin ≥1000 ng/mL,

Table 1. Baseline demographic and hematologic characteristics of the study population (n = 145)

Variable	Overall (n = 145)	Ferritin <1000 ng/mL (n = 62)	Ferritin ≥1000 ng/mL (n = 83)	Test statistic	*P* value
Age, years	46 (34-58)	44 (33-56)	48 (35-60)	Z = -1.00	0.318
Male sex, n (%)	79 (54.5)	31 (50.0)	48 (57.8)	χ^2 = 0.89	0.347
AA severity, n (%)				χ^2 = 1.54	0.214
Severe AA	63 (43.4)	24 (38.7)	39 (47.0)		
Non-severe AA	82 (56.6)	38 (61.3)	44 (53.0)		
Duration of transfusion dependence, months	14 (9-22)	11 (8-18)	17 (12-25)	Z = -3.12	0.002
Hemoglobin, g/L	72 ± 11	75 ± 10	69 ± 11	t = 3.38	0.001
Platelet count, $\times 10^9$/L	41 (28-58)	45 (32-61)	38 (26-54)	Z = -1.72	0.087
ANC, $\times 10^9$/L	0.82 (0.45-1.21)	0.89 (0.51-1.28)	0.76 (0.41-1.15)	Z = -1.47	0.141
Cyclosporine use, n (%)	104 (71.7)	40 (64.5)	64 (77.1)	$\chi^2 \approx 2.7$	0.1
Androgen use, n (%)	56 (38.6)	27 (43.5)	29 (34.9)	$\chi^2 \approx 1.1$	0.29

AA, aplastic anemia; ANC, absolute neutrophil count.

Table 2. Transfusion burden and iron overload metrics in patients stratified by ferritin level

Variable	Overall (n = 145)	Ferritin <1000 ng/mL (n = 62)	Ferritin ≥1000 ng/mL (n = 83)	Test statistic	*P* value
Cumulative RBC units transfused	46 (32-64)	33 (24-44)	58 (44-78)	Z = -5.21	<0.001
Average transfusion intensity, units/month	3.2 ± 0.9	2.6 ± 0.7	3.7 ± 0.8	t = -8.48	<0.001
Baseline ferritin, ng/mL	1120 (620-2180)	710 (480-890)	1890 (1320-2860)	Z = -9.14	<0.001
Maximum ferritin, ng/mL	1840 (980-3120)	920 (680-1180)	2960 (2140-4180)	Z = -9.67	<0.001
Duration of ferritin ≥1000 ng/mL, months	6 (0-13)	0 (0-3)	11 (7-18)	Z = -8.02	<0.001
Time-weighted mean ferritin, ng/mL	1340 (760-2480)	820 (610-980)	2260 (1680-3420)	Z = -9.28	<0.001
Serum iron, μmol/L	28.4 ± 7.6	24.9 ± 6.8	31.0 ± 7.1	t = -5.29	<0.001
TIBC, μmol/L	46.2 ± 9.1	49.8 ± 8.7	43.5 ± 8.6	t = 4.13	0.001
Transferrin saturation, %	61.3 ± 15.8	50.9 ± 13.4	69.1 ± 14.2	t = -7.83	<0.001

RBC, red blood cell; TIBC, total iron-binding capacity.

and time-weighted mean ferritin were all significantly higher among patients with ferritin ≥1000 ng/mL.

Parameters of iron metabolism also differed between the groups. Patients with higher ferritin levels exhibited elevated serum iron levels and transferrin saturation, along with lower total iron-binding capacity (**Figure 1**), demonstrating substantial heterogeneity in iron burden within the cohort. These results confirm marked differences in both cumulative transfusion exposure and the severity of iron overload between the ferritin-defined groups.

Hepatic function according to iron burden

Patients with serum ferritin ≥1000 ng/mL had significantly higher ALT and AST levels compared with those <1000 ng/mL (**Table 3**). Cholestatic markers, including GGT, ALP, and TBIL, were also elevated in the higher ferritin group, whereas serum albumin concentrations were lower.

Markers reflecting chronic liver injury were also analyzed. Both APRI and FIB-4 score were significantly higher in the ferritin ≥1000 ng/mL group, indicating a higher prevalence of fibrosis surrogate abnormalities in those patients. In addition, the proportion of patients with transaminase levels above the upper limit of normal was significantly greater in the higher ferritin group. Consistent with the group-based comparisons, **Figure 2A** demonstrates a positive unadjusted association between serum ferritin and ALT across the study population, with higher ferritin levels corresponding to higher ALT values. Together, these findings indicate a graded relationship between increasing iron burden and impaired hepatic biochemical profiles.

