Splenetic function in sickle-cell diseases

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Summary

1. Studies of splenic function were carried out on patients with sickle-cell diseases by the measurement of the clearance of autologous heat-damaged ⁹⁹ᵐTc-labelled erythrocytes from circulation and into the spleen, the spleen area by a scintillation scanning, the enumeration of pitted erythrocytes by direct-interference microscopy, and the percentage of irreversibly sickled cells (ISC) and of cells with Howell-Jolly bodies. All measurements were performed in seven HbS homozygotes, 10 patients with sickle cell β²-thalassaemia (S/β²-thalassaemia), three patients with sickle-cell disease (SC), four AS heterozygotes and 17 controls.

2. Three different patterns of splenic function were observed among the 20 patients with symptomatic sickle-cell diseases: six patients had enlarged hyperactive spleens, four had enlarged hypoactive spleens, and in 10 patients no splenic activity was detected.

3. The percentage of ISC was higher in sickle-cell anaemia than in S/β²-thalassaemia and very low in SC patients.

4. These results would suggest that the spleen goes through similar successive functional stages in the sickle-cell diseases, namely enlargement in the early years of life, which is followed by hypoactivity and finally atrophy. This evolution seems to be faster in sickle-cell anaemia than in S/β²-thalassaemia and SC disease.

Key words: sickle-cell anaemia, sickle-cell thalassaemia, spleen.

Abbreviations: ISC, irreversibly sickled cells; SC, Hb sickle-cell disease; SS, sickle-cell anaemia.

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Introduction

Apart from splenectomy and congenital asplenia, the most common causes of splenic hypofunction are sickle-cell anaemia, coeliac disease and ulcerative colitis [1-5]. Splenomegaly is regularly observed during the early years of life in sickle-cell anaemia, followed by atrophy of the spleen, which is reduced to a fibrotic nodule as a result of repeated episodes of infarction [6-8]. Accordingly, an enlarged spleen is extremely rare in adults with sickle-cell anaemia. By contrast, splenomegaly is a common finding in patients with sickle cell-β thalassaemia (S/β-thalassaemia), although the regression of an enlarged spleen occurs in this disease as well [9, 10]. In addition, hypofunction has been consistently demonstrated by the absence of splenic uptake of ⁹⁹ᵐTe–sulphur colloid in sickle-cell anaemia, even in the presence of an enlarged spleen (functional asplenia) [2, 4, 8, 11-13]. In contrast, few functional studies in S/β-thalassaemia patients have been reported; the results have been variable and no common pattern of splenic dysfunction can be established from the studies [4, 8, 13].

This paper presents the results of a comprehensive quantitative study of the splenic function carried out in a group of 24 patients with sickle-cell diseases, which includes AS heterozygotes, SS homozygotes, S/β²-thalassaemia patients and SC patients.

Material and methods

Subjects

The investigations were performed on 24 patients with sickle-cell diseases, including seven with sickle-cell anaemia (SS), 10 with sickle-cell β²-thalassaemia (S/β²-thalassaemia), three with SC disease and four AS heterozygotes. Some clinical
and haematological data for the patients are given in Table 1. For comparative purposes, 17 controls were also studied, including eight subjects (six males, two females) aged 13-51 years (median 21.5 years) with apparently normal spleens, with haemoglobin 10.5-16.4 g/dl (median 12.9 g/dl), five who had undergone splenectomy (two males, three females), aged 18-60 years (median 22 years), with haemoglobin 9.2-14.1 g/dl (median 13.0 g/dl), and four (two males, two females) with splenomegaly not related to HbS (one with hereditary spherocytosis, one with thalassaemia major and two with thalassaemia intermedia), aged 8-28 years with haemoglobin 6.2-13.2 g/dl. Diagnosis was established in each case by clinical, laboratory and family studies. Haemoglobin concentration was measured with a TOA electronic haemoglobinometer and HbF was quantified by alkali denaturation [14]. Two SS patients (nos. 1 and 2), four with S/β-thalassaemia (nos. 1-4) and all the three with SC disease had splenomegaly on physical examination. Only two SS patients (nos. 1 and 2) and two with S/β-thalassaemia (nos. 1 and 2) had received transfusions of erythrocytes in the 4-month period preceding the study. Consent was obtained from all adult patients after full explanation of the procedure. For the 11 children consent was obtained from one parent and in eight of them the procedure had also a diagnostic purpose when splenectomy was contemplated.

Morphological abnormalities of the erythrocytes

A blood smear was prepared immediately after collection of venous blood without anticoagulant, and stained by the Leishman method. At least 5000 cells were examined by each of two observers to determine the percentage of erythrocytes with Howell-Jolly bodies, and the percentage of irreversibly sickled cells (ISC) was determined by the examination of 2000 erythrocytes.

