THE PROGNOSTIC VALUE OF MATERNAL RH-ANTIBODY INHIBITION STUDIES AND LIQUOR AMNII INVESTIGATIONS*

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As a guide to the management of pregnancies complicated by Rh immunization, considerable progress was made with the introduction of amniotic fluid studies pioneered by Bevis,[1,2] and many investigators have now confirmed the relationship of bile pigment concentration in the amniotic fluid to the intensity of Rh-haemolytic disease *in utero*. However, in spite of such advances the spectrophotometric,[3] chemical[4] and pigment-protein ratio procedures[5] cannot always be accepted as being infallible, their shortcomings being too seldom emphasized while their advantages are simply taken for granted.[6]

Some investigators[7,8] believe that with the introduction of liquor amnii studies nothing useful can be gained by the inclusion of maternal Rh-antibody investigations. Others[5,9-11] emphasize that the prediction of the severity of Rh-haemolytic disease by amniotic fluid analysis can at times be most misleading. Thus, fully endorsed cautions about relying on the predictions obtained by amniotic fluid studies alone have led investigators to believe that bile pigment levels should be used only in conjunction with maternal Rh-antibody estimations.[12,13]

Since repeated abdominal paracentesis on every Rh-immunized mother can result in errors in the antenatal prediction of the severity of the disease,[9,14-16] selective amniocentesis has become an acceptable practice. The recording of low maternal Rh-antibody values is really not indicative of severe haemolytic manifestations of the disease.

In this report an analysis is presented which is intended to show that the application of Rh-antibody inhibition studies[6] can introduce a real measure of prognostic reliability in predicting the severity of Rh-haemolytic disease, particularly when the significance of liquor amnii values appears to be in some doubt. The routine use of maternal antibody studies is therefore designed as an effective procedure for supplementing the values obtained by spectrophotometric analysis. Rh-antibody studies can, in fact, be looked upon as a test to reveal whether a clinically favourable forecast of severity obtained by liquor examinations at 26 - 30 weeks of gestation continues to be the same at 34 weeks' gestation, especially when we take into account that more frequent episodes of re-immunization can be anticipated after 30 weeks of pregnancy.[17]

MATERIALS AND METHODS

All Rh-immunized mothers examined in this study were seen from the first trimester of pregnancy onwards. Mothers in whom the production of Rh antibodies may have been the result of previous Rh-incompatible blood transfusions were excluded; and only Rh-incompatible mother-infant combinations were used.

For the evaluation of the Rh-antibody inhibition index the procedure detailed in a previous communication[6] was used. Rh-antibody titre values were determined by the standardized method of indirect antiglobulin titration.[18]

Amniotic Fluid Studies

Amniotic fluid obtained by abdominal paracentesis was centrifuged immediately after collection at 4,000 r.p.m. for 30 minutes to remove cellular elements and was then placed in a light-proof container if an immediate analysis was not performed. Analysis of bile pigment concentrations in the specimen was carried out using the spectrophotometric procedure described by Fleming and Woolf,[19] while the total protein value was determined by the biuret reaction as described by Morris *et al.*[5] The calculation of pigment-protein ratio employed herein was also carried out as recommended by the same authors. Serum bilirubin estimations on the infants' cord bloods were determined by the method of Powell.[20]

RESULTS AND DISCUSSION

To present an objective analysis between the efficiency of amniotic fluid measurements and Rh-antibody inhibition studies, an investigation was carried out on 66 Rh-immunized mothers who had delivered Rh-incompatible infants. In the assessment of how closely these 2 procedures can forecast the severity of foetal red cell destruction *in utero*, it is important to realize that the Rh-antibody inhibition test determines the measured intensity of antibody implication up to the time of delivery. On the other hand, amniotic fluid studies (particularly when performed before 30 weeks of pregnancy) can only be considered to have measured the intensity of foetal red cell haemolysis known to be present at the time the amniotic fluid sample was obtained.

