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BLOOD COAGULATION STUDIES OF VARIOUS HEMATOLOGICAL DISORDERS

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ABSTRACT

Studies on blood coagulation were performed in the patients with hematopoietic disorders. Two hundred and twenty-three patients were investigated, consisting of 100 cases of leukemia, 36 of aplastic anemia, 7 of iron deficiency anemia and 80 of primary hemorrhagic diseases. The last consisted of 15 cases of purpura simplex, 32 of idiopathic thrombocytopenic purpura, 25 of hemophilia A and 8 of hemophilia B.

In each hemorrhagic disease there were found some differences among the characterisitic features of coagulation defects and also between hemorrhagic and non-hemorrhagic states.

In general platelet counts ranging from 30,000/cu. mm to 60,000/cu. mm could be considered to be the critical zone for manifesting hemorrhages in leukemia, aplastic anemia and idiopathic thrombocytopenic purpura. Functions of platelets were disturbed in some hemorrhagic diseases, and life span of platelets was shorten in many hematopoietic diseases. In studies on fibrinolysis there were noted increased activity of fibrinolysis in many cases, but no significant relationship was observed between increase of various factors of fibrinolytic activity and hemorrhagic manifestions.

There were performed analysis of the etiological factors that were classified into 5 elements of the blood vessel, platelet, clotting factors, anticoagulants and fibrinolysis. The combined abnormality of more than three elements were frequently seen in the bleeding states, than in the non-bleeding cases.

INTRODUCTION

There are many diseases which have hemorrhagic diathesis encountered in daily practice including primary and secondary hemorrhagic disorders. In this studies attention was drawn to the blood diseases and hematopoietic disorders in which hemorrhagic diathesis was the cardinal symptom or the important factor influencing the prognosis of the patient. Namely, the diseases mainly studied were leukemia, especially acute leukemia, aplastic anemia and primary hemorrhagic diseases that have been frequently seen in daily clinic.

MATERIALS

Two hundred and twenty-three patients were studied, consisting of 100

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cases of leukemia, 36 of aplastic anemia, 7 of iron deficiency anemia and 80 of primary hemorrhagic diseases. The last could be further classified into the following: 15 cases of purpura simplex, 32 of idiopathic thrombocytopenic purpura, 25 of hemophilia A and 8 of hemophilia B. All results indicated are those of tests made prior to commencing special therapeutic procedures.

METHODS OF EXAMINATION

(a) Blood was obtained between 11.00 a.m. and 12.00 noon by the two syringe method. As anticoagulant, 3.8% sodium citrated solution was used. To obtain citrated plasma siliconized glassware was used in all the procedures.

(b) The laboratory tests carried out were as follows;

1) Bleeding time: Duke's method.[1]

2) Vascular resistance;

i) Positive pressure method; Rumpel-Leede's method.

ii) Negative pressure method; von Borbély's method.

3) Whole blood clotting time: Lee-White's method.[2]

4) Recalcification time: (0.2 ml citrated platelet-rich plasma plus 0.2 ml 0.025 M $CaCl_2$ with 8×75 mm non-siliconized glass tube).

5) Thromboplastin generation test (TGT): Biggs-Douglas' method.[3]

6) Prothrombin time: Quick's method.[4]

7) Thrombin time: Lewis' method.

8) Fibrinogen: Ratnoff's method.[5]

9) Prothrombin in plasma: Alexander's method.[6]

10) Prothrombin in serum: Stefanini-Crosby's method.[7]

11) Factor V: Wolf's method.[8]

12) Factor VII+X: De Vries-Alexander's method.[9]

13) Factor VIII: Pitney's method.[10]

14) Factor IX: Fukui-Umegaki's method.[11]

15) Platelet count: Fonio's method.

16) Platelet aggregation: Anzai's method.[12]

17) Platelet adhesiveness: Tanaka's method.[13]

18) Platelet Factor 3: Biggs-Douglas' method.

