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Increase in the serum immunoglobulin fractions (IgG, IgA, IgM) of pregnant women with anti-Rh isoimmunization in relation to the neonatal fetal prognosis

by

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Although in haemolytic disease of the newborn (HDN) the fetal damage is related to the presence of anti-Rh antibodies formed in the mother, measurement of the antibody titre in the maternal serum by the indirect Coombs test (ICT) is not a significant index for an accurate asessment of the neonatal fetal prognosis ([1, 2]). Values equal to or below 1:32 are generally considered as indications of the absence of danger to the foetus ([3]), but beyond these limits it is not possible to assess the degree of fetal risk on the basis of the ICT antibody titre ([4]).

This is due to the fact that the ICT measures all the anti-Rh antibodies in this serum, irrespective of the group (IgG, IgA, IgM, IgD, IgE). Further, we know that only IgG is potentially harmful because it can pass through the placenta ([5]) and can lead to lysis of the fetal erythrocytes ([6]).

In the present study we therefore attempted to assess possible states of neonatal fetal danger more accurately than is possible with the ICT alone, by titrating the various groups of serum immunoglobulins (IgG, IgA, IgM) and comparing the ICT values obtained simultaneously.

MATERIALS AND METHODS

We examined 14 patients with anti-Rh isoimmunisation; at regular intervals throughout pregnancy (from the 24th-28th week to delivery) successive determination of the anti-Rh antibody titre were carried out, together with determination of the IgG, IgA, and IgM immunoglobulins.

The anti-Rh antibodies were titrated by the ICT as generally used and described elsewhere ([7]).

The various groups of immunoglobulins were determined by radial immunodiffusion on agar discs pre-treated with rabbit antibodies ([8]). For the quantitative determination of the various groups of antibodies the diameters of the precipitation rings were related to mg % ml, using a straight line obtained by a system of cartesian axes with three dilutions of a standard quantity of the immunoglobulin group to be tested (immunodiffusion discs by Partigen, Boeringswerke AG).

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The criteria used for assessment of the neonatal conditions were the clinical and laboratory ones commonly used for the diagnosis of HDN. Patients were divided into two groups:

1 - cases with a good prognosis (GP), where no signs of neonatal fetal risk were evident and where no treatment as given;

2 - those with a poor prognosis (PP), where there was serious neonatal foetal risk resulting in death of the newborn or the need for polytransfusion.

As normal controls for the IgG, IgA, and IgM values during a period of pregnancy roughly corresponding to that of our patients we used 9 primigravida with no pathological symptoms. The average values in mg % ml and the corresponding standard deviations found in this group for the three different groups of immunoglobulins were as follows: IgG (1031 ± 171); IgA (189 ± 66); IgM (146 ± 62).

RESULTS

In ten out of 14 cases (Table 1) the initial values of the ICT were equal to or below 1:32, i.e. thought not to be dangerous for the fetus. In eight of these cases the titre increased to 1:64, and in two the increase attained 1:256. Examination of the increases in the IgG level in two successive determinations shows that if a rise of 23.8% occurred (case 1, S.A., went from 1:32 to 1:256) or if this percentage was not exceeded, as occurred in another six cases (case 2 to 7), conditions for the newborn appeared to be satisfactory. When the percentual increase in IgG was higher, as occurred in three cases (cases 8, 9, 10), neonatal damage was such as to require one or more total blood transfusions, or the severe isoimmunisation led to death. The course of the IgA and IgM fractions differed from that of the IgG fraction; in the patients with a better neonatal foetal prognosis, although the final ICT values were high, the percentage of increase in relation to the initial determination varied from a minimum of 26% to a maximum of 70%, while in the cases with a poor prognosis this increase was much reduced.

These findings acquire a special significance if we note that they can be shown for each of the individual cases considered and do not emerge solely from an overall assessment of the case material.

Figures 1 and 2 show two typical cases in diagram form; in the first case, with a poor prognosis, the rising straight line of IgG crosses over those of IgA and IgM, while the opposite occurs in patients with a good neonatal foetal prognosis.

