

The Prevention of Rhesus Immunization*

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SUMMARY

The results of a rhesus prevention programme are reported, confirming that rhesus immunization is a preventable condition. The procedure of foeto-maternal transfusion using the Kleihauer technique did not appear to allow the detection of high risk cases. It is argued that anti-D immunoglobulin may be administered without the prior determination of foetal blood group and the detection of foetal red cells in the maternal circulation and that all rhesus-negative women should be given passive immunization as a routine shortly after delivery.

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Since Levine *et al.*¹ first recognized and described the pathophysiology of rhesus haemolytic disease of the newborn and its relationship to maternal iso-immunization, great progress has been made in the treatment and the prevention of this disease. Finn² suggested that Rhesus iso-immunization could be prevented by the administration of anti-D immunoglobulin shortly after the delivery of a rhesus-positive infant. Studies reported since then³⁻⁶ have proved that this hypothesis was correct. Rhesus prevention programmes are now an established part of obstetric practice in most major centres of the world.

In 1968 anti-D immunoglobulin became available in South Africa and a programme was established in those hospitals covered by the Peninsula Maternity Service in Cape Town. All patients who were rhesus negative and gave birth to rhesus-positive infants were considered for prophylactic therapy with anti-D immunoglobulin. During the earlier part of the trial the supplies of anti-D plasma were restricted and consequently it was possible to administer the immunoglobulin only to patients who were at high risk of immunization. Later, however, it became possible to administer anti-D globulin to all patients. This report compares the effects in cases of selective screening with those of over-all administration on the subsequent development of anti-D antibodies.

MATERIALS AND METHODS

Patient Selection

The study took place between September 1968 and September 1970. All known rhesus-negative women who were delivered in the hospitals of the Peninsula Maternity Service were included in the study, a total of 1172

patients. All had had blood taken in the last trimester of pregnancy and antibody screening had been performed. The tests were repeated at the time of delivery so that all the patients were known to be negative for anti-Rh₀(D). Of these 1172 women, 781 were found to have rhesus-positive infants and were considered for administration of anti-Rh₀(D) immunoglobulin; 207 women had rhesus-negative infants, and in the remainder the blood group of the infant was unknown.

Administration

During the first 6 months of the study the immunoglobulin was only administered following the finding of a significant degree of foeto-maternal bleeding at the time of delivery. In the second period, April 1969 - September 1970, anti-D immunoglobulin was given to nearly all patients who had a rhesus-positive infant. Throughout the entire study the immunoglobulin was given to the mother within 72 hours of delivery, when the results of the tests and the blood group of the infant were known.

Preparation Used

During the first period, commercially-prepared anti-Rh₀(D) (RhoGAM) was used. This preparation is made up so that 1 ml of solution contains not less than 300 µg of anti-Rh₀(D) immunoglobulin. During the second period, anti-D immunoglobulin was obtained from the Natal Blood Transfusion Service. This preparation contained 200 µg of anti-Rh₀(D) immunoglobulin in 1.2 ml of solution, and it was manufactured by the Netherlands Red Cross Blood Transfusion Service, Holland.

Estimation of Foeto-Maternal Transfusion

Blood samples taken from the mother immediately following delivery were examined for the presence of foetal red cells. Blood smears were made with undiluted blood and the film was treated according to a modification of the method of Kleihauer *et al.*⁷ Duplicate slides were made from each sample of blood and typical Hb F cells were counted in 50 low-power fields in each slide. Two control slides were also set up with each batch of specimens, one of normal adult blood and the other of cord blood. The results were reported in terms of the foetal cell score, i.e. the number of cells found in the 50 low-power fields, the mean of the counts for both slides being taken. Using this method, a foetal cell score of 20 corresponds approximately to the presence of 1 ml of foetal blood in the maternal circulation.

