B-lymphoproliferative disorders in patients with hepatitis C virus infection

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A cohort of 148 consecutive patients with hepatitis C virus infection were studied at the rheumatology outpatient clinic of a tertiary care teaching hospital. The diagnosis of hepatitis C virus infection was supported by detection of HCV RNA in the serum. Cryoglobulin screening was done in all patients and the presence of a monoclonal component was investigated when the cryocrit was higher than 1%. Patients with lymphoproliferative disorders were further investigated. Four patients had a B lymphoproliferative disorder, which represents a prevalence of 2.7% in this cohort of patients with hepatitis C virus infection. Mixed cryoglobulinemia (MC), with cryocrit higher than 1%, was found in 16 of 148 patients (11%). It was type III MC in 13 patients and type II MC in 3. All patients who developed a B lymphoproliferative disorder had mixed cryoglobulinemia, with a monoclonal component (type II MC) in two patients and without a monoclonal component (type III MC) in the other two. The incidence of B-lymphoproliferative disorders among this cohort of patients with hepatitis C virus infection seems to be significantly increased. However, the high frequency of asymptomatic, undiagnosed HCV infection among the apparently healthy general population may decrease the true significance of this association. Systematic screening of cryoglobulin production in patients with hepatitis C virus infection might clarify whether the risk of B lymphoproliferative disorders increases when type II or type III mixed cryoglobulinemia is present.

It has been established that patients with type II essential mixed cryoglobulinemia (EMC) may develop lymphoproliferative disorders (LPD).[1,2] EMC is presently kown to be associated with hepatitis C virus (HCV) infection in the majority of cases.[3-5] Certain types of LPD, namely non-Hodgkin's B cell lymphomas (NHBL) of the marginal zone, centrocytic-centroblastic follicular lymphomas, immunocytomas and diffuse large cell lymphomas, seem to be more frequently associated with HCV infection, with or without EMC.[6-10]

Recently, several studies have shown a high incidence (9-40%) of anti-HCV antibodies or HCV viremia in serum in patients with NHBL but not in other LPD such as Hodgkin's disease (HD) or T cell lymphomas.[6,7,11-13] Thus, a strong association between HCV infection, EMC and NHBL seems to exist. However, there are few published epidemiological data, to our knowledge, on the prevalence of NHBL or related LPD in patients with HCV infection; Ferry *et al.* reported 14 B-NHL among 500 patients with chronic hepatitis C.[14]

The purpose of this study is to report the incidence of lymphoproliferative processes in a cohort of 148 consecutive patients with HCV infection studied at the rheumatology outpatient clinic of a tertiary care teaching hospital from January 1993 through to January 1997. Patients attending the outpatient clinic or admitted to the hospital were included at the time when the diagnosis of HCV infection was made. Most of them were recruited from the Rheumatology Outpatient clinic and the Hepatology unit. The clinical protocol included determination of routine hematologic parameters, morphologic examination of the peripheral blood smear and cryoglobulin determinations. When lymphoma was suspected, we also did flow cytometry studies of blood, and bone marrow aspirate and biopsy examination.

The diagnosis of HCV infection was established by the finding of viral RNA in the serum. HCV-RNA was detected by the polymerase chain reaction (PCR) amplification of the 5'-untranslated region of the HCV genome using primers that define a sequence within this highly conserved region (Amplicor, Roche Diagnostic Systems, Branchburg, NJ, USA). HCV antibodies were studied in the serum by the 3.0 ELISA test (Ortho). Patients with human immune deficiency virus (HIV) or hepatitis B virus (HBV) infections, were excluded by 3rd generation EIA (Abbott Diagnostic) and enzyme immunoassay (Abbott Diagnostic), respectively.

The morphologic studies of bone marrow, peripheral blood and lymph node biopsies, as well as the cryoglobulin determinations were performed by conventional methods. Peripheral blood mononuclear cells were incubated with monoclonal antibodies (Coulter and Immunotech) for immunophenotype analysis by flow cytometry in an Epics Profile flow cytometer (Coulter, Hialeah, Fl. USA).

A diagnosis of B-LPD was made in 4 of the 148 consecutive patients included in the study; 3 of them had B-NHL and a fourth patient had LLC-B. No other B or T-LPD were seen. This represents a prevalence of 2.7 % B-LPD among these unselected patients with HCV chronic infection. MC, with a cryocrit higher than 1%, was detected in 16 of 148 patients (11%). It was type II in 3 patients and type III in 13 patients. All 4 B-LPD patients had MC, with a monoclonal component (type II) in 2 patients and a polyclonal (type III) in the other 2 patients.

