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Diagnostic Accuracy of Middle Cerebral Artery (MCA) Peak Systolic Velocity (PSV) in Detection of Neonatal Anemia in Rhesus Alloimmunisation Keeping Neonatal Hemoglobin (Hb) at Birth as Gold Standard

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ABSTRACT

Background and Aim: Rhesus Alloimmunisation can cause fetal and neonatal anemia with adverse perinatal outcomes. This study determined the diagnostic accuracy of middle cerebral artery peak systolic velocity (MCA-PSV) for detection of neonatal anemia in rhesus Alloimmunisation, using neonatal hemoglobin at birth as the reference standard. **Material and Methods:** A cross sectional study was conducted in the Department of Diagnostic Radiology, CMH, Lahore, over six months (October 2024 to March 2025). Consecutive non probability sampling was used. Rh-negative pregnant women aged 20–45 years, parity 1–5, singleton pregnancy, gestational age 32–35 weeks, and anti-D titers >1:16 were enrolled (n = 158). MCA-PSV was measured using a standardized Doppler protocol and converted to multiples of the median; MCA-PSV >1.5 MoM was taken as positive. Neonatal anemia was defined as hemoglobin ≤13.5 g/dL at delivery. A 2×2 table was used to calculate sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy. Stratification by age and parity was followed by chi square testing with p≤0.05. **Results:** Mean maternal age was 29.7 ± 5.2 years, gestational age 33.4 ± 0.9 weeks, and parity 2.6 ± 1.1. Neonatal anemia was present in 67 (42.4%). MCA-PSV was positive in 67 (42.4%). True positives were 58, true negatives 82, false positives 9, and false negatives 9 ($\chi^2 = 92.89$, $p < 0.001$). Sensitivity was 86.6% (95% CI 76.4–92.8), specificity 90.1% (95% CI 82.3–94.7), PPV 86.6%, NPV 90.1%, and accuracy 88.6% (95% CI 82.7–92.7). After stratification, associations remained significant across maternal age and parity strata ($p < 0.001$ in each). **Conclusion:** MCA-PSV demonstrated high diagnostic performance for neonatal anemia detection in rhesus Alloimmunisation and supported its use for non-invasive antenatal risk assessment.

INTRODUCTION

Fetal anemia represented an important cause of fetal morbidity and mortality, with adverse neonatal outcomes following birth. Early recognition was clinically valuable because timely intervention, including intrauterine transfusion and postnatal care, improved prognosis in appropriately selected cases [1,2]. Maternal Alloimmunisation occurred when a pregnant woman developed an immunologic response to paternally derived red cell antigens, with transplacental antibody passage leading to fetal erythrocyte sensitization and progressive haemolysis [3,4]. In severe disease, complications such as hydrops fetalis and fetal demise necessitated structured surveillance in high-risk pregnancies, particularly in resource-constrained settings [4,5].

Physiologically, anemic fetuses demonstrated increased cardiac output and reduced blood viscosity, producing higher blood flow velocities exploitable for anemia prediction using Doppler assessment [6,7]. Accurate anemia grading was pivotal for determining transfusion timing and requirement. Whilst fetal hemoglobin measurement through fetal blood sampling represented the most direct method, its invasive nature supported interest in reliable non-invasive alternatives [6,7]. Middle cerebral artery peak systolic velocity (MCA-PSV) emerged as a practical bedside parameter, with underlying rationale grounded in hemodynamic changes observed in fetal anemia [4,7].

Published diagnostic estimates demonstrated variability. Younas et al. reported MCA-PSV sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 90.2%, 92.9%, 86%, 95.1%, and 92%, respectively [8]. Shahbaz et al. reported corresponding values of 86.8%, 90.3%, 85.7%, 91.0%, and 88.8%, with neonatal anemia frequency of 42.2% [9]. This dispersion in performance, with sensitivity ranging from

approximately 50% to 90.2%, suggested heterogeneity related to operator experience, measurement timing, and baseline anemia prevalence across study settings [9,10].

Given limited local published evidence, the present study was conducted to evaluate the diagnostic accuracy of MCA-PSV for detecting neonatal anemia in rhesus alloimmunisation, using neonatal hemoglobin at birth as the reference standard, with the aim of informing safe, accessible risk stratification in routine practice.

MATERIAL AND METHOD

A cross sectional study was conducted in the Department of Diagnostic Radiology, CMH, Lahore, over a period of six months after approval of the synopsis. Prior to enrolment, ethical approval was obtained from the hospital ethical review committee, and written informed consent was taken from all eligible participants. Consecutive non probability sampling was used, and a total sample size of 158 participants was calculated at a 95% confidence level, assuming an expected sensitivity of 86.8% with 8% precision and specificity of 90.3% with 6% precision, while taking the prevalence of neonatal anemia as 42.2% on neonatal hemoglobin estimation [9].

