International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 5, Number 4, November 2026, *Page. 3903 - 3915*
Email : editorijhess@gmail.com

Diagnostic Utility Of Hemoglobin And Reticulocyte Parameters For Iron Deficiency In Children With Cyanotic Congenital Heart Disease: A Systematic Review

Moh. Fathur Rozi[1)] [*], Nidia Nursafitri[2)], Indiana Maharani Wijaya[3)]
[1,3)] Faculty of Medicine, Muhammadiyah Malang University East Java, Indonesia
[2)] Department of Emergency, Wijaya Kusuma Hospital, Lumajang, East Java, Indonesia

*Corresponding Author
Email: mohfathurrozi26@gmail.com

Abstract

Chronic hypoxemia and compensatory erythrocytosis complicate the interpretation of hematological parameters in children with cyanotic congenital heart disease (CCHD). The diagnostic utility of hemoglobin and reticulocyte-related parameters for assessing iron deficiency in this population remains uncertain. This review aimed to evaluate the diagnostic accuracy of hemoglobin and reticulocyte parameters for detecting iron deficiency in children with established CCHD. Two studies involving 169 children were included. Hemoglobin and hematocrit showed limited discrimination for iron deficiency, with area under the receiver operating characteristic curve (AUC) values of 0.57 and 0.61, respectively. Reticulocyte hemoglobin content (CHr) and reticulocyte hemoglobin equivalent (Ret-He) demonstrated better performance, with AUC values of 0.74-0.83. At cut-offs around 28 pg, sensitivity ranged from 64.3% to 75.0% and specificity from 84.1% to 88.9%. Red cell distribution width (RDW) showed potential, with 91.5% specificity, while a combined CHr-RDW model achieved an AUC of 0.794 and an accuracy of 85.4% in one study. Reticulocyte percentage performed poorly (AUC 0.47). CHr and Ret-He may complement conventional investigations for iron deficiency in children with established CCHD, whereas hemoglobin and hematocrit have limited discriminatory value. The evidence remains limited by the small number of studies, heterogeneous diagnostic criteria, and lack of external validation. Larger prospective studies with age-specific thresholds and appropriate comparison groups are needed.

Keywords: Cyanotic Congenital Heart Disease, Children, Hemoglobin, Reticulocyte, Iron Deficiency, Diagnostic Accuracy, Systematic Review.

INTRODUCTION

Congenital heart disease (CHD) is the most common birth defect worldwide, with an estimated birth prevalence of 9.41 per 1,000 births in 2010-2017. CHD is classified as cyanotic or acyanotic. In this review, cyanotic CHD (CCHD) refers to structural cardiac abnormalities associated with chronic arterial desaturation, including tetralogy of Fallot, transposition of the great arteries, and pulmonary atresia (Cheng et al., 2024; Prichayudh et al., 2026). Although prenatal ultrasound and postnatal pulse oximetry improve early detection. Diagnostic challenges are particularly important in settings with limited access to screening and echocardiography. Accessible laboratory parameters may therefore provide additional clinical information for children with CCHD. However, chronic hypoxemia and compensatory erythrocytosis may complicate the interpretation of hemoglobin and related hematological indices, particularly when assessing coexisting iron deficiency. Evaluating the diagnostic performance of hemoglobin and reticulocyte-related parameters may help clarify their clinical utility and identify priorities for future research (Cheng et al., 2024).

Cyanotic congenital heart disease (CCHD) may remain clinically silent during fetal life because oxygen is supplied through the placenta and fetal circulation maintains systemic blood flow. Right-to-left shunting allows deoxygenated venous blood to enter systemic circulation, reducing arterial oxygen saturation. The degree of hypoxemia depends on anatomical and hemodynamic factors, including pulmonary outflow obstruction and changes in pulmonary vascular resistance (Taçoy, 2021; Tobis et al., 2017). Chronic hypoxemia triggers compensatory secondary erythrocytosis through increased erythropoietin production, raising red blood cell mass and oxygen-carrying capacity in children with CCHD. However, excessive erythrocytosis may increase blood viscosity and contribute to

complications, including hyperuricemia, iron deficiency, bleeding disorders, and thrombosis. Therefore, hematological parameters should be interpreted carefully in children with CCHD, particularly when assessing coexisting iron deficiency (Akbari et al., 2025; Kaba & Tadele, 2025).

Hemoglobin is essential for oxygen transport and tissue oxygenation. However, congenital heart defects may also lead to severe erythrocytosis when left untreated, particularly in conditions involving substantial intracardiac shunting, such as Eisenmenger syndrome (Borsani et al., 2024). Elevated hemoglobin and hematocrit do not always indicate absolute erythrocytosis. Relative erythrocytosis caused by plasma volume contraction, including dehydration, should first be excluded. Absolute erythrocytosis is then classified as primary or secondary. Primary erythrocytosis results from intrinsic bone marrow abnormalities, whereas secondary erythrocytosis is generally driven by increased EPO production. Its causes include chronic hypoxia, congenital oxygen-sensing abnormalities, EPO-producing tumors, and certain medications (Borsani et al., 2024). Therefore, hemoglobin and hematocrit should be interpreted alongside clinical findings and other hematological parameters when evaluating children with CCHD, particularly because iron deficiency may coexist with compensatory erythrocytosis.