A striking finding in a high percentage of liver biopsies of patients with EMC is the presence of lymphoid aggregates of the B cell linage.[15,16] When phenotyped, these lymphoid aggregates proved to be CD19 or CD20 positive and CD5 negative[2] and showed a strong expression of surface monotypic immunoglobulin light chains.[15] Some of these patients go on

to develop overt NHBL in the course of their evolution.[2] Type II EMC is currently kown to be associated with HCV infection in the majority of cases,[3-5] which raises the question of the role of the chronic HCV infection in the pathogenesis of these disorders. This role is supported by the finding of hepatitis C virus proteins in pathologic material from these patients.[6,17] These pathologic findings strongly point to a role of the HCV in the development of HCV and EMC associated LPD, and recently published data on the incidence of HCV antibodies or HCV viremia among patients with various types of NHBL support this notion. Silvestri *et al.* studied the prevalence of HCV antibodies in 537 patients with LPD;[7] it was similar to that of normal controls (4%) in patients with HD, acute lymphoblastic leukemia, multiple myeloma and T cell lymphomas. In contrast, it was twice as high (9%) in NHBL in general, much higher (30%) in the immunocytoma, and slightly higher (10%) in marginal zone and follicular centrocytic-centroblastic and diffuse centroblastic lymphomas. Most, but not all, of these patients with lymphoma had EMC. Ferri *et al.* found HCV antibodies and HCV viremia in 30% of a series of 50 unselected patients with NHBL, but only in 3% of patients with Hodgkin's disease, and in 1% of healthy controls.[11] Luppi *et al.* report somewhat similar results, detecting anti-HCV antibodies in 42% of 69 NHBL patients, in 4% of patients with HD, and in 1% of normal controls.[6] There are other recently published studies confirming these data and the role that chronic HCV infection and EMC have in the development of NHBL.[8,9,10,12,13,18,19] Lastly, among patients with monoclonal gammopathies, HCV positivity was restricted to patients with EMC, whereas it was not detected in multiple myeloma, Waldenström's macroglobulinemia and monoclonal gammopathy of undetermined significance (MGUS) without cryoglobulinemic activity.[20,21]

Although these reports clearly establish an association between HCV and LPD (NHBL), to our knowledge there are few published epidemiological data about the incidence of LPD among patients with HCV infection. Ferry *et al*. reported 14 B-cell NHL among 500 patients with chronic hepatitis type C.[14] We studied 148 consecutive patients referred to a diagnostic clinic during a four year period because of HCV positivity, as determined by detection of HCV in serum. Four of the 148 developed a B-LPD (3 B-NHL and 1 B-CLL). This would represent an incidence of 2.7% B-LPD among these HCV positive patients. These figures are similar to those reported by Ferry *et al.* referring to patients with chronic hepatitis type C (2.8%).[14]

Although our patient population was not included in the study prospectively, it represents an unselected group of patients referred to a single diagnostic unit because of HCV infection. Unfortunately, the true prevalence of NHL in patients with HCV infection is difficult to obtain due to the existence of a high number of asymptomatic HCV-positive patients among the general population.

Three of the four LPD cases in our report had low grade B cell lymphoma. In this regard, it is of interest that the highest rate of HCV positivity in low grade B cell lymphoma has been reported in patients with immunocytoma, follicular lymphoma and marginal zone or MALT-type lymphomas.[6-10,18] The fourth case was a LPD (CLL-B), not associated with HCV infection in previous reports;[6,22] we do not know whether this case represents a coincidental association of the two disorders. Involvement of the liver, spleen and salivary or lachrymal glands (classical targets of HCV infection), as one of our patients showed, is frequent in the lymphomas arising in patients with HCV infection.[2,8,17,18,19] This raises the question about a possible role of the chronic HCV infection in the pathogenesis of the neoplastic transformation, akin to what is known to occur with *Helicobacter pylori* and gastric MALT lymphoma.[17] It is also worth noting the high frequency of marginal zone or MALT-type lymphomas (believed to be closely related) reported in these patients.[6,8,18]

In this series, the four patients with B-LPD had type II or type III MC; this would suggest that among patients with C virus infection, those with MC have a higher risk of developing a B-LPD than those without MC. Silvestri *et al.* detected cryoglobulins (in most cases type II) in 50% of 42 patients with B-NHL associated to HCV infection.[10] De Vita *et al.* found serum cryoglobulins in all of 35 patients with B-NHL and HCV infection, but in only 50% of them was the cryocrit higher than 1%; in their series type II and type III MC were equally frequent.[8] Further studies to clarify the true incidence of LPD in HCV positive patients and its relationship to mixed cryoglobulinemia type II and type III are warranted.