Eligible participants were Rh negative pregnant women aged 20 to 45 years, with parity 1 to 5, having singleton pregnancy confirmed on ultrasound, presenting with rhesus alloimmunisation as per the operational definition, and having gestational age between 32 and 35 weeks confirmed on ultrasound. Patients were excluded if antenatal ultrasound identified fetal malformations, intrauterine growth retardation defined as estimated fetal weight less than the 10th percentile for gestational age, prior fetal blood transfusions in the current pregnancy, or ectopic pregnancy confirmed on ultrasound.

Rhesus alloimmunisation was operationally defined as pregnancies in which the mother had anti D titers greater than 1:16. Prediction of neonatal anemia on middle cerebral artery peak systolic velocity (MCA PSV) was defined as a positive test when MCA PSV exceeded 1.5 multiples of the median (MoM). Prediction of neonatal anemia on neonatal hemoglobin was defined as hemoglobin 13.5 g/dL or less on neonatal blood sampling taken at the time of delivery, which was treated as the reference standard. Diagnostic accuracy was defined as the ability of MCA PSV to discriminate between neonates with and without anemia on neonatal hemoglobin at birth, and was assessed using a 2 by 2 contingency table to derive sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. True positive was considered when anemia was positive on both MCA PSV and neonatal hemoglobin, true negative when both were negative, false positive when MCA PSV was positive but neonatal hemoglobin was negative, and false negative when MCA PSV was negative but neonatal hemoglobin was positive.

After approval, 158 eligible patients presenting to Diagnostic Radiology CMH, Lahore were enrolled. Basic demographic information was recorded. Ultrasound Doppler was performed using a Canon 100G Convex 5 MHz probe. The Doppler gate was placed in the center of the middle cerebral artery soon after its origin from the internal carotid artery. An angle of insonation of 0 degrees was maintained, MCA PSV was measured in the segment closest to the transducer, three consecutive waveforms were recorded in the absence of fetal body or breathing movements, and the highest value was taken. The PSV value was converted to MoM with reference to gestational age using an online calculator, and MCA PSV greater than 1.5 MoM was recorded as positive. After birth, neonatal blood was sent to the hospital pathology laboratory for hemoglobin estimation, and MCA PSV classification was compared with neonatal hemoglobin at birth. All ultrasound reporting was performed by a single consultant radiologist, and patient management was maintained under a single medical team to reduce measurement and management variability.

All collected data were entered and analysed using SPSS version 20. Maternal age, gestational age, and parity were summarised as mean with standard deviation, while categorical variables such as gender were presented as frequency and percentage. A 2 by 2 contingency table was generated to calculate sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for MCA PSV using neonatal hemoglobin at birth as the reference standard. Data were stratified for age and parity as effect modifiers, and after stratification, chi square testing was applied with p value of 0.05 or less considered statistically significant, followed by recalculation of diagnostic performance measures.

RESULTS

A total of 158 Rh-negative pregnant women with rhesus alloimmunisation (anti-D titer >1:16) who fulfilled the eligibility criteria were analysed. The mean maternal age was 29.7 ± 5.2 years, mean gestational age at assessment was 33.4 ± 0.9 weeks, and mean parity was 2.6 ± 1.1. Among neonates, 86 (54.4%) were male and 72 (45.6%) were female. Neonatal anemia on hemoglobin estimation at birth (hemoglobin ≤13.5 g/dL) was identified in 67 (42.4%) neonates. Middle cerebral artery peak systolic velocity (MCA-PSV) was positive (>1.5 multiples of the median) in 67 (42.4%) cases.

Table 1

Baseline characteristics and outcome frequencies (n = 158)

Variable	Category	n (%) / Mean ± SD
Maternal age (years)	Mean ± SD	29.7 ± 5.2
Gestational age (weeks)	Mean ± SD	33.4 ± 0.9
Parity	Mean ± SD	2.6 ± 1.1
Neonatal gender	Male	86 (54.4)
	Female	72 (45.6)
Neonatal anemia on hemoglobin at birth	Yes	67 (42.4)
	No	91 (57.6)
Neonatal anemia on MCA-PSV	Positive	67 (42.4)
	Negative	91 (57.6)

MCA-PSV showed a strong association with neonatal anemia on hemoglobin at birth. True positive results were observed in 58 cases, while 82 cases were true negative. False positive and false negative results were each observed in 9 cases. The association between MCA-PSV classification and neonatal hemoglobin status was statistically significant ($\chi^2 = 92.89$, $p < 0.001$). Diagnostic performance estimates were: sensitivity 86.6%, specificity 90.1%, positive predictive value 86.6%, negative

predictive value 90.1%, and overall diagnostic accuracy 88.6%.

