Splenectomy in Idiopathic Thrombocytopenic Purpura: Its Correlation with the Sequestration of Autologous Indium-111-Labeled Platelets

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We present a retrospective analysis of ^{111}In-platelet sequestration studies in 111 patients with the clinical diagnosis of idiopathic thrombocytopenic purpura (ITP). Fifty-one of these patients underwent splenectomy, independent of the results of the ^{111}In-platelet studies to determine if these isotopic results could accurately predict a beneficial response to splenectomy. Between January 1984 and June 1990, 111 patients who presented with ITP were subjected to a study of autologous ^{111}In-labeled platelets through autotransfusion. The platelet sequestration site was splenic (81%), mixed (12%), or hepatic (7%). Fifty-one patients with persistent drug-resistant thrombocytopenia underwent splenectomy regardless of the isotopic results: 33 patients beyond 6 mo after diagnosis and 18 with high hemorrhagic risks before this delay. The follow-up median duration was 2.9 yr. Thirty-three of the 38 patients with splenic sequestration showed a normalized platelet count, as opposed to 2 of the 13 with mixed or hepatic sequestration ($p < 0.001$). In addition, platelet survival extended beyond 8 days in six patients, with no apparent sequestration site. The platelet isotopic study performed with this technique appears to be indicated in ITP: it guides clinicians in their decision to perform splenectomy and relates to a more central mechanism certain thrombocytopenias that are inappropriately categorized as ITP.

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Splenectomy is seen as a therapeutic means to increase the incidence of definitive remission in patients with idiopathic thrombocytopenic purpura (ITP) (*1*). A correlation between the platelet sequestration site and the outcome of splenectomy is controversial. Usually, the splenectomy is restricted to chronic ITP, as characterized by persistent thrombocytopenia (platelet count $< 50 \times 10^9$/l for more than 6 mo) and to some severe or drug-resistant forms. The overall effectiveness after a 6-mo minimum follow-up has been evaluated as 72.2% (*2*), although primary failure or relapse are always possible (*2,3*). The predictive factors of short- and long-term success of splenectomy are still ill-defined and questioned. The lifespan and the sequestration site of ^{111}In-labeled autologous platelets was determined in a retrospective survey involving 111 patients. The predictive value of this examination was analyzed in a population of 51 splenectomized patients.

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MATERIALS AND METHODS

Patients

Between January 1984 and June 1990, 111 patients (see Table 1 for patient distribution) were classified as meeting the classically accepted ITP criteria (*4*):

- Isolated thrombocytopenia (platelets $< 50 \times 10^9$/l) with maintained leukocyte and erythrocyte lines, bone marrow aspirate with no quantitative cytological abnormality and normal or elevated megakaryocyte count.
- No clinical or echographic splenomegaly.
- No evidence of any situations that could be associated with thrombocytopenia: drugs, viruses (CMV, HBs, HIV, EBV), systemic lupus erythematosis, leukemia, lymphoma or disseminated intravascular coagulation.
- No evidence of hereditary abnormality.

One hundred and one patients presented with hemorrhagic signs at diagnosis. Thrombocytopenia was discovered from systematic blood counts in 10 asymptomatic patients. In all cases, thrombocytopenia was confirmed at several-day intervals, and in asymptomatic subjects (absence of purpura, ecchymosis, etc...) it was ascertained using certain anticoagulants (EDTA, citrate, heparin) in order to avoid platelet autoaggregation as a cause of apparent thrombocytopenia. Anti-platelet-antibody IgG (PA IgG) measurements performed in 94 patients were positive in 60% of cases. Of the 111 patients, 15 were followed without treatment, 3 received androgen alone (danazol) and 93 were treated with prednisone at 0.25 to 2 mg/kg/day for at least 30 days (Fig. 1). Primary failures (no increase in platelet count above 50×10^9/liter) and relapses led to one of the following treatments: resumption of steroid therapy, danazol, vincristine or high-dose immunoglobulins (400 mg/kg/day for 5 days). Persistence of severe and drug-resistant thrombocytopenia beyond 6 mo after diagnosis led to splenectomy in 33 patients. This period was shortened for 18 patients at risk, i.e., those with hypertension, over 65 yr old

TABLE 1
Distribution of Patients as to Age and Sex

	Number	Age 16–30 yr	30–50 yr	50–70 yr	>70 yr
Women	66	18	17	24	7
Men	45	11	7	19	8
Total	111	29	24	43	15

or in the presence of life-threatening thrombocytopenia (<20 × 10^9/liter).

Two late relapsing patients underwent splenectomy 60 and 100 mo after initial diagnosis. The median diagnosis to surgery lag period was 6.5 mo for the other 49 patients. Eighteen were operated on within 6 mo after diagnosis. The median follow-up period after surgery was 2.9 yr (4–72 mo). Response to splenectomy was assessed by platelet counts in the third postoperative month:

- Failure = platelet count <50 × 10^9/liter.
- Partial Remission = platelet count between 50 and 150 × 10^9/liter.
- Complete Remission = platelet count > 150 × 10^9/liter.