Reticulocytes are immature, anucleate red blood cells released from the bone marrow following erythroblast enucleation (Newton et al., 2024). Anemia and hypoxemia may stimulate stress erythropoiesis, increasing reticulocyte production as an adaptive response to impaired oxygen delivery (Borges & Sesti-Costa, 2022). Reticulocyte counts, reported as percentages or absolute counts, reflect bone marrow erythropoietic activity (Bracho, 2022). The immature reticulocyte fraction (IRF) indicates the proportion of the youngest reticulocytes. CHr is an early marker of iron-deficient erythropoiesis. These parameters may identify changes before conventional hemoglobin and red cell indices decline, and CHr has demonstrated predictive value for iron deficiency anemia in children. However, evidence in children with cyanotic congenital heart disease (CCHD) remains limited (Gelaw et al., 2019).

Critical congenital heart disease comprises cardiac lesions requiring intervention during the first year of life and may initially remain asymptomatic. Relevant lesions include tetralogy of Fallot, pulmonary atresia, transposition of the great arteries, and other severe structural abnormalities (van Vliet et al., 2024). Symptoms may emerge after birth as fetal circulation changes and the ductus arteriosus closes, potentially causing cardiovascular collapse, mortality, and hypoxic-ischemic injury (Wasserman et al., 2021). Prenatal ultrasound, physical examination (PE), and pulse oximetry (PO) are important screening approaches. However, prenatal screening is not universally available in low- and middle-income countries (LMICs). Combining PE and PO achieved pooled sensitivity and specificity of 0.93 and 0.98, respectively (van Vliet et al., 2024). PO detects hypoxemia through differences in light absorption by oxygenated and deoxygenated hemoglobin. Echocardiography remains the reference standard for confirming suspected CCHD. Iron deficiency may be overlooked because compensatory erythrocytosis can produce apparently elevated hemoglobin levels; ferritin assessment is therefore important in affected children (Mukherjee et al., 2018).

Chronic hypoxemia stimulates secondary erythrocytosis, complicating the interpretation of hemoglobin and hematocrit because standardized diagnostic cut-offs for anemia in CCHD remain lacking (Prichayudh et al., 2026). Existing studies mainly evaluate iron status in children with established CCHD rather than the ability of hematological parameters to identify CCHD itself (Cheng et al., 2024). Hemoglobin and hematocrit have shown inconsistent or limited discriminatory value, whereas Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) and

reticulocyte hemoglobin parameters may provide additional information. Evidence is also limited regarding differences by age, cardiac lesion, and hypoxemia severity. Studies have used different oxygen saturation thresholds, iron deficiency definitions, and CHr/Ret-He cut-offs, while confounding factors such as inflammation, iron supplementation, and thalassemia were handled inconsistently. Moreover, associations between laboratory values and iron status do not necessarily establish diagnostic accuracy. A systematic synthesis is therefore needed to distinguish clinical associations from verified diagnostic performance.

Iron deficiency may complicate the clinical management of children with CCHD, making its identification important in this population. Complete blood counts and reticulocyte indices are relatively accessible hematological parameters that may help evaluate iron status, although they should be interpreted alongside appropriate iron status markers and cannot replace definitive diagnostic assessment. This systematic review aimed to evaluate the diagnostic accuracy of hemoglobin and reticulocyte parameters for detecting iron deficiency in children aged 0-18 years with established CCHD.

RESEARCH METHODS

Eligibility Criteria

Eligibility criteria were defined according to the PIRD framework (Population, Index test, Reference standard, Diagnosis of interest). The population comprised children aged 0-18 years with an established diagnosis of cyanotic congenital heart disease (CCHD). The index tests included hemoglobin, hematocrit, reticulocyte count, reticulocyte hemoglobin content (CHr), reticulocyte hemoglobin equivalent (Ret-He), and related red blood cell indices, including red cell distribution width (RDW), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). The reference standard was iron deficiency defined by serum ferritin, transferrin saturation, or other iron status markers, according to the definitions used in each study. The diagnosis of interest was iron deficiency in children with confirmed CCHD. Cross-sectional, cohort, and other observational studies were eligible if they reported diagnostic accuracy measures for at least one index test (sensitivity, specificity, AUC, predictive values, or data allowing construction of a two-by-two table). Studies were excluded if they involved adults only, acquired heart disease, animal or in-vitro models, or children without a confirmed diagnosis of CCHD, or if they did not report iron status as the reference standard. Only studies published between January 2021 and October 2026 were considered.

Information Sources and Search Strategy

The literature search was conducted between September and October 2026 using three electronic databases and search platforms: PubMed/MEDLINE, Google Scholar, and ScienceDirect. The search was restricted to studies published between January 2021 and October 2026 and written in English or Indonesian. The reference lists of included studies and relevant review articles were also manually screened to identify additional potentially eligible publications.

The search strategy combined free-text terms representing three main concepts: (1) congenital heart disease and cyanotic congenital heart disease; (2) hemoglobin, hematocrit, reticulocyte parameters, and red blood cell indices; and (3) children and pediatric populations. Variations in British and American spelling were included where applicable. The following general search strategy was adapted for each database: (cyanotic congenital heart disease" OR cyanotic heart disease) AND (hemoglobin OR haemoglobin OR reticulocyte) AND (child OR pediatric OR paediatric). Database-specific search syntax and filters were adjusted according to the search functions available in PubMed, Google Scholar, and ScienceDirect. Publication date filters were applied to restrict the results to the period

from 2021 to 2026. Search results from all sources were compiled for deduplication and subsequent screening.

