Invest Clin 20(3): 162-177, 1979

PREDICTION OF HEMOPHILIC CARRIERS: A NEW STATISTICAL APPROACH USING SIMULTANEOUS ASSAYS OF FACTOR VIII COAGULANT ACTIVITY, FACTOR VIII RELATED ANTIGEN AND RISTOCETIN COFACTOR ACTIVITY

Eric Chun-Yet Lian*, Maria Diez-Ewald, Stephen D. Walter*, Roger Núñez*, Jean-Fern Lian*** and Donald R. Harkness***

ABSTRACT

The posterior probability of each individual for normal or hemophilic carrier was calculated by multivarite discriminant analysis. When both factor VIII coagulant activity and factor VIII-related antigen were used, all of the 30 normal females and 18 of 20 obligatory carriers were correctly classified. When factor VIII coagulant activity and ristocetin cofactor activity were used, 97% of the normal females and 75% of carriers were correctly predicted. When all three parameters were taken into consideration in discriminant analysis, all normals and 17 of 20 carriers were correctly classified. We conclude that concurrent determination of both factor VIII coagulant activity and ristocetin cofactor activity can be used as an alternate or adjunctive method for detecting carriers.

---

* *The Department of Medicine, Veterans Administration Hospital and the Miami Hemophilia Center, Jackson Memorial Hospital, University of Miami, School of Medicine, Miami, Florida, U.S.A.*

** *Instituto de Investigaciones Clinicas, Apartado 1151, Maracaibo, Venezuela.*

*** *Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, U.S.A.*

The work was supported by the Veterans Administration Hospital, the Jon Eric Hemophilia Research Fund, and a N.I.H. Biomedical Research Support Grant. The abstract was presented at the 16th International Congress of Hematology at Kyoto, Japan, 1976.

10- JENKINS CSP, MEYER D, DREYFUSS MD, LARRIEU MJ: Willebrand factor and ristocetin. I. Mechanism of ristocetin induced aggregation. Brit J Haemat 28: 561-578, 1974.

11- KURAMOTO AM, STEINER M, BALDINI MG: Lack of platelet response to stimulation in Wiskott-Aldrich syndrome. N Engl J Med 282: 475-479, 1969.

12- MALDONADO JE, PINTADO T: Ultrastructure of the megakaryocytes in refractory anemia and myelomonocytic leukemia. En: Platelets, production, function, transfusion and storage. Baldini MG and Shirley E, ed. p. 105, Grune and Stratton, N.Y. 1974.

13- MIELKE CH Jr, KANESHIRO MM, MAKER IA, WEINER JM, RAPAPORT SI: The standarized normal bleeding time and its prolongation by aspirin. Blood 34: 204-215, 1969.

14- MURPHY S, OSKI FA, NAIMAN JL: Platelet size and kinetics in hereditary and adquired thrombocytopenia. N Engl J Med 284: 499-504, 1972.

15- RABY C: Hemorragias y trombosis p. 89, Ed. Toray-Mason S.A. Barcelona 1968.

16- SCHAAR FE: Familial idiopathic thrombocytopenic purpura. J Pediat 62: 546-551, 1963.

17- STEINER M, KURAMOTO A: Energy metabolism of aggregating platelets. Ser Haemat 4: 98, 1971.

18- VESTERMARK B, VESTERMARK S. Familial sex-linked thrombocytopenia. Acta Paediat (Stockholm) 53: 365-370, 1964.

19- WEISS HJ, EICHELBERGER JW: Secondary thrombocitopathia: Pf_3 in various disease states: Arch Intern Med (Chicago) 112: 827-834, 1963.

20- WHITE JG, GUERRARD JM, WITROP CJ: Platelet-platelet interaction: a simple test for storage pool deficiency and defective prostaglandin synthesis. En: Platelet function testing. US Department of Health, Education and Welfare. Public Health Service. National Institutes of Health. Day HJ, Holmsen H and Zucker MB ed. pp. 374-385, 1976.

21- WISKOTT A: Familiarer angeborener Morbus Werhofii?. Mschr Kinderh 68: 212-216, 1937.

INTRODUCTION

The detection of hemophilic carriers is greatly improved by comparing the level of factor VIII coagulant activity to that of factor VIII-related antigen (27). Recently it was shown that ristocetin cofactor activity (quantitative ristocetin-induced platelet aggregation activity), which is decreased or absent in von Willebrand's disease, is quantitatively normal and is proportional to the amount of factor VIII-related antigen in both normal and hemophilic subjects (28). Whether or not the simultaneous assays of ristocetin cofactor activity and factor VIII coagulant activity can be used for detecting hemophilic carrier has not been explored. In this communication, we report a different statistical method for predicting hemophilia carriers by simultaneous measurements of factor VIII coagulant activity, factor VIII-related antigen, and ristocetin cofactor activity. We also demonstrate that concurrent determinations of both factor VIII coagulant activity and ristocetin cofactor activity can be used as an alternate or adjunctive method for detecting hemophilic carriers.