One drop of freshly collected blood without anticoagulant was added to 0.3 ml of phosphate-buffered sodium chloride solution (150 mmol/l saline), pH 7.4, containing 2% formaldehyde. After 30 min the suspension was examined as a wet preparation under direct-interference contrast microscopy (Zeiss microscope equipped with Nomarski optics). The percentage of erythrocytes having one or more pits was determined by counting 500 cells, regardless of the size or the number of pits per cell.

Removal of autologous heat-damaged erythrocytes from circulation

Erythrocytes were heat-damaged and labelled with 99mTc by the following modification of reported techniques [15-17]. Approximately 10 ml of blood was collected into ACD solution and after centrifugation the plasma was separated, the buffy coat was removed and the cells were resuspended in sterile sodium chloride solution (150 mmol/l) to give a PCV of 0.30. The cells were then heat-damaged by incubating them at 50 ± 0.5°C for 30 min. After centrifugation the packed cells were incubated with 1.5-2.0 mCi (5.5-7.4 x 107 Bq) of 99mTc for 10 min, and then 0.05 ml of a freshly prepared solution of SnCl2,2H2O (400 mg/ml of saline) was added to each millilitre of cells and incubated for 5 min. The cells were washed three times in sterile saline, resuspended in autologous plasma and re-injected. Blood samples were taken from the opposite arm 3, 6, 10, 15, 20, 30 and 45 min after injection. Radioactivity was measured in 2 ml samples of whole blood and the clearance half-time (T1/2 clearance) of disappearance of radioactivity from circulation was calculated directly from the clearance curve. At the same time, the surface radioactivity over the spleen and the liver was measured simultaneously for 90 min by placing two collimated scintillation counters (NUCLEOPAN-M, Siemens) over the organs. Measurements were corrected for the decay of 99mTc. Graphic analysis of the curves of radioactivity over the spleen permits the calculation of radioactivity at t = 0 (C0) and after the counts reached a plateau (Cmax.), between 20 and 70 min. The values of surface counts at different times (Ci) can be fitted to a regression line so that:

\[ \ln \left[ \frac{(C_t - C_0)}{(C_{max.} - C_t)} \right] = a + b \cdot \ln t \]

and the half-clearance time into the spleen (T1/2 spleen) can be calculated from

\[ T_{1/2} = e^{-b/a} \]

The Q14 coefficient was calculated as a measure of the uptake of radioactivity by the spleen. This represents the ratio of corrected c.p.m. over the spleen (C_S) and the liver (C_L) at 15 min and 1 min:

\[ Q_{14} = \frac{C_S}{C_L} \text{ at 15 min/(C_S/C_L) at 1 min} \]

This coefficient permits comparisons between patients since it corrects for differences in the efficiency of labelling and minimizes differences due to variations in the splenic erythrocyte volume.

Two hours after injection the area over the spleen was scanned with a rectilinear scintillation scanner (SCINTIMAT-2, Siemens) at a speed of 100 cm/min, both in the anterior and left lateral positions, and the area was measured with a planimeter. The average coefficient of variation for five
planimetric measurements taken randomly on each of five different scintillation tracings was 5.1%. All measurements were made by the same observer.

Results

The results are summarized in Table 1 and in Fig. 1.

Damaged erythrocytes were cleared from the circulation of control patients with $T_{1/2}$ 18.6–41.3 min (median 25.1 min). The cells accumulated in the spleen, where surface radioactivity increased with $T_{1/2}$ 6.9–16.8 min (median 10.5), and the ratio of spleen/liver counts at 15 min was 1.13–2.75 times that at the first minute. The spleen areas on lateral scans measured from 24 to 63 cm$^2$ (median 47 cm$^2$). Less than 3.2% of cells showed surface pits and none had Howell-Jolly bodies. The results obtained for four AS heterozygote patients were similar to those for the controls.

In the splenectomized patients the clearance from circulation was slow, with $T_{1/2}$ 67.8–180.3 min (median 123.6 min). At the same time, there was a slight increase of the radioactivity over the liver without change over the spleen; consequently, $Q_{15}$ varied from 0.81 to 0.95 (median 0.92). Both the pit counts (28.8–63.0%, median 56.8%) and the percentage of cells with Howell-Jolly bodies (0.1–1.1%, median 0.3%) were elevated. There was no superposition of the range of any of the measurements of splenectomized and the control subjects.

The results are summarized in Table 1 and in Fig. 1.