Study Selection

All records retrieved from PubMed/MEDLINE, Google Scholar, and ScienceDirect were imported into reference management software, and duplicate records were removed before screening. Three reviewers (MFR, NN, and IMW) independently screened titles and abstracts, assessed full texts, extracted data, and appraised risk of bias using QUADAS-2. Disagreements were resolved through discussion until consensus was reached. The study identification, screening, eligibility assessment, and inclusion processes were summarized using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

Data Extraction

Data were independently extracted by three reviewers using a piloted standardized data extraction form. Extracted study characteristics included the first author, publication year, country, study setting, study design, sample size, age range, and CCHD subtypes. Information regarding the definition of CCHD, the oxygen saturation (SpO_2) threshold, and the reference standard used for diagnosis was also collected.

Index test data included the laboratory parameters assessed, specimen collection procedures, analyzer or measurement methods, reported diagnostic cut-off values, and methods used to derive those cut-offs. Diagnostic accuracy outcomes included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (AUC), together with their corresponding 95% confidence intervals (CIs), where available. Two-by-two contingency table data, including true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN), were extracted when reported or derivable.

Potential confounding factors were also recorded when available, including iron status indicators such as serum ferritin and transferrin saturation, previous iron supplementation, thalassemia or other hematological conditions, and inflammatory markers such as C-reactive protein (CRP). These factors were considered because they may influence hemoglobin concentrations and reticulocyte-related parameters independently of cyanotic heart disease.

Risk of Bias and Applicability Assessment

The methodological quality of the included studies was independently assessed by three reviewers using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. The assessment covered four domains: patient selection, index test, reference standard, and flow and timing. Each domain was evaluated for risk of bias, while applicability concerns were assessed for patient selection, the index test, and the reference standard.

Particular attention was given to whether diagnostic cut-off values were prespecified or derived from the study data, whether the index test was interpreted without knowledge of the reference standard results, and appropriate for establishing iron deficiency. Disagreements were resolved through discussion.

Data Synthesis and Statistical Analysis

The findings were first synthesized narratively and presented in summary tables describing study characteristics, index tests, diagnostic thresholds, reference standards, and reported diagnostic accuracy outcomes. Quantitative synthesis was considered when at least four sufficiently comparable studies reported two-by-two diagnostic data for the same index test and threshold. In such cases, a bivariate random-effects model or a hierarchical summary receiver operating characteristic (HSROC) model would be used to estimate pooled sensitivity and specificity, together with their corresponding 95% CIs.

Between-study heterogeneity was examined using the summary receiver operating characteristic (SROC) curve and prediction regions, where applicable. If sufficient data were available, subgroup analyses were planned according to age group, CCHD subtype, SpO_2 threshold, analyzer or measurement method, and hemoglobin cut-off value. The clinical and methodological comparability of the included studies was considered before conducting any meta-analysis.

Studies reporting only adjusted associations, such as odds ratios, without sufficient diagnostic accuracy data or two-by-two contingency tables were summarized narratively and were not included in the pooled diagnostic accuracy analysis. If the available evidence was insufficiently comparable for quantitative synthesis, the findings were reported descriptively.

RESULTS AND DISCUSSION

Study Selection

The database searches identified 220 records (ScienceDirect, n = 73; Google Scholar, n = 120; PubMed, n = 27). After removal of 74 duplicates, 146 records were screened by title and abstract and 107 were excluded. Of 39 reports sought for retrieval, 8 could not be accessed in full text, and 31 were assessed for eligibility. Twenty-nine were excluded after full-text review. Two studies (Cheng et al., 2024; Prichayudh et al., 2026) were included (Figure 1).