19) Clot retraction: Macfarlane's method.[14]

20) Antithromboplastin: Suzuki's method.[15]

21) Antithrombin: Suzuki's method.[15]

22) Heparin like substances: Le Roy's method.

23) Fibrinolytic activity (diluted plasma, euglobulin fraction, streptokinase-activated euglobulin fraction, plasminogen activator and antiplasmin): Fibrin plate method.[16]

24) Life span of platelet: Aas-Gardner's method.[17]

RESULTS

a) General aspects of blood coagulation studies

In Fig. 1 are shown the coagulation findings of the hemorrhagic diathesis of the various blood diseases studied, the horizontal lines drawn for each of the items representing the means obtained from healthy persons, and the vertical bars in each of the columns indicating the means obtained in this studies for the respective diseases. However, when such means deviated from the normal range and were definitely abnormal, the bars are shown in white; and when the means were within normal range or though deviated somewhat from the normal range but not to the grade that appeared to hold pathologic significance, the bars are indicated in black; while the numerals in each of the columns indicate the actual values recorded. The last columns represent the normal means and the normal ranges based on the results obtained similarly.

It is clearly shown in Fig. 1 that the features of the hemorrhagic diathesis in acute leukemia, aplastic anemia and idiopathic thrombocytopenic purpura (ITP) resembled each other, showing that abnormality of platelets was the primary factor of hemorrhagic diathesis in all of three, evidenced by prolongation of bleeding time and depletion of clot retraction, platelet count, platelet-aggregation, platelet-adhesiveness and platelet-factor 3. Further, in connection

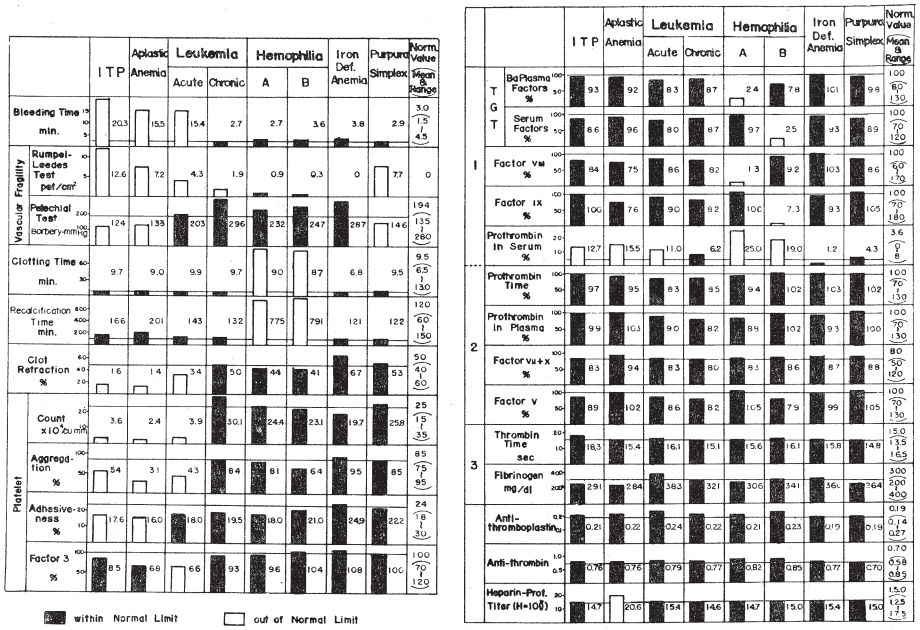

FIG. 1. Results of hemostatic tests in various hematological disorders (mean value).

with this there was recognized in common the impairment of prothrombin consumption. In addition, there was seen a marked increase of vascular fragility in ITP and aplatic anemia. In acute leukemia studies of the individual case indicated a fairly definite tendency for increase in vascular fragility, but in general was not so marked as in aplastic anemia and ITP. In aplastic anemia there was recognized an increase in heparin like-substances. And in 2 cases of this disease there was also seen an increase in anti-blood-thromboplastin.

