

661 THE ASPIRIN (ASA) BLEEDING TIME (BT) - A SCREENING TEST FOR EVALUATION OF BLEEDING DISORDERS. Marie J. Stuart, Merrill Miller, Joel Wolk & Frederick Davey.

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 In an attempt to elucidate the usefulness of the BT post ASA ingestion, this test was done with other tests of coagulation in 30 controls and 77 patients evaluated for a possible bleeding diathesis. Coagulation studies included PT, PTT, TT, VIII AHF & AGN, IX, XI, XIII, platelet retention and aggregation, and modified Ivy BTs pre and 2 hrs. post 600 mgms ASA. The mean control BT in 25 normals was $3.6' \pm 3.2'$ (3 SD). Following ASA the BT was $6.4' \pm 4.1'$ (3 SD). 5/30 "controls" without bleeding histories had abnormal BTs post ASA. In this group, 4 were proven to have unrecognized von Willebrand's disease (VWD) and one a platelet defect. Of the 77 patients studied, 23 had initial BTs that were abnormal (21 with VWD and 2 with platelet defects). Of the remaining 54 with initially normal BTs, 32 had abnormal BTs post ASA. 18/32 had VWD at their first evaluation. In 8/32, however, the abnormal BT post ASA was the only abnormality at initial evaluation. On repeated testing, these patients were also shown to have VWD. 6/32 with abnormal BTs post ASA were found to have platelet abnormalities. The remaining 22 patients had normal BTs pre and post ASA. 17/22 revealed no hemostatic abnormality. In 5 eventually proven to have VWD, the BT post ASA was normal. The use of the BT post ASA raised the sensitivity of the BT as a screening test from 35% to 92% in the abnormal patient population. We conclude that the BT post ASA is a valuable screen in the evaluation of possible hemostatic defects.

662 IMPROVED DETECTION OF β -THALASSEMIA HETEROZYGOES USING TWO TESTS TOGETHER. M. Jammis-Hadjopoulos, R.J.M. Gold, U.R. Maag, J.D. Metrakos and C.R. Scriver.

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Seven hematological parameters were examined singly and in their 21 possible pairs for their ability to discriminate between carriers of the β -thalassemia allele and normal homozygotes. When quadratic discriminants were constructed for each pair of parameters, % HbA₂ and MCV paired were found to yield the least classification error for carriers and for normal homozygotes. The "cost" of misclassification with this same pair is 1/30 of that with the best single test available. Furthermore, the discrimination achieved with the % HbA₂-MCV pair is of such high order that it can also be used to discriminate the genotype of sibs of homozygous probands whose prior probability of heterozygosity (0.67) would otherwise make classification less reliable. We surveyed 260 subjects of the Montreal Greek community; the apparent carrier frequency for β -thalassemia is 0.067. Surveys of knowledge and of attitudes toward genetic screening for β -thalassemia are also in progress, to guide initiation of mass screening, if it is wanted, in our community when facilities for prenatal diagnosis of thalassemia become available.

663 ERYTHROPOIETIC COLONY FORMING UNITS (CFU-E) IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). Kamran Tebbi, Samuel Gross.

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The extent of leukemic marrow (BM) involvement in children with ALL is often unrelated to the degree of anemia. This serial study was carried out to correlate marrow CFU-E with stages of disease. BMs from 35 normal children served as controls. In 29 ALL patients 64 cultures were done; 14 newly diagnosed and untreated; 32 in remission; 18 in relapse. BM cells were cultured with and without erythropoietin (EPO) and included EPO dose response curve. CFU-E at diagnosis was significantly lower than controls, and despite complete remission, values did not reach the normal range. The mean value of cultures obtained during remission (166 ± 47 S.E./ 10^6 nucleated cells) was markedly higher than the newly diagnosed (17 ± 8 S.E.) or relapse patients (25 ± 13 S.E., $p < 0.005$) but significantly lower than normals (326 ± 44 S.E., $p < 0.01$). An inverse correlation was found between CFU-E levels and % blasts. Also CFU-E did not appear to have predictive value in regard to impending relapse. The data indicate that the leukemic process in ALL involves the pluripotent stem cells and results in decreased numbers of early progenitors capable of differentiation to the erythrocytic series. Although achievement of morphological remission improves the flow rate to this pathway, unlike reported studies of granulocytic colonies in culture, it does not result in complete restoration of a normal erythroid pattern.

664 ENHANCING EFFECT OF RABBIT ANTI-MOUSE EOSINOPHILIC SERUM (AES) ON GRANULOPHOIETIC COLONY FORMING UNITS IN CULTURE (CFU-C). Kamran Tebbi, Adel A.F. Mahmoud, Samuel Gross.

