

All 10 control animals developed tumors. Two of 10 animals having excision of previously-inoculated CBP tumors developed tumors after subsequent challenge ($p < 0.01$ by χ^2 analysis as compared to controls). Four of 10 hamsters immunized with normal pancreas developed tumors after challenge ($p < 0.01$), indicating that a level of tumor immunity was induced presumably through determinants in the

normal pancreatic tissue. Further studies are necessary to characterize pancreatic-specific antigens and to determine whether similar classes of such antigens exist in various species. Examination of various pancreatic neoplasms is necessary before substantial inferences can be made as to whether the expression of normal tissue antigens is a characteristic of pancreatic cancers.

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ABO system incompatibility: evaluation of risk of hyperbilirubinaemia at birth by multivariate discriminant analysis¹

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Summary. A discriminant analysis was performed on a set of maternal and neonatal variables to predict at birth the serum bilirubin levels during the neonatal period in infants incompatible with their mothers in the ABO system. The results suggest that the rational and simultaneous utilization of clinical and laboratory parameters allows, a few hours after delivery, a useful classification of these infants in low or high risk for hyperbilirubinemia.

ABO feto-maternal incompatibility shows a high prevalence both in Caucasian and in Negro populations. Although severe ABO hemolytic disease is rare, milder forms are relatively frequent: in these cases jaundice may not be detected soon after birth and early discharge of the newborn may have serious consequences³. Therefore, in order to select infants which may be discharged in the very first days of life, the early identification of the newborns at risk of hyperbilirubinaemia is very important.

In the present paper we report a discriminant analysis performed on a set of maternal and neonatal variables to predict at birth the serum bilirubin levels during the neonatal period.

The analysis was performed according to Klecka⁴ on a IBM 370/158 computer. By this procedure a linear combination of independent variables (discriminant function) that best distinguishes between cases in the categories of the dependent variable (bilirubin level) is found. The most useful variables can be selected by stepwise procedure. Variables which are not able to contribute to discrimination according to a user-determined criterion (a fixed value of multivariate F ratio) are not included in the discriminant function. Several indexes of discriminating power of single variables and of the importance of discriminant functions are provided by SPSS Discriminant subprogram.

302 White newborns of European descent and 76 Black infants incompatible with their mother only in the ABO system were studied. The sample was collected at the Yale New Haven Hospital. Biochemical, immuno-hematological and sampling methods were reported in previous papers⁵⁻⁸.

Table 1. Variables used for the discriminant analysis

Variable included in the final analysis	Categories
Gestational age	
Birth weight	
Birth order	First or second Third or higher
ABO maternal phenotype	A or B O
Type of feto-maternal ABO incompatibility*	A B
Direct coombs test	Negative Positive
Presence of P1 ^{f1} allele of placental alkaline phosphatase** and of I ^B allele of ABO system	Both present Only one or none present
Dose of P1 ¹¹ or rare alleles of placental alkaline phosphatase**	Absent Heterozygous Homozygous

*Used only in Blacks. **Placental alkaline phosphatase (PAP) is a polymorphic enzyme which is produced by the fetus and is found in the maternal circulation during gestation. This polymorphic system is controlled by an autosomal locus with 3 common alleles (P1^{s1}, P1^{f1} and P1¹¹) and a high number of rare alleles. We had previously observed, in ABO incompatible newborn infants, a positive association between the direct Coombs test and the incidence of jaundice and a negative association between the latter and the simultaneous presence of I^B and P1^{f1} factors^{5,6}. Variables discarded. Maternal age. Previous spontaneous abortions. PGM₁ phenotype. Dose of P1^{f1} factor of placental alkaline phosphatase. Enzymatic activity of placental alkaline phosphatase phenotype.

Each series was subdivided into 2 groups according to the maximum serum bilirubin level (dependent variable) recorded during the 1st 4 days of life (< 10 mg/dl or ≥ 10 mg/dl).