Table 2

Diagnostic performance of MCA-PSV using neonatal hemoglobin at birth as reference (n = 158)

MCA-PSV Result	Neonatal Anemia Present (Yes)	Neonatal Anemia Absent (No)	Total
Positive (>1.5 MoM)	58 (TP)	9 (FP)	67
Negative (≤1.5 MoM)	9 (FN)	82 (TN)	91
Total	67	91	158

Table 3

Diagnostic performance of MCA-PSV using neonatal hemoglobin at birth as reference (n = 158)

Diagnostic Measure	Estimate (%)	95% Confidence Interval
Sensitivity	86.6	76.4 to 92.8
Specificity	90.1	82.3 to 94.7
Positive Predictive Value	86.6	76.4 to 92.8
Negative Predictive Value	90.1	82.3 to 94.7
Diagnostic Accuracy	88.6	82.7 to 92.7

Middle cerebral artery peak systolic velocity demonstrated strong diagnostic performance for detection of neonatal anemia in the studied population of 158 pregnancies complicated by rhesus Alloimmunisation. Sensitivity and specificity were 86.6% and 90.1%, respectively, with corresponding positive and negative predictive values of 86.6% and 90.1%. Overall diagnostic accuracy reached 88.6%, indicating reliable classification of both anemia-positive and anemia-negative cases. The chi-square association test yielded a highly significant result (χ^2 = 92.89, p < 0.001), confirming strong concordance between MCA-PSV measurement and neonatal hemoglobin status at birth. These findings supported MCA-PSV as a clinically dependable non-invasive parameter for anemia prediction in this high-risk population.

Table 4

Stratified Diagnostic Performance by Maternal Age and Parity

Stratification Factor	Subgroup	Sample Size	Sensitivity (%)	Specificity (%)	Accuracy (%)	χ^2	p Value
Maternal Age (years)	20 to 30	92	86.8	90.7	89.1	55.38	<0.001
	31 to 45	66	86.2	89.2	87.9	37.52	<0.001
Parity	1 to 2	96	87.2	91.2	89.6	59.02	<0.001
	3 to 5	62	85.7	88.2	87.1	33.90	<0.001

Stratified analysis demonstrated consistent diagnostic performance across maternal age and parity subgroups. When stratified by maternal age, women aged 20 to 30 years (n = 92) exhibited sensitivity of 86.8% and specificity of 90.7%, with diagnostic accuracy of 89.1% (χ^2 = 55.38, p < 0.001). Women aged 31 to 45 years (n = 66) demonstrated comparable results with sensitivity of 86.2% and specificity of 89.2%, achieving diagnostic accuracy of 87.9% (χ^2 = 37.52, p < 0.001). When stratified by parity, pregnancies with parity 1 to 2 (n = 96) yielded sensitivity of 87.2% and specificity of 91.2%, with diagnostic accuracy of 89.6% (χ^2 = 59.02, p < 0.001). Pregnancies with parity 3 to 5 (n = 62) showed sensitivity of 85.7% and specificity of 88.2%, achieving diagnostic accuracy of 87.1% (χ^2 = 33.90, p < 0.001). The consistency of MCA-PSV performance across both maternal age and parity strata, with all subgroup analyses demonstrating statistical significance (p < 0.001 for each), suggests that diagnostic reliability remained stable across these demographic and obstetric variables, supporting the utility of MCA-PSV as a broadly applicable risk stratification tool in routine clinical practice.

DISCUSSION

The present study evaluated the diagnostic performance of middle cerebral artery peak systolic velocity (MCA-PSV) for detection of neonatal anemia in rhesus alloimmunisation, using neonatal hemoglobin at birth as the reference standard. Neonatal anemia (hemoglobin ≤13.5 g/dL) was observed in 42.4% of neonates, closely aligning with the prevalence reported in a comparable regional diagnostic study that used the same gold standard framework [9]. In the current analysis, MCA-PSV >1.5 multiples of the median yielded sensitivity of 86.6%, specificity of 90.1%, positive predictive value of 86.6%, negative predictive value of 90.1%, and overall accuracy of 88.6%, with a strong association between Doppler classification and neonatal hemoglobin status (χ^2 = 92.89, p < 0.001). These estimates support MCA-PSV as a clinically useful discriminator under routine departmental conditions, with performance characteristics that are consistent with several published validation studies in alloimmunised pregnancies [11,12].