In order to reduce variations of this nature to a minimum it was important to use only the highest projected spectrophotometric measurement obtained from a series of amniotic studies on the same patient. It should also be noted that an average of 2·4 amniotic fluid samples were tested from the 66 mothers examined. Their time of collection ranged from 28 to 33 weeks of gestation and in no instance were single amniotic fluid studies included if they were performed after 33 weeks of pregnancy. A detailed analysis of the serological, biochemical and haematological findings of the 66 Rh-immunized mothers and their infants is given in Table I.

To determine how closely the Rh-antibody inhibition index and bilirubin protein values are related to the severity of the infant's anaemia at birth, a biserial *r* correlation coefficient analysis was carried out for the 2 testing procedures against (*a*) the haemoglobin concentration of the infant's cord blood at birth, (*b*) total serum bilirubin value observed at birth, and (*c*) the severity of Rh-haemolytic disease as determined by the infant's clinical condition. Table II sets out the results of this analysis and from the information obtained it can be seen that both procedures (amniotic fluid values and antibody inhibition studies) accurately predicted that an infant's cord-blood haemoglobin levels will be low when the bilirubin/protein ratio or Rh-antibody inhibition index values are high.

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TABLE I. SEROLOGY, BIOCHEMISTRY AND HAEMATOLOGY OF 66 RH-IMMUNIZED MOTHERS WHO DELIVERED RH-POSITIVE INFANTS