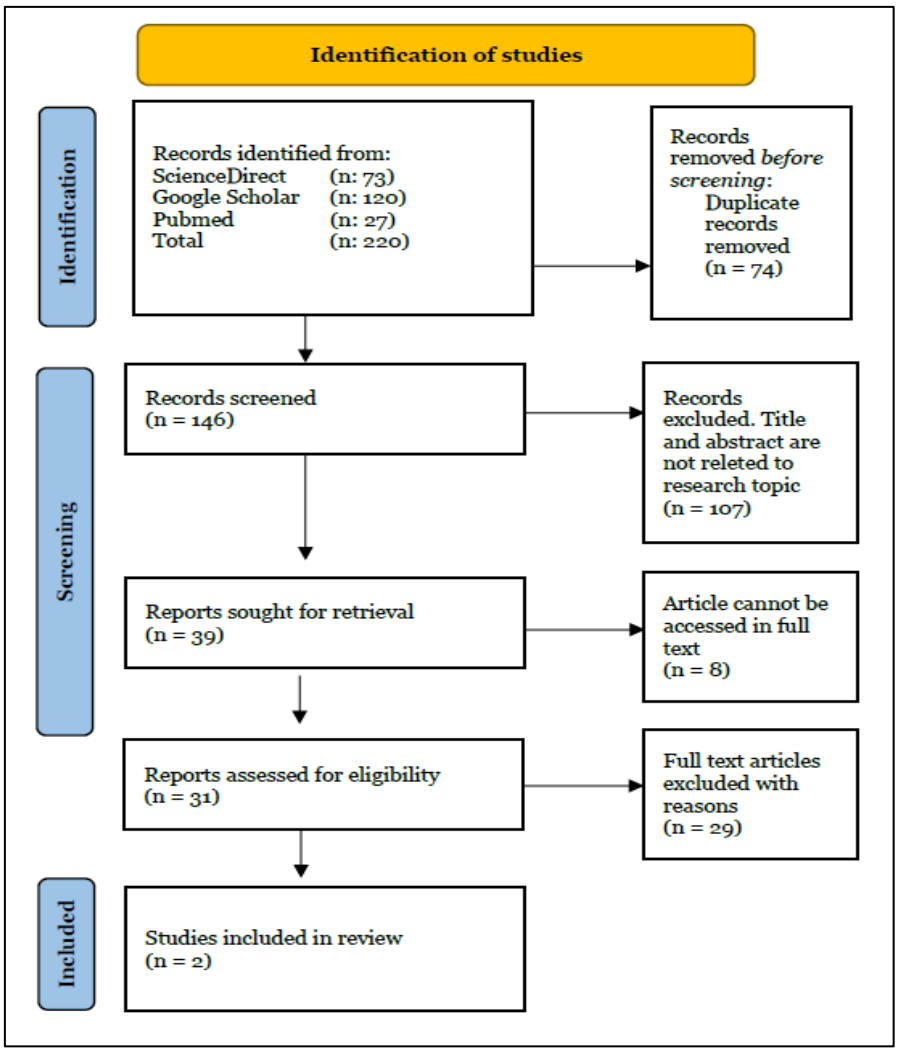


Figure 1. PRISMA Flow Chart

Characteristics of Included Studies

Both included studies enrolled children with established CCHD and evaluated iron deficiency. Cheng et al. (2024) was a prospective cross-sectional diagnostic test study conducted from December 2021 to March 2022 at two tertiary hospitals in Jakarta, Indonesia. It enrolled 59 children aged 3 months to 18 years (median 23 months) consecutively, after 6 of 65 eligible children were excluded; CCHD was confirmed by echocardiography with a mean daily SpO_2 below 90%. Prichayudh et al. (2026) was a study conducted from September 2022 to September 2023 at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, in 110 children aged 6 months to 15 years (median 5.2 years) with echocardiographically confirmed CCHD and SpO_2 below 95% in room air. The studies differed in index tests (Ret-He versus CHr, RDW, and other red cell indices) and in reference definitions. Cheng et al. defined iron deficiency as ferritin <12 ng/mL (<5 years) or <15 ng/mL (≥5 years) or transferrin saturation <15%, together with Haemoglobin (Hb) ≥16.5 g/dL and MCV <80 fL or MCH <27 pg, and iron deficiency anemia as Hb <16.5 g/dL with the same iron criteria; Prichayudh et al. defined iron depletion as ferritin <30 ng/mL or transferrin saturation <15% (Table 1).

Table 1. Characteristics and diagnostic accuracy results of the included studies

Author, Year	Country & Setting	Study Design	Sample Size (N)	Patient Population & Age	Index Test(s)	Reference Standard & Target Diagnosis	Diagnostic Cut-off Value	Diagnostic Performance (Sensitivity, Specificity, AUC)	Predictive Value & Odds Ratio	Key Findings & Authors' Conclusions
Cheng et al., 2024	Indonesia (2 Tertiary Hospitals in Jakarta: RSCM & Harapan Kita)	Prospective Cross-Sectional Diagnostic Study	59	59 children aged 3 months to 18 years (Median age: 23 months) with confirmed Cyanotic CHD (mean SpO2 < 90%). tetralogy of fallot (TOF) was the most common (33.9%).	Reticulocyte Hemoglobin Equivalent (Ret-He)	Reference Standard: • Serum Ferritin (<12 ng/mL for <5yo; <15 ng/mL for ≥5yo) or Transferrin Saturation (<15%) • Echocardiography for CCHD Target Diagnosis: 1) Iron Deficiency (ID without anemia) 2) Iron Deficiency Anemia (IDA)	• ID Cut-off: Ret-He < 28.8 pg • IDA Cut-off: Ret-He < 28.15 pg (Hb cut-off used: 16.5 g/dL)	1) ID (Ret-He < 28.8 pg): • Sensitivity: 75.0% • Specificity: 85.2% • AUC: 0.828 (95% CI: 0.664-0.934) 2) IDA (Ret-He < 28.15 pg): • Sensitivity: 75.0% • Specificity: 88.9% • AUC: 0.824 (95% CI: 0.691-0.916)	1) ID: • PPV: 60.0% • NPV: 92.0% • Likehood ratio (LR)+: 5.10, LR-: 0.30 2) IDA: • PPV: 85.7% • NPV: 80.0% • LR+: 6.75, LR-: 0.30	Ret-He is a dependable marker for assessing iron status in pediatric cyanotic CHD. However, Ret-He cannot be used as a standalone test to differentiate ID from IDA; it must be used in conjunction with Hemoglobin levels (e.g. ID: Ret-He <28.8 pg & Hb ≥16.5 g/dL; IDA: Ret-He <28.15 pg or Hb <16.5 g/dL).

Prichayudh et al., 2026	Thailand (King Chulalongkorn Memorial Hospital, Bangkok)	Descriptive / Cross-Sectional Diagnostic Study	110	110 children aged 6 months to 15 years (Mean age: 5.2 years) with confirmed CCHD (SpO2 < 95% in room air). Single ventricle & TOF most common.	Reticulocyte Hemoglobin Content (CHr) & Red Cell Distribution Width (RDW)	Reference Standard: • Serum Ferritin < 30 ng/mL or Transferrin Saturation < 15% • Echocardiography for CCHD Target Diagnosis: Iron Depletion Status (Prevalence: 25.5%)	• CHr Cut-off: ≤ 28.0 pg • RDW Cut-off: ≥ 18.8% • Combination: CHr ≤ 28 pg + RDW ≥ 18.8%	1) CHr (≤ 28 pg): • Sensitivity: 64.3% • Specificity: 84.1% • AUC: 0.74 (0.64-0.84) 2) RDW (≥ 18.8%): • Sensitivity: 57.1% • Specificity: 91.5% • AUC: 0.74 (0.65-0.84) 3) Combined CHr + RDW: • Model AUC: 0.794 (0.691-0.896) • Overall Accuracy : 85.4%	1) CHr (≤ 28 pg): • PPV: 58.1%, NPV: 87.3% • Adjusted OR: 4.97 (95% CI: 1.67-14.84, p=0.004) 2) RDW (≥ 18.8%) : • PPV: 69.6%, NPV: 86.2% • Adjusted OR: 7.79 (95% CI: 2.46-25.13, p=0.001)	Conventional parameters (Hb and Hct) fail to detect iron depletion due to secondary polycythemia. CHr (≤28 pg) and RDW (≥18.8%) are strong independent predictors of iron depletion. Combining CHr and RDW diagnostic accuracy to 85.4%, offering a cost-effective screening tool in limited-resource settings.

