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Originals

Influence of Splenectomy on Platelet Morphometry and Function

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Summary. Functional and morphometric platelet abnormalities may be influenced by splenectomy and thus contribute to postoperative thrombohaemorrhagic complications, especially in patients with splenomegaly and/or platelet defects. We investigated platelet function, platelet secretion, and platelet morphometry before and one week after splenectomy in seven patients with normal platelet production and normal spleen size (Hodgkin's disease) and five patients with splenomegaly and platelet abnormalities (4 with myeloproliferative disorders and 1 with chronic myelomonocytic leukemia).

Severe postoperative thrombohaemorrhagic complications occurred only in patients with myeloproliferative disorders, although platelet count and mean platelet volume increased in almost all patients after splenectomy. Four patients with myeloproliferative disorders had impaired platelet aggregation before splenectomy that improved in only one patient after surgery. Platelet buoyant density in this patient group was decreased before splenectomy and normalised thereafter. Concomitantly, intraplatelet concentrations of α-granular proteins increased. Before splenectomy, there was a positive correlation between platelet density and platelet volume in patients with Hodgkin's disease ($r=0.59$, $p<0.001$), but not in patients with myeloproliferative disorders. There was no correlation between platelet density and platelet volume after splenectomy in either patient group.

In conclusion, morphometric platelet abnormalities were found in all patients after splenectomy. In patients with myeloproliferative/myelodysplastic disorders, decreased platelet buoyant density normalised and intraplatelet concentrations of α-granule proteins were elevated after splenectomy. However, platelet function defects in this patient group were not corrected and may have been a major cause of thrombohaemorrhagic complications in the postoperative period.

Key words: Splenectomy – Platelet defects – Myeloproliferative disorders – Hodgkin's disease

Thrombocytosis after splenectomy has been regarded as a sign of predisposition to postoperative thrombotic complications. However, analysis of large patient groups has failed to demonstrate a significant association between postsplenectomy thrombocytosis and thromboembolism [3]. Although this may be true for the majority of patients, there is evidence that in certain disease states (e.g., myeloproliferative disorders) pre-existing platelet defects and high platelet mass may facilitate the development of thrombohaemorrhagic complications [8, 16]. Additional changes in platelet function and platelet morphology may be caused by splenectomy through loss of the splenic platelet pool or by a hypothetical regulatory function of the spleen on megakaryocytopoiesis. Because the splenic platelet pool depends on the size of the spleen, alterations of platelet function and morphology after splenectomy may be more pronounced in patients with splenomegaly [14].

The aim of this study was to determine whether

Abbreviations: ABVD = Adriamycin, bleomycin, vinblastine, dacarbazine; ADP = Adenosine diphosphate; Ara-C = Cytosine arabinoside; BS = Busulfan; CML = Chronic myelogenous leukemia; CMML = Chronic myelomonocytic leukemia; COPP = Cyclophosphamide, vincristine, procarbazine, prednisone; HD = Hodgkin's disease; HU = Hydroxyurea; MDS = Myelodysplastic syndrome; MOPP = Nitrogen mustard, vincristine, procarbazine, prednisone; MPD = Myeloproliferative disorders; MPV = Mean platelet volume; PF4 = Platelet factor 4; PRP = Platelet rich plasma; PV = Polycythemia vera; βTG = β-thromboglobulin

alterations in platelet morphometry and function were caused by splenectomy, and whether pre-existing platelet abnormalities were improved or aggravated. We investigated parameters of in vivo platelet α-granule secretion, platelet in vitro function, platelet volume, and platelet buoyant density in seven patients with Hodgkin's disease (HD), four with myeloproliferative disorders (MPD), and one with chronic myelomonocytic leukemia (CMML), before and after splenectomy.

Methods

Patients and Control Subjects

Clinical patient data are summarised in Table 1. In the group with Hodgkin's disease, five previously untreated patients were splenectomised during staging laparotomy. One patient had been treated with chemotherapy 4 years before surgery and one with chemotherapy and radiation 2 years before splenectomy. Both were splenectomised because of splenic relapse. Three of seven spleens were infiltrated by lymphoma and one was slightly enlarged.

