The Turkish Journal of Pediatrics
Volume 21 / Nos 2-4 / April-October 1979 / pp. 36 - 45

Splenic Functions in Non-negro Patients with Sickle-cell Anemia and Sickle-cell Beta Thalassemia*

Şinasi Özsoylu, M.D. / Necdet Altınöz, M.D.*** / Yahya Lâleli, M.D.*****

Studies in children with sickle-cell anemia have demonstrated that removal of Howell-Jolly bodies, phagocytic function of the spleen and antibody to the challenge of intravenous sheep erythrocytes are markedly impaired.[6, 19, 20, 25] However, the correlation of the filtering ability of the spleen to its phagocytic function is questionable.[4] There are also some studies in these patients concerning factor VIII and platelet reservoir function of the spleen.[6, 24] But these functions have not all been evaluated in the same patient and certainly not in ethnic groups other than Negroes with sickel-cell anemia, whose fetal hemoglobin concentrations are lower than Turks[14] and Saudi Arabians[22] with this disease.

Therefore the splenic functions as a whole were evaluated in 13 patients with sickle-cell anemia and in 7 with Hb-S.β-thalassemia, from Turkey.

Materials and Methods

A total of 39 children were studied by dividing them into 4 groups. Group I consisted of 11 healthy children (2 girls and 9 boys) 4 to 12 years of age with an AA hemoglobin pattern. Group 2 comprised 8 children, 3 to 12 years of age (2 girls and 6 boys), who had been splenectomized 1 to

* From Hacettepe University, Faculty of Medicine, Dept. of Pediatrics, Hematology Section and Dept. of Nuclear Medicine and Hacettepe Children's Hospital, Medical Center, Ankara, Turkey.
** Prof. of Pediatrics and Hematologist, Head of the Hematology Section.
*** Associate in Pediatrics.
**** Prof. of Biochemistry, and Nuclear Medicine.

3 years prior to the study because of β-thalassemia major (4 cases), idiopathic thrombocytopenic purpura (2 cases), spherocytosis (1 case) or severe α-thalassemia (1 case). Group 3 was formed of 13 sickle-cell anemia cases with an age range of 10 months to 20 years (3 girls and 10 boys). Seven children whose ages ranged from 2.5 to 15 years with sickle cell-β-thalassemia (4 girls and 3 boys) comprised the fourth group.[15] All children were free of infection and did not have any acute problem at the time that these tests were performed.

The hemoglobinopathy diagnosis was verified by Starch (pH: 8.6) and agar gel (pH 6,4) electrophoresis[14] in all patients and in both of their parents. Fetal hemoglobin concentrations were determined by Singer's alkali denaturation method.[27]

The phagocytic function of the spleen was studied in the last two groups one hour after intravenous injection of ^{99m}Tc sulfur colloid (1-3 mCi) by using an Anger scintillation camera and Pho-Gamma III with low energy collimator, and the images were recorded on Polaroid film and/or 35 mm Kodak PX402 film in present time. In addition to anterior and posterior projections, left posterior images were also obtained when the previous two projections did not give a splenic image. Scans were graded as normal, decreased and no uptake.

Reservoir function for platelets and F-VIII procoagulant activities were evaluated before and 10 minutes after intravenous injection of adrenalin (5 μg/kg adrenalin was diluted in 10 ml 5 % glucose and was perfused in 10 minutes). Platelets were enumerated by phase contrast microscopy[4] from a finger prick, and F-VIII procoagulant activity assays were performed according to McMillan et. al.[12] on fresh frozen samples of plasma which were stored at -30°C for no more than 3 weeks.

For the evaluation of the filtering function of the spleen the presence of Howell-Jolly bodies per 500 red blood cells was determined.

