On fetomaternal hemorrhage

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The incidence of large fetomaternal hemorrhage and the Kleihauer-Betke test

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Introduction

International guidelines for appropriate management of Rh D negative women at risk for rhesus immunization are based on large observational studies on the incidence of fetomaternal hemorrhage in patients undergoing obstetrical interventions.[1,2] A number of risk factors for fetomaternal hemorrhage have thus been identified.[1-4]

In a recently published study the incidence of fetomaternal hemorrhage after vaginal and Cesarean deliveries was much higher than previously reported in the literature.[5] We commented on this study by the following "Letters To The Editor", both published with a reply from the authors of the original article. We demonstrate that due to an incorrect formula in an obstetrical textbook to calculate the fetomaternal hemorrhage volume, the reported incidence of fetomaternal hemorrhage after vaginal and Cesarean delivery was incorrectly high.

To the editor *(by Pelikan et al.)*[6]

The study of Salim et al. entitled "The incidence of large fetomaternal hemorrhage and the Kleihauer-Betke test" reports on the frequency of large fetomaternal hemorhage (FMH) in caesarean sections compared with vaginal deliveries.[5] The quantification of fetal red cells was performed by the use of the Kleihauer-Betke test, which is based on resistance of fetal hemoglobin (HbF) to acid elution and widely used to determine the FMH volume.[7] Although this method is clinically useful in the detection of fetal red cells in maternal blood, a high inter-observer and inter-laboratory variability are reported, mainly due to the analysis of an insufficient number of microscopic fields.[8] Since many modifications of this method are known, it would have been meaningful, if the authors had provided information on which modification was applied, how many fields were counted and how the number of background cells was estimated. All these factors influence the fetal cell percentage.

More importantly, the authors' calculation that a volume of 30 ml fetal whole blood corresponds with 0.4% fetal red cells is incorrect. The formula (fetal red cells = (maternal blood volume x maternal hematocrit x % fetal red cells) / newborn hematocrit) calculates the FMH whole blood volume and not only the fetal red cell volume by assuming a maternal blood volume of 5000 ml at term, a maternal hematocrit of 35%, and a newborn hematocrit of 50%. The authors defined large FMH as 30 ml of fetal whole blood, the amount inactivated by 300 μg anti-D immunoglobulin. As the formula already adjusts for the fetal hematocrit, the computed FMH volume is the fetal whole blood volume and not the fetal red cell volume. A 30 ml fetal whole blood volume then corresponds with 0.85% fetal red cells and is

calculated as follows:

5000 ml x 0.35 x 0.0085 ≈ 15 ml (= fetal red cell volume) / 0.50 (= newborn hematocrit) ≈ 30 ml fetal whole blood. Based on an incorrect application of the formula, too many patients were included in the large FMH group, instead of only those with 0.85% fetal red cells. Therefore, the reported incidence of large FMH is too high and the calculated odds ratios are incorrect.

In reply *(by Salim et al.)*[9]

We appreciate Dr. Scherjon and colleagues' interest in our research.[5] Similarly, we are grateful for the opportunity to clarify our views about the concerns they raise. Regarding the method used for detecting fetal red blood cells in maternal blood, which is based on the Kleihauer-Betke test, 3 steps were performed. First, the total number of erythrocytes in 5 fields was counted, and the average number per field was determined. Then the number deeply stained fetal hemoglobin-containing erythrocytes in about 30 fields was counted, and the average number per field was determined. Finally, the percentage of fetal hemoglobin-containing erythrocytes was calculated on the basis of the total number erythrocytes per field. On each day of our testing, blood from a newborn and blood from an adult male (presumed not to have any hemoglobinopathy) were used and inspected on the same slide and served as positive and negative controls, respectively.