Three patients with Philadelphia-chromosome-positive chronic myelogenous leukemia (CML) in the accelerated disease phase, one with osteomyelofibrosis secondary to polycythemia rubra vera (PV), and one with chronic myelomonocytic leukemia (CMML) were subjected to splenectomy because of high transfusion requirements due to hypersplenism and pain in the upper left abdomen. Two patients with CML and the patient with CMML were treated with cytosine arabinoside 4 weeks before splenectomy, and one CML patient (H.R.) was on continuous therapy with hydroxyurea until one week before surgery. Four patients in the HD group were treated with low-dose heparin and one with aspirin after splenectomy. One patient in the MPD group (W.K.) was on aspirin at the time of postoperative investigation but not at the time of postoperative bleeding. Platelet aggregation data under aspirin were not included in the evaluation.

Sixteen healthy subjects, mostly from the hospital staff, served as the control group. Control subjects had not taken platelet-inhibitory drugs in the two weeks prior to the investigation.

Platelet Count

A blood count was routinely obtained using the Coulter Counter S plus.

Table 1. Clinical data of splenectomised patients

	HD	MPD/CMML
Number of patients	7	5
Sex	6 male, 1 female	2 male, 3 female
Median age	27	51
Pretreatment	5 × none 1 × MOPP 6 cycles 1 × COPP/ABVD 3 cycles and radiation	1 × Ara-C 1 × HU/Ara-C 1 × BS/HU 1 × BS/HU/Ara-C 1 × venesection
Complications after surgery	4 × none 3 × fever	1 × bleeding upper left abdomen 1 × nasopharyngeal bleeding and bleeding upper left abdomen 1 × bleeding upper left abdomen and right thigh, thrombosis V. axillaris 1 × abdominal bleeding, pneumonia 1 × ileus, sepsis
Spleen weight	6 × < 300 g 1 × 450 g	Median: 3000 g (range: 2000–3300 g)
Spleen infiltrated	3	5

Abbreviations: HD = Hodgkin's disease; MPD = Myeloproliferative disorders; CMML = Chronic myelomonocytic leukemia; Ara-C = cytosine arabinoside; BS = busulfan; HU = hydroxyurea; MOPP = nitrogen mustard, vincristine, procarbazine, prednisone; COPP = cyclophosphamide, vincristine, procarbazine, prednisone; ABVD = adriamycin, bleomycin, vinblastine, dacarbazine

Platelet Aggregation

Aggregation was performed according to the method of Born [2] in citrated blood (0.38%) using a Chrono Log aggregometer (Model 530, Havertown, Pa, USA). Agonists used were ADP 10^{-6} M, collagen 2 μg/ml, and adrenaline 10^{-4} M. Blood was centrifuged at 120 g for 10 min to obtain platelet rich plasma (PRP), and PRP was adjusted to a platelet count of 250×10^9/l with autologous platelet poor plasma (centrifugation for 15 min at 2200 g). Results were expressed as percent of maximal aggregation response.

Platelet Retention

Retention to glass beads was performed as described by Hellem [6] using commercially available glass bead columns (Adeplat S test, Semmelweis, Milano, Italy). Retention was expressed in percent of platelets passing through the column.

Platelet α-Granule Secretion

Plasma levels of β-thromboglobulin (βTG) and platelet factor 4 (PF4) were determined by radioimmunoassay as previously described [10]. In addition, the intraplatelet concentration of these proteins was measured after lysis of platelets with Triton X 100 (1% final concentration). Before lysis, platelet number in PRP was counted, and platelet concentrations of βTG and PF4 were expressed as $\mu g/10^9$ platelets.

Platelet Volume Distribution

Platelet volume was measured in whole blood and in samples obtained from the density gradients using the impedance method. Platelet sizing was performed by the Ultra Flo 100 (Becton Dickinson), and the signals were linearly amplified, digitized, and evaluated with a microcomputer. Mean platelet volume (MPV) was calculated from the distribution between 1.2 and 22 fl. For determination in whole blood, platelet volume was stabilised using glutaraldehyde 0.125% in addition to citrate (0.38%) as previously described [18].

Platelet Density Centrifugation

For determination of the platelet density distribution, we employed the method of Martin et al. [9]. In summary, blood was centrifuged over a continuous, iso-osmotic Percoll gradient to separate platelets from the other blood constituents (recovery rate: $94 \pm 12\%$). The platelet layer was then transferred to another, linear Percoll gradient covering the density range from 1.030 to 1.080 g/cm^3. Platelet density equilibrium centrifugation was carried out (2800 g for 90 min) in a swing out rotor (Sorvall centrifuge RC-3B). The gradient was fractionated, and platelet count and MPV were determined from each of 21 fractions. Marker beads of known density were used to check the linearity of the gradients. Percoll and coloured density marker beads were obtained from Pharmacia (Uppsala, Sweden).