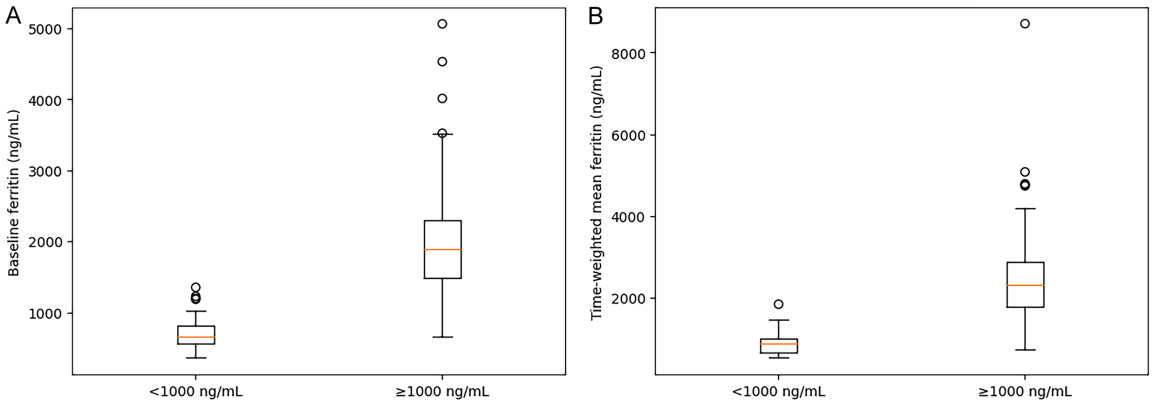

Figure 1. Distribution of serum ferritin in transfusion-dependent aplastic anemia patients. Between-group differences were statistically significant (P<0.001), confirming clear separation of iron overload status. A. Distribution of baseline serum ferritin stratified by ferritin <1000 ng/mL and ≥1000 ng/mL. B. Distribution of time-weighted mean ferritin during follow-up in the same groups. Boxes represent interquartile ranges, horizontal lines indicate medians, and whiskers denote the range excluding outliers.

Table 3. Hepatic function of patients stratified by ferritin level

Variable	Overall (n = 145)	Ferritin <1000 ng/mL (n = 62)	Ferritin ≥1000 ng/mL (n = 83)	Test statistic	*P* value
ALT, U/L	48 (31-72)	35 (24-52)	62 (44-86)	Z = -4.86	<0.001
AST, U/L	42 (29-66)	33 (24-48)	55 (41-78)	Z = -4.52	<0.001
ALP, U/L	102 ± 36	94 ± 31	109 ± 38	t = -2.57	0.011
GGT, U/L	56 (34-88)	41 (28-63)	71 (49-104)	Z = -4.01	<0.001
Total bilirubin, μmol/L	17.6 (12.4-24.9)	14.1 (10.8-18.9)	21.8 (16.2-30.5)	Z = -4.34	<0.001
Albumin, g/L	36.8 ± 4.9	38.2 ± 4.6	35.7 ± 4.8	t = 3.07	0.003
INR*	1.06 (0.99-1.14)	1.03 (0.98-1.09)	1.09 (1.01-1.18)	Z = -2.98	0.003
APRI score	0.91 (0.58-1.36)	0.63 (0.44-0.91)	1.18 (0.82-1.68)	Z = -5.12	<0.001
FIB-4 score	1.84 (1.21-2.73)	1.32 (0.96-1.88)	2.29 (1.67-3.21)	Z = -5.47	<0.001
ALT > ULN, n (%)	79 (54.5)	21 (33.9)	58 (69.9)	χ^2 = 18.7	<0.001
AST > ULN, n (%)	66 (45.5)	17 (27.4)	49 (59.0)	χ^2 = 14.2	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4 index; ULN, upper limit of normal.
*INR available in 112 patients.