MATERIAL AND METHODS

A. Subjects

Twenty healthy obligatory hemophilic carriers, including daughters of hemophiliacs, mothers with more than one hemophilic son and mothers with one hemophilic son and other hemophilic relatives, were examined. The controls, presumably normal subjects, were 30 healthy female laboratory and hospital personnel with no family or personal histories of bleeding disorders. Any subject, either normal or carrier, with any concurrent medical illness or menstruation was carefully excluded.

B. Blood Collection and Processing

Venous blood was drawn through an uncoated 19 gauge scalp vein needle by a double plastic syringe technique. The whole blood in the second syringe was transferred immediately into polyethylene tubes containing 3.8% sodium citrate (9 parts blood to 1 part citrate). Platelet poor plasma for determinations of factor VIII coagulant activity, factor VIII-related antigen and ristocetin cofactor activity was collected from the supernatant fraction after centrifugation at 2400 X g at 4°C for 20 minutes. Factor VIII coagulant activity was assayed always within four hours after procuring the blood sample. The plasma samples which were not used that day were immediately frozen in small aliquots at -80°C. Factor VIII-related antigen and ristocetin cofactor activity were assayed on fresh or frozen plasma samples within one week. No difference was found whether factor VIII-related antigen and ristocetin cofactor activity were

determined on fresh or frozen plasma. At least one normal control sample was run with each set of patient assays.

C. Normal Pooled Plasma

A normal pool of plasma was collected from 16 healthy laboratory and hospital personnel of each sex. The platelet poor plasma was collected as described above and further spun at 12,000 X g for 10 minutes at 4°C. The supernatant plasmas were pooled and immediately stored in small aliquots in plastic tubes at -80°C. One unit of factor VIII coagulant activity, factor VIII-related antigen, or ristocetin cofactor activity used in the test is equal to the amount of respective activity in one milliliter of this normal reference plasma.

D. Factor VIII Coagulant Activity

Factor VIII coagulant activity was assayed by the kaolin-activated partial thromboplastin time using factor VIII deficient plasma (21).

E. Factor VIII-related Antigen

The factor VIII-related antigen in plasma was determined directly on the plasma samples diluted with citrated rabbit plasma (28) by electrophoresis in 1% agarose gel (Calbiochem, San Diego, Calif.) containing rabbit antihuman-factor VIII-antiserum, according to the method of Laurell (13). The details of methods for assaying of factor VIII-related antigen and preparing rabbit antiserum have been described previously (14).

F. Ristocetin Cofactor Activity

Ristocetin cofactor activity in the plasma was assayed by a modification of the method described by Weiss et al (25). About 20 to 45 ml of normal platelet rich plasma was placed in a 50 ml conical plastic tube. A volume of 0.8 ml of 30% bovine albumin (Dade Reagent, Miami, Fla.) was introduced at the bottom of the tube as a cushion. After centrifugation of 2000 g for 12 minutes at room temperature, a platelet pellet was formed between the albumin cushion and the plasma. The pellet was removed and resuspended in 40 ml of Tris-saline buffer containing 1 part of 0.15 M Tris-HCl, pH 7.4, and 2 parts of 0.85% NaCl with 1% disodium EDTA in another conical plastic tube. Bovine albumin was again introduced at the bottom of the tube and the tube centrifuged as described above. The washing procedure was repeated four times using the Tris-saline EDTA buffer and twice using the same buffer without EDTA. The washed platelets were adjusted to a final concentration of 187,000/mm^3 with Tris-

saline buffer. To a cell containing 0.4 ml of washed platelets and 0.1 ml of undiluted or diluted test plasmas, ristocetin (Abbott) was added to make a final concentration of 1.2 mg/ml and the slope of platelet aggregation was recorded in a platelet aggregometer. The ristocetin-induced platelet aggregation activity of a test plasma was obtained by reading its slope against the normal curve constructed with the slopes from serially diluted normal pooled plasma samples.

G. Statistical Methods

For both normal females and obligatory carriers, statistical calculations were carried out on the logarithmic values of all three parameters, because a straight line was obtained when the log values of these parameters were plotted against cumulative per cent on a probability scale (5, 16, 21).

The 50% and 95% tolerance ellipses for each group in the graph were constructed according to the equation developed by Guttman (9) as described by Bouma et al (5). The equation is as follows:

$$\frac{1}{(1-R^2)V_1^2}(U-\bar{U})^2 - \frac{2R}{(1-R^2)V_1V_2}(U-\bar{U})(V-\bar{V})$$

$$+\frac{1}{(1-R^2)V_2^2}(V-\bar{V})^2 = \frac{2(N^2-1)}{N(N-2)}F_{2,\,N-2;\,\alpha}$$

R = Correlation coefficient between U and V, when U and V are factor VIII coagulant activity and factor VIII-related antigen respectively.

V_1 and V_2 = standard deviations of U and V respectively

$\bar{U}$ and $\bar{V}$ = mean value of U and V respectively

N = total cases in each group

α = significant level

$F_{2,\,N-2;\,\alpha}$ is the 100 $(1-\alpha)$% point on the F distribution with (2, N-2) degrees of freedom

The posterior probability for being normal or carrier is based on the squared Mahalanobis' distances of each case from the means of the normal and carrier groups using the discriminant analysis computer program BMDP7M (10).