When the initial ICT titre was 1:256 (cases 11-15 in Table I), which was a poor prognostic indication in itself, transition to a higher titre was accompanied by a rise in IgG values from a minimum of 45% to a maximum of 86% as compared with increases in IgA up to 45% and in IgM from a minimum of 22% to a maximum of 56%. All the infants showed some damage. In one case (M.D.) where the increase in IgG was less marked, and that of IgA and IgM scarcely appreciable, premature birth took place during the 26th week; the infant was dead, with severe signs of dropsy.

Examination, in Table II, of the average values of IgG, IgA, and IgM at the time of the first and second estimation of the ICT and their respective increases in the cases with a good and a poor neonatal prognosis brings out the following theree points:

Table 1. *Case material, initial and final indirect Coombs titre, corresponding level of immunoglobulins IgG, IgA, and IgM in mg %/ml, their percentage increase and neonatal clinical condition.*

N° Name	ICT titre	IgG (mg%ml)	Increase	IgA (mg%ml)	Increase	IgM (mg%ml)	Increase	Neonatal Condition
1. S.A.	1:32	640	200	125	120	200	122	No neonatal fetal damage
	1:256	840	(23,8%)	245	(48,9%)	322	(37,8%)	
2. G.A.	1:16	880	174	112	278	118	138	No neonatal fetal damage
	1:64	1054	(16,5%)	380	(70,5%)	260	(54,6%)	
3. R.L.	1:8	1020	80	158	57	85	31	No neonatal fetal damage
	1:64	1100	(7,2%)	215	(26,5%)	116	(26,7%)	
4. F.G.	1:32	797	175	118	77	112	64	No neonatal fetal damage
	1:256	972	(18%)	195	(65%)	176	(57%)	
5. M.O.	1:4	791	79	135	67	160	73	No neonatal fetal damage
	1:64	870	(9%)	202	(50%)	233	(46%)	
6. R.T.	1:4	835	195	140	45	105	29	No neonatal fetal damage
	1:64	1030	(19%)	185	(32%)	134	(28%)	
7. S.V.	1:16	672	118	155	64	98	35	No neonatal fetal damage
	1:64	790	(15%)	219	(41%)	133	(36%)	
8. E.T.	1:32	626	474	145	3	155	3	Newborn polytransfused
	1:64	1160	(46%)	148	(2%)	158	(1,5%)	
9. F.G.	1:4	584	536	152	3	107	−6	Newborn dead
	1:64	1120	(47,8%)	158	(3,8%)	101	(−5,6%)	
10. C.G.	1:4	486	794	120	8	180	7	Newborn dead
	1:64	1280	(62%)	128	(7%)	187	(4%)	
11. C.M.	1:256	80	520	100	42	50	65	Newborn polytransfused
	1:512	600	(86,6%)	142	(29,5%)	115	(56,5%)	
12. M.E.	1:256	480	720	117	97	140	179	Newborn polytransfused
	1:512	1200	(60%)	214	(45,3%)	319	(56,1%)	
13 M.D.	1:256	490	553	320	115	160	74	Newborn dead
	1:512	1043	(53%)	435	(36%)	234	(46%)	
14. B.G.	1:256	283	604	190	7	220	59	Newborn dead
	1:1024	887	(68%)	197	(4%)	279	(27%)	
15. M.D.	1:256	880	720	412	−22	165	47	Fetal death as a result of
	1:1024	1600	(45%)	390	(−5,3%)	212	(22,1%)	dropsy (26 week)

In cases 1-10 the initial ICT titre was = 1:32, in cases 11-15 the initial ICT titre was = 1:256.

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1) In the cases with a good prognosis the change seen in the average values of IgG is much less marked than that observed in the cases with a poor neonatal prognosis (+ 145.85 mg % ml against + 621.3 mg % ml). The difference is highly significant. ($p < 0.01$).
2) The average IgG level at the first ICT determination was significantly lower