b) Hemorrhagic and non-hemorrhagic states

The observation was made of the changes in the factors constituting the hemorrhagic diathesis, comparing the results obtained at the time of hemorrhagic and non-hemorrhagic states, of the respective disorders. In Fig. 2 the values of the non-hemorrhagic state are taken to be 100% (shown as white bars of the histogram), and those of the hemorrhagic state are compared as black bars. In ITP, aplastic anemia and acute leukemia there was noted prolongation of bleeding time, decreased platelet count, marked depletion of clot retraction and a tendency for decrease of platelet factor 3 in the hemorrhagic state when compared with the non-hemorrhagic. And there was also noted depletion of platelet aggregation in ITP and, moreover, in these three diseases factor VIII tended to decrease, in hemorrhagic states. In relation to the above mentioned changes, tendency for impairment of prothrombin consumption was found in these three diseases at hemorrhagic state.

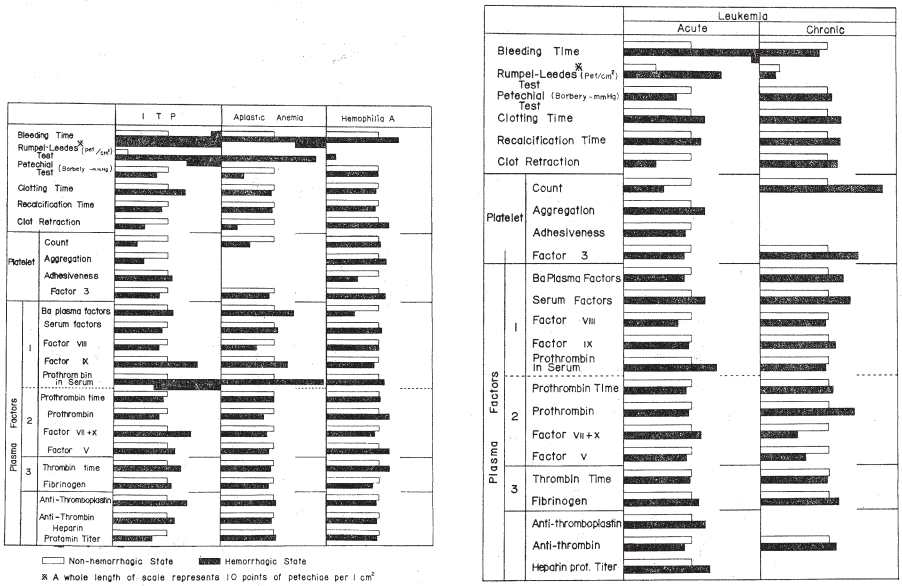

FIG. 2. Results of hemostatic tests in various hematological disorders in comparison of hemorrhagic and non-hemorrhagic state.

Tests of vascular resistance by the positive pressure method also showed enhancement of fragility in hemorrhagic state.

A comparison of the hemorrhagic state with the non-hemorrhagic state in chronic leukemia showed rather an increase of platelet count and decreases of factor V and factor VII+X in the former, but no other marked difference in general.

In hemophilia A the amount of factor VIII showed a decrease at both hemorrhagic and non-hemorrhagic states, but no further decrease in factor VIII was recognized during the hemorrhagic state when compared with the non-hemorrhagic. At the hemorrhagic state, however, a decrease was noted in the adsorbed plasma activity in thromboplastin generation test. It is, therefore, presumed that a decrease in factors other than factor VIII occurred.

c) Platelets

1) Platelet count: A study was made of platelet count in relation to hemorrhages in acute leukemia, aplastic anemia and ITP. In Fig. 3, the bars of the histogram shaded by oblique lines represented the number of hemorrhagic cases, the white unshaded bars the non-hemorrhagic cases, and the horizontal lines (abcissa) the platelet count.