Relapses were characterized by platelet counts falling below 50 10^9/liter after the third postoperative month when initial response was either full or partial.

Isotopic Study and Platelet Kinetics

All 111 patients underwent autologous platelet isotopic study as an inclusion criterion in this study. Platelet counts were between 10 × 10^9/liter and 60 × 10^9/liter at the time of the trial, and patients were individually stable. Platelet lifespan was measured in 95 patients in the absence of treatment, either at the time of diagnosis, upon relapse or after failure of the medical treatments implemented. The other 16 patients were treated medically (prednisone, danazol). The labeling procedure was previously described in detail in the ICSH recommendations (*5*) based on the method of Thakur et al. (*6*). Platelet-rich plasma (PRP) was obtained from blood samples (42 ml blood, 8 ml ACDA) after 1 or 2 slow centrifugations (150 g, 15 min). A platelet button was isolated from PRP after rapid centrifugation (900 g, 20 min). Three to 5 MBq of ^{111}In-oxine were added to this platelet button which had been preserved in a small volume of plasma (<1 ml). After incubation for 5 min, the platelet solution was washed with platelet-poor plasma (PPP). Label efficiency averaged 68% (±18%).

Survival time was determined from platelet counts in 5–10 blood samples from 15 min after injection until radioactivity had totally disappeared from the blood. The method was tested on six patient volunteers, whose normal platelet lifespan was 8.7 ± 0.8 days using a linear model. During the first 30 min following the injection, the amount of ^{111}In radioactivity in the liver, spleen and heart were measured from the posterior view with a wide angle gamma camera peaked for 173 and 247 keV and interfaced with a computer. Thirty minutes after injection and daily thereafter, the organs were visualized and the counts were computed. The splenic platelet pool (SPP) was measured from the splenic activity at 30 min in relation to the total activity injected. The depth of the spleen was computed from the lateral view. The following S and L ratios calculated each day were used to assess late sequestration:

- S represented late splenic sequestration equal to the radioactivity of the spleen of the last day in relation to the radioactivity of the spleen at 30 min.
- L represented late liver sequestration, equal to the ratio of radioactivity of the liver on the last day in relation to the radioactivity of the liver at 30 min.

Late sequestration was considered normal when S and L: 1 ± 0.2 Late sequestration was considered "splenic" when S > 1.2, "hepatic" when L > 1.2, and "mixed" when S and L > 1.2. The values used in the ratios were corrected for physical decay. Platelet recovery was computed for each patient. Platelet turnover was computed by a standard formula (*7*) as follows:

$$\text{Platelets}/\mu\text{l}/\text{hr} = \frac{\text{platelet count (per } \mu\text{l)}}{\text{lifespan (hr)}} \times \frac{90\%}{\text{initial recovery}}.$$

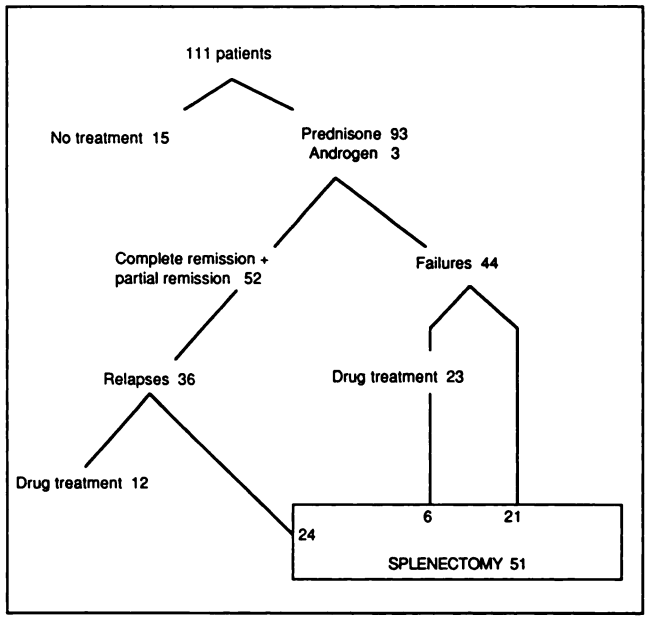

FIGURE 1. Treatment protocol.