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Follow-up

All rhesus-negative women who had given birth to a rhesus-positive infant were asked to return 6 months after delivery for reassessment. At that time a sample of blood was taken from the patients and was tested for the presence of anti-D antibody. Of the 225 patients in the first period of the study, 121 (58.6%) returned for follow-up. During the second period anti-D immunoglobulin had been given to 554 patients, of whom 283 (51.1%) returned. Information was therefore available about the development of antibodies in 404 patients.

RESULTS

During the first period anti-D immunoglobulin was only administered to the patient if foetal cells were counted in both slides. Although Kleihauer tests were done on all patients, anti-D immunoglobulin was given irrespective of the results, only during the second period of this study.

Table I shows the incidence of foeto-maternal bleeding in the two groups. During the first period, 32.5% of the

TABLE I. INCIDENCE OF TRANS-PLACENTAL BLEEDING IN ALL PATIENTS

Trans-placental bleeding	Foetal cell score			
	0	1-4	5-20	20+
Period I 32.5%	81	26	11	2
Period II 22.9%	158	32	12	13

$\chi^2 = 3.694$ $P = 0.296$ (not a statistically significant difference)

patients were found to have foetal cells compared with 22.9% of those in the second period. The difference between these results was not statistically significant. The over-all incidence of foeto-maternal transfusion was similar to that found by other workers.

Selective Administration Group

Of the 121 patients in this group who returned for follow-up, 26 had been treated with RhoGAM. No patients with foetal cell scores of zero had received anti-D immunoglobulin and of those patients with cell scores of 1 to 4, approximately half had been treated (Table II).

TABLE II. INCIDENCE OF IMMUNIZATION IN TREATED AND NON-TREATED GROUPS BY THE FOETAL CELL SCORE

Foetal cell score	Not treated		Treated	
	No.	Immunized	No.	Immunized
0	81	3	0	0
1-4	13	3	14	1
5-20	1	0	11	0
20+	0	0	2	0
Total	95	6 (6.1%)	27	1 (3.8%)

$\chi^2 = 0.206$ $P = 0.206$

The over-all incidence of sensitization in the untreated group was 6.4% and in the treated group 3.8%. This difference is not statistically significant. Among those patients with no evidence of foeto-maternal bleeding on screening with the Kleihauer test, 3 were found to have anti-Rh(D) antibodies. The single case of immunization in the treated group is difficult to explain, as there had been a low foetal cell score.

Universal Administration Group

Of the 283 patients in this group who returned for follow-up, 277 had been given anti-D immunoglobulin. (Six patients had not been treated because the information about the baby had not been received in time.) In the treated group no patients were immunized, whereas 2 patients were found to have antibodies among those who had not received anti-D immunoglobulin (Table III).

TABLE III. INCIDENCE OF IMMUNIZATION DURING THE SECOND PERIOD OF THE STUDY

Group	No.	Immunized at 6 months
Treated	277	0
Not treated	6	2 (33.3%)

$\chi^2 = 69.74$ (highly significant difference)

This difference is highly statistically significant. Both these patients had foetal cell scores of zero at the time of delivery.

If the over-all figures are taken into account, then in that group who were considered to be at low risk and were not treated (Table IV), 7.9% were found to be

TABLE IV. COMPARISON OF TREATED AND NON-TREATED PATIENTS IN THE WHOLE STUDY

Group	No.	Immunized	Incidence
Non-treated	101	8	7.9%
Treated	303	1	0.3%

$\chi^2 = 18.498$ $P < 0.001$

immunized. Among those who were given anti-D immunoglobulin, only 1 out of 303 patients (0.3%) developed antibodies. The difference between these results is highly statistically significant. During the first period (selective administration) 5.8% of the patients developed antibodies and 0.7% of the patients in the second period (universal administration) developed antibodies (Table V). Again this difference is statistically significant.