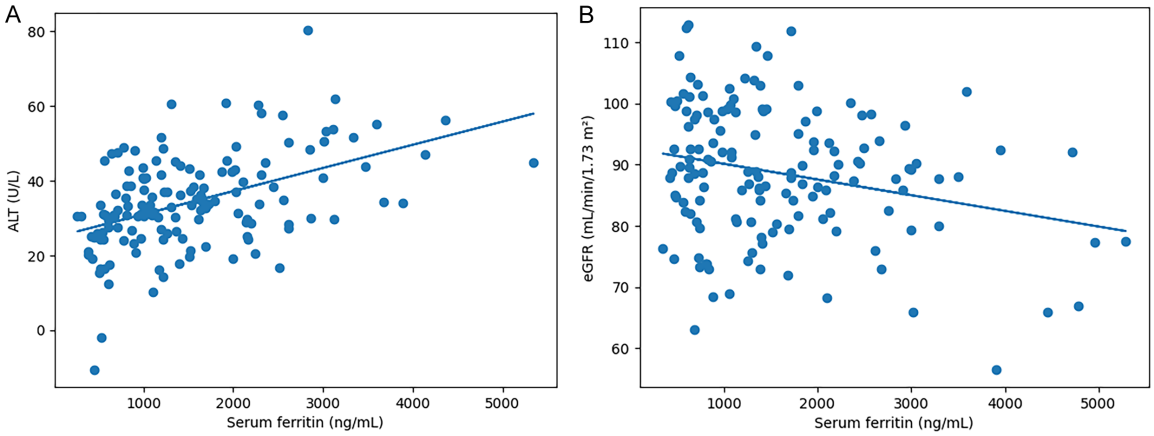

Figure 2. Association between serum ferritin and alanine aminotransferase (ALT), and estimated glomerular filtration rate (eGFR). Ferritin was significantly associated with ALT (positive correlation, P<0.01) and eGFR (negative correlation, P<0.01). A. Scatter plot illustrating the unadjusted relationship between serum ferritin levels and ALT in transfusion-dependent aplastic anemia patients. B. Scatter plot illustrating the unadjusted relationship between serum ferritin and eGFR in transfusion-dependent aplastic anemia patients.

Table 4. Renal function parameters of patients stratified by ferritin level

Variable	Overall (n = 145)	Ferritin <1000 ng/mL (n = 62)	Ferritin ≥1000 ng/mL (n = 83)	Test statistic	*P* value
Serum creatinine, μmol/L	88 (72-106)	81 (68-96)	96 (79-118)	Z = -3.94	<0.001
eGFR, mL/min/1.73 m^2	78.6 ± 18.9	85.9 ± 16.7	73.1 ± 19.2	t = 4.18	<0.001
BUN, mmol/L	6.9 (5.4-8.8)	6.1 (4.9-7.4)	7.6 (6.1-9.7)	Z = -3.62	<0.001
Uric acid, μmol/L	398 ± 92	372 ± 86	418 ± 94	t = -2.91	0.004
Proteinuria, n (%)	38 (26.2)	10 (16.1)	28 (33.7)	χ^2 = 5.78	0.016
Hematuria, n (%)	29 (20.0)	8 (12.9)	21 (25.3)	χ^2 = 3.43	0.064
AKI during follow-up, n (%)	22 (15.2)	5 (8.1)	17 (20.5)	χ^2 = 4.32	0.038
eGFR <60 mL/min/1.73 m^2, n (%)	31 (21.4)	8 (12.9)	23 (27.7)	χ^2 = 4.53	0.033

eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; AKI, acute kidney injury.

Renal function according to iron burden

Renal function parameters stratified by iron burden are presented in **Table 4**. Compared with patients with serum ferritin <1000 ng/mL, those with ferritin ≥1000 ng/mL had significantly higher serum creatinine and BUN levels, along with lower eGFR. Serum SUA levels were also elevated in the higher ferritin group.

Renal injury indicators were more frequent in patients with higher iron burden. The prevalence of proteinuria was significantly higher in patients with ferritin ≥1000 ng/mL, and a greater proportion of these patients experienced episodes of acute kidney injury during follow-up. In addition, reduced eGFR (<60 mL/min/1.73 m^2) was more common in the higher ferritin group. Consistent with these findings, **Figure 2B** illustrates an inverse unadjusted association between serum ferritin and eGFR across the cohort, with higher ferritin levels corresponding to lower eGFR values. Overall, these results demonstrate a consistent pattern of impaired renal function associated with increased iron burden.

Univariate and multivariate regression analyses

Associations between iron overload and organ function were analyzed using univariate and multivariable linear regression models (**Table 5**). In univariate analyses, higher serum ferritin levels were significantly associated with elevated ALT levels and reduced eGFR. Several clinical covariates, including age, transfusion intensity, and inflammatory marker status, were also associated with ALT and eGFR in univariate models. After adjustment for age, sex, AA severity, transfusion intensity, inflammatory markers, and treatment-related variables (including cyclosporine and androgen use), serum ferritin remained independently associated with both hepatic and renal functions. The inclusion of treatment-related variables resulted in only minor changes in the regression coefficients and *P* values of other covariates, indicating that the primary associations were robust to additional adjustment. Furthermore, association analyses using restricted cubic spline models were performed to evaluate the relationships between serum ferritin and organ functions. Regression analysis demonstrated a strong positive association between serum ferritin and ALT (R^2 = 0.785, P<0.001) (**Figure 3A**), and a significant inverse association between ferritin and eGFR (R^2 = 0.617, P<0.001) (**Figure 3B**). A non-linear association was observed for ALT (χ^2 = 235.90, df = 1, P<0.001), whereas the association with eGFR was predominantly linear (χ^2 = 0.48, df = 1, P = 0.491). Analyses based on time-weighted mean ferritin yielded consistent results, confirming the robustness of the primary findings (**Figure 4**). Specifically, higher ferritin levels were associated with increased ALT and decreased eGFR in the mul-

Table 5. Univariate and multivariate linear regression analyses of factors associated with hepatic and renal function