Key words

Lymphoproliferative disorders, non-Hodgkin's lymphomas, mixed cryoglobulinemia, hepatitis C virus infection

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Circulating hematopoietic progenitors are not altered in patients with post-transplant erythrocytosis

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Granulocyte / macrophage (GM), mixed colony and erythroid burst forming unit assays were performed in 9 post-transplant erythrocytosis (PTE) patients, 18 non-PTE kidney transplant recipients and 12 healthy volunteers. The number of GM precursors was lower in PTE patients than in normal subjects. This indicates that hematopoietic stem cell potential is not altered in PTE.

The pathogenesis of erythrocytosis, which occurs in up to 20% of patients after renal transplantation, is unclear. Possibilities include transplant rejection, transplant artery stenosis, hydronephrosis, resetting of the erythropoietin threshold, resolution of hyperparathyroidism, immunosuppressants, hepatic or native kidney erythropoietin hypersecretion). In this study, hematopoietic stem cell reserve was investigated by *in vitro* assays for circulating granulocyte-erythroid-monocyte-megakaryocyte (CFU-GEMM), granulocyte-monocyte colony (CFU-GM) and erythroid burst forming units (BFU-E).

The study group comprised 27 kidney transplant recipients aged 27-68 (mean 44) years. Nine PTE patients had had symptoms of hyperviscosity, relieved by phlebotomies, in the previous year with maximal hematocrit values 0.52-0.64 (mean 0.55). Renal artery stenosis was not specifically looked for, but there was no suspicion that any patient had this condition. The remaining 18 non-PTE patients had serum creatinine values < 200 μmol/L.[1] The number of rejection episodes, hemodialysis duration and time after transplantation did not differ significantly between the two groups (data not shown). Twelve healthy male volunteers (22-45 years, mean 36) served as a control group.

Blood samples were collected in the morning and used immediately for cell counts and cultures. Sera for erythropoietin were frozen until assayed (EPO ELISA kit, Boehringer, Mannheim, Germany). *In vitro* assay was assessed in a methylcellulose-using modified technique suggested by the manufacturer (Stem Cell Inc., Vancouver, Canada). Briefly, separated mononuclear cells (MNC) were plated in triplicate in two concentrations, 0.5 and 1×10^5 cells/mL of standardized mixture medium (MethoCult H4433, Stem Cell) containing 30% fetal bovine serum, 1% bovine serum albumin, 10^{-4} mol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 5% PHA-LCM in serum and 3 U/mL of human recombinant erythropoietin,[2] and

Table 1. Data (median and range) on indicators of hematopoiesis for patients and controls. A significant difference (p<0.05) between patients and controls is marked by an asterisk.

Indicator#°	*Patients*		*Controls*
	with erythrocytosis (n=9)	*w/o erythrocytosis (n=18)*	*(n=12)*
BFU-E	14 (4-85)	13 (1-41)	13 (3-27)
CFU-GM	7 (3-29)*	11 (2-37)	21 (9-37)
CFU-GEMM	1 (0-5)	2 (0-10)	3 (0-9)
Erythropoietin	199 (136-406)	146 (114-281)	–

#All committed progenitors were counted and expressed per 10^5 mononuclear cells: BFU-E, erythroid burst forming units, CFU-GM, granulocyte colony-forming units, CFU-GEMM, granulocyte-erythroid-monocyte-megakaryocyte colony forming units; °erythropoietin was expressed in IU/L.

scored without staining on day 14.[2,3]

The differences in laboratory parameters between groups were analyzed using the Kruskal-Wallis nonparametric test. If the difference was significant, a Mann-Whitney test was performed to assess the difference between patients and controls.

Stem cell assays showed that only the CFU-GM colony number was significantly lower in PTE patients than in controls. (Table 1, p<0.05). Erythropoietin in all patients was above the assay manufacturer's reference range (4-90 IU/L) with no difference between groups. Results were also evaluated according to the immunosupressive therapy (cyclosporin and corticoids with or without azathioprine), but no differences in colony numbers or erythropoietin were found (not shown), except a lower blood leukocyte count in patients receiving triple therapy (p<0.05, data not presented).