Results

Complications after Surgery

All removed organs in the MPD/CMML group were massively enlarged and infiltrated. Four of five patients had serious bleeding complications necessitating corrective surgery in three patients. One CML patient (H.R.) was re-operated the day after splenectomy and was found to be bleeding from a small pancreatic vessel. He needed 13 blood units in the postoperative period, and died in blast crisis 5 months later. Another patient with CML (W.-R.R). bled from a small mesenteric vessel following drain removal 6 days after splenectomy. He needed 17 units of blood and developed pneumonia two weeks later. The third CML patient (W.K.) was re-operated the day after splenectomy because of diffuse abdominal bleeding. He received 18 blood units over the two days following splenectomy. Two weeks later he developed thrombosis of the left axillary vein at the location of an intravenous catheter. After another three weeks he suffered a massive hematoma in the right thigh. He died 4 months after surgery from intracerebral bleeding and was shown at autopsy to have multiple pulmonary emboli. The patient with PV suffered from severe nasopharyngeal bleeding during the operation and developed a left subdiaphragmatic hematoma, but was not re-operated because of the bleeding tendency. She needed 16 blood units in 3 days. The patient with CMML developed septicemia with a paralytic ileus on the third postoperative day. Among the patients with Hodgkin's disease there were three episodes of fever of unknown origin in the postoperative period but no serious complications (Table 1).

Platelet Function and Platelet Secretion

After splenectomy, ADP-induced aggregation increased in the HD group, but aggregation with the other agonists was largely unchanged. In patients with MPD/CMML, platelet aggregation defects in response to all agonists increased in two patients after splenectomy, and improved in one patient. Despite high postoperative platelet counts, platelet retention to glass beads decreased slightly in both the HD and MPD group (Table 2).

Plasma levels of α-granule proteins were higher in seven patients after splenectomy. In MPD patients, there was an increase in the intraplatelet concentrations of α-granule proteins after surgery.

Platelet Size and Platelet Density

After splenectomy, the platelet count was elevated in both patient groups, and mean platelet volume increased in MPD patients (Table 2).

The platelet density distribution was unimodal and symmetrical in all patients. Whereas patients with HD had almost identical platelet density distributions before and after splenectomy (Fig. 1), platelet density in the MPD/CMML group was

Table 2. Platelet parameters (1) before and (2) after splenectomy

	HD ($n=7$)		MPD/CMML ($n=5$)		Control subjects ($n=16$)
	1	2	1	2	
Platelet count (10^9/l)	264 ±107	555 ±200[b]	274 ±196	508 ±478	257 ±73
Mean platelet volume (fl)	6.7± 1.2	7.1± 0.9	7.1± 0.7	8.4± 1.0[a]	6.4± 0.8
βTG plasma (ng/ml)	44 ± 18	58 ± 34	88 ± 55	105 ± 27	27 ± 9
PF4 plasma (ng/ml)	11 ± 6	19 ± 8[a]	19 ± 15	56 ± 45[a]	7 ± 3
βTG platelet (μg/10^9)	37 ± 11	34 ± 10	29 ± 13	43 ± 18[a]	31 ± 9
PF4 platelet (μg/10^9)	13 ± 6	11 ± 2	12 ± 2	17 ± 5[a]	13 ± 5
Aggregation ADP 10^{-6} M (%)	24 ± 27	37 ± 26[a]	16 ± 16	10 ± 10	47 ±29
Aggregation collagen 2 μg/ml (%)	87 ± 11	77 ± 22	31 ± 30	8 ± 7	73 ±16
Aggregation adrenaline 10^{-4} M (%)	44 ± 40	40 ± 39	42 ± 27	27 ± 19	51 ±31
Retention (%)	77 ± 21	66 ± 9	68 ± 30	55 ± 22	76 ±12

Abbreviations: HD = Hodgkin's disease; MPD = Myeloproliferative disorders; CMML = Chronic myelomonocytic leukemia; βTG = β-thromboglobulin; PF4 = Platelet factor 4. All values are given as Mean ± 1 SD.
[a] $p<0.05$,
[b] $p<0.01$ (paired Student's t-test)