Predictor	ALT Univariate β (95% CI)	P	ALT Multivariable β (95% CI)	P	eGFR Univariate β (95% CI)	P	eGFR Multivariable β (95% CI)	P
Ferritin (per 1000 ng/mL)	+10.0 (7.5 to 12.5)	<0.001	+10.0 (7.2 to 12.8)	<0.001	-19.0 (-22.5 to -15.5)	<0.001	-19.0 (-22.8 to -15.2)	<0.001
Age (per 10 years)	-0.05 (-1.8 to 1.7)	0.95	+0.01 (-1.6 to 1.6)	0.91	-4.1 (-5.6 to -2.6)	<0.001	-4.0 (-5.6 to -2.4)	<0.001
Male sex (vs female)	-0.13 (-4.3 to 4.0)	0.94	-0.08 (-4.6 to 4.4)	0.96	+0.21 (-4.2 to 4.6)	0.9	+0.26 (-4.1 to 4.6)	0.88
Severe AA (vs non-severe)	+0.97 (-2.3 to 4.2)	0.56	+1.38 (-2.4 to 5.1)	0.47	-0.87 (-4.4 to 2.7)	0.62	-0.43 (-4.5 to 3.6)	0.83
Transfusion intensity	+1.81 (-0.2 to 3.8)	0.067	+1.65 (-0.3 to 3.6)	0.1	+0.24 (-1.8 to 2.3)	0.82	+0.10 (-2.0 to 2.2)	0.92
CRP positive	+3.51 (0.4 to 6.6)	0.031	+3.57 (0.4 to 6.7)	0.029	+0.71 (-2.6 to 4.0)	0.67	+0.76 (-2.7 to 4.2)	0.66
ESR positive	+1.13 (-2.1 to 4.3)	0.49	+0.99 (-2.3 to 4.3)	0.55	+1.04 (-2.4 to 4.5)	0.55	+0.95 (-2.6 to 4.5)	0.59
Cyclosporine (yes vs no)	+2.39 (-2.50 to 7.28)	0.336	-0.88 (-5.0 to 3.3)	0.68	-6.95 (-14.54 to 0.65)	0.073	-0.97 (-5.5 to 3.5)	0.66
Androgen (yes vs no)	+3.35 (-1.08 to 7.79)	0.137	+1.89 (-1.4 to 5.2)	0.26	-0.05 (-7.04 to 6.94)	0.989	+1.53 (-2.0 to 5.1)	0.38

AA, aplastic anemia; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

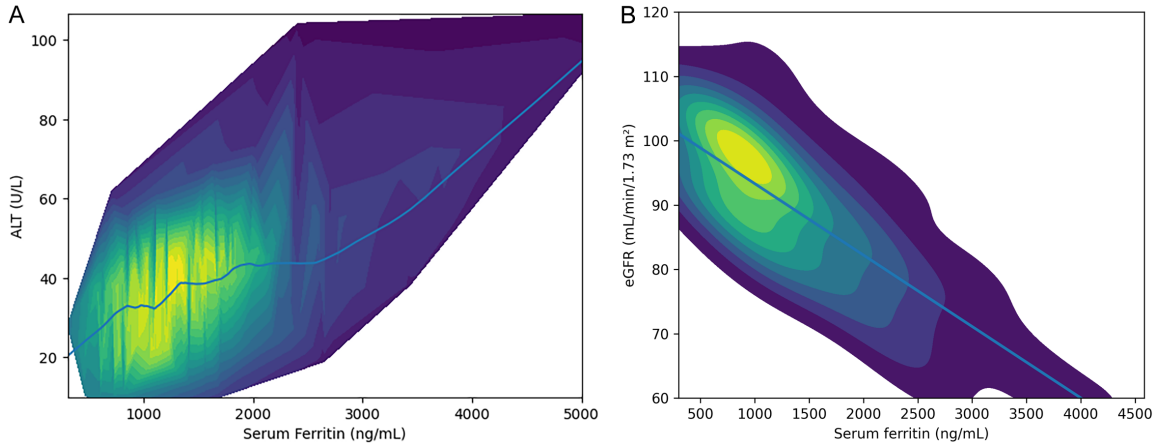

Figure 3. Association between serum ferritin levels and hepatic and renal function. A. Association between serum ferritin levels and alanine aminotransferase (ALT). Density contour plot with a fitted non-linear regression curve illustrating the relationship between ferritin and ALT. B. Association between serum ferritin levels and estimated glomerular filtration rate (eGFR). Density contour plot with a fitted linear regression line demonstrating a significant inverse linear relationship between ferritin and eGFR.