One PTE patient had an exceptionally high BFU-E count (85 colonies/10^5 MNC) at 227 µmol/L of serum creatinine.[1]

In normal hematopoiesis stem cells in bone marrow and peripheral blood are in a steady state, and presumed to be by 1-2 logarithms lower than those in the bone marrow.[4] In PTE the erythroid progenitors may have increased proliferative capacity or could be more sensitive to erythropoietin.[5,6] Our results indicate that circulating progenitors are not altered in patients after kidney transplantation, as there was no difference between patients' groups and controls. Normal CFU-E count with normal responsiveness to erythropoietin in PTE has been reported.[7] This is at variance with findings in polycythemia vera.[8] The stimulation of erythroid precursors may be achieved by cyclosporin,[9] although this drug can impair endogenous erythropoietin production.[10] Lower CFU-GM counts in patients after kidney transplantation, (significant only for PTE patients) might suggest disruption of differentiation towards myeloid cell lineage, but further investigations are needed to prove this.

In conclusion, no alteration of circulating hematopoietic stem cell reserve or disruption in the early phases of erythropoiesis in PTE patients was observed.

Key words

Erythrocytosis, kidney transplantation

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Mucormycosis and hemopoietic transplants

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Mucormycosis is becoming recognized as a serious complication in patients undergoing hemopoietic transplantation (HT), because it is a major cause of morbidity and mortality. In our institution 4 cases of mucormycosis in post-HT period among 345 patients undergoing HT were diagnosed between 1984 and 1997. We studies the clinical characteristics of these cases and we conclude that mucormycosis is not a common infection in patients undergoing HT but that it is followed by a high morbidity and mortality. Maintained neutropenia is the most important risk factor.

Mucormycosis is becoming recognized as a serious complication in patients undergoing hemopoietic transplantation (HT), because it is a major cause of morbidity and mortality. Mucormycosis is a term used to describe the diseases caused by fungi of the family *Mucoraceae*, which includes the genera *Absidia, Apophysomyces, Mucor, Rhizomucor and Rhizopus*.[1] Its wide clinical spectrum includes sinonasal, rhinocerebral, pulmonary, disseminated, gastrointestinal, cutaneous, and miscellaneous disease forms.[2,3] The histologic findings are broad, non-septate hyphae that branch at right angles, vascular invasion, tissue necrosis and infarction. Prolonged neutropenia, extended steroid treatment and immunosupression are implicated as risk factors for post-BMT mucormycosis.[2]

In our institution 32 cases of invasive mycosis (candidiasis 13, aspergillosis 14, mucormycosis 4 and cryptococcosis 1) were diagnosed between 1984 and 1997 in the post-HT period among 345 patients undergoing HT. The clinical characteristics of the four cases of mucormycosis are summarized in Table 1. The median age of the patients affected was 22 years (range 19-25). Two were male and two female. Primary disease diagnosis was CML (chronic phase), AML, aplastic anemia, and Fanconi's anemia (without previous treatment with deferoxamine). All had received allogeneic HT, three of them, bone marrow from an HLA-identical sibling donor, and the other umbilical cord blood. The diagnosis of mucormycosis was established by identifying the fungi in histologic samples and in one case (UPN 108), *Rhizopus spp.* was isolated in microbiological cultures. Autopsies were performed in two cases (UPN 20 and 314-partial). In three cases, the diagnosis was made in the early post-HT period (days: +4, +11, and +21). These patients had sustained neutropenia before HT. The remaining case was diagnosed at the same time as the development of acute GVHD and steroid therapy. The clinical presentations were: rhinocerebral

Table 1. Clinical characteristics and evolution of patients.

UPN	*Age-Sex diagnosis*	*Hematopoietic source*	*Conditioning regimen / GVHD prophylaxis*	*Post-transplant evolution*	*Days after transplant / site of infection*	*Neutropenia*	*Treatment*	*Outcome*
108	25-M CML	HLA-identical sibling donor	TBI (12GY) + CY (120 mg/kg) / MTX+CsA	Cytogenetic relapse + 4 years post-transplant IFN treatment with partial. cytogenetic response IDL with aGVHD development grade II and complete remission (morphologic and cytogenetic)	+6 years and 2 months /rhinocerebral	No	Liposomal amphotericin B (total dose 12 g) Surgical debridement	Alive
314	22-F Fanconi's anemia	UCB-UR	TBI (12 Gy) + CY (40 mg/kg) + ATG (90 mg/kg) PDN + CsA		4 days / thoracic wall + lung	Yes (> 6 months)	Liposomal amphotericin B Granulocytic infusion Surgical debridement	Dead +13 days post-transplant
20	23-F AML	HLA-identical sibling donor	TBI (12 Gy) + CY (120 mg/kg) / MTX		21 days / thoracic wall surrounding central venous access	Yes (+76 days)	Amphotericin B Surgical debridement	Dead +78 days post-transplant (CMV pneumonia)
38	19-M AA	HLA-identical sibling donor	TBI (12 Gy) + CY (120 mg/kg) / CsA		11 days / rhinocerebral	Yes (> 6 months)	Amphotericin B	Dead +20 days post-transplant