RESULTS

The results of factor VIII coagulant activity (U) and factor VIII-related antigen (V) in 30 normal females and 20 obligatory carriers are shown in Fig. 1. The inner ellipses contain 50% and outer ellipses 95% of normals and carriers for each group for factor VIII coagulant activity and factor VIII-related antigen. Seventeen out of 20 (85%) carriers are outside the 95% tolerance region of the normals. The regression lines of both normal and carrier group appear to cross each other at higher levels. The equations of the tolerance ellipses on the U-V plane for normals and carriers are different as shown below:

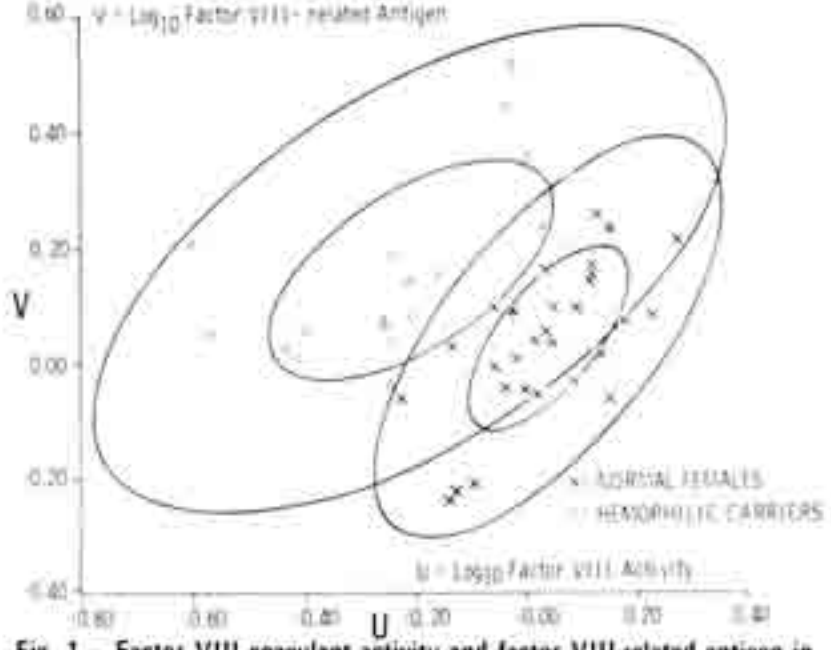

Fig. 1.– Factor VIII coagulant activity and factor VIII-related antigen in normals and carriers.

For normals:

$$133.407\,(U-\bar{U})^2 - 164.066\,(U-\bar{U})\,(V-\bar{V}) + 109.243\,(V-\bar{V})^2 = \frac{2\,(N^2-1)}{N\,(N-2)}\,F_{2,\,N-2;\,\alpha}$$

For carriers:

$$36.960\,(U-\bar{U})^2 - 59.066\,(U-\bar{U})\,(V-\bar{V}) + 67.683\,(V-\bar{V})^2 = \frac{2\,(N^2-1)}{N\,(N-2)}\,F_{2,\,N-2;\,\alpha}$$

Fig. 2, shows the results of factor VIII coagulant activity (U) and ristocetin cofactor activity (T) of both normals and carriers. Again the inner and outer ellipses represent the 50% confidence intervals of the respective groups. There are 14 out of 20 (70%) carriers outside the 95% tolerance region of normals; only 1 normal out of 30 (3%) is outside the 95% region of carriers. The regression lines of the two groups are not parallel. The equations of tolerance ellipses for normals and carriers on the U – T plane are also different as shown below:

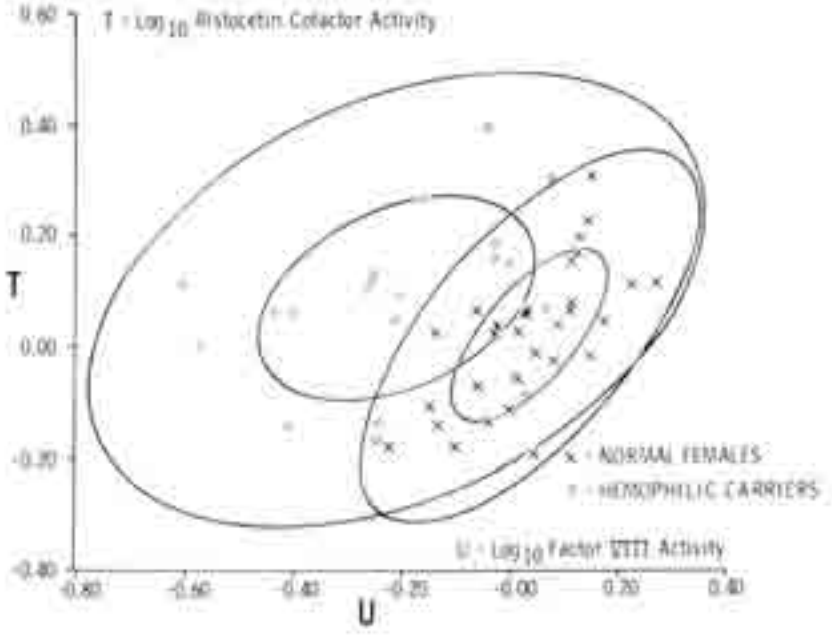

Fig. 2.– Factor VIII coagulant activity and ristocetin cofactor activity in normals and carriers.