When compared with studies that used fetal hemoglobin obtained by cordocentesis as the comparator, the observed sensitivity and specificity were within the expected range but were not identical, which is biologically and methodologically plausible. Mari et al. demonstrated that an elevated MCA-PSV detected moderate to severe fetal anemia with very high sensitivity in non-hydropic fetuses, supporting MCA-PSV as a robust screening approach when invasive confirmation was being considered. Scheier et al. similarly reported high detection of severe anemia using a 1.5 standard deviation threshold, with strong correlation between MCA-PSV and fetal hemoglobin deficit [11,12]. Deren and Onderoglu further supported the role of MCA-PSV for timing initial and repeat invasive procedures in fetal anemia management, including recognition that threshold behaviour could differ after transfusion exposure [13]. In contrast, the present work used neonatal hemoglobin at delivery rather than fetal hemoglobin at the time of Doppler assessment, and this distinction may be expected to attenuate apparent test performance because hemoglobin status could change between the antenatal Doppler measurement window (32 to 35 weeks) and birth, particularly in actively monitored high-risk pregnancies where clinical decisions and obstetric timing may influence neonatal hemoglobin [11].

The current findings were closely aligned with local studies that employed the same reference standard.

Shahbaz et al. reported sensitivity and specificity of 86.8% and 90.3%, with accuracy of 88.8% for neonatal anemia detection using MCA-PSV, which closely mirrored the present estimates and supported reproducibility in similar practice environments [9]. The Pakistan Armed Forces Medical Journal diagnostic study evaluating fetal MCA-PSV for prediction of fetal anemia also supported clinical utility of this Doppler parameter in routine radiology workflows, reinforcing the practical role of MCA-PSV in settings where invasive fetal testing may be constrained [14]. Together, these regional data suggest that, when neonatal hemoglobin at birth is selected as the reference, MCA-PSV can provide high sensitivity with clinically acceptable specificity, allowing risk stratification and planning for delivery and neonatal support.

The observed false positive and false negative counts were symmetrical (each n = 9), which merits clinical interpretation. False positive classification (MCA-PSV positive with non-anemic neonatal hemoglobin) could plausibly arise from physiological or technical factors that increase cerebral blood flow velocity without sustained anemia at delivery, including transient hemodynamic variation, measurement timing relative to fetal activity, or conservative obstetric decision-making that curtailed ongoing hemolysis through earlier delivery [15]. False negative classification (MCA-PSV negative with anemic neonatal hemoglobin) can occur when anemia develops or worsens after Doppler assessment, or when the relationship between blood viscosity and velocity is less pronounced at a given severity threshold. This phenomenon has been discussed in the literature, including reports that performance may differ by anemia severity and by transfusion status, and that a single universal cut-off may not optimally detect milder anemia [16].

The present results also align with evidence that overall diagnostic performance of MCA-PSV depends on whether fetuses are untransfused or transfused and on gestational age at assessment. The diagnostic test accuracy meta-analysis by Martinez-Portilla et al. specifically evaluated MCA-PSV ≥1.5 MoM for prediction of moderate to severe anemia in untransfused and transfused fetuses, supporting the established role of MCA-PSV while recognising clinically relevant modifiers of test accuracy [17]. The current protocol excluded patients with prior fetal blood transfusions, which likely reduced one important source of measurement uncertainty and may partially explain the high specificity observed. This is consistent with prior evidence that performance can be altered after transfusion exposure due to changes in hematocrit-velocity coupling and evolving fetal hemodynamics [13].

Comparison with studies reporting divergent performance further supports the interpretation that case-mix and operational definitions shape accuracy. Alshimmiri et al. reported comparatively low sensitivity at 1.5 MoM in their dataset despite high specificity, highlighting that sensitivity may fall when anemia spectrum differs, when repeated procedures dominate the sample, or when timing between Doppler and hemoglobin ascertainment is variable [10]. Ahmed et al. evaluated alloimmunised pregnancies without ultrasound evidence of hydrops and concluded that MCA-PSV Doppler was effective for prediction of fetal anemia and could reduce invasive procedures, reinforcing the principle that the method performs best within clearly defined clinical contexts and surveillance protocols. Parviainen et al. described “real-world” clinical performance of MCA-PSV for detection of fetal anemia in isoimmunised pregnancies, supporting external validity of MCA-PSV in routine care rather than narrowly controlled research conditions [15]. Taken together, the totality of evidence supports the current findings as credible and consistent with both seminal validation and pragmatic practice studies, while also illustrating that reported sensitivity may vary materially across studies because of differences in anemia severity distribution, timing of Doppler relative to hemoglobin measurement, and local thresholds for intervention.

CONCLUSION

MCA-PSV assessment provided clinically meaningful discrimination for neonatal anemia in pregnancies complicated by rhesus Alloimmunisation when neonatal hemoglobin at birth was used as the reference standard. The findings supported MCA-PSV as a practical non-invasive tool that can aid antenatal risk stratification and perinatal planning, particularly where invasive fetal testing is limited or selectively applied. Standardized Doppler acquisition and interpretation strengthened consistency and suggested that the method remained reliable across common maternal age and parity categories. Incorporation of MCA-PSV into structured surveillance pathways may facilitate timely decision-making and improve preparedness for neonatal management in high-risk pregnancies.

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