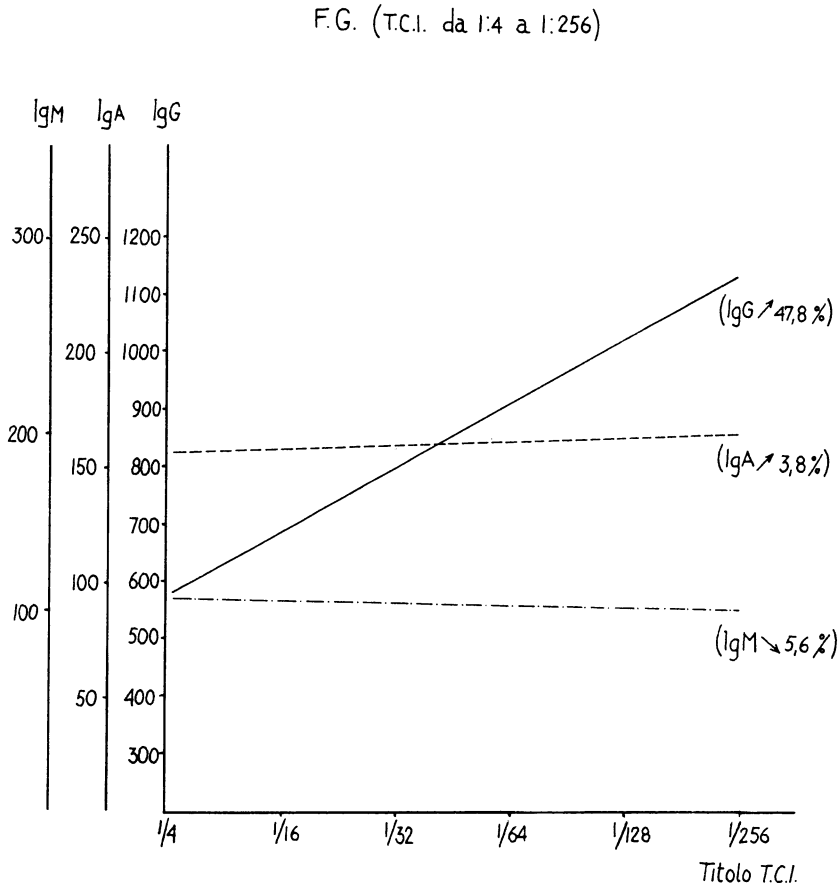

Fig. 1

than the average found in the course of normal pregnancy taken as a standard (565.33 mg % ml against 1031 mg % ml) in the cases with a poor neonatal prognosis. ($p < 0.01$).
3) In the cases with a good prognosis the IgA and IgM level at the second estimation of the ICT were higher, both in comparison with the first estimation and as an absolute value in relation to the same measure carried out in the cases with a poor prognosis. In these cases the difference for IgM was significant ($p < 5\%$).

We can thus say that the IgG level shows a considerable increase in the cases with a poor prognosis, while in the cases with a good prognosis it is largely IgA and IgM which increase.

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DISCUSSION

Two points which require discussion emerge from our results:
1) The low IgG values at the time of the first ICT determination in the cases with a poor neonatal prognosis.
2) The rise in IgG in the cases with a poor prognosis in comparison with its