Case No.	Maternal Rh-antibody inhibition index at delivery	*Liquor amnii studies*			*Cord-blood estimations*		Time of delivery, treatment, and outcome*	Clinical evaluation of the disease†
		Bilirubin µg./ml.	Protein mg./ml.	Ratio bilirubin µg./mg. of protein	Haemoglobin G/100 ml.	Bilirubin total mg./100 ml.		
1	450	2·5	6·4	0·39	11·8	5·4	35/52, Ex. Tr. A & W	2
2	560	3·2	3·6	0·89	—	—	Stillborn 28/52	4
3	640	12·4	6·8	1·82	—	—	Stillborn 30/52	4
4	204	1·4	5·2	0·26	16·2	2·2	36/52, A & W	1
5	620	10·7	6·7	1·56	—	—	Stillborn 35/52	4
6	570	1·9	6·3	0·31	6·4	8·6	36/52, Ex. Tr. A & W	3
7	312	2·3	5·7	0·40	11·6	3·7	35/52, Ex. Tr. A & W	3
8	36	1·1	5·4	0·20	12·3	3·3	36/52, A & W	1
9	144	1·2	3·8	0·31	12·0	3·0	36/52, Ex. Tr. A & W	2
10	896	6·2	4·9	1·26	—	—	Stillborn 31/52	4
11	206	2·9	7·1	0·40	10·8	4·8	35/52, Ex. Tr. A & W	2
12	428	1·4	3·4	0·44	9·4	3·1	36/52, Ex. Tr. A & W	3
13	68	1·2	5·8	0·20	13·6	3·4	36/52, Ex. Tr. A & W	1
14	560	1·9	3·1	0·61	10·2	2·5	35/52, Ex. Tr. A & W	2
15	488	2·4	3·8	0·63	6·8	5·2	35/52, Ex. Tr. A & W	3
16	1,120	3·9	6·2	0·63	8·0	5·7	35/52, Ex. Tr. A & W	3
17	10	1·1	3·8	0·28	17·8	2·1	40/52, A & W	1
18	72	0·8	2·8	0·28	13·3	3·1	38/52, A & W	1
19	24	0·6	2·9	0·20	15·0	3·3	40/52, A & W	1
20	180	3·3	5·0	0·66	10·3	3·9	38/52, Ex. Tr. A & W	2
21	280	1·0	2·7	0·37	9·3	2·6	36/52, Ex. Tr. A & W	3
22	320	2·0	2·8	0·71	10·7	3·8	35/52, Ex. Tr. A & W	2
23	700	12·0	7·0	1·70	—	—	Stillborn 31/52	4
24	220	1·6	5·4	0·29	15·1	2·4	37/52, Ex. Tr. A & W	1
25	800	2·0	5·8	0·34	5·1	7·2	36/52, Ex. Tr. A & W	3
26	350	2·6	6·1	0·42	10·8	2·9	36/52, Ex. Tr. A & W	3
27	54	1·3	6·3	0·20	13·8	2·7	36/52, A & W	1
28	400	2·2	5·8	0·37	11·0	5·0	36/52, Ex. Tr. A & W	2
29	600	3·0	3·4	0·88	—	—	Stillborn 30/52	4
30	820	8·4	7·3	1·15	—	—	Stillborn 29/52	4
31	840	9·7	7·2	1·34	—	—	Stillborn 34/52	4
32	150	1·5	4·8	0·31	13·4	2·6	37/52, Ex. Tr. A & W	2
33	230	2·4	5·4	0·44	11·7	4·3	36/52, Ex. Tr. A & W	2
34	454	1·6	3·8	0·42	9·1	3·7	36/52, Ex. Tr. A & W	3
35	80	0·9	4·2	0·21	14·0	2·7	37/52, Ex. Tr. A & W	1
36	540	2·0	3·1	0·64	11·9	2·9	36/52, Ex. Tr. A & W	3
37	522	2·8	4·2	0·66	7·0	5·6	35/52, Ex. Tr. A & W	3
38	980	4·2	5·8	0·72	6·7	5·0	35/52, Ex. Tr. A & W	3
39	26	1·7	7·3	0·23	15·4	2·1	38/52, A & W	1
40	92	0·5	2·4	0·20	14·3	2·4	39/52, A & W	1
41	78	0·9	4·3	0·21	13·9	3·1	40/52, A & W	1
42	200	4·1	6·8	0·60	12·0	4·8	37/52, Ex. Tr. A & W	2
43	310	1·5	4·4	0·34	10·1	3·7	36/52, Ex. Tr. A & W	2
44	420	1·9	2·4	0·79	9·4	3·9	35/52, Ex. Tr. A & W	3
45	640	3·4	3·8	0·89	—	—	Stillborn, 29/52	4
46	500	2·8	6·6	0·42	12·0	6·1	36/52, Ex. Tr. A & W	2
47	180	1·2	5·8	0·20	14·3	2·7	36/52, Ex. Tr. A & W	1
48	620	2·3	5·2	0·44	8·7	4·8	35/52, Ex. Tr. A & W	3
49	300	1·8	4·1	0·43	12·1	3·9	36/52, Ex. Tr. A & W	2
50	68	1·4	6·1	0·26	14·2	2·4	36/52, A & W	1
51	96	1·0	3·3	0·30	15·1	2·7	37/52, A & W	1
52	190	2·0	5·2	0·38	11·7	3·9	36/52, Ex. Tr. A & W	2
53	720	11·4	6·5	1·75	—	—	Stillborn, 30/52	4
54	400	1·3	4·1	0·31	10·7	4·2	36/52, Ex. Tr. A & W	3
55	72	0·8	3·8	0·21	13·7	2·1	38/52, A & W	1
56	510	1·8	3·0	0·60	12·1	3·8	36/52, Ex. Tr. A & W	2
57	480	2·3	4·3	0·53	9·7	4·9	36/52, Ex. Tr. A & W	3
58	846	4·7	7·4	0·63	8·4	5·5	35/52, Ex. Tr. A & W	3
59	42	2·1	9·4	0·22	16·3	2·0	39/52, A & W	1
60	960	5·2	4·8	1·08	—	—	Stillborn, 33/52	4
61	864	9·1	6·4	1·57	—	—	Stillborn, 30/52	4
62	78	1·3	5·4	0·24	14·7	2·4	39/52, A & W	1
63	88	0·7	3·1	0·22	16·4	2·0	40/52, A & W	1
64	210	3·9	6·3	0·61	11·8	3·9	38/52, Ex. Tr. A & W	2
65	248	1·9	4·6	0·41	12·6	3·7	37/52, Ex. Tr. A & W	2
66	310	1·7	3·2	0·53	10·7	4·3	36/52, Ex. Tr. A & W	3

* Ex. Tr. = exchange transfusion; A & W = alive and well.
† 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.
For comparative statistical analysis of the Rh-antibody inhibition index against other values, scored results were divided by a factor of 1,000; for example, case 1, index of 450 = 0·45.