For normals:

$$134.875\,(U-\bar{U})^2 - 174.382\,(U-\bar{U})\,(T-\bar{T}) + 120.543\,(T-\bar{T})^2 = \frac{2\,(N^2-1)}{N\,(N-1)}\,F_{2,\,N-2;\,\alpha}$$

For carriers:

$$28.148\,(U-\bar{U})^2 - 30.019\,(U-\bar{U})\,(T-\bar{T}) + 55.298\,(T-\bar{T})^2 = \frac{2\,(N^2-1)}{N\,(N-1)}\,F_{2,\,N-2;\,\alpha}$$

The mean value of each variable and $F_{2,\ N-2;\ \alpha}$ values are shown as follows:

	$\bar{U}$	$\bar{V}$	$\bar{T}$	N	$F(\alpha = 0.50)$	$F(\alpha = 0.95)$
Normal	0.0417	0.0483	0.0184	30	0.71	3.34
Carriers	-0.2046	0.1647	0.0847	20	0.72	3.55

The posterior probability of each individual calculated from the squared Mahalanobis' distances from the respective means of normals and carriers by stepwise discriminant analysis is shown in Tables I, II, and III. When both factor VIII coagulant activity and factor VIII-related antigen were used, all of the normals and 18 of 20 (90%) obligatory carriers are correctly classified. When factor VIII coagulant activity and ristocetin cofactor activity were used, 97% of normals and 75% of carriers are correctly predicted. One of two carriers misclassified by factor VIII coagulant activity and factor VIII-related antigen is correctly diagnosed by factor VIII coagulant activity and ristocetin cofactor activity. When all three parameters are taken into consideration in discriminant analysis, all normals and 17 of 20 (85%) carriers are correctly classified.

DISCUSSION

Hemophilic carriers have an average level of factor VIII coagulant activity of approximately 50% of normal. However, because of the wide overlap between carriers and normal females, only about 25 to 55% of carriers can be identified by this measurement alone (6, 20).

In 1971 Zimmerman et al (26) demonstrated that the level of factor VIII-related antigen is decreased in patients with von Willebrand's disease but normal in hemophiliacs. It was found that the ratio of factor VIII coagulant activity to factor VIII-related antigen in hemophilic carriers is significantly lowered (27). By comparing factor VIII coagulant activity to factor VIII-related antigen, the sensitivity of detecting hemophilic carriers has been increased to 70 to 95% (4, 5, 6, 7, 27). Our results reported here are similar to those of other investigators.

Howard and Firkin (12) observed that ristocetin caused platelet aggregation in platelet-rich plasma from normal persons but not from patients with von Willebrand's disease. In 1973 Weiss and his associates (25) demonstrated that the ristocetin cofactor activity in the plasma can be quantitated by using washed normal platelets. They found that the level of ristocetin cofactor activity generally parallels the level of factor VIII-related antigen in normal subjects and in patients with hemophilia and von

TABLE I

CARRIER VS NORMAL DISCRIMINATION ANALYSIS BY FACTOR VIII ACTIVITY AND FACTOR VIII-RELATED ANTIGEN

Case	Classification	Factor VIII coagulant activity μ/ml	Factor VIII related antigen μ/ml	Posterior Probability for normal group	Posterior Probability for carrier group
Normal					
1	normal	1.09	1.15	0.971	0.029
2	normal	1.32	1.51	0.942	0.058
3	normal	1.32	1.44	0.961	0.039
4	normal	1.00	0.91	0.992	0.008
5	normal	1.12	1.10	0.984	0.016
6	normal	0.74	0.60	0.997	0.003
7	normal	0.72	0.58	0.997	0.003
8	normal	1.42	0.88	1.000	0.000
9	normal	0.92	0.91	0.983	0.017
10	normal	1.89	1.66	0.995	0.005
11	normal	1.22	0.94	0.998	0.002
12	normal	1.24	1.26	0.979	0.021
13	normal	0.88	1.00	0.941	0.059
14	normal	1.42	1.73	0.901	0.099
15	normal	0.88	1.26	0.663	0.337
16	normal	0.96	1.05	0.965	0.035
17	normal	1.36	1.05	0.998	0.002
18	normal	1.09	1.49	0.780	0.222
19	normal	1.44	1.75	0.903	0.097
20	normal	1.35	1.83	0.776	0.224
21	normal	1.13	1.26	0.953	0.047
22	normal	1.51	1.30	0.998	0.002
23	normal	1.31	1.40	0.968	0.032
24	normal	1.70	1.23	0.999	0.001
25	normal	1.05	0.89	0.996	0.004
26	normal	0.95	1.25	0.812	0.188
27	normal	0.60	0.87	0.622	0.378
28	normal	0.74	1.08	0.619	0.381
29	normal	1.04	1.11	0.967	0.033
30	normal	0.80	0.62	0.998	0.002
Carrier					
31	carrier	1.21	2.15	0.227	0.773
32	carrier	0.93	2.80	0.002	0.998
33	carrier	0.57	0.92	0.384	0.616
34	carrier	0.40	1.15	0.003	0.997
35	normal	0.95	1.22	0.845	0.155
36	carrier	0.57	1.10	0.110	0.890
37	carrier	0.70	1.44	0.068	0.932
38	carrier	0.62	1.40	0.029	0.971
39	normal	1.18	1.63	0.742	0.258
40	carrier	0.95	3.30	0.001	0.999
41	carrier	0.55	1.21	0.037	0.963
42	carrier	0.25	1.61	0.000	1.000
43	carrier	1.03	1.74	0.411	0.589
44	carrier	0.58	1.54	0.007	0.993
45	carrier	0.27	1.13	0.000	1.000
46	carrier	0.39	1.03	0.007	0.993
47	carrier	0.37	1.07	0.003	0.997
48	carrier	0.63	1.22	0.110	0.890
49	carrier	0.56	1.17	0.056	0.944
50	carrier	1.01	2.29	0.029	0.971