Results

Peripheral platelet count. In the healthy control subjects the baseline platelet count varied between 220-464 (mean ± S. D.: 320.3 ± 71.6) X 10^3 / μl, The corresponding values for the five asplenics ranged from 44-744 (376.8 ± 311.1) X 10^3 / μ 1, the patients with sicklecell 112-920 (395.1 ± 235.3) X 10^3 / μl, and in the patients patients with Hb-S-β-thalassemia 124-560 (329.1 ± 145.6) X 10^3 / μl. The elevation in the thrombocyte count following adrenalin perfusion was highly significant in the control group (8.82 %; $P < 0.001$) and in patients with Hb-S-β-thalassemia (6.71 %; $P < 0.05$) but not in the splenectomized group nor

TABLE I

RESULTS OF PLATELET ELEVATION FOLLOWING ADRENALİN PERFUSION (X 103 / μ l)

	Initial	Post Perfusion	Mean of Each	Post Perf. Initial	P
Control [11]*	320.3 ± 71.6 (220-464)**	349.0 ± 79.1 (228-484)		1.09	< 0.001
Splenectomized [5]	376.8 ± 311.1 (44-744)	376.8 ± 310.1 (48-560)		0.98	> 0.05
Sickle-Cell Anemia [12]	395.1 ± 235.3 (112-920)	405±216.2 (100-800)		1.04	> 0.05
Hb S-β-Thalassemia [7]	329.1 ± 145.6 (124-560)	345.7 ± 138.1 (136-556)		1.07	< 0.05

* Numbers in brackets indicate the number of cases
** Numbers in parentheses indicate the range

TABLE II

INITIAL F-VIII LEVELS WITH THEIR ELEVATIONS FOLLOWING EPINEPHRINE PERFUSION (%)

	Initial	Post Perfusion	Mean of Each	Post Perf. Initial	P
Controls [11]	132.8 ± 71.2 (62-286)	267.3 ± 160 (85-565)		1.93	< 0.0001
Splenectomized [8]	110.8 ± 47 (56-194)	189.5 ± 104.6 (111-428)		1.71	< 0.01
Hb SS [8]	170 ± 90.9 (80-375)	263.1 ± 132 (121-511)		1.58	< 0.01
Hb S-β-Thalassemia [7]	165.5 ± 72.8 (77-258)	230.2 ± 107.9 (103-361)		1.38	< 0.01

in patients with sickle-cell anemia ($P > 0.05$ for both). The means of individual values for the ratio of post perfusion initial platelet count are also given in Table I with detailed findings.

AHG activity. The mean basal AHG level in the healthy controls was 132.8 ± 71.2 % (range 62-286 %). Corresponding values for the asplenics were 110.8 ± 47 %, in patients with Hb-S-β-thalassemia 165.5 ± 72.8 %, and in 8 patients with sickle-cell anemia in whom it could be determined, 170 ± 90.9 %. Following adrenalin perfusion the elevation in Factor-VIII activity was significant in all groups, the details are indicated in Table II.

Splenic scanning and Hb F determinations were performed in all children with sickle-cell anemia and Hb S-β-thalassemia. The spleen could not be visualized in 10 patients with sickle-cell anemia, 3 of whom had splenomegaly. Splenic image was shown in the remaining 3 patients with clinically enlarged spleen (being decreased in a 3 year old boy and normal in two girls, 8 and 12 years of age) (Table III). Splenic scanning of the 8 year old girl was repeated a year later and was still visible. The spleen could not be visualized in one of the 5 patients with Hb-S-β-thalassemia with splenomegaly, and phagocytic dysfunction was shown in two of the cases without clinical splenomegaly (no uptake in one and decreased in one) Table IV.

TABLE III

SPLENIC SIZE Hb F LEVELS AND SCANNING RESULTS IN PATIENTS WITH SICKLE-CELL ANEMIA

Patient's Initials	AGE and Sex		Spleen (cm)	Hb F (%)	Splenic Scanning*
1. A.A.	10/12	M	3.5	14	N.U.
2. M.Y.F.	3	M	7	28	D
3. K.K.	8	F	3	4.6	N
4. M.S.M.	9	M	1	14	N.U.
5. F.C.	12	F	5	—	N
6. S.C.	16	M	1	3.1	N.U.
7. N.T.	7	M	N.P.**	9.5	N.U.
8. E.L.	7	M	N.P.	29	N.U.
9. L.T.	8	M	N.P.	6	N.U.
10. D.C.	8	M	N.P.	8.4	N.U.
11. A.G.	13	M	N.P.	26	N.U.
12. M.S.Y.	14	F	N.P.	13.1	N.U.
13. M.Y.	20	M	N.P.	12	N.U.