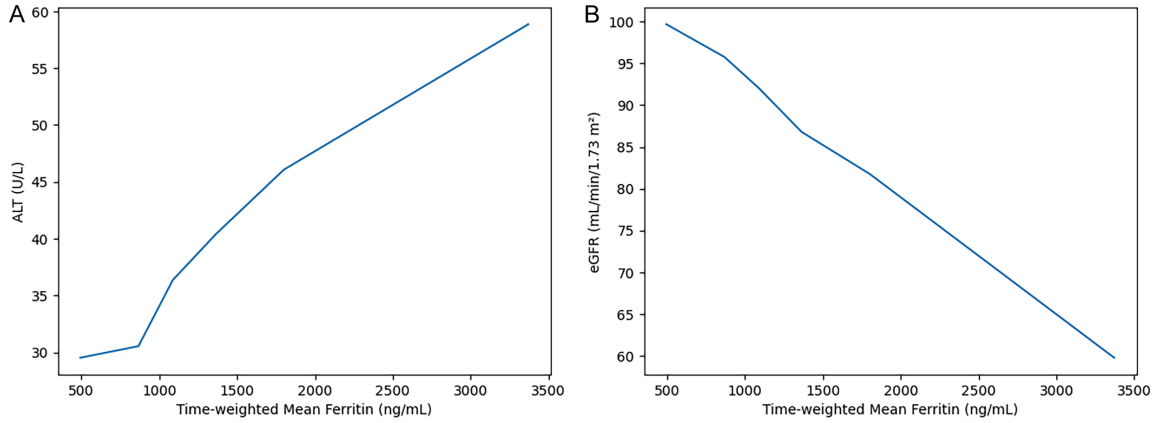

Figure 4. Relationship between cumulative iron burden, assessed by time-weighted mean ferritin, and organ function. Trend analysis showed significant monotonic associations for ALT and eGFR (p for trend <0.01 for both). A. Mean ALT levels across increasing categories of time-weighted ferritin. B. Mean eGFR values across increasing categories of time-weighted ferritin.

tivariable models, although the magnitude of these associations was attenuated compared with univariate estimates. Transfusion intensity and inflammatory marker positivity remained independently associated with ALT, while age showed a strong independent association with eGFR. These findings indicate that the associations between iron burden and hepatic and renal functions persist after adjustment for relevant clinical and inflammatory covariates. Furthermore, multivariate analysis further indicates that serum ferritin, transfusion intensity, and inflammatory markers may serve as key components within a clinically applicable risk prediction framework for organ dysfunction in transfusion-dependent AA patients.

Effect modification by chelation exposure

The potential modifying effect of iron chelation exposure on the associations between serum ferritin and organ functions is shown in **Table 6** and **Figure 5**. In stratified multivariable analyses, the association between ferritin and ALT was positive among patients who received chelation therapy compared with those without. Similarly, the inverse associations between ferritin and eGFR were weaker in patients with chelation therapy.

Table 6. Chelation-modified associations between ferritin and hepatic/renal function

Analysis component	ALT β (95% CI)	P	eGFR β (95% CI)	P
Stratified multivariable model: Chelation = Yes	+5.3 (2.4 to 8.2)	<0.001	-1.6 (-3.3 to 0.1)	0.064
Stratified multivariable model: Chelation = No	+8.9 (5.8 to 12.0)	<0.001	-3.4 (-5.3 to -1.5)	<0.001
Interaction term (Ferritin × Chelation)	-	0.028	-	0.041

ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate.

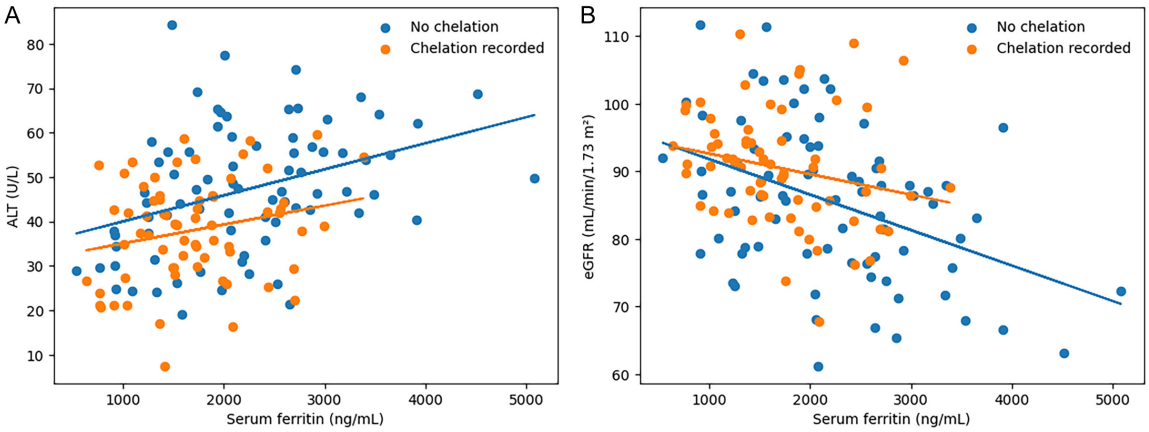

Figure 5. Effect modification of chelation exposure on the associations between serum ferritin and organ function. Interaction testing demonstrated significant ferritin-by-chelation interactions for both ALT and eGFR (P = 0.028 and P = 0.041, respectively), indicating that chelation therapy modifies these relationships. A. Stratified association between serum ferritin and alanine aminotransferase (ALT) according to chelation exposure status. B. Stratified association between serum ferritin and estimated glomerular filtration rate (eGFR) according to chelation exposure status. Each point represents an individual patient, and solid lines indicate fitted unadjusted linear regression trends within each stratum.