Hemoglobin and Hematocrit Findings

Hemoglobin (Hb) and hematocrit (Hct) showed limited ability to discriminate iron deficiency. Hb and Hct did not differ significantly by iron, while another reported the highest mean Hb in children with iron deficiency without anemia (20.2 g/dL), followed by those with normal iron status (17.1 g/dL) and iron deficiency anemia (IDA; 13.6 g/dL). These findings suggest that compensatory erythrocytosis may mask iron deficiency. SpO_2 did not differ significantly by iron status, and arterial oxygen content showed poor discrimination (AUC 0.51). Neither study established a direct relationship between SpO_2 and Hb (Cheng et al., 2024; Prichayudh et al., 2026).

The association between CCHD lesion type and iron status was not significant (P=0.85), and Ret-He performance was not evaluated across lesion types. Age-related effects also remain uncertain: Hb was highest in older children with iron deficiency without anemia and lowest in younger children with IDA, but adequate age-adjusted analyses were not performed. Hematological findings may be confounded by hypoxemia severity, age, cardiac lesion type, iron supplementation, inflammation, hemoglobinopathies, nutritional status, and altitude. Malnutrition was common, but its effects were not assessed using multivariable analysis. Differences in anemia definitions and iron deficiency criteria may also explain the variation in reported prevalence. These limitations highlight the need for standardized diagnostic criteria and adjusted analyses in future studies (Cheng et al., 2024; Prichayudh et al., 2026).

Reticulocyte-Related Parameters

Reticulocyte percentage showed poor diagnostic performance for iron deficiency, with an AUC of 0.47, sensitivity of 50%, and specificity of 43.9% (Prichayudh et al., 2026).

Neither study reported absolute reticulocyte counts or the immature reticulocyte fraction (IRF). In contrast, reticulocyte hemoglobin content (CHr/Ret-He) showed better diagnostic performance**.** Both studies found lower values in children with iron deficiency. CHr ≤28 pg had an AUC of 0.74, while Ret-He cut-offs of <28.8 pg and <28.15 pg yielded AUCs of 0.828 and 0.824, respectively. Sensitivity ranged from 64.3% to 75%, while specificity ranged from 84.1% to 88.9%. These findings suggest that CHr/Ret-He may help identify iron deficiency, although a normal result cannot reliably exclude it. Other blood indices also showed potential. RDW ≥18.8% had 91.5% specificity, and combining RDW with CHr achieved 85.4% accuracy in Prichayudh et al. The findings should be interpreted cautiously because the studies used different definitions of iron deficiency, included different populations, and did not validate their cut-offs in independent samples. Although CHr/Ret-He cut-offs were similar, further studies are needed to establish reliable thresholds for children with cyanotic CHD (Prichayudh et al., 2026).

Risk of Bias and Applicability

Table 2. Risk of Bias and Applicability Assessment (QUADAS-2 Tool)

Study ID	RISK OF BIAS (QUADAS-2 DOMAINS)				CONCERNS REGARDING APPLICABILITY			Overall Risk of Bias
	Domain 1: Patient Selection	Domain 2: Index Test	Domain 3: Reference Standard	Domain 4: Flow and Timing	Domain 1: Patient Selection	Domain 2: Index Test	Domain 3: Reference Standard	
Cheng et al., 2024	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW RISK
Prichayudh et al., 2026	UNCLEAR	LOW	LOW	LOW	LOW	LOW	LOW	UNCLEAR RISK

The risk of bias and applicability of the included studies were assessed using the QUADAS-2 tool, which evaluates four domains of risk of bias and three domains of applicability concerns. As presented in Table 2, Cheng et al. (2024) demonstrated a low risk of bias across all four domains: patient selection, index test, reference standard, and flow and timing. Furthermore, all three applicability domains were rated as low concern, indicating good applicability of the study findings to the review question. Therefore, the overall risk of bias for this study was classified as low.

In contrast, Prichayudh et al. (2026) showed an unclear risk of bias in the patient selection domain, while the index test, reference standard, and flow and timing domains were all rated as low risk. All three applicability domains were also assessed as having low concern. The unclear rating for patient selection suggests that the available information was insufficient to determine whether the participant recruitment process might have introduced bias. Consequently, the overall risk of bias for this study was classified as unclear risk. Overall, the assessment indicates that Cheng et al. (2024) had a more favorable methodological profile, whereas the findings of Prichayudh et al. (2026) should be interpreted with some caution due to uncertainty regarding patient selection.

