

# Non-invasive diagnosis of fetal anemia due to maternal red-cell alloimmunization

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## ABSTRACT

**Objectives:** To determine the value of measurement of velocity of the middle cerebral artery (MCA) in the prediction of fetal anemia in alloimmunized pregnancies without ultrasound evidence of hydrops and to see if this could replace the invasive techniques in the diagnosis and management of this condition.

**Methods:** In a prospective cohort study, 65 non-hydrotic fetuses with red blood cell alloimmunization were evaluated with ultrasound and Doppler imaging. This study was carried out at Feto Maternal Medicine Unit at Women's Hospital, Hamad Medical Corporation, Doha, Qatar from January 2003 to December 2003. Middle cerebral artery-peak systolic velocity (PSV) was measured. We interfered only if there is a sign of fetal anemia as indicated by changes in blood velocity in MCA using the table designed by Giancarlo Mari or the fetus developed signs of hydrops fetalis. Immediately after delivery, the blood was taken from umbilical cord for full blood count, blood group, bilirubin and antibodies level. Results before first fetal blood sampling (FBS) or delivery were analyzed.

**Results:** Sixty-five patients met the inclusion criteria for the study and were managed. Sixty-three women were

alloimmunized with rhesus (Rh)-D antibodies and 2 with anti-C. With the conventional management, all 65 patients undergone amniocentesis to determine bilirubin levels. However, with non-invasive management, 60 women were safely prevented from undergoing invasive testing or invasive procedures. Amniocentesis was performed for only one (1.54%) patient, despite having a normal MCV-PSV which was carried out solely due to the care provider was insisting on this procedure and the result was normal. Planning delivery based on MCA-PSV allowed us to deliver 97% (63/65) of these babies without any signs of severe anemia between 28-38 weeks. Two (3%) of the fetuses were diagnosed as severely anemic with MCA-PSV. In both cases, anemia was confirmed by FBS and intra uterine transfusion was carried out immediately to the test. Only 2 babies were diagnosed anemic after birth and underwent blood transfusion.

**Conclusion:** Doppler evaluation of MCA-PSV is effective in the prediction of fetal anemia in red blood cell alloimmunization. Using this technique will reduce the number of invasive procedures needed with the added risks of these procedures with good fetal outcome.

**Saudi Med J 2005; Vol. 26 (2): 256-259**

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With the established use of postnatal anti-D prophylaxis for rhesus (Rh) negative women, together with its increasing use for routine antenatal prophylaxis, the incidence of Rh-D sensitization has dramatically fallen.<sup>1,2</sup> However, this condition, together with sensitization against other red cell antigens, still affects a large number of pregnancies every year, with significant health and financial

implications. Women with rising red cell antibody levels, with or without a history of a previously affected pregnancy, are usually referred to tertiary fetal medicine units for specialized management. The main challenge facing fetal medicine specialists today is not the skill required for invasive therapy, but rather the non-invasive monitoring of the disease so that its progress can be predicted to guide

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Received 24th May 2004. Accepted for publication in final form 25th July 2004.

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the need and timing of intra-uterine transfusions (IUT) to minimize unnecessary invasive testing. In conventional management, the gold standard test for the detection of fetal anemia remains fetal blood sampling (FBS),<sup>3</sup> which carries a fetal loss rate of 1.4% per procedure in experienced hands.<sup>4</sup> Furthermore, invasive testing is associated with fetomaternal hemorrhage, which in turn may increase antibody levels,<sup>5</sup> and thus exacerbate the disease. Amniocentesis for amniotic fluid optical density at 450 nm (OD450) measurement is still considered an essential management tool in Rh disease.<sup>6,7</sup> This test is also invasive, with inherent risks, and its predictive value has been questioned.<sup>8</sup>

In recent years, several non-invasive methods for the prediction of fetal anemia have been assessed, aiming to defer the use of invasive testing until transfusion is expected to be necessary.<sup>9,10</sup> The association of increased blood flow velocity in the fetal middle cerebral artery (MCA)-peak systolic velocity (PSV) with fetal anemia<sup>11-13</sup> is the most promising of these non-invasive tests as has subsequently been reported.<sup>1,14-19</sup> This management does not carry the risks associated with amniocentesis but involves multiple ultrasound examinations, which must be performed with strict adherence to proper technique. Published studies have reported impressive sensitivities (88-100%),<sup>1,20</sup> although clinical experience with MCA-PSV Doppler is far less than with conventional management.

The aim of this study was to determine the value of measurement of velocity of MCA in the prediction of fetal anemia in alloimmunized pregnancies and to see if this could replace the invasive techniques in the diagnosis and management of this condition.

