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Predicting the severity of fetal anemia using time-domain measurement of volume flow in the fetal aorta

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KEYWORDS: fetal anemia; fetal aorta; cordocentesis; volume flow

ABSTRACT

Objectives *To assess the value of fetal aortic time-domain measurement of volume flow (using color velocity imaging quantification (CVI-Q)) in predicting the severity of fetal anemia.*

Methods *This was a prospective observational study, in which 24 pregnant women with suspected fetal anemia due to rising anti-red blood cell antibody titers underwent cordocentesis. The fetal aortic time-domain volume flow was measured before fetal blood sampling for fetal hemoglobin investigation. We examined the correlation between increased fetal aortic time-domain volume flow (> 2 SD for gestational age) and fetal anemia (hemoglobin level < 2 SD for gestational age).*

Results *Seventeen fetuses had anemia, and seven had normal hemoglobin. There was a strong correlation between the increase in fetal aortic time-domain volume flow and the drop in hemoglobin value* ($r = 0.81$; $P < 0.01$). *The sensitivity of this technique to predict fetal anemia was 81.3% and the specificity was 71.4%. The mean increase over time in aortic CVI-Q in anemic fetuses was 323.2 mL/min (95% CI, 200.1 to 446.4) compared with 86.9 mL/min (95% CI, −17.7 to 191.5) in the non-anemic group* ($P = 0.004$).

Conclusion *Fetal aortic time-domain measurement of volume flow is significantly increased in cases of fetal anemia due to red-cell alloimmunization. These findings can be used to improve the sensitivity, specificity and positive predictive value of the non-invasive techniques used to predict fetal anemia, and may help in the selection of pregnancies that require cordocentesis and transfusion.*

INTRODUCTION

Fetal anemia remains an important cause of perinatal morbidity and mortality. In recent years, antenatal serology screening and the widespread use of anti-D immunoglobulin injection have reduced the incidence of immune anemia. The number of cases referred with severe hemolytic disease has dropped as a result. This has been accompanied by an increase in the number of women referred with mild disease and low antibody titers[1]. The management of these women is still controversial, as invasive testing can be associated with serious complications.

Doppler flow studies in the anemic fetus aim to detect compensatory hemodynamic changes with sufficient sensitivity and specificity to serve as a reproducible marker of significant hemolysis. The results to date have been variable. A systematic review of the diagnostic value of fetal sonography and Doppler flow velocity in the evaluation and prediction of fetal anemia found that no diagnostic test generated significant likelihood ratios consistent with a highly accurate test[2]. According to this review, the most recent study by Mari *et al.*[3], measuring peak systolic velocity in the middle cerebral artery (MCA) Doppler flow wave as a predictor of fetal anemia, was the study of the highest quality among the literature published on this condition. This study, however, did not produce a significant positive likelihood ratio for the prediction of fetal anemia. This clearly demonstrates our current lack of rigorous evidence about the best non-invasive test for the assessment of these fetuses. We recently reviewed the different ultrasound and Doppler techniques used to predict fetal anemia[4].

There is little doubt that color Doppler imaging provides valuable information about the fetal circulation but is not a direct measure of blood flow. Doppler ultrasound measures the frequency shift in red blood cells

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in motion, thereby providing indirect evidence of blood volume flow rate (VFR) and resistance to flow. In the past, direct volume flow rate measurement using conventional Doppler techniques was hampered by large margins of error in two principal components: the diameter of the vessel under study, and true velocity. Unlike color Doppler imaging, however, color velocity imaging quantification (CVI-Q™, Philips) measures velocity using a time-domain cross-correlation processing technique[5]. Using shorter ultrasound pulses, unlike the long pulses employed in conventional color Doppler imaging, permits high resolution of the intravenous flow profile, providing simultaneous measurement of the velocity profile and effective vessel diameter[6].

CVI-Q therefore combines the accuracy of M-mode imaging in the measurement of vessel diameter, with time-domain correlated measurements of blood flow velocity within a vessel. This combination provides the most accurate non-invasive ultrasound assessment of blood volume flow rate currently available for clinical use, with a documented intraobserver error of $< 10\%$ for large vessels, although limitations exist with smaller vessels[7,8]. The technique has been validated both *in vitro* and *in vivo*[6,9]. The aim of this study was to investigate the value of CVI-Q measurement in the human fetal aorta in predicting the severity of fetal anemia.

