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AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR FACTOR VIII INHIBITORS. C.H. Miller, M.W. Hilgartner, and D.R. Lopez. Division of Pediatric Hematology/Oncology, New York Hospital-Cornell University Medical Center, New York, N.Y. U.S.A.

ELISA microtechniques are suitable for measurement of a variety of antigens and antibodies. They are sensitive, safe, and easy to perform. Enzyme-labeled antibodies to factor VIII and factor IX can be used to measure antigen in a "sandwich" assay analogous to the immunoradiometric assay (IRMA). Likewise, specific antibodies can be easily quantitated in plasma samples. Quantitation of F. IX inhibitors by ELISA has been performed by Orstavik and colleagues. We have studied use of the ELISA system for measurement of inhibitors to factor VIII. Purified factor VIII prepared from concentrate is bound to plastic microtiter plates, which are then incubated with plasma dilutions. After washing to remove non-bound material, heterologous anti-human IgG labeled with alkaline phosphatase is added. It binds to any hyman IgG remaining on the plate. Following incubation and washing, the enzyme substrate, p-nitrophenyl phosphate, is added. Degradation of the substrate produces a color change which is read spectrophotometrically. A "through-the-plate" reader allows rapid OD measurements without transfer of the well contents. Ten or more samples in dilution series can be tested simultaneously. The inhibitor titer may be quantitated by comparison with a standard inhibitor measured in Bethesda units by a clotting assay or by direct comparison with the OD curve produced by known quantities of human IgG. Twelve known inhibitors from hemophilia patients and two acquired non-hemophilic inhibitors showed good correlation with the Bethesda unit method. Five normal individuals and five hemophiliacs with no history of inhibitor by clotting assay showed no anti-IX inhibitor by this test.

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BLOOD GROUP (BG) PREVALENCE IN END-STAGE (ES) RENAL DISEASE (RD): RELATIONSHIP TO RISTOCETIN WILLEBRAND FACTOR (VIII: RWF) AND PLATELET SURVIVAL (PS). V. Torres, V. Fuster, B. Moore, J. Donadio, F. Kazmier, E. Bowie. Mayo Clinic and Foundation, Rochester, Minnesota, U.S.A.

BG O patients (pts) have lower risk for cardiovascular-thromboembolic disease than A-AB-B pts, particularly males. This might be related to blood hypocoagulability (lower Factor VIII). We report BG frequencies in our Caucasian renal transplant population divided in two groups, ES reflux nephropathy (RN) and ESRD of other etiologies excluding diabetes mellitus.

BG	ESRN			ESRD			Control		
	M+F	M	F	M+F	M	F	M+F	M	F
	61	25	36	335	220	115	1905	882	1023
O	28*	16**	36	41	37*	47	43	45	41
A+B+AB	72*	84**	64	59	63*	53	57	55	59

Percents; vs control, * $p<0.05$, ** $p<0.01$

BG O male pts are protected, especially for ESRN. To determine whether this sex dependency could be related to different VIII:RWF levels or PS, VIII:RWF levels (n=82) and ^{51}Cr-labelled PS (n=74) were analyzed in controls according to sex and BG.

BG	VIII:RWF (%)			Platelet (^{51}Cr) half-life (hrs)		
	M+F	M	F	M+F	M	F
O	99±4	93±7	101±6	98±3	92±3	104±5†
A+B+AB	121±5*	121±7*	123±6*	99±2	94±3	106±3††

Mean ± SEM; * vs O, $p<0.02$; †,†† vs M, $p<0.05$, $p<0.01$

VIII:RWF is sex independent and lower in BG O. PS is BG independent and shortened in males. A hypercoagulable state reflected by high VIII:RWF and short PS might explain the increased risk of non-BG O males to develop ESRD. Non-BG O male pts with RN may be more susceptible to develop progressive RD.

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RISTOCETIN INDUCED PLATELET FACTOR 3 AVAILABILITY, A POSSIBLE NEW TOOL IN THE DIAGNOSIS OF VON WILLEBRAND'S DISEASE. L. Muszbek, H. Losonczy*, J. Hársfalvi and I. Nagy*. Department of Clinical Chemistry, University School of Medicine, Debrecen, Hungary and *I. Department of Medicine, University School of Medicine, Pécs, Hungary.

Ristocetin induced platelet aggregation (RIPA) is a highly valuable technique in the laboratory diagnosis of von Willebrand's disease (vWD) and together with the estimation of bleeding time, F VIII procoagulant activity and F VIII related antigen in most cases it makes also division of vWD into phenotypic subgroups possible. We have shown that ristocetin makes platelet factor 3 available and ristocetin induced platelet factor 3 availability (RIPF3) similarly to RIPA also depends on a plasma factor. In the present paper it was examined if RIPF3 can be applied as a laboratory test in the diagnosis of vWD. PRP from normal controls, patients with various release defects and von Willebrand patients was incubated and continuously stirred with ristocetin or phys. saline and after 20 min the RVV clotting times were determined. RIPF3 activity was expressed as the ratio of the clotting times obtained following incubation with phys. saline and ristocetin.

In healthy controls and patients with release defect (n=21) this ratio was higher than 1.5, and in most cases between 1.8-2.5. In patients with vWD (n=10) the ratio was well below 1.5 (with one exception it ranged between 0.95-1.2). In 9 of the 10 patients the absence or strong diminution of RIPA and RIPF3 showed close paralellism. In one patient only the slope of first vawe of ristocetin aggregation was decreased while at the same time an almost total inhibition of RIPF3 could be observed. The results indicate that RIPF3 is a valuable quantitative diagnostic test in von Willebrand disease. It can be used if expensive aggregometer is not available and it may also have an importance as additional test to RIPA in the division of vWD into subgroups. An attempt was made to develop this test further by using chromogenic substrate for the measurement of RIPF3.

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THE BINDING OF ^{125}I-von WILLEBRAND FACTOR TO HUMAN PLATELETS IN THE PRESENCE OF RISTOCETIN. P. Silber and T.H. Finlay. Department of Obstetrics and Gynecology, N.Y.U. Medical Center, New York, N.Y. 10016.

The effect of ristocetin on the binding of ^{125}I-porcine von Willebrand factor to human platelets was studied. Previously, we had shown that ^{125}I-porcine von Willebrand factor binds to human platelets in the absence of ristocetin. The present work demonstrates that binding is stimulated by ristocetin and this stimulation is maximal at a ristocetin concentration of 2 mg/ml. At a ristocetin concentration of 0.5 mg/ml, Scatchard analysis indicates a binding constant of 5.13×10^{-9}M and the presence of 105,000 binding sites. This compares with our previous finding, in the absence of ristocetin, of a binding constant of 2.92×10^{-7}M and 4760 binding sites. These binding data assume the porcine von Willebrand factor to be a tetramer with a molecular weight of 9×10^5. This study indicates that ristocetin causes tighter binding and increases the number of binding sites on human platelets for porcine von Willebrand factor. Unlabelled porcine von Willebrand factor competitively inhibits the specific binding of the labelled protein and gives a binding constant of 0.17×10^{-9}M. Similar results were obtained using human von Willebrand factor.

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