RESULTS

Platelet Kinetics

The lifespan of ^{111}In-labeled autologous platelets was shortened by 1 to 4 days in 105 of the 111 tests performed (Table 2). In contrast, normal 8–9-day lifespan and concomitant absence of noticeable sequestration sites corrected the initial ITP diagnosis in six patients. With a

TABLE 2
Results of Lifespan of ^{111}In-Labeled Autologous Platelets

Patients	Lifespan (days) 1	2	3	4	>8
Splenectomized patients (n = 51)	9	16	11	15	0
Nonsplenectomized patients (n = 60)	9	13	18	14	6

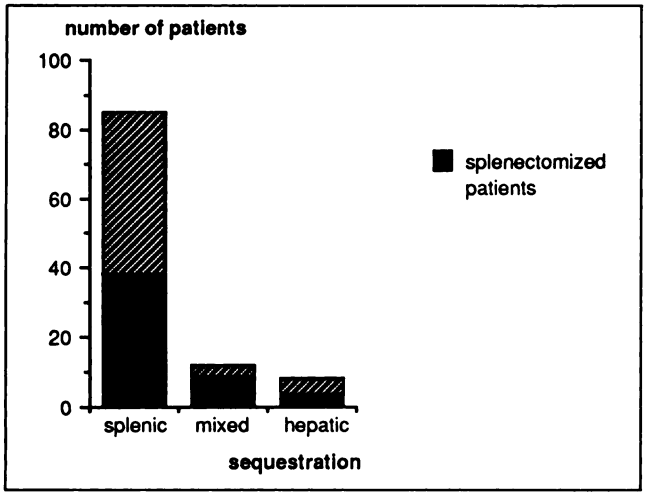

FIGURE 2. Result of sequestration site of 105 patients with shortened lifespan platelets.

follow-up of 6 yr, these six patients with nongenetic thrombocytopenia had stable platelet counts (<50 × 10^9/liter) without development of myelodysplasia.

The sequestration site in the other 105 patients appeared splenic (85/105; 81%), mixed (12/105; 12%), or hepatic (8/105; 7%) (Fig. 2). The values of sequestration ratios are summarized in Table 3.

Correlation with Splenectomy

In this series, the indication for splenectomy did not take into account the respective sites of isotopic sequestration (Table 4). The overall proportion of complete remission was 68.6%. Among the 38 splenic sequestration cases, 33 complete remissions, 1 partial remission stable after 3 yr and 4 failures were recorded with a median follow-up of 2.9 yr; one relapse was recorded during pregnancy after a 15-mo remission. Among the 13 cases of mixed or hepatic sequestration, 11 failures were recorded ($p < 0.001$). The four patients with hepatic sequestration had splenectomy failure. There was no correlation between shortened platelet survival and the results of the splenectomy.

Turnover

The turnover rate was not statistically different between the responder (mean value 1142 ± 155 platelets/μl/hr) and the nonresponder patients to splenectomy (mean value 1240 ± 188 platelets/μl/hr) (p = n.s.).

TABLE 3
Results of Sequestration Ratio (105 Patients with Shortened Lifespan Platelets)

Sequestration site	Patients (n = 105)	Sequestration ratio* Spleen (S)	Liver (L)
Splenic	85	3 (1.5)	0.9 (0.2)
Mixed	12	1.8 (0.8)	1.6 (0.7)
Hepatic	8	1 (0.2)	2.2 (0.5)

* Mean ± 1 s.d.

TABLE 4
Outcome 3 Months after Splenectomy (51 Patients)

Sequestration site	Number	Complete remission	Partial remission	Failures
Splenic	38	33	1	4
Mixed	9	2	0	7
Hepatic	4	0	0	4
Platelet counts 10^9/liter*		322 (110)	85	32 (9)

* Mean ± 1 s.d.

DISCUSSION

The incidence of relapses or medical treatment failures observed in adult ITP gives an idea of the frequency at which splenectomy may be indicated, 48% in our 105 patient population. A number of studies seeking predictive factors of effectiveness yielded conflicting results; subjects' young age (*8–11*) and the quality of initial response to steroid therapy (*10,12*) are considered to be favorable factors among others. The interest of isotopic studies of platelet kinetics remains a subject of debate. It is probably due to the labeling technique (^{51}Cr-sodium chromate or ^{111}In-oxine or tropolone) and the different methods used for monitoring platelet sequestration. Some reports have indicated their value in obtaining total remission, depending on the sequestration site (*11,13–17*). Other studies contradict this conclusion (*18–21*).

This study focused on analyzing the predictive value of autologous platelet kinetics after ^{111}In-labeling. This is a retrospective, single center series that is homogeneous in terms of diagnosis, therapeutical protocol and isotopic techniques. Relapses and splenectomy failures appeared to be unlikely when splenic sequestration was observed (4/38). There were no differences in the splenic ratio between these four patients and the responders to splenectomy, respectively S = 2.8 (±0.5) versus S = 3.2 (±1.4) (p = ns). Conversely, the proportion of failures appeared to be larger (11/13) when mixed or hepatic sequestration was found ($p < 0.001$).