Discussion

Principal Findings

This systematic review included two studies involving 169 children with cyanotic congenital heart disease (CCHD). Hemoglobin and hematocrit showed limited ability to identify iron deficiency, whereas reticulocyte hemoglobin content (CHr) and reticulocyte hemoglobin equivalent (Ret-He) demonstrated better diagnostic performance. Hemoglobin and hematocrit showed poor discrimination, with AUC values of 0.57 and 0.61, respectively. Chronic hypoxemia may maintain elevated hemoglobin levels through compensatory erythrocytosis, potentially masking iron deficiency. In contrast, CHr and Ret-He achieved AUC values of 0.74-0.83, with moderate sensitivity (64.3-75.0%) and relatively high

specificity (84.1-88.9%). RDW also showed potential, and combining RDW with CHr achieved an accuracy of 85.4%. However, this model was not externally validated. These findings suggest that CHr, Ret-He, and RDW may complement conventional tests when assessing iron status. Nevertheless, differences in study populations, iron deficiency definitions, and laboratory methods limit comparability. Both studies derived cut-offs from their own samples without external validation. Future studies should include children with suspected CHD, appropriate comparison groups, standardized reference standards, and independent validation (Cheng et al., 2024; Prichayudh et al., 2026).

Interpretation of Hemoglobin and Hematocrit

Chronic hypoxemia stimulates secondary erythrocytosis to improve oxygen-carrying capacity. However, iron deficiency may impair this response and contribute to complications associated with excessive erythrocytosis and increased blood viscosity. In Prichayudh et al. (2026), median hemoglobin was similar in iron-depleted and iron-sufficient children (17.8 vs. 17.3 g/dL; $P = 0.90$). Cheng et al. (2024) also reported the highest hemoglobin and hematocrit in children with iron deficiency without anemia, followed by lower values in those with iron deficiency anemia. This suggests that elevated hemoglobin does not exclude iron deficiency. Iron deficiency may also affect oxygen delivery despite similar hemoglobin and oxygen saturation (Cheng et al., 2024).

Age, clinical characteristics, and other factors may influence these findings. The iron-depleted group was older in Prichayudh et al. (2026), whereas iron deficiency anemia was more common among children under two years in Cheng et al. (2024). Oxygen saturation did not differ significantly between iron-status groups, and neither study adequately assessed diagnostic performance by hypoxemia severity or cardiac lesion. Iron supplementation, nutritional status, and thalassemia may also affect hematological indices, particularly Ret-He (Kadegasem et al., 2019). Hemoglobin and hematocrit showed poor discrimination for iron deficiency in Prichayudh et al. (2026), with AUC values of 0.57 and 0.61, respectively. Earlier studies also found these parameters less informative than MCV, MCH, and RDW. In Cheng et al. (2024), 35% of children with hemoglobin below 16.5 g/dL had normal iron status. Moreover, because hemoglobin was included in the study's case definition, its relationship with iron status should not be interpreted as independent evidence of diagnostic accuracy (Cheng et al., 2024).

Interpretation of Reticulocyte Parameters

Reticulocyte hemoglobin content (CHr) and Ret-He reflect iron availability for recent red blood cell production, allowing earlier detection of iron restriction than conventional erythrocyte indices (Hönemann et al., 2020; Ogawa et al., 2020). CHr has also been identified as a useful predictor of iron deficiency in children (Gelaw et al., 2019). Cheng et al. (2024) proposed that hypoxia-related hepcidin suppression and increased erythrocyte destruction may further increase iron requirements in CCHD. Both included studies reported lower CHr/Ret-He in iron-deficient children. Cut-offs around 28 pg yielded AUC values of 0.74-0.83, specificity of 84-89%, and sensitivity of 64-75%. CHr remained independently associated with iron deficiency, while combining CHr and RDW achieved 85.4% accuracy (Prichayudh et al., 2026). However, reticulocyte percentage performed poorly (AUC 0.47). These findings suggest that CHr/Ret-He may help identify iron deficiency but cannot reliably exclude it when results are normal. The findings require cautious interpretation because CHr and Ret-He are measured using different analytical platforms, and their cut-offs may not be interchangeable. Ret-He also could not distinguish iron deficiency without anemia from iron deficiency anemia (Cheng et al., 2024). Similar limitations were reported in children without heart disease, where Ret-He performed better for iron deficiency anemia than for iron deficiency without anemia (Poventud-Fuentes et al., 2024). Neither included

study assessed these parameters for diagnosing CCHD, and neither reported absolute reticulocyte counts or immature reticulocyte fraction.

Clinical Implications

CHr/Ret-He and RDW may complement conventional investigations when assessing iron deficiency in children with CCHD, particularly where standard iron studies are less accessible (Prichayudh et al., 2026). Identifying iron deficiency is clinically relevant because it has been associated with cyanotic spells and may contribute to viscosity-related complications (Mukherjee et al., 2018). Combining reticulocyte hemoglobin with hemoglobin has also been proposed to predict response to iron therapy, although evidence comes from adults without heart disease (Auerbach et al., 2021). However, moderate sensitivity means that normal CHr/Ret-He values do not exclude iron deficiency. These parameters should support, rather than replace, confirmatory iron studies (Auerbach et al., 2021).

Comparison with Previous Literature

The findings are consistent with previous studies showing limited usefulness of hemoglobin and hematocrit for identifying iron deficiency in cyanotic heart disease, while supporting the potential value of CHr and RDW (Mukherjee et al., 2018). Reticulocyte hemoglobin may detect iron deficiency before hemoglobin decreases (Urrechaga Igartua et al., 2017). Some findings differed from previous research. MCV showed poor discrimination in Prichayudh et al. (2026). Ret-He also could not distinguish iron deficiency without anemia from iron deficiency anemia in Cheng et al. (2024), consistent with findings in a pediatric population without heart disease (Poventud-Fuentes et al., 2024). These differences may reflect variations in iron deficiency definitions, age ranges, iron supplementation, inflammatory criteria, analytical platforms, and anemia thresholds. The inclusion of hemoglobin in the reference definition used by Cheng et al. (2024) may also have affected comparability.