A stepwise discriminant analysis was carried out on 13 variables; 6 variables in Caucasians and 5 in Blacks were discarded since their independent contribution to discrimination was negligible (see tables 1, 2 and 3 for variables included). Therefore discriminant functions for Whites and Blacks were based on 7 and 8 variables respectively. Individual discriminant scores were obtained by introducing in the calculated discriminant function the pertinent values of each subject. The individual scores were standardized. In this way the average value and the SD of all scores become 0 and 1 respectively and the individual scores may assume a negative or a positive value.

Tables 2 and 3 show the stepwise discriminant analysis. The separation obtained between infants with a serum bilirubin

level < 10 mg/dl (LB) and those with a level ≥ 10 mg/dl (HB) is statistically significant both in Caucasians ($p < 0.001$) and in Blacks ($P < 0.025$). Variables which contribute most to differentiation are Coombs test, gestational length and birth weight in Caucasians, and Coombs test, dose of $P1^{fl}$ and $P1^{rare}$ factors, and gestational length in Blacks. Tables 4 and 5 show the distribution of infants according to the sign of discriminant score and for each class the number of infants attaining a serum bilirubin level higher than 10 mg/dl. It appears that the probabilities of false positives (i.e. the proportion of infants which did not develop hyperbilirubinaemia among those classified as high risk) are quite large whereas the probabilities of false negatives (i.e. the proportion of infants which developed hyperbilirubinaemia among those classified as low risk) are very low, especially among females.

A simple discrimination between infants with bilirubin level of less than 10 mg/dl from those with bilirubin over

Table 2. Stepwise discriminant analysis by Wilks' method. White newborn infants

Step number	Variable entered	F to enter	Wilks' lambda	Significance	Raos' V	Change in Raos' V	Significance of change
1	Direct Coombs test	24.07	0.92	0.00	24.07	24.07	0.00
2	Gestational age	12.82	0.88	0.00	38.00	13.93	0.00
3	Birth weight	3.70	0.87	0.00	42.22	4.22	0.04
4	ABO maternal phenotype	1.98	0.87	0.00	44.52	2.30	0.13
5	Presence of $P1^{fl}$ allele of PAP and of I^B allele of ABO	0.65	0.86	0.00	45.28	0.76	0.38
6	Dose of $P1^{fl}$ or $P1^{rare}$ alleles of PAP	0.38	0.86	0.00	45.72	0.44	0.51
7	Birth order	0.02	0.86	0.00	45.74	0.02	0.89
Standardized discriminant function coefficients							
Gestational age			0.38	Eigenvalue	0.157		
Birth weight			0.33	Canonical correlation	0.369		
ABO maternal phenotype			0.19				
Dose of $P1^{fl}$ or $P1^{rare}$ alleles of PAP			0.09	χ^2	41.975		
Presence of $P1^{fl}$ allele of PAP and of I^B allele of ABO			0.12	D.F.	7		
Birth order			-0.02	Significance	$p < 0.001$		
Direct Coombs test			0.68				

Table 3. Stepwise discriminant analysis by Wilk's method. Black newborn infants

Step number	Variable entered	F to enter	Wilks' lambda	Significance	Raos' V	Change in Raos' V	Significance of change
1	Direct Coombs test	8.29	0.90	0.00	8.29	8.29	0.00
2	Dose of $P1^{fl}$ or $P1^{rare}$ alleles of PAP	4.05	0.85	0.00	12.87	4.58	0.03
3	Gestational age	3.56	0.81	0.00	17.17	4.31	0.04
4	Presence of $P1^{fl}$ allele of PAP and of I^B allele of ABO	1.47	0.79	0.00	19.07	1.90	0.17
5	Birth weight	1.21	0.78	0.00	20.69	1.62	0.20
6	ABO maternal phenotype	0.54	0.77	0.00	21.44	0.75	0.39
7	Birth order	0.14	0.77	0.01	21.64	0.20	0.66
8	Type of feto-maternal ABO incompatibility	0.07	0.77	0.02	21.74	0.11	0.74
Standardized discriminant function coefficients							
Gestational age			0.29	Eigenvalue	0.302		
Birth weight			0.30	Canonical correlation	0.482		
Type of feto-maternal ABO incompatibility			-0.06	χ^2	17.948		
ABO maternal phenotype			0.17	D.F.	8		
Dose of $P1^{fl}$ or $P1^{rare}$ alleles of PAP			-0.49	Significance	$p < 0.025$		
Presence of $P1^{fl}$ allele of PAP and of I^B allele of ABO			0.34				
Birth order			0.09				
Direct Coombs test			0.84				