TABLE V. INCIDENCE OF IMMUNIZATION IN EACH OF THE TREATMENT PERIODS

	Immunized	Not immunized	Incidence
Period I	7	114	5.8%
Period II	2	281	0.7%

$\chi^2 = 10.4$ $P < 0.005$

DISCUSSION

The results of this study, although in agreement with other reports that rhesus iso-immunization can be prevented using anti-D immunoglobulin, raise some interesting problems. Selective screening, using the Kleihauer technique to detect foeto-maternal bleeding, does not appear to be effective, and the relationship between foetal cell score and immunization is not clear. This does not mean that foetal cell-counts are without value, as some of the women with antibodies and low foetal cell scores may have become immunized during the course of the pregnancy.⁸ Possibly the antibodies present at delivery were too weak to be detected by the screening tests currently in use. There was a single case of immunization following treatment with anti-D immunoglobulin and in this case there was a low foetal cell score. The finding of antibody in this patient could have been due to the persistence of passive antibody in the maternal circulation following its administration after delivery although this is unlikely as it has been shown that the anti-D immunoglobulin decays exponentially with a half-life of between 22 and 25 days.⁵

The over-all incidence of immunization in the untreated cases was similar to that found in other series. This is surprising, as the patients had been selected as being at low risk and it could be expected that the incidence of immunization would be lowered. The over-all incidence of immunization found 6 months after delivery may be falsely low because the incidence of immunization in subsequent pregnancies with rhesus-positive infants has been shown to be about 17%.^{5,9} This is a disadvantage of testing at 6 months but unfortunately there is as yet no way of confirming the absence of immunization apart from awaiting the next pregnancy.

The incidence of transplacental bleeding, which was demonstrated in these patients, is similar to the range for term infants reported by other workers.^{30,31} The relationship between foetal cell score and the amount of foeto-maternal bleeding has been established empirically and it appears that a foetal cell count of 5 is equivalent to about 0.25 ml of foetal blood in the maternal circulation. Zipursky *et al.*¹² suggested that the primary immunizing dose is 1.3 ml of rhesus-positive foetal blood which would be equivalent to a foetal cell score of 30. As all the patients in our study who became immunized had foetal cell scores of less than 5, this again raised doubts about the relationship between the detection of foeto-maternal transfusion using the Kleihauer technique and the appearance of immunization.

It has now been firmly established that rhesus iso-immunization is a preventable disease, and the experience in Cape Town is in line with that found abroad. This study has established that selective screening of the patients using the Kleihauer technique to detect significant foeto-maternal bleeding is not a satisfactory method of preventing immunization. In order to prevent the appearance of antibodies the immunoglobulin must be given to all rhesus-negative mothers with rhesus-positive infants. The Kleihauer test is, however, still useful to detect large transplacental bleeds at the time of delivery, when further doses of immunoglobulin may be required to clear blood

of foetal cells. As there were only two cases in this series that required further administration of anti-D immunoglobulin it would seem reasonable to argue that administration of anti-D immunoglobulin without screening for foetal cells in the maternal circulation is reasonable in any clinical prevention programme.

Recent work¹¹ has suggested that passive immunization may be performed as an intrapartum measure. With this method, the blood group of the infant is unknown at the time of administration of the immunoglobulin. Is it therefore necessary to establish the blood group of the infant? In the present series 27% of the infants were found to be anti-D rhesus negative, an incidence which did not differ from that reported by Zipursky and Israels.¹¹ If every rhesus-negative woman was to be given anti-D immunoglobulin at the time of delivery then it would be unnecessary in one-quarter of cases. However, where technical facilities are limited, the wasteful nature of treating patients in this manner may be outweighed by practical considerations. Where every patient is screened at delivery for foetal red cells and the infant's blood group is determined before administering immunoglobulin (assuming a 17% incidence of rhesus immunization in subsequent pregnancies in unprotected mothers), the cost of preventing one case of rhesus iso-immunization is approximately R70. To treat each Rhesus-negative mother without knowing the infant's blood group would cost the same amount. As the collection and analysis of specimens is costly in terms of personnel, a strong case can be made for universal administration of anti-D immunoglobulin to all rhesus-negative mothers shortly after delivery. The only practical objection to such a policy is that anti-D immunoglobulin is still in short supply, but if this problem can be overcome then rhesus iso-immunization could almost be eliminated and become a rarity in obstetric practice.

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