In the splenomegaly and without structural haemoglobinopathies, clearance from circulation ($T_{1/2}$ 1.8–12.7, median 3.9 min) and into the spleen ($T_{1/2}$ 1.5–7.0, median 3.2 min) were rapid and $Q_{15}$ (1.71–4.81, median 2.73) was elevated. These results were significantly different from those for the controls and AS heterozygotes by the rank sum test ($P < 0.01$ for the first two measurements, and $0.05 < P < 0.10$ for $Q_{15}$) [18]. Howell-Jolly bodies were absent or rare and the percentage of pitted erythrocytes was similar to that in controls in three cases (0.8–3.7%) but elevated in one patient with β-thalassemia intermedia (26.7%).

Three different patterns of splenic function were observed among the 20 patients with symptomatic sickle-cell diseases, of which ten patients had splenomegaly. The enlarged spleen was hyperactive in two SS homozygotes aged 5 and 7 years (cases 1 and 2), in three with S/β-thalassaemia aged 4–10 years (cases 1–3) and one 12-year-old girl with SC disease (no. 1). The values of splenic function tests for these patients were similar to those for other patients with splenomegaly and without struc-

**Table 1. Clinical and laboratory data for 20 patients with sickle-cell diseases**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Haemoglobin (g/dl)</th>
<th>HbF (%)</th>
<th>Howell-Jolly (%)</th>
<th>ISC (%)</th>
<th>Spleen area on lateral scan* (cm$^2$)</th>
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<td>1.0</td>
<td>0.0</td>
<td>0.3</td>
<td>87</td>
</tr>
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</table>

* Range in eight normal controls 24–63 cm$^2$ (median 47 cm$^2$); range in four patients with splenomegaly 92–206 cm$^2$ (median 171 cm$^2$).
Fig. 1. Values of half-time of clearance from circulation ($T_{1/2}$ clearance) of damaged erythrocytes, $Q_{15}$ coefficient and percentage of pitted erythrocytes in four controls with splenomegaly (H), eight normals and four AS heterozygotes (N), five splenectomized (S) and 20 patients with sickle-cell diseases (P). •, Subjects with a functioning spleen; ○, subjects with a hypoactive spleen; ●, subjects without a detectable spleen.

tural defects of haemoglobin: short clearance time from the circulation ($T_{1/2}$ 0.8-5.6 min) and into the spleen ($T_{1/2}$ 2.7-5.5 min) of the damaged erythrocytes, elevated $Q_{15}$ (1.91-4.69) and low pit counts (1.0-4.8%). An enlarged hypoactive spleen was observed in two patients aged 9 and 19 years with S/βthalassaemia (cases 4 and 5), and two with SC disease 23 and 26 years old (cases 2 and 3). The $T_{1/2}$ values for the clearance from circulation (47.5-94.5 min) and the pit counts (19.1-22.6%) for these four patients were intermediate between the values for normal and asplenic patients. The spleen area on scintillation scanning was enlarged (87-204 cm$^2$) but the increase of radioactivity over the organ was slower than in normals, with $T_{1/2}$ 22.2-35.2 min and $Q_{15}$ 1.20-1.46. Finally, the spleen was not detected by scintillation scanning in five SS homozygotes (cases 3-7) and in five with S/βthalassaemia (cases 6-10). All measurements of splenic function for these patients were similar to those for the splenectomized, with slow clearance of heat-damaged erythrocytes, high values of pit counts and of Howell-Jolly bodies, and $Q_{15}$ of 1.00 or less.

The percentage of ISC was higher in sickle-cell anaemia than in S/βthalassaemia, and very low in SC disease. The value of ISC had no relationship to the splenic function as measured by the pit counts and clearance of heat-damaged erythrocytes, at least for patients with S/βthalassaemia or SC disease. The low values found in the two SS homozygotes with splenomegaly was probably the consequence of recent blood transfusion.

The Spearman's rank correlation coefficients ($r_s$) [18] were significant ($P<0.01$) for the correlations between $T_{1/2}$ values of clearance and pit counts, $T_{1/2}$ values and $Q_{15}$, and pit counts and $Q_{15}$, either for the entire group of 41 subjects ($r_s = 0.698, -0.807$ and $-0.727$ respectively) or for the 20 symptomatic patients ($r_s = 0.750, -0.680$ and $-0.851$ respectively). The $r_s$ coefficient of correlation between $T_{1/2}$ clearance and $T_{1/2}$ spleen was 0.874 ($P<0.01$) for the 26 subjects who had a functioning spleen and therefore had measurable values of $T_{1/2}$ (spleen).