TABLE II

CARRIER VS NORMAL DISCRIMINATION ANALYSIS BY FACTOR VIII ACTIVITY AND RISTOCETIN COFACTOR ACTIVITY

Case	Classification	Factor VIII coagulant activity μ/ml	Factor VIII related antigen μ/ml	Posterior Probability for normal group	Posterior Probability for carrier group
Normal					
1	normal	1.09	1.15	0.847	0.153
2	normal	1.32	1.42	0.859	0.141
3	normal	1.52	1.20	0.939	0.061
4	normal	1.00	0.77	0.967	0.033
5	normal	1.12	0.64	0.994	0.006
6	normal	0.74	0.72	0.852	0.148
7	normal	0.72	0.78	0.751	0.249
8	normal	1.42	0.96	0.989	0.011
9	normal	0.92	0.73	0.958	0.042
10	normal	1.59	1.30	0.991	0.009
11	normal	1.22	0.94	0.973	0.027
12	normal	1.24	1.09	0.946	0.054
13	normal	0.88	1.16	0.558	0.442
14	normal	1.42	1.68	0.795	0.205
15	normal	0.88	0.85	0.877	0.123
16	normal	0.96	1.09	0.762	0.238
17	normal	1.36	1.56	0.817	0.183
18	normal	1.09	1.17	0.833	0.167
19	normal	1.44	2.02	0.604	0.396
20	normal	1.35	1.47	0.854	0.146
21	normal	1.13	0.97	0.948	0.052
22	normal	1.51	1.11	0.983	0.017
23	normal	1.31	1.16	0.947	0.053
24	normal	1.70	1.29	0.982	0.018
25	normal	1.05	1.06	0.872	0.128
26	normal	0.95	1.05	0.787	0.213
27	normal	0.60	0.66	0.693	0.307
28	carrier	0.74	1.06	0.397	0.603
29	normal	1.04	0.88	0.948	0.052
30	normal	0.80	0.66	0.939	0.061
Carrier					
31	carrier	1.21	2.00	0.337	0.663
32	carrier	0.93	2.48	0.026	0.974
33	carrier	0.57	1.39	0.029	0.971
34	carrier	0.40	1.15	0.007	0.993
35	carrier	0.95	1.44	0.388	0.612
36	normal	0.57	0.68	0.577	0.423
37	carrier	0.70	1.84	0.021	0.979
38	carrier	0.62	1.11	0.135	0.865
39	normal	1.18	1.17	0.895	0.105
40	carrier	0.95	1.53	0.310	0.690
41	carrier	0.55	1.25	0.035	0.965
42	carrier	0.25	1.29	0.000	1.000
43	normal	1.08	0.82	0.971	0.029
44	normal	0.58	0.73	0.508	0.492
45	carrier	0.27	1.00	0.001	0.999
46	carrier	0.39	0.72	0.074	0.926
47	carrier	0.37	1.15	0.004	0.996
48	carrier	0.63	1.23	0.090	0.910
49	carrier	0.56	1.31	0.030	0.970
50	normal	1.01	1.40	0.525	0.475