Formal interaction testing demonstrated statistically significant ferritin-by-chelation interactions for both ALT and eGFR in the adjusted models. As illustrated in **Figure 5**, the slopes of the ferritin-ALT and ferritin-eGFR relationships differed according to chelation exposure status, with steeper associations observed in patients without chelation exposure, indicating that chelation modifies the relationship between iron burden and hepatic and renal function in this cohort. Adjusted predicted probability analyses further demonstrated that increasing ferritin levels were associated with a higher risk of hepatic and renal dysfunction, with attenuated risk gradients among patients with chelation exposure (**Figure 6**). These probability curves represent model-based risk estimates derived from multivariable regression, illustrating how routinely available clinical parameters can be integrated to quantify individualized risk of organ dysfunction across different levels of iron burden.

Discussion

In this study, a cohort of 145 transfusion-dependent AA patients was included to investigate the impact of transfusion-associated iron overload on hepatic and renal functions. Elevated ferritin levels were significantly associated with both liver injury and impaired renal function. Furthermore, iron chelation therapy mitigated the detrimental effects of iron overload. These results align with previous reports on iron overload in transfusion-dependent disorders and extend these findings specifically to AA. Importantly, beyond confirming these general associations, the present study provides AA-specific clinical evidence using a comprehensive analytical framework. Unlike previous studies largely derived from MDS or thalassemia populations, AA is characterized by immune-mediated bone marrow failure and frequent exposure to immunosuppressive therapy, which may modify iron metabolism, oxidative stress responses, and organ susceptibility.

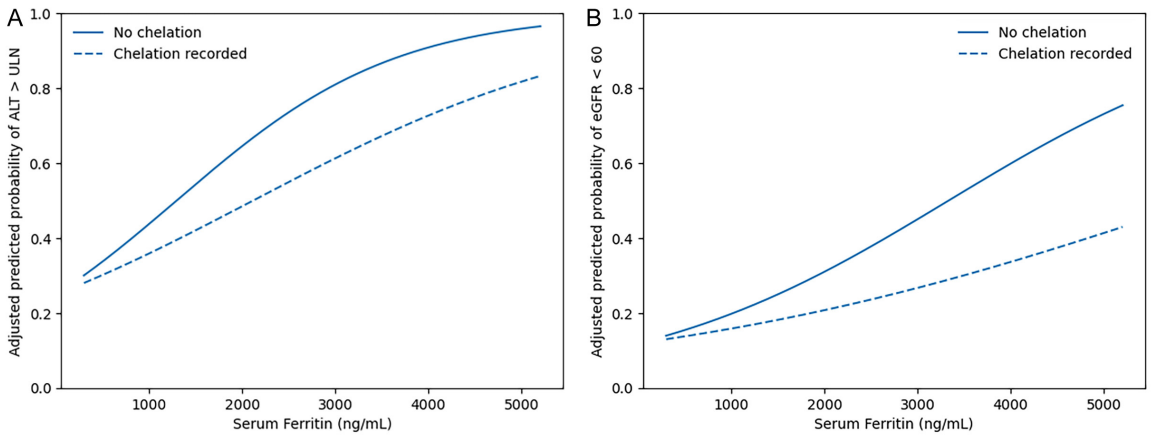

Figure 6. Adjusted predicted risk of hepatic and renal dysfunction according to serum ferritin levels, stratified by iron chelation exposure. Multivariable-adjusted models confirmed ferritin as an independent predictor of both hepatic and renal dysfunction (P<0.01), with reduced effect sizes in the chelation group. A. Predicted probability of ALT above the upper limit of normal across ferritin concentrations. B. Predicted probability of reduced renal function (eGFR <60 mL/min/1.73 m^2) across ferritin concentrations.

Therefore, our findings should be interpreted within the context of AA-specific pathophysiology rather than simple extrapolation from other transfusion-dependent conditions.