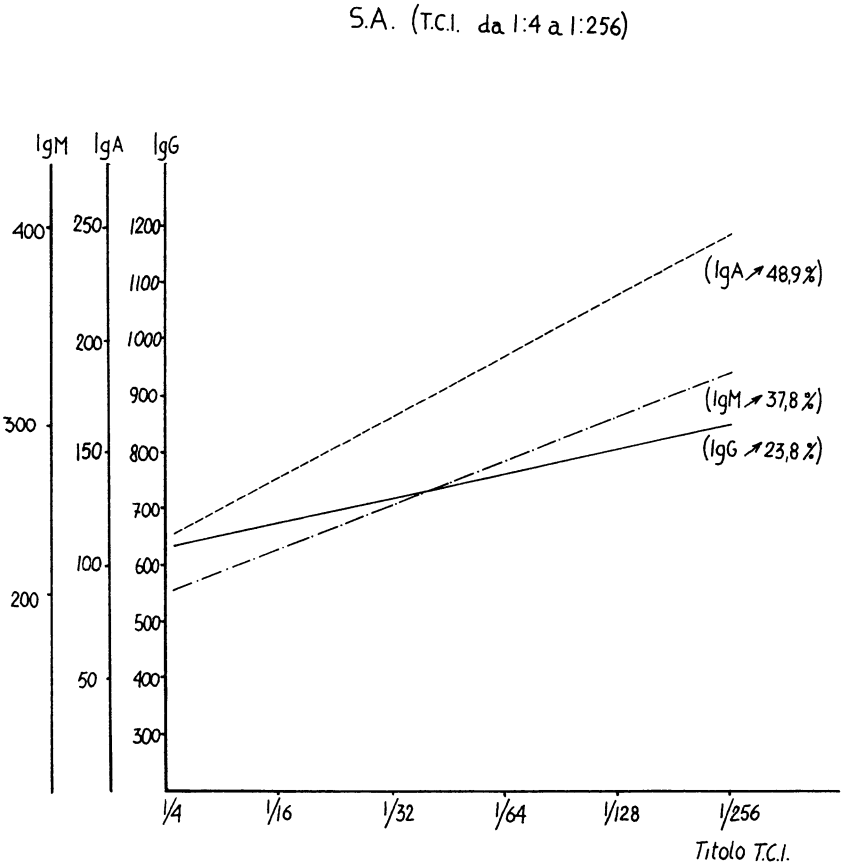

FIG. 2

lack of increase and the simultaneous rise in IgA and IgM in the cases with a good prognosis.

In regard to the first point, it may be that the low IgG level depends on: a) the fixation of a significant quantity of anti-Rh IgG immunoglobulins (which cross through the placenta) to the foetal erythrocytes. With the increase in the production of anti-Rh antibodies as a result of persistent antigenic stimulation, the quantity of free IgG in the maternal serum also rises; b) a certain degree of central inhibition (by the maternal immunocompetent cells) against the production of anti-Rh IgG. The possible presence of a similar mechanism acting through the antigen/antibody immunocomplexes present in the maternal serum cannot

Table 2. *Comparison between the averages of the increases in mg%/ml of IgG, IgA and IgM from ICT values = 1:32 to values = 1:64 in cases with good (GP) and poor (PP) neonatal fetal prognosis.*

Groups of immunoglobulins	IgG				IgA				IgM			
Neonatal foetal prognosis	GP		PP		GP		PP		GP		PP	
ICT Titre	1:32	1:64	1:32	1:64	1:32	⇒1:64	1:32	⇒1:64	1:32	⇒1:64	1:32	⇒1:64
Average of the immunoglobulins values in mg %/ml	805	950	565,33	1186,66	134,71	234,42	139	144,66	125,42	196,28	147,33	148,66
Average of the increase in immunoglobulins in mg %/ml	+145,85		+621,3		+99,71		+5,66		+70,86		+1,33	
	→								←			
Student's t.	7,95308				2,01980				2,41536			
Significance (p)	Significant (p <0,01)				Not significant				Significant (p <0,05)			

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be entirely excluded. Its existence is suggested by the fact that the IgA levels found at the first ICT determination in the cases with a poor prognosis are lower than the normal values. The development of a general initial depression of the anti-Rh immune response present in the cases with a poor neonatal foetal prognosis may therefore be involved here.

As to the rise in IgG in the cases with a poor prognosis, it must first be emphasized that this does not signifiy an increase in the total IgG level in relation to the normal average in pregnancy; indeed, the final IgG values do not differ significantly from this average (1186 against 1031 mg % ml). We see here a marked rise from a value lower than normal to an almost normal value.

The finding that IgA and IgM behave in a contrasting manner to IgG and increase especially in the cases with a good prognosis can only be explained by the ability of IgG to cross the placental barrier, and its fixation on the fetal erythrocytes. Because of the size of their molecule or because of reasons related to their particular molecular structure ([9]), IgA and IgM cannot cross the placenta actively. According to Gitlin ([10]), only moderate transfer by diffusions is possible for them. Thus, if it is the IgG which rises, the prognosis is less favourable because this antibody can cross the placenta and damage the foetus. If, on the contrary, IgA and IgM increase, the prognosis is better because these fractions cannot cross the placenta and harm the fetus.

For diagnostic purposes it is important to establish whether the rise is initiated from the « basal » values of the ICT equal to or less than 1:32. When the initial ICT is already suspect in itself (= 1:64), the results may be more inconsistent. Indeed, the increase in harmful IgG may already have occurred without being discovered; under these circumstances, the possible increase in all the groups of immunoglobulins only assumes the function of an indicator for an alarming situation which already prevails.