METHODS

Twenty-four women with rhesus alloimmunization were referred to the fetal medicine unit at King's College Hospital, London, UK. All women were referred for cordocentesis as they had rising anti-D antibody titer indicating significant hemolysis. The gestational age ranged from 18 to 35 weeks. All women gave their informed consent and local ethical committee approval was granted for the study.

Ultrasound measurements of volume flow rate in the fetal descending aorta by CVI-Q were obtained in all 24 patients. The CVI-Q measurements were obtained before performing fetal blood sampling by cordocentesis. All measurements were performed by a single operator (K.H.) using a Philips CVI (Philips, Santa Ana, California, USA), ultrasound machine with a 5.0-MHz linear array transducer. The details of the CVI-Q technique have been previously reported[6,9]. Briefly, each woman lay on a couch and a long straight segment of the fetal descending aorta was identified in the gray-scale mode (without color) at an optimum angle and depth of insonation. The CVI-Q function was activated and the cursor was placed at a central point in the vessel.

All measurements were made with the angle of insonation kept between 30° and 70° for an optimized frequency display. Blood flow velocities were initially assessed using the pulsed Doppler ultrasound function, to ascertain the peak systolic flow velocity at the same angle of insonation as that used later in the CVI-Q mode. A computerized calculation (using internal software supplied with the ultrasound machine) of the volume flow was made over a minimum of three cardiac cycles. The velocity and volume flow waveforms were checked to ensure the information obtained reflected blood flow within the vessel accurately and the average of three measurements was recorded. All the fetuses studied were assessed prior to their first transfusion. Three of the fetuses studied had hydrops diagnosed by ultrasound prior to their assessment.

In our previous study, we showed that normal CVI-Q values in the human fetal aorta increase in a monotonic pattern with advancing gestational age[10]. The difference in CVI-Q (ΔCVI-Q = measured CVI-Q–normal CVI-Q (50^{th} centile)) was estimated for each fetus examined in the present study. The CVI-Q value was considered increased if the measurement was more than 2 SD above the mean for gestational age, according to nomograms produced by Thompson *et al.* from 20 to 40 weeks' gestation[10].

Fetal hemoglobin estimation was then performed by cordocentesis from the umbilical vein at the placental cord insertion; blood was ready for intrauterine transfusion if necessary. The first 1 mL was discarded to avoid possible mixing with maternal blood, and then 1–2 mL fetal blood was taken for fetal hemoglobin determination. Intravascular transfusion was then performed if fetal anemia was confirmed. All procedures were performed by one operator (K.N.), who was blinded to the results of the CVI-Q measurement. Significant fetal anemia was defined as a hemoglobin value of > 2 SD below the mean for gestational age[11]. Hemoglobin deficit (ΔHb = normal Hb (50^{th} centile)–measured Hb) was estimated for each fetus. The normal Hb value for each gestational age was obtained from Nicolaides[11], and the 50^{th} centile was used as the normal reference range. CVI-Q measurement had no bearing on the clinical management of the fetal condition. Statistical analysis was performed using the SPSS package (SPSS for Windows, version 10.0; SPSS Inc, Chicago, IL, USA).

RESULTS

Among the 24 pregnancies referred for cordocentesis, 17 fetuses had fetal anemia and seven had normal hemoglobin values (Table 1). The median gestational age at examination was 27 (range, 18–35) weeks. Table 2 illustrates ΔCVI-Q and ΔHb in the 17 fetuses suffering from anemia. Using Pearson's correlation coefficient, there was a significant correlation between the increase in CVI-Q and ΔHb ($r = 0.62$; $n = 17$; $P < 0.01$) (Figure 1). Intraobserver variation for CVI-Q estimation had an overall mean of 19% (95% CI, 11–26%) for the absolute volume flow rate (not adjusted for estimated weight for gestation). This is higher than the intraobserver variation recorded during previous *in-vitro* and *in-vivo* validation studies[6]. This could be due to the fact that these were conducted on larger vessels compared with the current study. Of the three measurements taken at each sitting, the latter two were consistently about 7% lower than was the first one (95% CI, 3–11%). This difference in measurements was not affected by gestational age, despite

Table 1 Hemoglobin (Hb) level and color velocity imaging quantification (CVI-Q) values in the 24 fetuses enrolled in the study compared with the normal means for gestational age[10,11]

