Detection of Trace Amounts of Fetal RhD-Positive Red Cells with Flow Cytometry after Multiple Rh-Negative Intrauterine Transfusions and RhD-Gene Determination with PCR

R.		Lynen ^a
T.J.		Legler ^a
J.H.		Maas ^a
A.		Suren ^b
R.		Osmer ^b
B.		Behring ^c
W.		Schroeter ^c
M.		Koehler ^a

^aAbteilung Transfusionsmedizin ^bZentrum Geburtshilfe und Gynäkologie ^cZentrum Pädiatrie, Universitätskliniken, Göttingen

Key Words

RhD fetal cells
Trace analyses
Flow cytometry
Polymerase chain reaction

Summary

In 2 cases of RhD-sensitized women, hemolytic disease of the newborns (hdn) was mitigated by intrauterine transfusions of O Rh-negative donor blood during pregnancy. In both cases preterm delivery was performed by cesarean section in week 32 of pregnancy. With cord blood the direct antiglobulin test (DAT) was negative using the gel test. The blood groups corresponded to the transfused donor blood. With flow cytometry (FC), 2% of IgG-coated red cells were found in the cord blood in case 1 and 5% in case 2. Using FC, these cells were RhD positive. Polymerase chain reaction (PCR) analyses showed the presence of RhD-gene sequences in both cases. Therefore, FC and PCR seem to be suitable methods for the diagnosis and the monitoring in cases of imminent hemolytic disease due to anti-D alloimmunization. With further transfusions, both children developed well after delivery.

Dr. med. Rainer Lynen Arzt für Labormedizin/Transfusionsmedizin Akademischer Rat Abteilung für Transfusionsmedizin, Universitätskliniken Robert-Koch-Straße 40, D-37075 Göttingen (Germany)¹

Schlüsselwörter

Fetale RhD-Zellen · Spurenanalysen · Durchflußzytometrie · Polymerase-Kettenreaktion

Zusammenfassung

In zwei Fällen Rh-negativer Mütter mit Anti-D-Antikörpern wurde durch intrauterine Transfusionen von O Rh-negativem Spenderblut ein Morbus haemolyticus abgewendet. Die Kinder wurden durch Sectio caesarea in der 32. Schwangerschaftswoche entbunden. Mit dem Geltest war der direkte Coombs-Test in beiden Fällen am Nabelschnurblut negativ. Die Blutgruppe entsprach der transfundierten Spenderblutgruppe. Durchflußzytometrisch ließen sich 2% (Fall 1) bzw. 5% (Fall 2) IgG-beladene Erythrozyten im Nabelschnurblut finden. Diese Zellen waren mittels Durchflußzytometrie RhD-positiv. Die DNS-Analysen aus Amnionzellen ergaben in beiden Fällen den Befund RhD-positiv. Somit scheinen Durchflußzytometrie und Polymerase-

Kettenreaktion in Fällen eines drohenden Morbus haemolyticus bei Anti-D-Aloimmunisierung für die Diagnostik und das Monitoring geeignet zu sein. Nach weiteren Transfusionen entwickelten sich beide Kinder gut.

We report on two cases of hemolytic disease of the newborn (HDN), caused by anti-D in case I and by anti-D + anti-s in case II. In case I, there was a history of stillbirth due to anti-D (IgG1 and IgG3) two years before. In case II the first pregnancy was uneventful, but in spite of anti-RhD prophylaxis after delivery progressively rising titers of anti-D were detected in the first trimester of the second pregnancy. Furthermore, anti-C, anti-E and anti-s were found later on in case II. (The anti-C in this case might be proven to be anti-G. Red cells for respective tests are very rare and were not available.) Table 1 shows the mothers' preterm results and the blood groups of the fathers.

In case I, 5 intrauterine transfusions of O Rh-negative donor red cells had been given, and 3 such transfusions had been administered in case II. In both cases the newborns were delivered at week 32 of gestation by cesarean section. Exchange transfusion were performed immediately after delivery. The blood groups in the cord blood samples were (after multiple intrauterine transfusions) exclusively O Rh negative with a negative DAT, also when using the gel test method. However, an acid eluate prepared from the cord red cells contained high amounts of anti-D (gel test) in case I, and of anti-D + anti-s in case II.

The discrepancies were further investigated by additional flow cytometric (FC) investigations with FITC-labeled sheep antibodies (Binding Site, Heidelberg, Germany) against human IgG and its subclasses, as described recently [1]. The results are illustrated in figure 1. In case I, the cord blood could be shown to contain about 2% of fluorescent red cells coated with IgG (A1 in fig. 1), and in case II, 5% of IgG-coated red cells were

KÄUJER

© 1996 S. Karger GmbH, Freiburg Fax (0761) 4520714 <http://www.karger.ch>

Table 1. The mothers' preterm results and the blood groups of the fathers

Case I

Case II

The mothers'

blood group (gel test)

Antibodies (gel test)

O Rh negative

Anti-D 1:256 Anti-C 1:2

A Rh negative, SS

Anti-D 1:1024 Anti-C 1:64 Anti-E 1:256 Anti-s 1:32

“i* ‘ ““i*””

The fathers' blood groups

A CCD.ee

O ccD.Ee, Ss

found (B1 in fig. 1). These cells obviously represented the source of the eluted anti-D. After incubation with monoclonal IgG1-anti-D (clone BS221 from Biotest, Dreieich, Germany) the

References

- 1 Lynen R, Neuhaus R, Schwarz DWM, Simson G, 2 Legler TJ, Köhler M, Mayr WR, Panzer S, Ohto H, 4 Cherif-Zahar B, Le Van Kim C, Rouillac C, Raynal Riggert J, Mayr WR, Köhler M: Flow cytometric analyses of the subclasses of red cell IgG antibodies. *Vox Sang* 1995;69:126-130.
- Fischer GF: Genotyping of the human platelet antigen systems 1 through 5 by multiplex polymerase chain reaction and ligation-based typing. *Transfusion* 1996;36:426-431. 3 Le Van Kim C, Mouro I, Cherif-Zahar B, Raynal V, Cherrier C, Cartron JR Colin Y: Molecular cloning and primary structure of the human blood group RhD polypeptide. *Proc Natl Acad Sci* 1992;89:10925-10929.
- V, Cartron JP, Colin Y: Organization of the gene (RhCE) encoding the human blood group RhCcEe antigens and characterization of the promotor region. *Genomics* 1994;19:68-74.

Trace Amounts of Fetal Red Cells

InfusionstherTransfusionsmed 1996;23:260-261