From the ratio of hemorrhagic to non-hemorrhagic cases shown in Fig. 3, it appeared possible to classify these cases into three groups. Namely, there are a group with platelet count of "less than 30,000/cu. mm", a group with "more than 60,000/cu. mm", and a group with counts ranging from "30,000/cu. mm to 60,000/cu. mm" in cases of acute leukemia. Aplastic anemia was classifiable into three groups, one with "less than 20,000/cu. mm", one with "more than 70,000/cu. mm" and the third with ranging from "20,000/cu. mm

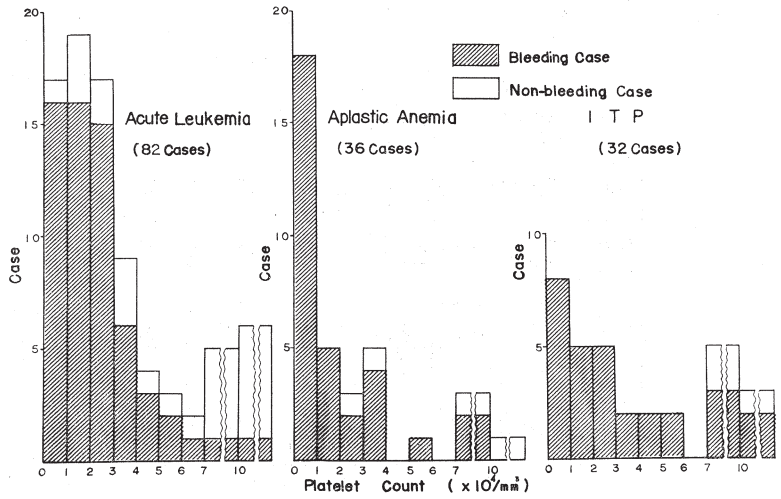

FIG. 3. Relation between platelet counts and hemorrhage.

to 70,000/cu. mm", while in case of ITP into two groups of "less than 70,000/cu. mm" and "more than 70,000/cu. mm".

Based on the results described above, it became possible to classify them into a group with platelet count of less than 20,000/cu. mm to 30,000/cu. mm associated with severe hemorrhage, a group with platelet count more than 60,000/cu. mm to 70,000/cu. mm associated with mild hemorrhage and an intermediate group with platelet count ranging from 30,000/cu. mm to 60,000/cu. mm. Hence a platelet count ranging from 30,000/cu. mm to 60,000/cu. mm can be considered to be the critical zone for transition from a non-hemorrhagic to a hemorrhagic state.

2) Function of platelets: In Fig. 4 are shown the results of functional tests of platelet in the hemorrhagic diseases. The areas shaded by oblique lines represent the normal range, while the white circles represent the values in the non-hemorrhagic state (non-bleeding cases), and the black circles those in the hemorrhagic state (bleeding cases).

It is assumed that ITP, aplastic anemia and acute leukemia have a tendency for depletion of adhesiveness, aggregation, clot retraction and platelet factor 3, though the values showed a wide distribution at both hemorrhagic and non-

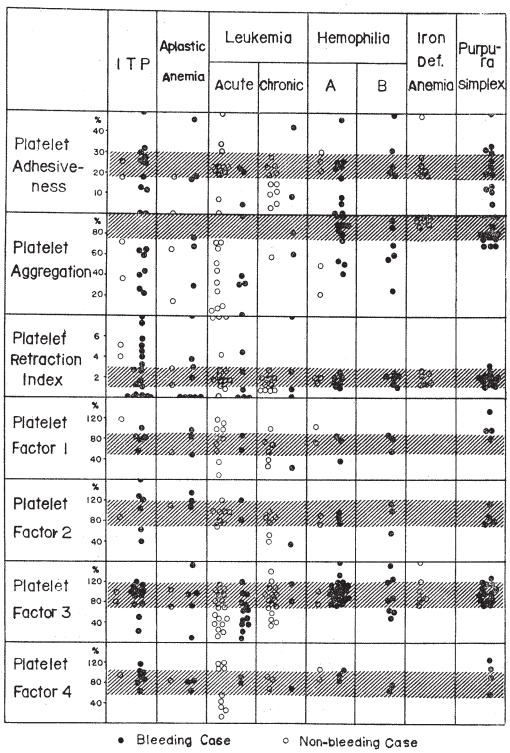

FIG. 4. Platelet factors in various hematological disorders.