TABLE III

CARRIER VS NORMAL DISCRIMINATION ANALYSIS BY FACTOR VIII ACTIVITY, ANTIGEN AND RISTOCETIN COFACTOR ACTIVITY

Case	Classification	Posterior Probability for normal group	Posterior Probability for carrier group
Normal			
1	normal	0.969	0.031
2	normal	0.934	0.066
3	normal	0.965	0.035
4	normal	0.995	0.005
5	normal	0.994	0.006
6	normal	0.997	0.003
7	normal	0.997	0.003
8	normal	1.000	0.000
9	normal	0.989	0.011
10	normal	0.996	0.004
11	normal	0.999	0.001
12	normal	0.982	0.018
13	normal	0.925	0.075
14	normal	0.872	0.128
15	normal	0.767	0.233
16	normal	0.961	0.039
17	normal	0.997	0.003
18	normal	0.800	0.200
19	normal	0.835	0.165
20	normal	0.773	0.227
21	normal	0.966	0.034
22	normal	0.998	0.002
23	normal	0.972	0.028
24	normal	0.999	0.001
25	normal	0.995	0.005
26	normal	0.833	0.167
27	normal	0.735	0.265
28	normal	0.601	0.399
29	normal	0.977	0.023
30	normal	0.999	0.001
Carrier			
31	carrier	0.157	0.843
32	carrier	0.001	0.999
33	carrier	0.231	0.769
34	carrier	0.002	0.998
35	normal	0.777	0.223
36	carrier	0.195	0.805
37	carrier	0.031	0.969
38	carrier	0.030	0.970
39	normal	0.787	0.213
40	carrier	0.001	0.999
41	carrier	0.026	0.974
42	carrier	0.000	1.000
43	normal	0.637	0.363
44	carrier	0.015	0.985
45	carrier	0.000	1.000
46	carrier	0.010	0.990
47	carrier	0.002	0.998
48	carrier	0.086	0.914
49	carrier	0.036	0.964
50	carrier	0.036	0.964

Willebrand's disease. It is reasonable to suspect that the simultaneous measurement of ristocetin cofactor activity and factor VIII coagulant activity might also enhance the detection rate for hemophilic carriers. Indeed, this is the case. In the current study 75% of carriers were accurately predicted by comparing factor VIII coagulant activity to ristocetin cofactor activity. We conclude that comparison of factor VIII coagulant activity with ristocetin cofactor activity can be used as an alternate or adjunctive method for detection of hemophilic carriers.

Both arithmetic and logarithmic values of factor VIII coagulant activity and factor VIII-related antigen have been used for statistical analysis. We, as well as others (6, 21), have observed that logarithmic values of these parameters for normal controls give a better fit to a symmetric distribution. This probably arises from the fact that the levels of these biological parameters are usually obtained by reading against time (for factor VIII coagulant activity), migration distance (for factor VIII-related antigen), or slope (for ristocetin cofactor activity) on double log paper. Therefore, it is most logical to use logarithmic rather than arithmetic values of these three parameters for statistical analyses.

Several statistical methods have been used for analyzing factor VIII coagulant activity and factor VIII-related antigen to discriminate carriers from normals. These include simple confidence region of the arithmetic ratio (4, 6, 22), confidence interval around the regression line (1, 23, 27), linear discriminant function analysis (7), and elliptical tolerance region (6). With the exception of the last two, these methods fail to treat carriers as a group contrary to the normals. Furthermore, these above mentioned methods can provide an "all or none" statement only. From a logical point of view, a probability rather than an "all or none" statement should be offered to a person seeking genetic counseling, especially if the laboratory test results are borderline.

Bouma and his associates (6) were the first to use elliptical tolerance confidence regions for both normal and carrier groups. Using the same methods for statistical analysis of factor VIII coagulant activity and factor VIII-related antigen, we obtained 85% of carriers outside the 95% tolerance confidence region for the normal group. But 73% of normals fell within the 95% tolerance region of the carrier group. Apparently our results demonstrated that there was a wide overlapping of these two tolerance confidence ellipses, especially when factor VIII coagulant activity and ristocetin cofactor activity were used for the calculations. From the tolerance ellipses alone, we were not as successful as Bouma in separating carriers from normals and were also unable to provide carrier versus normal probability based on the results of the tests. However, when these

data were subjected to discriminant analysis (10), a posterior probability of being normal or carrier for each case could be obtained from the squared Mahalanobis' distances from the means of both normal and carrier groups. According to this method, 90% of carriers were correctly classified when factor VIII coagulant activity and factor VIII-related antigen were used; 75% of carriers were accurately predicted when factor VIII coagulant activity and ristocetin cofactor activity were used; and 85% of carriers and 94% of all subjects were correctly predicted when all three parameters were used in the computation simultaneously.

Most of the multivarite techniques including regression and discriminate analyses estimate parameters which are optimal for the data actually observed in the sample. When the results of these analyses are applied to new data, the prediction rate will be somewhat poorer than with the original sample.

Other than technical and statistical problems there are several factors which might affect the accurate detection of hemophilic carriers. First, it was observed by Rizza et al (22) that carriers with higher levels of factor VIII coagulant activity and factor VIII-related antigen are difficult to differentiate from normals. Our data show the same phenomenon since the two regression lines of normal and carrier groups crossed each other at higher levels of factor VIII. Conditions which elevate factor VIII levels, such as exercise, estrogens, pregnancy and adrenalin infusion, might make the discrimination between normals and carriers more difficult. Second, as in other sex-linked inherited diseases (2, 17), the random inactivation of either the maternal or paternal X-chromosome early in embryonic life produces a mosaic resulting in considerable variability of factor VIII activity in carriers. This phenomenon, called "lyonization" may explain the variability of ratios between factor VIII coagulant activity and factor VIII-related antigen in carriers. Third, there is increasing evidence that ristocetin cofactor activity and factor VIII-related antigen determined with rabbit antiserum are more directly related to the primary gene product of the autosomal von Willebrand locus than to the sex-linked locus associated with factor VIII coagulant activity. It has been demonstrated that factor VIII can be dissociated into two components, a high molecular weight component containing ristocetin cofactor activity and factor VIII-related antigen devoid of factor VIII coagulant activity and a low molecular weight component containing only factor VIII coagulant activity (18, 24). Though these factor VIII related properties are closely related, the comparison of two biological properties on separate polypeptides from two separate genes would not be theoretically as sound as their being on the same polypeptide and therefore controlled by a single gene. Finally, other than hemophiliacs and hemophilic carriers, a decreased