Table 5 shows the main series in which a correlation between the results of platelet isotopic studies and those of splenectomy in ITP was investigated. Platelet kinetic studies with ^{51}Cr can only be performed in autologous transfusion when thrombocytopenia is moderate (platelets >50 × 10^9/l), leading most often to exploration with donor platelets (and the risk of isoimmunization and viral transmission). With ^{51}Cr, however, the labeling efficiency is low (<15%) and the activity injected and its physical properties do not permit biodistribution studies with a gamma camera. Generally, external counts are performed with a probe which preclude quantitative measurements of the uptake in the spleen, liver or heart. As early as 1976, platelet

TABLE 5
Results of the Other Series Analyzing the Correlation of Sequestration Sites with Effectiveness of Splenectomy

No. of patients	Method (isologous platelets vs. autologous platelets)	Splenectomized patients	Duration of assessment (mo)	%Complete remission (CR) according to the sequestration site			Reference
				Splenic	Mixed	Hepatic	
563	^{51}Cr:iso	206	≥6	81	38	9	14
34	^{51}Cr:iso	34	3–56	92	87		19
197	^{51}Cr:iso:164	111	≥12	80		45	13
181	^{51}Cr:iso:133 ^{111}In:48	181	≥3	83		42	11
59	^{111}In:auto	21	<1	60	66	66	18
222	^{111}In:auto	103	6	86	55	7	15
96	^{111}In:auto:78 iso:18	36	?	CR 29/36 6/7 failures without splenic sequestration			16

kinetics were studied by labeling with a liposoluble marker, ^{111}In-oxine (*6*) or tropolonate (*22*). There are many advantages to this method: kinetic studies with autotransfusion, even in the presence of severe thrombocytopenia (platelets 10×10^9/liter), high labeling efficiency (50%–90%) and the ability to perform a biodistribution study with a gamma camera.

Authors agree on using a gamma camera to study platelet sequestration, but the measuring methods still vary. An absolute quantitation according to the dose injected appears more satisfying, but more difficult to perform in a routine hospital setting (*23*). In our study, we evaluated the sequestration of ^{111}In-labeled platelets by examining the activity ratios of the spleen and liver on each day of the study in relation to the activity recorded in the early dynamic stages, i.e., from 25 to 30 min visualization of the hepatic and splenic areas of interest is easier.

The results from different centers on the study of sequestration sites appear to be somewhat contradictory. The spleen appeared to be the preferential site of platelet sequestration, although in variable proportions (29%–76%) (*16,19*). Hepatic sequestration was found in at least 20% of cases. The sequestration site is not always related to the spleen or liver (diffuse or undetermined) in significant proportions of patients (up to 28%) (*16*). This seems surprising since this parameter could always be assessed in a population of 246 ITP patients studied according to our protocol (*24*). A correlation seemed to appear between the type of sequestration and the results of splenectomy in five of seven studies. In Ries' study (*19*), none of the 34 splenectomized patients had hepatic sequestration. In Siegel's study (*18*), 5 of 21 patients had normal or slightly reduced platelet lifespan (>6.5 days): four of these were splenectomy failures. In addition, the results of splenectomy were assessed very early (4–6 wk after surgery) and relapse incidence could not be evaluated.

The predictive value of the increase in platelet turnover reported by Siegel was not evident in our study. This parameter has to be analyzed according to the medical treatment under way at the time of the study, especially prednisone (14 of 21 patients in Siegel's series), since this therapy may influence the rate of platelet production (*17*).

In our series, a group of six patients who satisfied the initial ITP criteria differentiated itself by the existence of normal platelet kinetics: the number of megakaryocytes was normal, even slightly increased, in bone marrow with no signs of myelodysplasia. Direct antiglobulin test on platelets was positive in two cases. No hemorrhagic complication was recorded after a substantial follow-up period (over 6 yr), and thrombocytopenia was stable in these six patients. There was no familial history of thrombocytopenia. In a retrospective analysis of 3600 platelet kinetic studies in ITP, Najean reported 54 cases of thrombocytopenia apparently genetically determined in a dominant autosomal manner (*25*). The risk of acute leukemia in the study appeared to be high, 4 of 54 patients. Thrombocytopenia in our six patients could be linked to a mechanism different from genetic or a platelet production defect: the results of the isotopic study performed after failure of steroid therapy and/or danatrol treatment led us to rule out splenectomy as a treatment likely to succeed.

During ITP, studying the lifespan of ^{111}In-labeled platelets, as described in this work, appears very useful on two counts. First, this study shows that the thrombocytopenias, that still meet the usual clinical and biological criteria of ITP are not necessarily caused by excessive destruction. Second, it guides clinicians in deciding whether or not splenectomy should be performed, as chances of success are greatly reduced in cases of mixed or hepatic sequestration.

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