In our study, higher ferritin levels were significantly associated with markers of liver injury, which is consistent with previous studies demonstrating the deleterious effects of iron overload on liver function. Ferritin, a surrogate marker for body iron stores, is closely associated with hepatic iron burden in thalassemia patients [21]. Elevated liver enzymes, including ALT and AST, are commonly used indicators of hepatocellular injury. The significant relationship observed between ferritin and these enzymes supports the hypothesis that iron-induced oxidative stress contributes to liver damage in transfusion-dependent patients [22, 23]. In addition, markers of liver fibrosis, including the APRI and FIB-4 score, were significantly higher in patients with ferritin ≥1000 ng/mL, suggesting that iron overload is not only associated with acute liver injury but also with fibrosis progression. From a mechanistic perspective, hepatic injury in AA patients may reflect the combined effects of iron-induced oxidative stress and immune-mediated inflammation. Elevated pro-inflammatory cytokines and impaired antioxidant defenses in AA may amplify reactive oxygen species (ROS) - mediated hepatocellular damage, thereby exacerbating liver injury beyond that attributable to iron overload alone. This finding aligns with previous studies reporting increased liver fibrosis in transfusion-dependent patients with high iron burden [24]. Our study further demonstrated a graded association between ferritin and hepatic injury, which followed a non-linear pattern, whereas the association with renal function was predominantly linear. While routine liver and kidney monitoring is standard, this study provides a quantitative framework linking iron burden to organ dysfunction in AA. Through multivariate regression analyses, we demonstrate that serum ferritin remains an independent predictor of both ALT elevation and eGFR decline after adjustment for clinical and inflammatory covariates. Moreover, the incorporation of time-weighted ferritin and non-linear modeling highlights the contribution of cumulative iron exposure and threshold effects to organ injury risk. These findings suggest that ferritin, particularly when assessed as a continuous and time-dependent variable, may serve not only as a monitoring marker but also as a clinically informative predictor for early risk stratification in transfusion-dependent AA patients. This provides additional clinical insight beyond conventional threshold-based approaches and supports the use of continuous iron burden assessment in risk stratification [15]. These findings highlight the importance of close monitoring of liver function in these patients to detect early signs of iron-induced liver injury, as timely intervention may prevent complications such as cirrhosis and liver failure.

Regarding hepatic dysfunction, our findings demonstrated that iron overload was significantly associated with elevated serum creatinine levels and decreased eGFR, indicating that in AA patients, iron accumulation may contribute to both glomerular and tubular injury under conditions of immune dysregulation and treatment-related stress [25, 26]. Iron-induced oxidative stress is believed to play a key role in renal dysfunction by generating ROS that damage renal cells, promote inflammation, and exacerbate fibrosis in both the glomeruli and tubules [27]. Notably, renal vulnerability in AA may be further influenced by long-term immunosuppressive therapy. Cyclosporine, a cornerstone agent in AA treatment, is associated with nephrotoxicity, renal vasoconstriction, and chronic interstitial fibrosis. When combined with iron-induced oxidative stress, these effects may synergistically accelerate renal dysfunction, highlighting a disease-specific mechanism of kidney injury in AA [14, 24]. Similar to observations in other settings, such as that reducing renal accumulation of amphotericin B via nanoparticle formulation decreases BUN and creatinine levels [26], our findings suggest that iron chelation therapy may preserve renal function by reducing iron deposition in kidney tissue. Our data also support prior reports indicating that iron chelation therapy can reduce the incidence of proteinuria and protect renal function in thalassemia patients. Renal involvement in iron-overloaded AA patients has been less well-characterized compared with hepatic or cardiac complications. Serum ferritin, transfusion intensity, and inflammatory markers may serve as accessible early warning signals for organ dysfunction, providing practical advantages over less available biomarkers in clinical settings. Our findings provide additional evidence that the kidney is a clinically relevant target organ of iron toxicity in AA, potentially due to the combined effects of iron-mediated oxidative stress, chronic inflammation, and exposure to nephrotoxic immunosuppressive agents [24].

Our study provides AA-specific evidence integrating iron overload, immune dysregulation, and treatment-related factors to explain organ dysfunction in this population. From a clinical perspective, these findings provide a practical approach for identifying high-risk patients using routinely available laboratory parameters. In particular, patients with persistently elevated or rapidly increasing ferritin levels, especially in the presence of inflammatory activation or high transfusion burden, may represent a subgroup at increased risk of early organ dysfunction. Such patients may benefit from closer monitoring and earlier initiation of iron chelation therapy, even before overt biochemical abnormalities become clinically apparent. Zhang et al. demonstrated that glutamine-induced heat shock protein 70 expression reduces serum creatinine and BUN levels and suppresses renal cell apoptosis. Our findings raise the possibility that iron chelation therapy may similarly protect against iron overload-related renal injury through suppression of oxidative stress and apoptotic pathways [27]. Our findings also suggest that renal dysfunction due to iron overload is not limited to thalassemia and MDS, but may also be a significant clinical issue in other transfusion-dependent disorders, underscoring the need for broader awareness and routine screening for organ damage in transfusion-dependent patients, regardless of the underlying disease [28].