* N : Normal
D : Decreased
N.U. : No uptake
** N.P. : Not palpable

TABLE IV

SPLENIC SIZE Hb F LEVELS AND SCANNING RESULTS IN PATIENTS WITH Hb S-β-THALASSEMIA

Patient's Initials	AGE and Sex		Spleen (cm)	Hb F (%)	Splenic Scanning*
1. M.G.	2.5	M	3	14	N
2. H.K.	8	F	3	19	N
3. A.A.	9	M	4	6.3	N.U.
4. E.H.	15	F	15	22	N
5. G.S.	15	F	4	18	N
6. Y.C.	9	M	N.P.**	11.3	D
7. F.S.	10	F	N.P.	20	N.U.

* N : Normal
D : Decreased
N.U. : No uptake
** N.P. : Not palpable

Howell-Jolly bodies were present on the peripheral smears of all splenectomized children and of patients with Hb SS and Hb-S-β-thalassemia but not in those of normal children.

Discusssion

Phagocytosis of sulfur colloid particles by the spleen could be shown in only 3 of 13 patients (23 %) with sickle-cell anemia; all three had clinically enlarged spleens. This finding fits well with the previous studies supporting the presence of "functional asplenia" in most sickle-cell anemia cases,[5, 6, 19, 20, 24, 25] but it does not seem to correlate with the Hb F levels (Table III), which do not support O'Brien et. al. conclusion.[13] The impairment of this reticuloendothelial activity was shown in the youngest of the patients, a 10 month old baby, but not in a 12 year old child. However, it should be emphasized that some children with sickle-cell anemia, despite "functional asplenia", may develop the so-called splenic sequestration crises[23, 26, 28, 30] and thrombocytopenia due to hypersplenism.[9, 23]

"Functional asplenia" has not been reported in Negroes with Hb-S-β-thalassemia[20, 24] but was shown in at least 2 of our 7 patients (28.6 %). With one exception, all of our Hb-S-β-thalassemia combinations had β°-thalassemia and thier Hb S concentrations were comparable to those of the patients with sickle-cell anemia. Neither the mean fatel hemoglobin (Hb F values of the patients with Hb SS 13.98 % and Hb-S-β-thalassemia 15.8 %) nor the hemoglobin, hematocrit and ages of the patients were significantly different. Therefore, these factors, including Hb S concentrations, are probably not the sole explanation for

the differences of the phagocytic function in the spleen of these patients and improvement of phagocytic function of the organ in the newborn period cannot be considered in our patients because of their ages.[16, 29]

Baseline AHG activity was found to be higher in patients with Hb SS, as reported previously,[11] and in those with Hb-S-β-thalassemia. Although it was lowest in the splenectomized group, it ranged within normal limits. AHG procoagulant activity elevation following adrenalin perfusion in all groups was statistically singificant as compared to their original values, but relatively low in patients with Hb-S-β-thalassemia. AHG values and the elevation in controls in this study were comparable to our previous results obtained from blood donors.[17] Our findings in splenectomized children as in Falter and co-workers[6] differed from those of Libre et. al.[11] Therefore, we do not believe that the changes in peripheral blood AHG activity following adrenalin perfusion is a meaningful measure of splenic function derangement in sickle-cell anemia.

Penny and associates[21] have reported that the spleen contains about one-third of the platelet mass. It has been shown by Aster[2] and Branehög et al.[3] that the blood platelets in the spleen are exchangeable and available to the circulation with epinephrine infusion, which is abolished by splenectomy as in our splenectomized controls. There was also no statistically singnificant platelet elevation after epinephrine perfusion in patients with sickle-cell anemia and in 3 patients with Hb-S-β-thalassemia in functional hyposplenism was shown by scanning. These results agree in general with Schwartz's findings.[24] However, in 3 sickle cell anemia patients, marked platelet elevations were observed without splenomegaly and thrombocytopenia, which could not be explained by hypersplenism, in at least two cases. Although the platelet reservoir function of the spleen which seems to be related to adrenergic alpha receptor stimulation,[7] was impaired in patients with sickle-cell anemia, it was not correlated with Hb S concentrations.