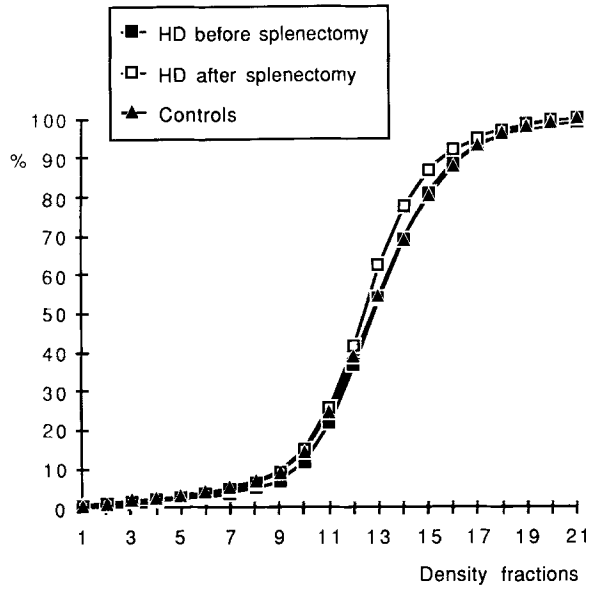

Fig. 1. Cumulative mean platelet buoyant density before and after splenectomy in patients with Hodgkin's disease (HD, $n=7$) in relation to control subjects ($n=16$)

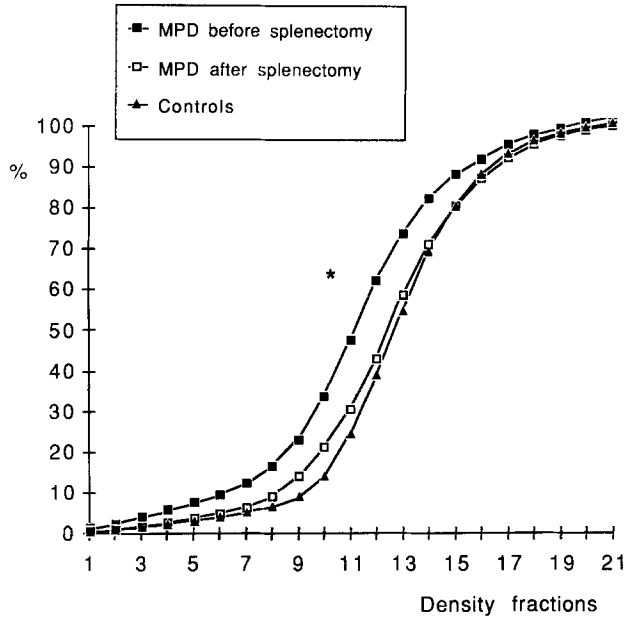

Fig. 2. Cumulative mean platelet buoyant density before and after splenectomy (* $p<0.001$, paired Student's t-test) in patients with myeloproliferative disorders and chronic myelomonocytic leukemia (MPD, $n=5$) in relation to control subjects ($n=16$)

low before, and normalised after splenectomy (Fig. 2).

Before splenectomy, platelet volume was correlated to platelet density (density fractions 11–16, $r=0.59$, $p<0.001$) in HD patients. However, there was no such correlation after splenectomy, and platelet volume was higher than before in all density fractions. One HD patient was tested relatively late after splenectomy (32 days). In this patient, the correlation between platelet density and platelet volume was comparable to the pre-splenectomy state, although platelet volume was increased. In the MPD/CMML group, platelet volume was not related to density in 2/5 patients before splenectomy, and there was no correlation between the two parameters after surgery.