Discussion

Splenic function is usually evaluated on the basis of the ability of the spleen to remove particles from the circulating blood. This includes the removal of inclusion bodies from erythrocytes or removal of the entire cell when it is abnormal. Consequently, when the spleen is absent, target cells and cells with Howell-Jolly bodies accumulate in the blood [2, 19]. Also, elevated numbers of erythrocytes with surface pits can be observed by direct-interference contrast microscopy [11, 20]. The spleen can be demonstrated by scintillation scanning of the abdomen after the phagocytosis of
intravenously injected particles tagged with radioactive isotopes, usually $^{51}$Cr or $^{99m}$Tc. The isotope may be incorporated into sulphur colloidal particles, in which case both the spleen and the liver will be delineated, or it may be incorporated into autologous heat-damaged erythrocytes, and in this case only the spleen will be demonstrable, provided the cells are not too extensively damaged by heating. The uptake of the sulphur colloid by the spleen is almost immediate, but the uptake of damaged erythrocytes is slower and the rate of clearance from circulation and accumulation into the spleen is an additional measure of function. The latter method was employed in the present study together with quantification of erythrocyte abnormalities to evaluate the phagocytic function of the spleen in sickle-cell diseases.

The results of the present study showed considerable variations in both spleen size and function in sickle-cell diseases. Whereas splenic volume and function of the AS heterozygotes were similar to those of normal controls, the 20 symptomatic subjects with sickle-cell anaemia, S/β²-thalassaemia or SC disease showed three patterns of abnormalities: (a) an enlarged hyperactive spleen, (b) an enlarged hypoactive spleen or (c) asplenia.

In five of the seven SS homozygous patients the spleen was not demonstrable by scintillation scanning of the abdomen, and the other parameters of splenic function were similar to those for the splenectomized patients. These findings agree with previously published reports [4, 8, 11–13, 21]. However, two patients with sickle-cell anaemia had splenomegaly and hyperfunction similar to patients with hereditary spherocytosis and thalassaemia.

The proportion of patients with splenomegaly is larger among those with S/β-thalassaemia than among SS homozygotes [9, 10]. Studies of splenic function in patients with S/β-thalassaemia are rare. Discordant results have been reported for seven patients by Pearson et al. [8] and by Sills & Oski [21], but the type of thalassaemia gene, i.e. β⁰ or β⁺, was not reported by either group. Splenic scans with $^{99m}$Tc-sulphur colloid were normal in five other cases [4, 13]. All 10 patients in the present study had S/β¹-thalassaemia. Therefore they do not produce HbA, and thus have a form of disease which is more severe than S/β⁺-thalassaemia. The association of pit counts and quantitative measurement of the removal of heat-damaged erythrocytes showed that splenic function is heterogeneous in patients with S/β¹-thalassaemia. In half the cases no splenic phagocytic activity was detected. On the other hand, among the patients with splenomegaly there were cases with hyperactive spleens and others with hypoactive spleen. This demonstrates that asplenia occurs not only in sickle-cell anaemia but in S/β⁰-thalassaemia as well, and suggests that the functional impairment precedes the anatomical regression. This suggestion is supported by the fact that the hypoactive spleens of these patients, which were palpable 7 and 9 cm below the costal margin when the test was carried out, were not detected on physical examination 1 year later.

All three patients with SC disease had splenomegaly and the spleen was hyperactive in one and hypoactive in two. Abnormal splenic scans have been found in four of the eight cases previously reported in the literature [11, 13], and in one case the abnormality was transient [22]. Also pit counts were abnormal in all six cases previously described [11, 21]. In three cases the counts were very high, similar to those in splenectomized patients, and in the remaining three the values were intermediate as in our two cases with hypoactive spleens. No case with hyperactive spleen has been documented in the literature. Actually, the methods used thus far to study splenic function in sickle-cell diseases, namely splenic scanning with sulphur-colloid $^{99m}$Tc and pit counts, are not appropriate for the detection of hyperfunction of the organ.

This study differs from others in the literature because all patients were studied by both morphological and functional quantitative methods, which permit the detection of an increase, a decrease or the absence of splenic function. It also includes a homogeneous group of patients with S/β-tw-thalassaemia which is the largest studied thus far.

Our results and those reported in the literature show a similar spectrum of splenic function in patients with sickle-cell anaemia, S/β²-thalassaemia and SC disease. The lower age of patients with a hyperactive spleen as compared with those with asplenia, and the observation of anatomical regression of enlarged spleens, would suggest an evolution of the organ through successive functional changes, starting with enlargement and hyperactivity in the early years of life, which would be followed by hypoactivity and finally atrophy. The evolution seems to be faster in sickle-cell anaemia than in S/β²-thalassaemia and SC disease. This slower progression to atrophy could be the result of a reduced tendency to sickling in vivo. The lower percentage of irreversibly sickled cells in patients with S/β²-thalassaemia and SC disease is in accordance with this view.

Acknowledgment

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References