Higher platelet counts in patients with sickle-cell anemia have been reported by Schwartz[24] and Freedman-Karpatkin,[9] as was our finding, more markedly in those without enlarged spleen. Marked thrombocytosis was also present in two patients with Hb-S-β-thalassemia without palpable spleen. The age of our patients with Hb SS and Hb-S-β-thalassemia did not seem to affect splenic function either. Very recently Freedman and his colleagues[8] have shown the presence of a nonsplenic platelet, pool, but it could not be considered in our study because of the lengthened time interval.

Casper and colleagues[5] concluded that the percentage of Howell-Jolly bodies does not correlate well with the ^{99m}Tc sulfur colloid visualization of the spleen, and pitted red cells are more sensitivite for splenic reticuloendothelial dysfunction in Hb SS disease. The presence of Howell-Jolly bodies in our patients with Hb SS and Hb-S-β-thalassemia was not correlated with ^{99m}Tc scanning results either; but since they were present in all patients, it was interpreted as an early evidence of filtering function derangement of the spleen.

From these studies it is concluded that splenic functions of these patients are dissociated as indicated by Schwartz,[24] and functional hyposplenism is present in most non-negro patients with Hb SS disease and in more than one-third of those with Hb-S-β-thalassemia but not in children with beta thalassemia major.[18] Although AHG level changes is not a useful test of "functional hyposplenism," platelet count alterations following adrenalin infusion is a simple test for the evaluation of this condition without correlation of splenic scanning.

Summary

Platelet and F-VIII reservoir and phagocytic functions of the spleen have been studied in 7 patients with Hb-S-β-thalassemia and 13 cases of Hb SS disease. Eight splenectomized patients and 11 healthy children served as controls. F-VIII elevation following administration of adrenalin was not found to be a meaningful index in the evaluation of "functional hyposplenism" which was suspected by ^{99m}Tc scanning in 42.8 and 77 % of the patients with Hb-S-β-thalassemia and Hb SS disease, respectively. A high platelet count was observed in patients with sickle-cell anemia and Hb-S-β-thalassemia without palpable spleen. The independence of the splenic functions of these patients as emphasized.

REFERENCES

1. Abildgaard, C. F., Simone, J. V. & Shulman, L.: Factor VIII (anti haemophilic factor) activity in sickle-cell anemia Brit. J. Haemat 13: 19, 1967.
2. Aster, R. H.: Pooling of palatelets in the spleen: Role in the pathogenesis of "hypersplenic" thrombocytopenia J Clin. Invest. 45: 645, 1966.
3. Branehog, L., Weinfeld, A. & Ross, B.: The exchangeable splenic platelet pool studied with epinephrine infusion in idiopathic thrombocytopenic purpura and in patients with splenomegaly Brit. J. Haemat. 25: 239, 1973.
4. Brecker, G. & Cronkite, E. P.: Morphology and enumaration of human blood platelets J Appl Physiol. 3: 365, 1951.
5. Casper, J. T., Koethe, S., Rodey, G. E. & Thatcher, L. G.: New Method for studying splenic reticuloendothelial dysfunciton in sickle-cell disease patients and its clinical application. A brief report Blood 47: 183, 1976.