		Hb level (g/dL)		*CVI-Q value (mL/min)*	
Case	*GA (weeks)*	*Current study*	*Normal for GA*	*Current study*	*Normal for GA*
1	22	10.9	12.0	208	117
2	28	6.6	13.2	628	183
3	33	8.7	14.2	795	235
4	34	8.7	14.6	1131	252
5	18	9.1	11.4	125	87
6	20	7	11.8	436	100
7	23	7.5	12.2	370	122
8	34	8.7	14.6	401	252
9	32	9.8	14.0	480	226
10	32	9.6	14.0	642	226
11	35	8.6	14.8	815	270
12	19	2.0	11.6	1376	96
13	19	10.3	11.6	106	96
14	23	11	12.2	198	122
15	26	6.1	12.8	331	157
16	26	5.1	12.8	646	157
17	31	9.0	13.8	572	209
18	32	9.5	14.0	491	226
19	35	6.9	14.8	646	270
20	30	12.7	13.6	210	200
21	34	13.2	14.6	301	252
22	24	11.3	12.4	473	139
23	26	2.9	12.8	811	157
24	26	4.4	12.8	862	157

GA, gestational age.

Table 2 Relationship between increase in fetal aortic color velocity imaging quantification (ΔCVI-Q) and hemoglobin deficit (ΔHb) in anemic fetuses

Case	*GA (weeks)*	*ΔCVI-Q (mL/min)*	*ΔHb (g/dL)*
1	28	445	6.6
2	33	560	5.5
3	34	879	5.9
4	20	336	4.8
5	23	248	4.7
6	34	149	5.9
7	32	254	4.2
8	32	416	4.4
9	35	545	6.2
10	19	1280	9.6
11	26	174	6.7
12	26	489	7.7
13	31	363	4.8
14	32	265	4.5
15	35	376	7.9
16	26	654	9.9
17	26	705	8.4

GA, gestational age.

a progressive increase in the aortic diameter from 2.8 mm at 20 weeks' gestation to 6.9 mm at 40 weeks' gestation.

The mean aortic ΔCVI-Q in non-anemic fetuses was 86.9 mL/min (95% CI, −17.7 to 191.5). This was significantly lower than was the mean corresponding value

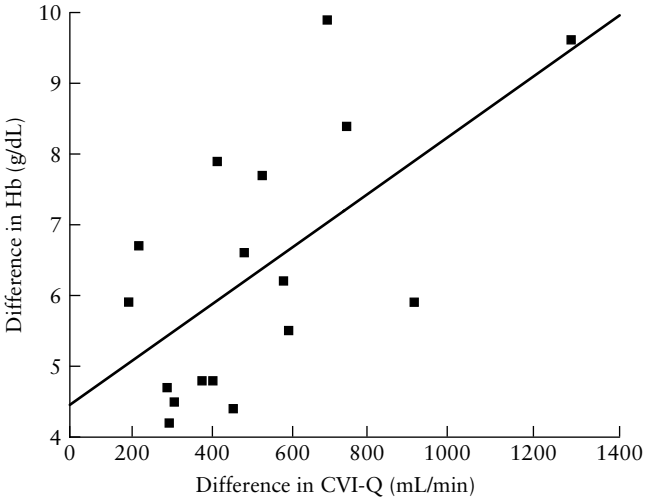

Figure 1 Significant correlation existed between the difference in fetal aortic color velocity imaging quantification (CVI-Q) and the difference in fetal hemoglobin (Hb) in anemic fetuses ($r = 0.62$; $n = 17$; $P < 0.01$).