ratio of factor VIII coagulant activity and factor VIII-related antigen has been observed in conditions associated with intravascular coagulation and fibrinolysis (2, 11, 18), in which factor VIII coagulant activity is selectively inactivated and factor VIII-related antigen and ristocetin cofactor remain intact. Diseases such as disseminated carcinoma, leukemia, hemolytic anemia, thrombotic thrombocytopenic purpura, pulmonary embolism, severe burns, liver disease, and myocardial infarction, have been shown to have a significantly decreased ratio between factor VIII coagulant activity and factor VIII-related antigen. Accordingly, when carrier detection is to be studied, persons with possible intravascular coagulation or fibrinolysis should be excluded.

F.N.: While preparing this manuscript, comparable results were published by H.M. Reissner et al in Brit J Haemat 40: 339, 1978.

RESUMEN

Predicción de Portadoras de Hemofilia. Un nuevo enfoque estadístico, utilizando la determinación simultánea, de la actividad coagulante del factor VIII, el antígeno asociado y el cofactor ristocetina. *Chan-Yet Lian E. (Department of Medicine, Veterans Administration Hospital, 102 N.W. 16th Street, Miami, Florida 33125, U.S.A.), Diez-Ewald M., Walter S.D., Nuñez R., Jean-Fern L., and Harkness, D.R.* **Invest Clín** *20(3): 162-177, 1979.*— Se utilizó el análisis discriminante multivariado en un grupo de mujeres, para calcular la posibilidad futura de ser normales o portadoras de hemofilia. Cuando se utilizaron la actividad coagulante del factor VIII y el antígeno asociado a este factor, todas las mujeres normales (30) y 18 de 20 portadoras obligatorias, se clasificaron correctamente. Cuando en lugar del antígeno, se utilizó la actividad del cofactor ristocetina se hizo una predicción correcta en el 97% de las mujeres normales y en el 75% de las portadoras obligatorias. Al tomar en consideración los tres parámetros en análisis discriminante, se clasificaron correctamente todas las mujeres normales y 17 de las 20 portadoras obligatorias. Concluímos en que se puede utilizar la determinación conjunta de la actividad coagulante del factor VIII y la del cofactor ristocetina, como un método alterno o adjunto para detectar las portadoras de hemofilia.

REFERENCES

1- BENNETT B, RATNOFF OD: Detection of the carrier state for classic hemophilia. N Engl J Med 288: 342-345, 1973.

2- BENNETT B, OXNARD SC, DOUGLAS AS, RATNOFF OD: Studies on antihemophilic factor (AHF), (factor VIII) during labor in

normal women, in patients with premature separation of the placenta and in a patient with von Willebrand's disease. J Lab Clin Med 84: 851-860, 1974.

3- BENTLER E, YEH M, FAIRBANKS VF: The normal female as a mosaic of X-chromosome activity: Studies using the gene for G-6-PD as a marker. Proc Natl Acad Sci 48: 9-16, 1962.

4- BIGGS R, RIZZA CR: The sporadic case of hemophilia A. Lancet 2: 431-433, 1976.

5- BOUMA BN, VAN DER KLAAUW MM, VELTKAMP JJ, STARKENBURG AE, VAN TILBURG NH, HERMANS J: Evaluation of the detection rate of hemophilic carriers. Throm Res 7: 339-350, 1975.

6- EKERT H, HELLIGER H, MUNTZ RH: Detection of carriers of hemophilia. Thromb Diath Haemorrh 30: 255-262, 1976.

7- EYSTER ME, JONES MB, MOORE T, DELLI-BOVI L: Carrier detection in classic hemophilia by combined measurement of immunologic (VIII AGN) and procoagulant (VIII AHF) activities. Am J Clin Pathol 65: 975-981, 1976.

8- GOMPERTS ED, WHITBREAD P, FEESEY M: Factor VIII-related antigen in the detection of the haemophilia carrier state. S Afr Med J 49: 1005-1007, 1975.

9- GUTTMAN I: Statistical tolerance regions. McGriffin (London), 1970.

10- Health Sciences Computing Facility, Department of Biomathematics, School of Medicine, University of California, Los Angeles, Calif., Biomedical Computer Program, University of California Press, 1975.

11- HOLMBERG L, NILSSON IM: AHF related protein in clinical praxis. Scand J Haematol 12: 221-231, 1974.

12- HOWARD MA, FIRKIN BCT: Ristocetin – A new tool in the investigation of platelet aggregation. Thromb Diath Haemorrh 26: 362-369, 1972.

13- LAURELL CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15: 45-52, 1966.