Platelet Parameters in Patients with Bleeding Complications

Platelet count was elevated in only one patient after splenectomy, but mean platelet volume in-

Table 3. Platelet parameters in patients with bleeding complications (1) before and (2) after splenectomy

Patient, Disorder	E.F., PV		W.K., CML		W-R.R., CML		H.R., CML	
	1	2	1	2*	1	2	1	2
Platelet count (10^9/l)	336	195	73	347	625	1184	130	145
Mean platelet volume (fl)	7.07	9.6	7.57	8.09	5.78	7.02	7.44	7.55
βTG plasma (ng/ml)	90	81	36	78	179	145	110	128
PF4 plasma (ng/ml)	16	47	2	16	40	124	32	87
βTG platelet (µg/10^9)	–	41	29	39	46	63	32	60
PF4 platelet (µg/10^9)	12	23	12	18	14	18	14	16
Aggregation ADP 10^{-6} M (%)	0	5	13	64	25	0	0	–
Aggregation collagen 2 µg/ml (%)	0	16	34	74	80	0	0	–
Aggregation adrenaline 10^{-4} M (%)	57	54	15	78	88	13	20	–
Retention (%)	95	85	60	–	94	49	–	31

* patient was on acetylsalicylic acid (100 mg) at the time of re-investigation

creased in all four patients (Table 3). Platelet density was still low after splenectomy in the patient with PV but it had normalised in the other patients. Intraplatelet concentrations of α-granular proteins increased in all patients. Secretion of β-thromboglobulin remained unchanged after splenectomy, whereas platelet factor 4 levels increased. There were no clear-cut changes in platelet aggregation; while response to all inducing agents decreased in patient W.-R.R, it improved in patient W.K. despite low-dose ingestion of acetylsalicylic acid.

Discussion

Bleeding complications after splenectomy were observed only in patients with MPD in this study. All 4 MPD patients were in an advanced stage of their disease (they had either developed myelofibrosis or were in the accelerated phase), and three of them had platelet hypofunction in vitro before surgery. Splenectomy resulted in improved platelet function in only one of these patients. Brenner et al. [4] also observed impaired platelet aggregation persisting after splenectomy in a group of patients with myelofibrosis. We found increased PF4 plasma levels after splenectomy that may partly be due to platelet activation in the early postoperative state of the patients, as abdominal drains and intravenous lines had not been removed in most cases. In contrast, the increase in intraplatelet α-granule concentrations we observed was probably due to an alteration in the properties of the circulating platelet population. This was also reflected by the postoperative normalisation of platelet density in 4 of 5 patients in the MPD/CMML group. It is known from the work of Vicic and Weiss [13] and van Oost et al. [12] that α-granules are a major determinant of platelet density. Although low platelet density is a characteristic feature of MPD [7, 17], it may be the result of either a dense granule storage pool defect, or reduced α-granules, or both. Labelling experiments by Watson and Ludlam [15] have sugested that high-density platelets are preferentially retained in the spleen. This effect may be increased in patients with hypersplenism. In our study, patients with normal spleen size and normal megakaryocytopoiesis (as in the HD group) did not show changes in platelet density after splenectomy.

A correlation between platelet density and platelet volume is characteristic of a normal platelet population [5]. We found this correlation in all patients with HD before splenectomy. However, it is not always expressed in patients with advanced myeloproliferative disorders [17]. In addition to the advanced disease state, chemotherapy with cytosine arabinoside or hydroxyurea in the four weeks prior to the investigation could explain the altered platelet density/volume relation in four patients in this study. After splenectomy, there was no correlation between platelet density and platelet size in either patient group except in one HD patient who was reinvestigated 32 days postoperatively. This may indicate a disturbed equilibrium of platelet subpopulations soon after splenectomy with subsequent normalisation of the density/volume relation. It cannot be concluded from these data whether the changes in platelet subpopulations are a direct consequence of splenectomy or of a regulatory mechanism in platelet production. Tanum et al. [11] observed that megakaryocyte DNA content and platelet number increased after both splenectomy and sham operation in a rat model, and slowly returned to normal within 30 days after surgery. They concluded that these were reactive changes due to the stress of the operation. How-

ever, the changes in both megakaryocyte DNA content and platelet number were more pronounced following splenectomy than after sham operation. In addition, when total or partial splenectomy was performed in a murine model [1], the postoperative increase in the platelet count was higher in completely splenectomised animals. These findings suggest that aside from the operative procedure the removal of the spleen may directly contribute to the changes in the circulating platelet population.

In summary, splenectomy has a profound influence on both platelet number and platelet morphometry. Patients with splenomegaly due to myeloproliferative disorders may have normalised platelet density and increased storage of α-granule proteins after splenectomy, whereas these parameters did not change in patients with normal spleen size. However, platelet function does not seem to be improved by splenectomy, and underlying platelet defects in myeloproliferative disorders were not corrected by splenectomy.

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