Strengths

This review addresses an underexplored clinical question by evaluating several hematological parameters in children with CCHD. Diagnostic performance was assessed using sensitivity, specificity, predictive values, AUC, and cut-offs, alongside methodological quality and applicability using QUADAS-2. The review identifies important evidence gaps, including the lack of appropriate comparison groups, external validation, and analyses by age, cardiac lesion, and hypoxemia severity. These findings can guide future diagnostic accuracy studies.

Limitations

This review was limited by the restriction to studies published since 2021, a limited number of databases (excluding Embase and Scopus), language restrictions to English and Indonesian, and eight inaccessible full-text reports. No protocol was registered, and evidence certainty was not assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. Only two small observational studies (n = 110 and n = 59) were included, precluding quantitative pooling and meaningful assessment of publication bias. Differences in study populations, designs, and reference standards, along with the lack of external validation, may have overestimated diagnostic performance. Data inconsistencies included a negative likelihood ratio of 0.30 versus 0.28 upon recalculation in Cheng et al. (2024) and inconsistent cut-off notation in Prichayudh et al. (2026). These discrepancies were considered during interpretation. Potential incorporation bias and residual confounding from iron supplementation, nutritional status, thalassemia, and hypoxemia severity also remain concerns.

Implications for Future Research

Cheng et al. employed a prospective diagnostic design, whereas Prichayudh et al. conducted a descriptive study. This methodological heterogeneity limits cross-study comparability and the robustness of diagnostic accuracy estimates. Future investigations should adopt prospective diagnostic accuracy designs to strengthen the validity and generalizability of findings. Neither study established age-stratified cut-off values for CHr/Ret-He, hemoglobin, or red cell distribution width (RDW). Future research should determine age-specific thresholds using predefined pediatric age groups, including infants, preschool children, school-age children, and adolescents. Furthermore, both studies exclusively enrolled children with cyanotic congenital heart disease (CCHD), without including healthy controls or children with acyanotic CHD. Consequently, the specificity and broader applicability of the proposed thresholds remain uncertain. Future studies should incorporate appropriate comparison groups to determine whether these thresholds are specific to cyanotic physiology or generalizable to broader pediatric populations.

CONCLUSION

Hemoglobin and hematocrit showed limited ability to discriminate iron deficiency in children with CCHD, potentially because hypoxia-driven erythrocytosis maintains elevated levels despite depleted iron stores. In contrast, CHr and Ret-He demonstrated moderate diagnostic performance, with relatively high specificity but only moderate sensitivity. RDW may provide additional diagnostic information when combined with CHr, whereas reticulocyte percentage showed poor performance. These findings suggest that reticulocyte hemoglobin parameters may complement iron status assessment but should not replace confirmatory iron studies. Interpretation is limited by the inclusion of only two studies, heterogeneous diagnostic criteria, and the absence of external validation. Future prospective studies should establish age-specific thresholds, include healthy and acyanotic CHD comparison groups, standardize reference criteria, and validate diagnostic performance in independent populations.

REFERENCES

Akbari, M., Beiranvand, R., Bahrami, M., & Ghandi, Y. (2025). Investigation of platelet-related parameters and BMI in children with cyanotic and non-cyanotic congenital heart disease. *Journal of Kerman University of Medical Sciences, 32*(1). https://doi.org/10.34172/jkmu.3896

Auerbach, M., Staffa, S. J., & Brugnara, C. (2021). Using reticulocyte hemoglobin equivalent as a marker for iron deficiency and responsiveness to iron therapy. *Mayo Clinic Proceedings, 96*(6), 1510–1519. https://doi.org/10.1016/j.mayocp.2020.10.042

Borges, M. D., & Sesti-Costa, R. (2022). Macrophages: Key players in erythrocyte turnover. In *Hematology, Transfusion and Cell Therapy* (Vol. 44, Number 4, pp. 574–581). Elsevier Editora Ltda. https://doi.org/10.1016/j.htct.2022.07.002

Borsani, O., Varettoni, M., Riccaboni, G., & Rumi, E. (2024). Erythrocytosis in congenital heart defects: Hints for diagnosis and therapy from a clinical case. *Frontiers in Medicine, 11*. https://doi.org/10.3389/fmed.2024.1419092

Bracho, F. J. (2022). Reference intervals of automated reticulocyte count and immature reticulocyte fraction in a pediatric population. *International Journal of Laboratory Hematology, 44*(3), 461–467. https://doi.org/10.1111/ijlh.13776

Cheng, W., Yanuarso, P. B., Wahidiyat, P. A., Rohimi, S., Trihono, P. P., Kadim, M., & Muktiarti, D. (2024). The role of reticulocyte hemoglobin equivalent on the evaluation

of iron deficiency and iron deficiency anemia in pediatric cyanotic heart disease: A diagnostic study in Indonesia. *BMC Pediatrics, 24*(1). https://doi.org/10.1186/s12887-024-05000-w

Gelaw, Y., Woldu, B., & Melku, M. (2019). The role of reticulocyte hemoglobin content for diagnosis of iron deficiency and iron deficiency anemia, and monitoring of iron therapy: A literature review. In *Clinical Laboratory* (Vol. 65, Number 12, pp. 2211–2218). Verlag Klinisches Labor GmbH. https://doi.org/10.7754/Clin.Lab.2019.190315