6. Falter, M. E., Robinson, M. G., Kim, O. S., Go. S. G. & Taubkin, S. P.: Splenic function and infection in sickle-cell anemia Acta Haemat. 50: 145, 1973.
7. Freden, K., Olsson, L-B., Suurkula, M. & Kutti, J.: The exchangebale splenic platelet pool in response to intravenous infusion of isoprenaline Scand, J. Haemat. 20: 335, 1978.
8. Freedman, M., Altszuler, N. & Karpatkin, S.: Presence of nonsplenic platelet pool Blood 50: 419, 1977.
9. Freedman, M. L. & Karpatkin, S.: Elevated platelet count and megathrombocyte number in sickle-cell anemia Blood 46: 579, 1975.
10. Jenkins, M. E., Scott, R. B. & Baird, R. L.: Studies in sickle-cell anemia XVI Sudden death during sickle-cell anemia crises in young children J. Pediatr, 56: 30, 1960.
11. Libre, E. P., Cowan, D. H., Watkins, S. S. & Shulman N. R.: Relationships between spleen platelet and factor VIII levels Blood 31: 358, 1968.
12. McMillian, C. W., Diamond, L. K. & Surgenor, D. M.: Treatment of classic hemophilia. The use of fibrinogen rich in factor VIII for hemorrhage and for Surgery N. Engl. J. Med. 265: 224, 277, 1961.
13. O'Brien, R. T., McIntosh, S., Aspnes, G. T. & Pearson, H. A.: Prospective study of sickle-cell anemia in infancy J Pediatr 89: 205, 1976.
14. Özsoylu, S. & Altınöz N.: Sickle-cell anemia in Turkey, Evaluation of 97 cases (with parents findings) Scand. J. Haemat. 19: 85, 1977.
15. Özsoylu, S., Hiçsönmez, G., Altay, C. & Altınöz, N.: Sickle-cell-β-thalassemia International İstanbul Symposium on abnormal hemoglobin and thalassemia (Ed M Aksoy) İstanbul 1975. p. 329.
16. Özsoylu, S., Houssain, F. & McIntyre, P.: Functional development of phagocytic activity of the spleen J. Pediatr. 90: 560, 1977,
17. Özsoylu, S., Kuranel, K., Pirnar, A. & Kanra, T.: Factor VIII procoagulant activity and adrenalin infusion Haemophilia (Eds O N Ulutin, I R Peake) Excerpta Medica 1975, p. 183.
18. Özsoylu, S., Laleli, Y. & Munipoglu, G.: Splenic functions in thalassemia major Turk J. Pediatr. 18: 90, 1976.
19. Pearson, H. A., Cornelius. E. A., Schwartz, A. D., Nelson, J. H., Wolfson, S. L. & Spencer, R. P.: Transfusion reversible asplenia in Young children with sickle-cell anemia N. Engl. J. Med. 283: 334, 1970.
20. Pearson, H. A., Spencer, R. P. & Cornelius, E. A.: Functional asplenia in sickle-cell anemia N. Engl. J. Med. 281: 923, 1969.
21. Penny, P., Rozenberg, M. C. & Firkin, B-G.: The splenic platelet pool Blood 27: 1, 1966.
22. Perrine, R. P., Brown, M. J., Clegg, J. B., Weatherall, D. J. & May, A.: Benign sickle-cell anemia Lancet 2: 1163, 1972.
23. Rossi, E. C., Westiring, D. W., Santoz, A. S. & Gutierrez, J.: Splenectomy for hypersplenism in sickle-cell anemia Arch. Intern. Med. 114: 408, 1964.
24. Schwartz, A. D.: The splenic platelet reservior in sickle-cell anemia Blood 40: 678, 1972.
25. Schwartz, A. D. & Pearson, H. A.: Impaired antibody response to intravenous immunization in sickle-cell anemia Peditar Res. 6: 145, 1972.

26. Seeler, R. A. & Shwiaki, M. Z.: Acute splenic sequestration crises (ASSC) in young children wit sickle-cell anemia Clin. Pediatr. 11: 701, 1972.
27. Singer, K., Chernoff, A. E. & Singer, L.: Studies of abnormal hemoglobinis I. Their demonstration in sickle-cell anemia and other hematologic disorders by mean of alkali denaturation Blood 6: 413, 1951.
28. Sommer, A. & Kontras, S. B.: Splenomegaly with hypersplenism in sickle-cell anemia treted by radiation Case Report Pediatr. 48: 457, 1971.
29. Spirer, Z., Shalit, I., Zakuth, V., Svirsky-Fein S., Milbaurer, B. & Bogair, N.: Decreased antihaemophilic globulin and leukocyte response to epinephrine in preterm infants Arch. Dis. Child. 51: 231, 1976.
30. Stevens, A. R., Jr.: Splenectomy in sickle-cell anemia Arch Intern. Med. 125: 883, 1970.