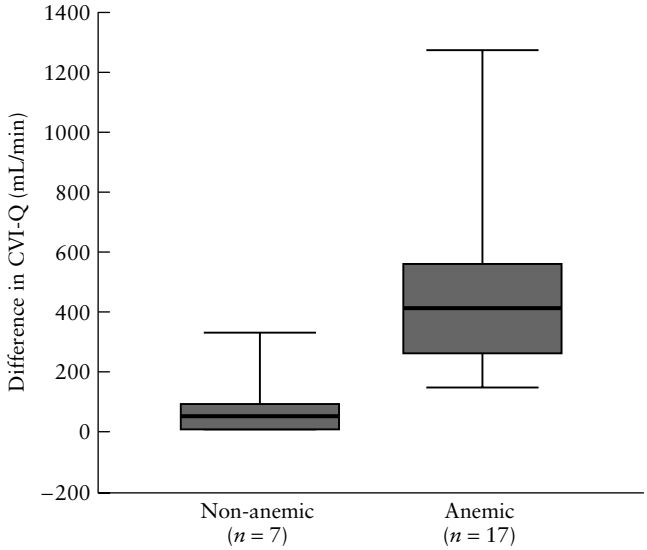

Figure 2 Distribution of the difference in fetal aortic color velocity imaging quantification value (ΔCVI-Q) in anemic and non-anemic fetuses showed minimal overlap between the two groups. Box plots represent median and interquartile range of each group. Anemic fetuses: median, 416; interquartile range, 280; maximum value, 1280; minimum value, 149 mL/min); non-anemic fetuses: median, 49.0; interquartile range, 81; maximum value, 334; minimum value, 10 mL/min).

in anemic fetuses, 478.1 mL/min (95% CI, 332.4–625.0) ($P = 0.002$, independent samples t-test). Figure 2 shows the distribution of the fetal aortic CVI-Q (mL/min) in anemic and non-anemic fetuses and the median, range and interquartile ranges for each group. There was only minimal overlap between the two groups. The sensitivity and specificity for increased CVI-Q in predicting fetal anemia were 81.3% and 71.4%, respectively. There were two false-positive cases and three false-negative cases, giving a positive and negative predictive value of 86.6% and 62.5%, respectively. Figure 3 illustrates

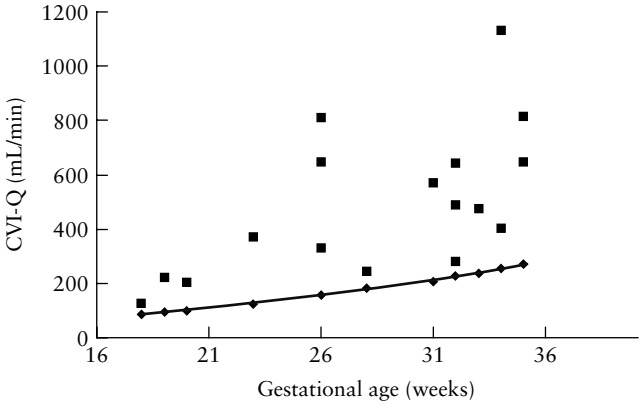

Figure 3 Fetal aortic color velocity imaging quantification (CVI-Q) in anemic fetuses (■) compared with gestational age-matched controls (♦).

the CVI-Q values in the 17 anemic fetuses enrolled in the study compared with gestational-age matched controls. The latter were obtained from our previous study[10].

DISCUSSION

Fetal anemia can be a result of immune, non-immune or idiopathic causes. Among the immune causes, anti-D rhesus antibodies are the most common. In most Western countries, the incidence remains stable at around 1 per 1000 live births[12]. Other immune causes include antibodies to K in the Kell system and to Fya in the Duffy system. Non-immune causes include homozygous alpha-thalassemia (most common in South-east Asia), parvovirus B19 infection, chronic fetomaternal hemorrhage, twin–twin transfusion and erythroleukemia[13].

Fetal anemia may develop slowly over several months in association with a normal reticulocyte count and bilirubin level, or within a week with associated hyperbilirubinemia and high reticulocyte count[13,14]. Significant fetal anemia is associated with increased erythropoeitin and extramedullary erythropoiesis in the fetal liver and spleen, which become enlarged, in part due to red-cell production and in part to congestion resulting from cardiac dysfunction.

In general, the fetus tolerates mild to moderate anemia well, but when ΔHb is extreme, fetal hydrops develops[15]. The exact mechanism underlying fetal hydrops is still unclear, but cardiac dysfunction secondary to insufficient oxygen carrying capacity is thought to be the most likely etiology. Other less important contributing factors include hepatomegaly, portal hypertension and hypoalbuminemia. The importance of the latter in the development of hydrops has recently been questioned[16].