It is thus necessary to take a « basal » measure of the immunoglobulins in those cases where the ICT is = 1:32, to follow their possible increase in time, simultaneously with their rise on the ICT. The significance of the increase can be adequately assessed only if the patient is used as her own control.

The possibility of determining the neonatal fetal prognosis by measuring the groups of immunoglobulins matched to the ICT may also help to provide an indication for the use of amniocentesis, which may be reserved solely for the cases in which the rise in the ICT is accompanied by a typical increase in IgG.

SUMMARY

Determination of the antibody titre alone by the indirect Coombs test (ICT) in pregnant patients with anti-Rh isoimmunisation provides an inaccurate indication for assessment of the neonatal foetal prognosis.

It was the purpose of this study to ascertain whether a more accurate assessment might be obtained by measuring the individual groups of serum immunoglobulins (IgG, IgA, IgM) in correlation with the ICT.

Fifteen pregnant patients with isoimmunisation were observed, and the following points were investigated:

1) The variations in the ICT between an initial determination and a final determination.

2) The variations in IgG, IgA, and IgM between these two determinations. On the basis of the neonatal conditions assessed at birth by the usual clinical and

laboratory methods, the patients were divided into cases with a good prognosis (GP), i.e. without neonatal foetal compromise, and cases with a poor prognosis (PP), with neonatal fetal risk.

Results were as follows:

1) In the cases with PP the average IgG level at the time of the first determination with the ICT was lower (with high statistical significance) than the average found during normal pregnancy (565.33 mg % ml against 1031 mg % ml, $p < 0.01$).

2) In the same cases (PP) we found a much more marked increase in IgG between the two ICT determinations thans shown by the cases with GP (+ 621.3 mg % ml against + 145.85 mg % ml, $p < 0.01$).

3) IgA and IgM behaved in an inverse manner, increasing in the cases with GP rather than in those with PP.

The increase in IgA had a 5% statistical significance.

The authors discussed their findings and suggest that the greater increase in IgG in the cases with PP is explained by the fact that these antibodies can cross the placenta and damage the foetus. If, on the contrary, IgA and IgM are increased, the prognosis is better because these are incapable of crossing the placenta.

The authors also emphasise that the usefulness of this investigation is increased if the measurement of the rise of the globulins begins with the « basal » ICT values of 1:32, with successive determinations in the same patient.

This method may also be useful as an indicator for the use of amniocentesis.

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BIBLIOGRAPHY

1. Hofmann D., Hollander N.J., Mast N., Quackernak K. and Shellong G.G.: *Geburtsh. Frauenheilk.*, 31, 797, 1971. - 2. Tovey L. A. D.: *J. Obst. Gynaec. Brit. Cwlth.*, 76, 117, 1969. - 3. Bartsch F. K.: *Atti del Convegno sui problemi attuali della MEN.* Genova 17-19 Dicembre 1971, pag. 139. A cura di G. Sansone ed F. D'Ambrosio. - 4. Robertson J. R. e D'Ambrosio F.: *The Rh problem - Proceedings Int. Symp.* Milan, Oct. 9-11, 1969. Ann. Ost. Ginec., special number 1970. - 5. Carretti N.: *Attual. Ost. Gin.*, 14-20, 1° suppl. 1968. - 6. Carretti N. and Ovary Z.: *Proc. Soc. Exp. Biol and Med.* 130, 509, 1969. - 7. Giaquinto M., Mega M. e Bertolin A.: *Attual. Ost. Gin. suppl.* al vol. X, 1964. - 8. Mancini G., Carbonara A. O. e Heremans J. F.: *Immunochemistry* 2, 235, 1965. - 9. Ovary Z.: *Proceedings from the 1st. Int. Congress of Immunology in Obst. and Gynec.* Padua 7-9 June 1973, A. Centaro, N. Carretti Ed. (in press) Excerpta Medica, Amsterdam. - 10. Gitlin D.: *Proceedings from the 1st. Int. Congress of Immunology in Obst. and Gynec.* Padua 7-9 June 1973, A. Centaro, N. Carretti Ed. (in press.) Excerpta Medica, Amsterdam.