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Although eosinophilia is reported in parasitic and other infections the total leukocyte count frequently is not elevated. This suggests a possible inhibitory effect of eosinophiles on granulopoiesis. This study was undertaken to examine the effects of monospecific rabbit anti-mouse anti-eosinophilic sera (AES) on the colony forming ability of *Trichinella spiralis* infected (30% marrow eos) and normal (2% marrow eos) mice. Marrow from inbred C₅₇ Black mice infected with *Trichinella spiralis* was cultured in semi-solid media with 1) Human Colony Stimulating Activity (CSA), 2) CSA+AES, 3) CSA+normal rabbit serum (RS), 4) AES alone, and 5) α -media alone. Identical experiments were repeated with normal mice. In infected mice, the number of CFU-C/10⁵ cells cultured with CSA was 72 and with RS, 52. With AES+CSA, the colony number rose to 157 and with AES+RS to 110 ($P < 0.001$ for both). The addition of AES alone or α -media alone produced < 5 colonies. These results are in keeping with the fact that mice, unlike the human, lack CSA producing cells in the marrow and thus require exogenous CSA to elicit the effects of AES. The CFU-C promoting effects of AES with CSA did not occur in normal mice. Examination of the colonies by light microscopy revealed neutrophilic series. It thus appears that the effective reduction in eosinophiles and the parallel increase in neutrophilic colonies indicates an inhibitory effect of eosinophiles in maintaining granulopoietic homeostasis.

665 ENHANCING EFFECT OF RABBIT ANTI-HUMAN ANTI-EOSINOPHILIC SERA (AES) ON GRANULOPHOIETIC COLONY FORMING UNITS IN CULTURE (CFU-C) OF PATIENTS WITH EOSINOPHILIA. Kamran Tebbi, Marjorie Krause, Steven Polmar, Samuel Gross.

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Agranulocytosis with eosinophilia is known to occur. We examined the *in vitro* effects of AES on bone marrow (BM) cultures of 2 patients with eosinophilia (Immune deficiency, Felty's syndrome - 35% and 60% eosinophilia, respectively) in combination with neutropenia. BM's were cultured in a methyl cellulose system using combinations of human colony stimulating activity (CSA), normal rabbit serum (RS), patient's sera (PS), AES, and α -media (α). Following are the results reported as CFU-C/10⁵ cells.

	AES	RS	NS	α	RS				
Patient A	36	66	28	40	7	52	13	4	18
Patient B	21	119	18	24	5	32	32	8	12
Normal	50	58	54	48	40	42	40	38	43

The results indicate that the addition of AES to CSA enhances the colony forming ability of eosinophilic patients but has no effect on normal controls. In another experiment, the addition of AES to non-adherent marrow cells (lacking CSA) failed to produce colonies, indicating the CSA requirements for the AES "enhancing" effect. The significant increase in CFU-C, promoted by AES, in BM cultures of these patients supports the likelihood of an eosinophile-inhibiting effect on neutrophile production. This suggests a role for eosinophiles in maintaining granulopoietic homeostasis.

666 PROBLEMS WITH LEAD (Pb) SCREENING IN SICKLE CELL DISEASE (SSD). Linda Tigner-Weekes, Charles H. Pegelow, Darleen R. Powers.

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Serious Pb toxicity in 4 children with sickle cell anemia (SS) with blood Pb $< 80 \mu\text{g/dl}$ prompted a search for asymptomatic Pb burden in a group of 30 children, age 3-18, with SSD (27 SS, 3 SC). Blood Pb and whole blood free erythrocyte protoporphyrin (FEP) were determined and the patients divided into 4 major Pb poisoning classes by correlating the results. (J. Pediatr. 87:824) 21 patients fit the criteria for class I, 6 for Ia and 1 for class IV. Two children with Pb levels $< 29 \mu\text{g/dl}$ but FEP values $> 190 \mu\text{g/dl}$ do not fit the numerical classification. Pb/EDTA mobilization was done on 6 patients with FEP $> 60 \mu\text{g/dl}$ and blood lead $< 40 \mu\text{g/dl}$ who were demonstrated not iron deficient. Preliminary data shows abnormal Pb excretion in those children. In normal children blood Pb levels correlate with the FEP and clinical toxicity. However, in SSD an FEP $> 60 \mu\text{g/dl}$ can be associated with an increased Pb burden despite a blood Pb less than $40 \mu\text{g/dl}$.