There is a definite survival advantage if the anemic fetus is identified and intrauterine transfusion is started before hydrops develops. A recent review of 18 published studies reported a mean mortality rate of 31% for hydropic fetuses undergoing transfusion (range, 9–50%)[12]. This reflects the greater state of fetal morbidity by the time hydrops has developed, with the fetal heart less able to cope with the strain of the additional volume load of transfusion. The compensatory hemodynamic responses of the unborn fetus to anemia include an increase in cardiac output and changes in peripheral vascular resistance. This, along with the reduced blood viscosity due to the anemia itself, lead to an increased blood supply to vital organs such as brain, heart and adrenals[17,18]. The increase in cardiac output seems to result primarily from an increase in stroke volume, rather than a significant increase in fetal heart rate.

In a multicenter prospective study using MCA peak systolic velocity, Mari *et al.* studied 112 fetuses referred for cordocentesis and blood transfusion[3]. None of these fetuses had been transfused before. They found that this technique has a sensitivity of 100% for the prediction of moderate and severe anemia, with a false-positive rate of 12% and 15%, respectively. The major strength of this study was the masking of the diagnostic test results from the person interpreting the reference standard (ΔHb). The results, however, should be interpreted with caution because the authors, instead of using cut-off levels proposed by an earlier study[16], used new data to improve the cut-off levels; this led to excellent results in terms of the value of MCA peak systolic velocity in predicting fetal anemia. Furthermore, MCA peak systolic velocity measurements suffer from lack of reproducibility and consistency, with significant inter- and intraobserver variation. This should be taken into consideration before incorporating this test into a management protocol for such pregnancies.

Abdel-Fattah *et al.* recently evaluated MCA time-averaged mean velocity (TAMV) by color flow Doppler in 17 hydropic fetuses at risk of fetal anemia before fetal blood sampling by cordocentesis. They found a strong negative correlation between the MCA-TAMV and hemoglobin level ($r = -0.9$, $P < 0.0001$) and concluded that the MCA-TAMV is significantly increased in cases of fetal hydrops caused by anemia, including cases other than red-cell alloimmunization[19].

CVI-Q produces volume flow information throughout the cardiac cycle, with simultaneous vessel diameter measurements. This is in contrast to standard continuous Doppler imaging methods that calculate volume flow rate using averaged flow velocity measurements with independently estimated vessel diameter usually taken at peak systole. Clearly there are significant errors inherent in the application of such a diameter calculation.

CVI-Q estimation in the fetal aorta in cases of significant fetal anemia measures the compensatory hemodynamic response of the fetus to this condition. This includes the development of hyperdynamic circulation, which, along with reduced blood viscosity, increases cardiac output and results in a significant rise in the volume flow in major blood vessels. This can be assessed using this technique. To our knowledge, the present study is the first to investigate the potential use of this technique in predicting significant fetal anemia.

Our study, despite being cross-sectional, suggests a significant potential for fetal aortic CVI-Q measurement in the prediction of significant fetal anemia. When using a cut-off value of 100mL/min for ΔCVI-Q, this technique had a sensitivity of 88.2%, specificity of 83.3% and a positive predictive value of 93.3%. The major strength of this study is the blinding of the investigator, who was interpreting the results of fetal hemoglobin obtained by cordocentesis, to the results of the CVI-Q measurement, as standardized reference values for fetal hemoglobin[11] were used to determine the significance of the anemia.

CVI-Q measurement in the fetal aorta needs further evaluation in a prospective study to determine its usefulness in the prediction of significant fetal anemia. It also has potential in the non-invasive evaluation of other conditions in which there is alteration in circulating blood volume (e.g. twin–twin transfusion syndrome). The major disadvantage of the technique is the difficulty in obtaining a good insonation angle of the fetal aorta due to the anatomical position of the vessel in the body parallel to the spine. Furthermore, it is important that the transducer is placed in the middle of the insonated vessel; otherwise misleading results will be obtained.

Our experience with this technique shows that the operator bias is successively lower with increasing operator experience[10], emphasizing the importance of the operator learning curve. Measurements were also more reproducible with increasing gestation, possibly due in part to the progressive increase in the maximum measured diameter of the blood vessels. Other limitations in the use of CVI-Q involve attenuation defects in later pregnancy, transducer frequency and fetal behavior.

There is a need to improve the baseline accuracy of this technique along with developing newer versions of ultrasound machines that will allow CVI-Q measurement in a more user-friendly way. A study is needed to evaluate whether combining different non-invasive techniques for the prediction of fetal anemia, in parallel or in sequence, might possibly improve the performance of each individual test. This would have a great impact on the non-invasive evaluation of this condition, an emerging necessity in current clinical practice.

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