UPN: unique patient number; M: male; F: female; CML: chronic myeloid leukemia; AML: acute myeloid leukemia; AA: aplastic anemia; UCB-UR: umbilical cord blood - unrelated; GVHD: graft-versus-host disease; TBI: total body irradiation; CY: cyclophosphamide; MTX: methotrexate; ATG: antithymocyte globulin; CsA: cyclosporin; IDL: infusion of donor lymphocytes.

(n=2), thoracic wall and lung (n=1), cutaneous involvement (surrounding a central venous access) (n=1). All patients received high doses of amphotericin B and surgical debridement was performed in three. One patient is alive, three are dead (two died of mucormycosis and one of CMV pneumonia).

We conclude that mucormycosis is not a common infection in patients undergoing HT but a high morbidity and mortality follow it. Sustained neutropenia is the most important risk factor. The early diagnosis followed by prolonged treatment with amphotericin B and surgical debridement, when possible, can improve the survival of these patients.

Key words

Mucormycosis, transplantation, hemopoiesis

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Transfusion-related acute lung injury associated with an NA1-specific antigranulocyte antibody

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Transfusion-related acute lung injury (TRALI) is an infrequent complication of hemotherapy. Antigranulocyte antibodies, most of them present in donor's serum, have been implicated in its pathogenesis. We describe a case of TRALI, following red blood cell transfusion, associated with an antigranulocyte antibody with NA1 specificity in the patient's serum.

A 70-year-old female with a history of previous transfusions was admitted for an elective prosthetic hip implant. Following surgery a single unit of non-buffy-coat deprived packed red blood cells with saline, adenine, glucose, mannitol, (SAG-M) was transfused. Thirty minutes later the patient developed acute respiratory failure. A chest X-ray revealed bilateral alveolar infiltrates with a non-dilated heart, findings consistent with acute pulmonary edema. A Swan-Ganz catheter was placed, showing pulmonary and central venous pressures suggestive of TRALI. Systemic corticosteroids (prednisolone 2 mg per kg) were started, and the patient required mechanical ventilatory support. The clinical course was favorable with resolution within 48 hours. In order to establish a serologic diagnosis, antileukocyte antibodies were searched for in both the patient's and donor's serum. Anti-HLA antibodies were ruled out with a lymphocytotoxic test using the patient's serum and a lymphocyte panel (n=18) of known HLA phenotypes. The presence of specific antigranulocyte antibodies was studied with granuloagglutination and an indirect immunofluorescence (GIFT) test. Both tests showed the presence of an antigranulocyte antibody in the patient's serum, and when tested against granulocytes of known phenotype, the antibody was shown to be specific for NA1 (Table 1). The patient's and donor's granulocyte phenotypes were established by an immunofluorescence technique with flow cytometry (FACScan, Becton Dickinson, San José, CA, USA) using monoclonal antibodies specific for NA1, NA2 and CD16. The patient's phenotype was NA2/NA2, $CD16^+$, while the donor's was found to be NA1/NA2, $CD16^+$. Finally, once the antibody's specificity had been established, a confirmatory bidirectional crossmatch was perfomed with a positive reaction with the patient's serum and the donor's granulocytes (Table 1). The diagnosis was TRALI associated with an antigranulocyte antibody with NA1 specificity in the patient's serum.

TRALI is a relatively infrequent transfusion-related complication, although it ranks second in transfusion-related mortality.[1] TRALI has been described following the transfusion of the majority of blood components;[2-9] its incidence has been estimated as 1 in 5000 transfusions.[2] Clinically TRALI presents as an adult respiratory distress syndrome. Diagnosis requires a high index of suspicion and is made by exclusion. With appropriate supportive treatment, 80% of patients can be expected to recover fully, and mortality ranges from 5 to 10% in most studies.[2] The pathogenesis of TRALI is not fully understood,

Table 1.