Grzegorzewski, W., Czerniecka-Kubicka, A., Gołda, K., Niedźwiedzka, A., Wollocko, H., Majewski, M. S., & Wojtkiewicz, J. (2025). Hemoglobin-based oxygen carriers: Selected advances and challenges in the design of safe oxygen therapeutics (A focused review). In *International Journal of Molecular Sciences* (Vol. 26, Number 19). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijms26199775

Hönemann, C., Hagemann, O., Doll, D., Luedi, M. M. L., Ruebsam, M. L., & Meybohm, P. (2020). Reticulocyte hemoglobin equivalent as a diagnostic marker for the current iron deficiency: Old wine in new bottles. In *Anaesthesist* (Vol. 69, Number 12, pp. 919–925). Springer Medizin. https://doi.org/10.1007/s00101-020-00870-y

Itiola, A. Y., Animasahun, B. A., & Njokanma, O. F. (2019). Serum iron status of children with cyanotic congenital heart disease in lagos, Nigeria. *Sultan Qaboos University Medical Journal, 19*(4), e345–e351. https://doi.org/10.18295/squmj.2019.19.04.010

Kaba, L., & Tadele, H. (2025). Polycythemia and its determinants among children with unoperated cyanotic congenital heart disease at Tikur Anbessa specialized hospital, Ethiopia: Observational cross-sectional study. *BMC Pediatrics, 25*(1). https://doi.org/10.1186/s12887-025-06072-y

Kadegasem, P., Songdej, D., Lertthammakiat, S., Chuansumrit, A., Paisooksantivatana, K., Mahaklan, L., Wongwerawattanakoon, P., Tangbubpha, N., & Sirachainan, N. (2019). Reticulocyte hemoglobin equivalent in a thalassemia-prevalent area. *Pediatrics International, 61*(3), 240–245. https://doi.org/10.1111/ped.13775

Liu, Q., Wu, X., Li, Y., Wang, H., An, R., Dou, D., Bie, D., Jia, Y., Yuan, S., Yan, F., & Ding, J. (2023). Effect of hemoglobin and oxygen saturation on adverse outcomes in children with tetralogy of fallot: A retrospective observational study. *BMC Anesthesiology, 23*(1). https://doi.org/10.1186/s12871-023-02290-y

Mukherjee, S., Sharma, M., Devgan, A., & Jatana, S. K. (2018). Iron deficiency anemia in children with cyanotic congenital heart disease and effect on cyanotic spells. *Medical Journal Armed Forces India, 74*(3), 235–240. https://doi.org/10.1016/j.mjafi.2017.07.003

Newton, L. M., Fowler, V. M., & Humbert, P. O. (2024). Erythroblast enucleation at a glance. *Journal of Cell Science, 137*(19). https://doi.org/10.1242/jcs.261673

Ogawa, C., Tsuchiya, K., & Maeda, K. (2020). Reticulocyte hemoglobin content. In *Clinica Chimica Acta* (Vol. 504, pp. 138–145). Elsevier B.V. https://doi.org/10.1016/j.cca.2020.01.032

Poventud-Fuentes, I., Chong, T. H., Dowlin, M., Devaraj, S., & Curry, C. V. (2024). Reticulocyte hemoglobin equivalent as a marker to assess iron deficiency: A large pediatric tertiary care hospital study. *International Journal of Laboratory Hematology, 46*(1), 148–155. https://doi.org/10.1111/ijlh.14188

Prichayudh, H., Chiengthong, K., Whaidee, K., Phiphattanatikanunt, N., La-Orkhun, V., & Lertsapcharoen, P. (2026). Red cell distribution width and reticulocyte hemoglobin content as predictors of iron depletion in children with cyanotic congenital heart disease. *Clinical and Experimental Pediatrics, 69*(9), 706–712.

https://doi.org/10.3345/cep.2026.00241

Taçoy, G. (2021). Congenital heart disease and air travel. *Anatolian Journal of Cardiology, 25*, S18–S19. https://doi.org/10.5152/AnatolJCardiol.2021.S107

Tobis, J. M., Narasimha, D., & Abudayyeh, I. (2017). Patent foramen ovale closure for hypoxemia. In *Interventional Cardiology Clinics* (Vol. 6, Number 4, pp. 547–554). Elsevier Inc. https://doi.org/10.1016/j.iccl.2017.05.003

Urrechaga Igartua, E., Hoffmann, J. J. M. L., Izquierdo-Álvarez, S., & Escanero, J. F. (2017). Reticulocyte hemoglobin content (MCHr) in the detection of iron deficiency. *Journal of Trace Elements in Medicine and Biology, 43*, 29–32. https://doi.org/10.1016/j.jtemb.2016.11.001

van Vliet, J. T., Majani, N. G., Chillo, P., & Slieker, M. G. (2024). Diagnostic accuracy of physical examination and pulse oximetry for critical congenital cardiac disease screening in newborns. In *Children* (Vol. 11, Number 1). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/children11010047

Wasserman, M. A., Shea, E., Cassidy, C., Fleishman, C., France, R., Parthiban, A., & Landeck, B. F. (2021). Recommendations for the adult cardiac sonographer performing echocardiography to screen for critical congenital heart disease in the newborn: From the American Society of Echocardiography. *Journal of the American Society of Echocardiography, 34*(3), 207–222. https://doi.org/10.1016/j.echo.2020.12.005