**Methods.** From January 2003 to December 2003, we prospectively collected ultrasound and Doppler data on all red blood cell alloimmunized pregnancies referred to the Fetal Maternal Medicine Unit at Women's Hospital, Hamad Medical Corporation, Doha, Qatar, the only regional referral center for this condition serving 99% of women in Qatar. For the purpose of this study, we included only pregnancies at true risk for fetal anemia, not previously transfused, with RhD, Kell (K1), or c antibodies and titres of at least 32 at the time of referral. This cut-off value is based on the hospital protocol at which amniocentesis is performed for detection of bilirubin level and the findings from 2 recent studies on maternal serum testing.<sup>21,22</sup> Patients with signs of fetal hydrops on referral were excluded. Antibody titres were also assessed weekly. At every visit, Doppler measurements of MCA-PSV was performed according to previously described methods.<sup>1,18,23</sup> Briefly, color Doppler imaging was used to identify the circle of Willis and

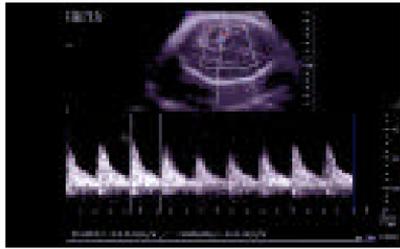


Figure 1 - Ultrasound image of cross-section through fetal brain, with color Doppler visualization of circle of Willis and measurement of peak flow velocity in fetal middle cerebral artery (MCA).

the MCAs in a cross-section through the fetal head. The pulsed Doppler sample volume was placed in the proximal part of the MCA, with the closest possible to a zero-degree angle between the ultrasound beam and the vessel. If this could not be obtained, on-screen angle correction was applied, to ensure that the angle of insonation was less than 30 degrees. The gate size was adjusted to match the size of the vessel. When at least 5 identical consecutive waveforms were observed, the image was frozen and an on-screen caliper was used to measure the PSV (Figure 1).

All measurements were performed or supervised by the first author. All ultrasound and Doppler studies were performed with an ATL 5000 or 3000 machines with a 5-MHz curved array transducer. The results were plotted on previously published graphs.<sup>1,18,23</sup> The cut-off value of 1.5 multiples of median was used for the MCA measurement. The decision to perform amniocentesis for bilirubin levels or FBS was left to the discretion of the responsible clinician, using all available information such as history, fetal movements, antibody titres, and included results of fetal ultrasound and Doppler findings.

**Results.** Sixty-five patients met the inclusion criteria for the study. Sixty-three women were alloimmunized with RhD antibodies and 2 with anti-C. With the conventional management all 65 women under study would have undergone amniocentesis to determine bilirubin levels. However with non-invasive management, 60 women were safely prevented from undergoing invasive testing or invasive procedures. Amniocentesis was performed for only one (1.54%) patient, despite having a normal MCV-PSV, which was carried out solely because her care provider was insisting on this procedure and the result was

normal. Planning delivery based on MCA-PSV allowed us to deliver 97% (63/65) of these babies without any signs of severe anemia between 28-38 weeks. Two (3%) of the fetuses were diagnosed as severely anemic with MCA-PSV. In both cases, anemia was confirmed by FBS and IUT was carried out immediately. Only 2 babies were diagnosed anemic after birth and underwent blood transfusion.

**Discussion.** This study confirms the earlier retrospective studies<sup>17,22</sup> suggesting that Doppler measurements of fetal blood flow velocities are effective tests in the prediction of fetal anemia in red blood cell alloimmunization. These results may be explained by the underlying pathophysiologic mechanisms. Velocity changes are thought to result from increased cardiac output and decreased viscosity of fetal blood (namely a hyperdynamic circulation).<sup>24</sup> In addition, the MCA blood flow may be increased further, as the brain circulation is known to respond quickly to hypoxemia.<sup>1</sup> Although flow velocities in all fetal vessels will be increased, the MCA is particularly suitable for assessment due to its easy visualization with color Doppler imaging (even with grey scale, it can usually be visualized as it courses directly above the greater wing of the sphenoid bone), and usually an angle of insonation close to zero degree can readily be obtained. Because velocity is a quantitative measurement, angle correction or preferably a zero-degree angle is critical to its correct interpretation. Intra-observer and inter-observer variability has been shown to be low.<sup>12</sup> Clinicians and sonographers working in units caring for high-risk pregnancies will be familiar with the measurement because it is now widely used to evaluate growth-restricted fetuses for "brain-sparing" effects.<sup>25</sup>

Although our numbers are limited, these findings in a prospective study is important. This means that invasive diagnostic techniques can safely be avoided when normal MCA flow velocity is found. Specificity was also 100% in our series. In the series by Mari et al,<sup>16</sup> specificity was 88%, which means that in 12% of fetuses at risk, FBS would have been performed in the absence of severe anemia. In one prospective evaluation of MCA Doppler imaging to predict fetal anemia by Teixeira et al,<sup>13</sup> a sensitivity of only 67% was found, their 10% false-positive rate was comparable to the results by Mari et al.<sup>1,10</sup> Another recently published multi-centre study found a sensitivity to detect moderate-to-severe anemia of MCA Doppler combined with B-mode ultrasound of 88%, with a sensitivity of 87%.<sup>15</sup>

In conclusion, this prospective study confirms that MCA-PSV measurement is an effective non-invasive test in the management of pregnancies at risk for fetal anemia. Its use may prevent unnecessary invasive procedures (amniocentesis or FBS). We believe that this non-invasive test has the

potential to safely replace amniocentesis for OD450 measurement, and appropriate comparative studies to evaluate this are ongoing.

**Acknowledgment.** We would like thank Dr. Nagla Elysed, Dr. Faten El-Taher, Dr. Z. Almansori and Dr. Fareed Abun-Ahia for translating the abstract to Arabic.

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