14- LIAN EC-Y, DEYKIN D: In vivo dissociation of factor VIII (AHF) activity and factor VIII-related antigen in von Willebrand's disease. Am J Hematol 1: 71-78, 1976.

15- LIAN EC-Y, NUÑEZ RL, HARKNESS DR: In vivo and in vitro effects of thrombin and plasmin on human factor VIII (AHF). Am J Hematol 1: 481-491, 1976.

16- LIAN EC-Y, DEYKIN D: Diagnosis of von Willebrand's disease. A comparative study of diagnostic tests on 9 families with von Willebrand's disease and its differential diagnosis from hemophilia and thrombocytopathy. Am J Med 60: 344-356, 1976.

17- LYON ML: Gene action in the X-chromosome of the mouse. Nature 190: 372-373, 1961.

18- OWEN WG, WAGNER RH: Antihemophilic factor: separation of an active fragment following dissociation by salts or detergents. Thromb Diath Haemorrh 27: 502-515, 1972.

19- PITNEY WR, ARNOLD BJ: Plasma antihaemophilic factor (AHF) concentrations in families of patients with hemorrhagic states. Br J Haematol 5: 184-193, 1959.

20- RAPAPORT SI, PATCH MJ, MOORE FJ: Antihemophilic globulin levels in carriers of hemophilia A. J Clin Invest 39: 1619-1625, 1960.

21- RAPAPORT SI, SCHIFFMAN S, PATCH MJ, WARE AG: A simple specific one stage assay for plasma thromboplastin antecedent (PTA) deficiency. J Lab Clin Med 51: 771-780, 1961.

22- RIZZA CR, RHYMES IL, AUSTEN DFG, KERNOFF PBA, ARONI SA: Detection of carriers of haemophilia: a blind study. Br J Haematol 30: 447-456, 1975.

23- THOMOPOULOS D, SCLIROS P, LYBERATOS C: Detection of carriers of hemophilia A. Acta Haematol 54: 32-35, 1975.

24- WEISS HJ, HOYER LW: Von Willebrand's factor: dissociation from antihemophilic factor procoagulant activity. Science 182: 1149-1151, 1972.

25- WEISS HJ, HOYER LW, RICKLES FR, VARMA A, ROGERS J: Quantitative assay of a plasma factor deficient in von Willebrand's disease that is necessary for platelet aggregation. Relationship to factor VIII procoagulant activity and antigen content. J Clin Invest 52: 2708-2716, 1973.

26- ZIMMERMAN TS, RATNOFF OD, POWELL AE: Immunologic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand's disease. J Clin Invest 50: 244-254, 1971.

27- ZIMMERMAN TS, RATNOFF OD, LITTLE AS: Detection of carriers of classic hemophilia using an immunologic assay for anti-hemophilic factor (Factor VIII). J Clin Invest 50: 255-258, 1971.

28- ZIMMERMAN TS, HOYER LW, DICKSON L, EDGINGTON TS: Determination of the von Willebrand's disease antigen (factor VIII-related antigen) in plasma by quantitative immunoelectrophoresis. J Lab Clin Med 86: 152-159, 1975.

Invest Clin 20(3): 178-187, 1979

DEVELOPMENT OF AVIAN LIVER LIPOGENIC ENZYMES DURING THE PERINATAL PERIOD. MINIREVIEW.

Elena Ryder and Gilberto Campos*

Contrary to the mammalian embryo, lipid synthesis is minimal in the liver of the chick embryo. During this period, the animal is consuming a fatty diet from where it derives its energy in the last part of its embryonic life. It is understandable that there is no necessity to elaborate these enzymes, since the embryo environment is rich in lipids. After hatching, a high carbohydrate-low fat diet is installed and the animal has to adjust to this new nutritional state developing the hepatic lipogenic activity with a concomitant coordinate increase of the enzymes involved in this pathway.

It has been shown (5, 7) that the activity of **citrate lyase** (CL) increases slightly but significantly during the hatching period and the starvation period following hatching. After 24 h of feeding, the values are elevated 4.5 fold reaching the maximum activity 5-6 days after feeding. The **fatty acid synthetase** (FAS) activity, as found by Joshi and Sidbury (14) in the newly hatched chicks, goes up even if the animal is starved. After feeding, there is an additional increase. The maximum activity is found at 2-4 days, going down to adult values in ten days. **Malic enzyme** (ME) behaves like the other two enzymes (7), however there is no increase in activity before feeding is established. We showed (24) at the same time as did Arinze and Mistry (1), that the **acetyl CoA carboxylase** (ACCx) activity is very low during the embryonic period. During the hours after hatching there is a rise and with the onset of feeding the activity increases 8-10 times, peaking at 15 days. This enzyme is activated by citrate, and at any point there was a citrate requirement (5mM) for maximal activity (26). It seems then

* *Instituto de Investigaciones Clínicas. Facultad de Medicina. Universidad del Zulia. Apartado 1151. Maracaibo, Venezuela.*
This paper was presented at the Symposium "Regulation of Enzyme Activity". 2nd. PAABS Meeting. Caracas, Venezuela. September 3-9, 1978.