Table 4. Prediction results of discriminant analysis in Whites. Newborn infants with a negative score (standardized value assumed by discriminant function) are considered at 'low risk'

Cases classified as	Number of cases which actually developed hyperbilirubinaemia ≥ 10 mg/dl	Number of cases which actually did not develop hyperbilirubinaemia ≥ 10 mg/dl
Low risk: females	1	89
males	3	88
High risk: females	17	44
males	13	47
Total: females	18	133
males	16	135

10 mg/dl could be of questionable relevance to clinicians. As a first approach to the problem, however, the subdivision is reasonably justified on the basis of the general indications for phototherapy. It is very likely that the discriminating power could sensibly increase by adding other variables such as cord bilirubin level⁹, hematocrit, hemoglobin, reticulocyte count and clinical conditions at birth, and informations on drugs given during pregnancy and on anesthesia during labor. Unfortunately, these data were not available for the present analysis. The results indicate that the multivariate approach is feasible. The main advantage of the procedure consists in the optimal, rational and simultaneous utilization of clinical and laboratory parameters available for the care of ABO incompatible infants. These data can be obtained within 12 h after birth. Even at the present preliminary stage, the analysis might allow, few hours after delivery, a useful classification of low and high risk infants regarding the level of serum bilirubin in the neonatal period. Overall, in fact, babies with a negative score, representing in our sample more than 50% of all ABO incompatible infants, show a risk of hyperbilirubinaemia lower than 3%. However, since the aim of the study is to select ABO incompatible infants which would be discharged very early, the inclusion of false negative may have deleterious consequences. Therefore, to have practical utilization, the discriminant function should be improved in order to further reduce the probabilities of false negative to negligible values.

Table 5. Prediction results of discriminant analysis in Blacks. Newborn infants with a negative score (standardized value assumed by discriminant function) are considered at 'low risk'

Cases classified as	Number of cases which actually developed hyperbilirubinaemia ≥ 10 mg/dl	Number of cases which actually did not develop hyperbilirubinaemia ≥ 10 mg/dl
Low risk: females	0	21
males	1	22
High risk: females	6	8
males	8	10
Total: females	6	29
males	9	32

The differences observed between Caucasians and Blacks concerning the pattern of factors predisposing to clinical jaundice and those between sexes may be of major theoretical and practical importance and deserves further investigations to be elucidated.

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Studies on the Dd antigen-antibody system. II. Antigen Dd reactivity in some North Indian populations

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Summary. The frequency of antigen Dd-reactors has been recorded in Muslims and Buddhists from Ladakh, in Rana Tharus from Uttar Pradesh and in two samples of largely Jat Sikh origin from Punjab, all in India. The results show a wide range of variation, from 0% in the Rana Tharus to 25% in the Punjabi blood donors, of incidence of antigen Dd-reactivity in these populations.

Antigen Dd is a component of certain specimens of human dandruff and precipitates some but not all human sera³. The nature of this antigen is not precisely known and it cannot as yet be definitely said that it is not of extra-human origin. The antibodies reacting with antigen Dd have not been detected in human cord serum but they appear to be present permanently in the sera of adult antigen Dd-reactors.

Shrivastava³ found 3.97% Polish blood donors from Warsaw to have antibodies against antigen Dd. In contrast, the frequency of antigen Dd-reactors in Punjab, in North India, was estimated to be 24.17% in one sample⁴ and 19.28% in

another⁵. In yet another sample, this time drawn from the Gaddi tribals of Himachal Pradesh in India, we did not come across a single antigen Dd-reactor in 87 sera examined⁶.

Here we present results of our further studies on the distribution of antigen Dd-reactors in some more populations from North India.

Materials and methods. Antigen Dd was prepared as described earlier³. About 5 ml blood was drawn i.v., under aseptic conditions, from each individual and the sera extracted were stored at -20°C until they were used. Immunoelectroosmophoresis was performed on agarose gels at