A corresponding correlation is also confirmed for the potentiality of these 2 procedures to measure the over-all severity of the disease. However, no real correlation could be established between the bilirubin/protein ratio results and the cord-blood bilirubin values, whereas a significant association between Rh-antibody inhibition results and the cord-blood bilirubin values is evident. Such variations would seem to lend support to the argument that the bilirubin/protein ratio test (being recorded at least 4 weeks before delivery of the infant) is only able to determine the intensity of *in utero* haemolysis noted at the time of collection, and does not necessarily constitute an indicative measurement of the true intensity of red cell haemolysis to be anticipated at birth.

TABLE II. STATISTICAL EVALUATION OF ACCURACY IN PREDICTING SEVERITY OF RH-HAEMOLYTIC DISEASE BEFORE BIRTH OF RH-INCOMPATIBLE INFANTS BY RH-ANTIBODY AND AMNIOTIC FLUID STUDIES*

Comparative analysis		*r correlation and significance*
Maternal Rh-antibody inhibition index value obtained at delivery	Infant cord-blood haemoglobin value	−·764†
	Infant cord-blood bilirubin value	·581†
	Severity of Rh-haemolytic disease	·847†
Highest bilirubin protein ratio values obtained between 28 and 32 weeks gestation	Infant cord-blood haemoglobin value	−·696†
	Infant cord-blood bilirubin value	·248n.s.
	Severity of Rh-haemolytic disease	·799†

* See details of recorded values on 66 Rh-immunized mothers listed in Table I.
† Denotes significance at ·001 level.

Realizing that frequent episodes of antibody stimulation can be expected as pregnancy progresses towards term,[17] it would appear that the lack of positive correlation between bilirubin/protein ratio values and cord-blood bilirubin is often influenced by an increased rate of red cell destruction seen after the amniotic fluid samples have been taken. The possibility that raised Rh-antibody production, induced by the disruption of foetal-placental circulation at amniocentesis, is a contributing factor cannot of course be ignored, and like the practice of external version[21] can be considered as a factor which may further the intensity of the disease.[14]

It is obvious from the findings that a real measure of confidence in the prognosis of Rh-haemolytic disease can be attained when 2 different testing procedures are employed. However, it would also appear that even if a statistical correlation between the testing procedures and the severity of the disease is evident in a combined analysis of many cases, the same may not necessarily be true for some individual cases. Marked differences can occasionally be anticipated due to the condition of an infant being influenced by factors other than Rh immunization.[22]

With the knowledge that these variations can be anticipated at the individual level it appears that the prognosis of the disease should ultimately be based on the assessment of a combination of parameters (previous history, father's Rh genotype, Rh-antibody value and amniotic fluid results) concurrent with any additional experience gained from other statistical studies.

In an effort to help women to avoid pregnancies with a high risk of stillbirth, prenatal counselling and examination is advocated until the mother can be assured that the Rh-antibody value indicates that there is a fair measure of safety for her to conceive again.

Liquor examinations are of practical significance in determining the presence of an Rh-negative foetus.

CONCLUSIONS

From the findings reported in Parts I and II of this study[6,17] it is apparent that the effect of Rh antibodies on the foetus differs in almost every Rh-immunized mother. In some immunized pregnancies the antibody value remains the same throughout pregnancy, while in others significant changes can be anticipated which increase foetal mortality. If variations in maternal responses to Rh-antibody formation can be established, it must be agreed that transplacental bleeds from the foetus to the mother are the primary cause.

Failure to correlate raised Rh-antibody production with the severity of Rh-haemolytic disease has led many investigators to believe that Rh-antibody titre values cannot be considered as true indicators of the intensity of the disease or evidence of recent foeto-maternal bleeds. The former opinion is well founded, because sufficient information is available to show that although low Rh-antibody titre values are always associated with mild or unaffected conditions of haemolytic disease, the reverse is not always true for the prediction of high Rh-antibody titres. This situation has inevitably directed investigators away from the recognition that significant episodes of re-immunization, regardless of their prognostic use, can be considered as definite indicators of transplacental foetal bleeds, and as such must present a threat to furthering the process of immunization.