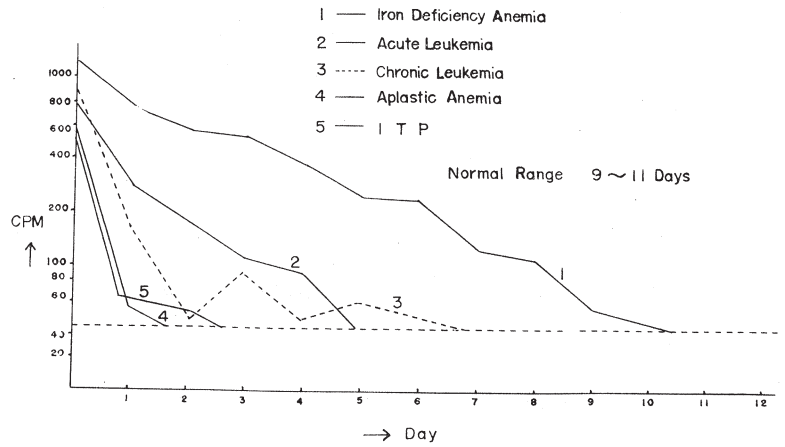

FIG. 5. Life span of platelets from various hematological disorders by means of $Na_2Cr^{51}O_4$ labeling.

hemorrhagic states. In diseases other than ITP, aplastic anemia and acute leukemia the distribution of the values was not relatively wide, and a tendency was noted for them to lie near the normal range.

3) Life span of platelets: The life span of $Na_2Cr^{51}O_4$ labelled platelets of the various hematological disorders are shown in Fig. 5.

In ITP (hemorrhagic state), aplastic anemia (non-hemorrhagic state) and leukemia (both acute and chronic, non-hemorrhagic state), shortening of the life span was noted, and this was especially marked in the former two disorders. It is of interest to note that the case of chronic leukemia shown in Fig. 5 had a platelet count of 284.000/cu. mm, so that despite the count being normal the life span was shortened.

d) Fibrinolysis

The activity of factors concerning fibrinolysis obtained from the cases with the various blood disorders were plotted in Fig. 6. The areas shaded by stippling represent the normal range, the white circles the values at no hemorrhagic states (non-bleeding cases) and the black circles those at hemorrhagic states (bleeding cases). All values showed a fairly wide scattering, but antiplasmin value was practically normal in all the cases.

According to the investigation of fibrinolytic activity of diluted plasma, euglobulin, Sk-activated euglobulin and plasminogen activators, increased activity of fibrinolysis was found in many cases of those diseases. But no significant relationship was observed between the increase of the said various factors of fibrinolytic activity and hemorrhagic phenomenon. However, from these results the view can be presented that in many cases of these diseases patients are kept in prehemorrhagic state in the presence of enhanced fibrinolytic activity.

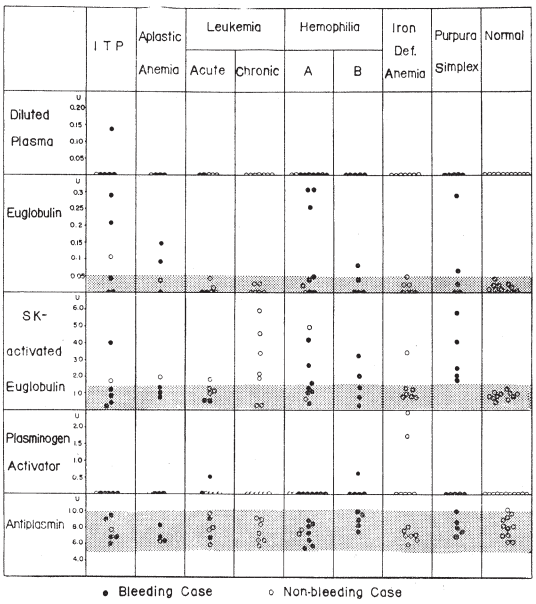

FIG. 6. Fibrinolytic activity in various hematological disorders.