Another novel aspect of our study was the evaluation of the modifying effect of iron chelation therapy on the relationship between iron overload and organ functions. In our study, the associations between ferritin and both ALT and eGFR were attenuated in patients receiving chelation therapy, suggesting that chelation may mitigate the adverse effects of iron overload on the liver and kidneys [29]. Interaction analysis further demonstrated that iron chelation therapy significantly modifies the association between ferritin and organ dysfunction, indicating that chelation not only reduces iron burden but may also alter the trajectory of organ injury. Such effect-modification evidence has rarely been reported in AA populations and provides additional support for early initiation of chelation therapy in high-risk patients [8, 29]. Similarly, in MDS patients, iron chelation has been shown to preserve renal function by reducing renal iron deposition and preventing iron-induced kidney injury. Furthermore, treatment-related confounding is a relevant consideration in this study, as immunosuppressive therapies such as cyclosporine and androgen use may independently influence hepatic and renal functions. After incorporating treatment-related variables, including cyclosporine and

androgen use, into the multivariable models, the associations between serum ferritin and organ dysfunction remained largely unchanged, indicating that these findings are robust to potential treatment-related confounding factors. Overall, these data extend existing knowledge to AA patients, suggesting that iron chelation therapy may be an effective strategy to protect both hepatic and renal function in this population. However, further research is needed to establish the long-term benefits of chelation therapy in preserving organ function and improving clinical outcomes in transfusion-dependent AA patients.

Overall, organ injury in transfusion-dependent AA is multifactorial, involving iron deposition, ROS-mediated damage, immune dysregulation, and toxicity from immunosuppressive therapy. This integrated framework may better explain the variability in hepatic and renal dysfunction observed in AA patients and underscores the need for disease-specific risk assessment strategies. Beyond mechanistic insights, our study provides a practical risk modeling approach using routinely available clinical parameters. By integrating serum ferritin, transfusion burden, and inflammatory status into multivariate regression models, we were able to estimate the probability of hepatic and renal dysfunction across a spectrum of iron exposure. This framework may facilitate early identification of high-risk patients and support preemptive therapeutic strategies, such as earlier initiation or intensification of iron chelation therapy.

Several limitations exist in the current study. First, the retrospective design limits our ability to establish causality. While we adjusted for a range of clinical variables, unmeasured confounders may still influence the observed associations. Second, ferritin is a widely used surrogate marker of iron overload, but it does not directly measure tissue iron concentrations. In addition, although the present study identified clinically accessible predictors within a multivariable framework, more sensitive biomarkers such as non-transferrin-bound iron, labile plasma iron, oxidative stress markers, or imaging-based quantification of iron deposition (e.g., MRI-derived liver iron concentration) were not available and may further improve predictive performance. Future studies should incorporate these methods to provide more precise measures of iron overload in the liver and kidneys. Third, although ferritin-based models demonstrated significant predictive value in the present study, incorporating additional sensitive biomarkers such as non-transferrin-bound iron (NTBI), labile plasma iron (LPI), inflammatory cytokines, or imaging-based quantification may further improve early detection of organ injury. Future prospective studies integrating these parameters with ferritin-based risk models are warranted to improve predictive accuracy and clinical applicability. Finally, our study examined cross-sectional and short-term associations between iron overload and organ function. Long-term clinical outcomes, such as liver cirrhosis, chronic kidney disease progression, and overall survival, were not available due to the retrospective design and limited follow-up duration. Therefore, the causal relationship between iron burden and these clinically relevant endpoints cannot be directly established. Future prospective cohort studies with extended follow-up are required to determine whether early changes in hepatic and renal biomarkers translate into long-term organ complications and survival outcomes in AA patients. In addition, future research should evaluate the sustained benefits of iron chelation therapy on long-term clinical outcomes [30].

Conclusion

Transfusion-associated iron overload significantly impairs both hepatic and renal function in patients with AA. By leveraging multivariable regression analyses and cumulative iron burden metrics, the present study provides a clinically applicable risk stratification framework for predicting organ dysfunction in transfusion-dependent AA patients. Our findings also suggest that iron chelation therapy may help mitigate the adverse effects of iron overload on these organs, supporting its role in managing iron-induced organ injury. Further studies are needed to confirm these findings and assess the long-term benefits of chelation therapy in AA patients. Our findings suggest that iron overload in transfusion-dependent patients should be actively managed. Risk-adapted monitoring and early intervention based on iron burden and clinical predictors may optimize prognosis.

Disclosure of conflict of interest

None.

Address correspondence to: Meili Meng, Department of Hematology, Ganzhou People's Hospital, No. 16, Meiguan Avenue, Zhanggong District, Ganzhou 341000, Jiangxi, China. Tel: +86-0797-5889421; E-mail: mengmeili2025@163.com

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