Investigations have also confirmed that external version, abdominal paracentesis and the management of the third stage of labour can bring about an increase in foetal red cell transfer across the placenta, but little is known about other defects of the placenta which cause maternal bleeds. The findings of frequent episodes of Rh-antibody formation suggest that these are not uncommon, and this raises the problem that an apparently satisfactory prediction of the severity of Rh haemolytic disease established at 30 weeks' gestation can be completely misleading 6 weeks later.

Considering that in more than half of the Rh-immunized mothers significant variations in Rh-antibody responses can be anticipated to alter the prognosis of the disease, it is not surprising that the introduction of repeated abdominal paracentesis emerged as an attractive mode of management while single determinations were often deemed unreliable. However, since the frequent practice of abdominal paracentesis is not warranted in at least 30% of Rh-immunized mothers (and definitely constitutes a risk in furthering the intensity of immunization) selective amniotic fluid studies based on a combination of parameters has become more acceptable.

With the introduction of more sensitive techniques of Rh-antibody determination,[6] selective amniocentesis can be accepted as a very sound complementary test for assisting the obstetrician in selecting infants requiring intrauterine transfusions. The added advantage of this approach is the considerable assurance it affords that the risk of furthering the intensity of Rh immunization will be kept to a minimum.

SUMMARY

The present analysis has shown that the Rh-antibody inhibition test can be accepted as a reliable procedure for the antenatal determination of the severity of Rh-haemolytic disease of the newborn. This was established by a comparative study of 66 Rh-immunized mothers examined by the Rh-antibody inhibition test and liquor amnii investigations. Of particular significance was the observation that a positive correlation between Rh-antibody inhibition index values and cord-blood bilirubin could be established. The absence of a similar positive correlation between amniotic fluid bilirubin/protein ratio values and cord-blood bilirubin indicates that liquor amnii studies cannot always establish the occurrence of an increased rate of foetal red cell destruction after the amniotic fluid samples have been taken.

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REFERENCES

1. Bevis, D. C. A. (1950): Lancet, **2**, 443.
2. *Idem* (1952): *Ibid.*, **1**, 395.
3. Walker, A. H. C. (1957): Brit. Med. J., **2**, 376.
4. Mackay, E. V. and Watson, D. (1962): Med. J. Aust., **2**, 942.
5. Morris, E. D., Murray, J. and Ruthven, C. R. J. (1967): Brit. Med. J., **2**, 352.
6. Vos, G. H. (1969): S. Afr. Med. J., **43**, 241.
7. Pirofsky, B. (1965): Amer. J. Obstet. Gynec., **92**, 720.
8. Robertson, S. E. J. (1961): Med. J. Aust., **1**, 398.
9. Liley, A. W. (1963): Amer. J. Obstet. Gynec., **86**, 485.
10. Walker, W. (1967): Brit. Med. J., **1**, 175.
11. Hyslop, R. S. and Whiley, W. (1960): Med. J. Aust., **1**, 622.
12. Nilsson, B. A. (1965): Acta obstet. gynec. scand., suppl. 2, 44.
13. Crawford, J. W., Cameron, C. and Walker, C. H. M. (1966): Lancet, **2**, 887.
14. Zipursky, A., Pollock, J., Chown, B. and Israels, L. G. (1963): *Ibid.*, **2**, 493.
15. Queenan, J. T. and Adams, D. W. (1964): Obstet and Gynec., **24**, 530.
16. Misenhimer, H. R. (1966): Amer. J. Obstet. Gynec., **94**, 1133.
17. Vos, G. H. (1969): S. Afr. Med. J., **43**, 130.
18. Vos, G. H. and Kirk, R. L. (1958): Amer. J. Clin. Path., **29**, 284.
19. Fleming, A. F. and Woolf, A. J. (1965): Clin. chim. Acta, **12**, 67.
20. Powell, W. N. (1944): Amer. J. Clin. Path., **14**, 55.
21. Vos, G. H. (1967): Vox Sang. (Basel), **12**, 390.
22. Mollison, P. L. (1961): *Blood Transfusion in Clinical Medicine*. Oxford: Blackwell Scientific Publications.