e) Hemorrhage and factors underlying hemorrhagic diathesis

Many of the etiological factors connected with the hemorrhagic diathesis were classified into 5 elements, namely, the blood vessel, platelet, clotting factors, anticoagulants and fibrinolysis. And in the above mentioned disases it has been found that abnormality of one or more of these elements was frequently seen. A study of the states of combination of such abnormality in elements was made, according to whether bleeding is present or not, in 50 cases, consisting of 26 bleeding cases and 24 non-bleeding cases where sufficient data were available (Fig. 7).

In the bleeding cases (Fig. 7, B) the abnormality of only one element was rarely seen (3.8%), but that of two elements was observed in 30.8% and that of three elements or more in 65.4%. In the non-bleeding cases (Fig. 7, C), on the other hand, 29.2% of the cases showed abnormality of only one element. And in 37.5% of the cases abnormalities in two elements and in 33.3% of the cases in three or more elements were recognized. On the basis of the above analysis it became clear that the abnormalities in three and more elements were more frequent in the bleeding cases than in the non-bleeding cases and at the time of actual bleeding the abnormalities in many elements are involved simultaneously.

But in the state of non-bleeding, though not so frequently seen as in that of bleeding, the abnormality of either one or more elements could be noticed.

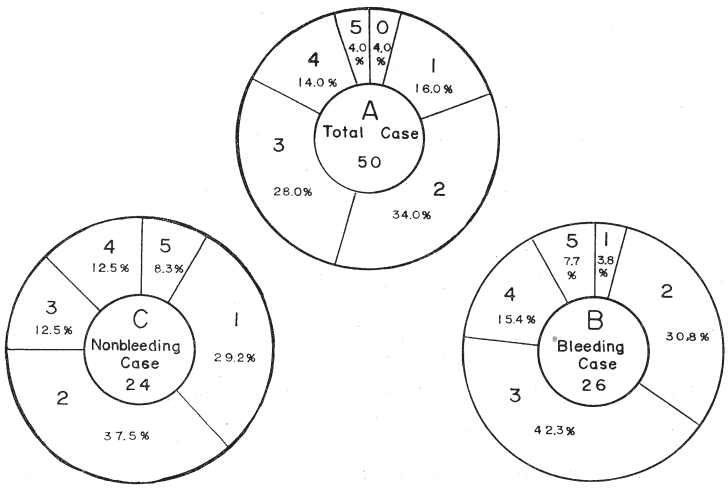

FIG. 7. Number of element of hemorrhagic diathesis in various hematological disorders.

The abnormality in the elements underlying the hemorrhagic diathesis will not necessarily indicate the immediate occurrence of bleeding, and for the manifestation of the hemorrhagic states the combined abnormality of the many elements should be regarded as extremely important.

SUMMARY AND CONCLUSION

The study was made of the hemorrhagic diathesis in blood and hematopoietic disorders and primary hemorrhagic diseases, especially leukemia, aplastic anemia, ITP and hemophilias.

1) In each of the hemorrhagic diseases there were found some differences among the characteristic features of coagulation defects and also between hemorrhagic and non-hemorrhagic states.

2) A survey of platelet counts showed a ranges from 30,000/cu. mm to 60,000/cu. mm could be considered to be the critical zone for hemorrhages. Life span of platelets was shorten in many hematopoietic diseases.

3) In studies on fibrinolysis there were noted prehemorrhagic states in many cases.

4) Analysis of the elements of hemorrhagic diathesis was performed. The combined abnormalities of the elements were frequently seen in the bleeding states.

5) There is need to watch constantly the fluctuation in the individual factors connected with the hemorrhagic diathesis and the states of their combination, in order to arrest the establishment of a hemorrhagic state.

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