The formula used in our research for calculating the amount of fetomaternal hemorrhage is adopted from a well-respected obstetric textbook.[10] Moreover, raising the cut-off to 0.85%, as suggested, leaves nearly a rate of 4% of large fetomaternal hemorrhage in both the vaginal and the cesarean delivery groups. This rate is still higher than the rate reported in the literature. (0.23-1%).[11-13] Our results show a rate of large fetomaternal hemorrhage that is substantially higher than previously reported, with no difference between vaginal and cesarean deliveries. This may reflect inaccuracies with the current method used to estimate the degree of fetomaternal hemorrhage. Further studies, preferably using immunological identification measures, are needed to verify also whether fetomaternal hemorrhage truly occurs before delivery and whether current preventive strategies should be modified.

To the editor *(by Pelikan et al.)*[14]

The Green Journal recently published our letter to the editor[6] commenting on the article "The incidence of large fetomaternal hemorrhage and the Kleihauer-Betke test"[5] together with a reply by the authors.[9] After reading the authors' reply we now understand the source of the serious error in the study by Salim et al., leading to falsely high incidences of fetomaternal hemorrhage.[9] The error lies in the formula the authors adopted from the 21st edition of Williams Obstetrics[10] to calculate the fetomaternal hemorrhage volume. In the recent 22nd edition of Williams Obstetrics[15] the correct formula to calculate the transfused fetal *whole blood* volume is given: fetal blood volume (ml) = the fetal *whole blood* volume = (maternal blood volume x maternal hematocrit x fetal red cell %) / newborn hematocrit.

In the 21st edition the result of the formula, which represents the fetal *whole blood* volume, is divided wrongly by the fetal hematocrit once again. Based on the correct formula in the 22nd edition, a fetal red cell percentage of 0.85% instead of 0.4% corresponds to a fetal whole blood volume of 30 ml. A maternal blood volume of 5000 ml, a maternal hematocrit of 0.35 and a newborn hematocrit of 0.50 are assumed in this calculation, as recommended.[10,15] By referring to the 21st edition and thus using the wrong formula the authors have not realized that this has led to falsely high incidences and incorrect confidence intervals of fetomaternal hemorrhage in all their study groups. We have our concerns and disagree with the reply by the authors that even when the cut-off level is raised to 0.85% the incidence of large fetomaternal hemorrhage is still higher than reported before in literature. Nevertheless, all the incidences and confidence intervals of fetomaternal hemorrhage in their study are incorrect because of the wrong formula and must be recalculated.

It is important for clinical practice and for investigators who will be referring to this article in the future that the correct incidences and confidence intervals will be available.

In reply *(by Salim et al.)*[16]

We appreciate Dr. Pelikan and colleagues' interest in our research,[5] and at the same time we are grateful for the opportunity to clarify again our views on the concerns they raise. Even though the formula we used appears in both the 20th and 21st editions of the textbook,[10] we recalculated our results according to the new formula. Raising the cut-off to 0.85%, according to the formula recently reported in the 22nd edition,[15] still leaves, according to our results, an incidence of nearly 4% of large fetomaternal hemorrhage in the vaginal and cesarean delivery groups. This rate is

still higher than reported in the literature (0.23-1%),[11-13] with no significant difference between the groups. The similarity among the groups has no relationship to which formula was used.

We therefore stand behind our ultimate outcome that shows a rate of large fetomaternal hemorrhage, which is substantially higher than previously reported, with no difference between vaginal and cesarean deliveries. More clarifications are provided in the original study.[5]

Comment

Quantification of fetomaternal hemorrhage is important for routine clinical practice. The amount of anti-D immunoglobulin, which needs to be administered to Rh negative women with a Rh positive child to prevent rhesus immunization is based on the calculation of the fetomaternal hemorrhage volume. Several formulas to calculate the transfused fetal blood volume have been described in literature. An error in the formula adopted from the 21st edition of Williams Obstetrics[10] has led to falsely high incidences of fetomaternal hemorrhage in the cited study.[5] In the recent 22nd edition of Williams Obstetrics the correct formula to calculate the transfused fetal whole blood volume is given.[15] In this comment we aimed to demonstrate the understanding of basic physiological principles is needed to apply the formula, which transforms the fetal to maternal red cell ratio detected in maternal blood into a transfused fetal whole blood volume correctly.

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