Phenotype of tested granulocytes	*NA1NA1*	*NA2NA2*	*NA1NA2 (Donor)*	*NA1NA2*
Patient's serum	+++	–	++	++
Multispecific anti-HLA antiserum	+++	+++	+++	+++
AB serum	–	–	–	–

although there are two plausible hypotheses: an immune-mediated reaction or direct lung injury by biologically active lipids generated during the storage of the blood product.[10] Classically it has been attributed to the presence of antileukocye antibodies in the patient's or donor's serum, which are found in less than 50% of cases with specific tests.[12] In 90% of these cases the antibodies are, however, found in the donor's serum,[11] unlike our case. Previously described antigranulocyte antibodies have shown specificities for the NB and 5b antigens.[4,6,8] Other antibodies implicated have been anti-HLA class I antibodies,[11] anti-HLA A2[4] and B35-specific antibodies.[6] Currently the role of these antibodies in the pathogenesis of TRALI is controversial, since anti-HLA antibodies are found in 1-2% of the general population.[2] Recently, Silliman *et al.* suggested that TRALI may be mediated by biologically active lipids generated during the storage of blood products, particularly when transfused to patients with certain predisposing factors, leading to a *syndrome of neutrophil overactivation* with endogenous cytokine release, indiscriminate endothelial neutrophil adhesion and activation.[10] Although unproven, the concept of a multifactorial pathogenic mechanism, including the effect of specific antigranulocyte antibodies, active lipids and other as yet undefined factors, emerges as a more rational explanation of this complex syndrome.

Key words

Transfusion-related injuries, antigranulocyte antobodies

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Plasma and urinary endothelin-1 titers and plasma von Willebrand activity in *Pseudoxanthoma Elasticum*

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We found high endothelin-1 and von Willebrand factor plasma titers not only in two individuals (daughter and father) affected with *Pseudoxanthoma elasticum* but also in a young unaffected relative. These findings raise the possiblity that these molecules could be the first biochemical fingerprints of this, still not clinically evident, rare inherited disorder of elastic tissue.

Pseudoxanthoma elasticum (PXE) is a rare inherited disorder of elastic tissue characterized by progressive calcification of the elastic fibers in the skin, retina and cardiovascular system;[1] the estimated prevalence of this disease is 1 in 70,000-100,000. A more common autosomal recessive and a less common autosomal dominant pattern of inheritance, with high penetrance, have been described. Recently, an area on the long arm of chromosome 16 (16p13.1) was identified as the single gene that accounts for both the recessive and dominant forms of PXE.[2] The most characteristic clinical manifestations of PXE are yellowish grouped papules and plaques on the skin of flexure areas, angioid streaks in Bruch's membrane of the retina, calcified cardiovascular lesions, and severe hemorrhagic diatheses.[1] Diagnosis of PXE is based on clinical evaluation, histologic demonstration of abnormal, calcified elastic fibers in skin biopsy, and fundoscopic examination showing the presence of the typical angioid streaks.[1]

We recently cared for a 41-year-old woman affect-

Table 1. Plasma and urinary ET-1 levels, and plasma vWF activity in two relatives affected by PXE and in three other unaffected relatives. Plasma vWF activity was expressed as percentages of normal pooled plasma, the antigen levels of which were defined as 100%.

	Father (affected)	*Patient (affected)*	*Sister (unaffected)*	*Brother (unaffected)*	*Son (unaffected)*
Plasma ET-1 *(n.v. 0.5-1.2 pg/mL)*	2.78	2.98	0.98	0.67	2.66
Urinary ET-1 *(n.v. 0.3-1.2 µg/h)*	1.5	3.34	0.58	0.68	4.56
Plasma vWF activity	212%	188%	100%	98%	176%

ed by PXE, who, besides presenting all the required diagnostic criteria, also developed, as a rare complication of her disease, an atrial septal aneurysm. Both the patient's father and one of her brothers, who had died of acute myocardial infarction, had been diagnosed as having PXE, and her paternal grandfather was also supposed to have had it. Neither her mother nor any other relative from the materal lineage showed signs of the disease, thus suggesting a dominant autosomal inheritance. Two of the patient's brothers and her 15 year-old son do not present clinical evidence of PXE.

Endothelin-1 (ET-1), a potent vasoconscriptor, and von Willebrand factor (vWF), have been demonstrated to be markedly increased when the vascular endothelium is damaged.[3] Furthermore, some authors have suggested that both ET-1 and vWF could contribute to the progression of vascular lesions in patients with PXE.[4] Plasma and urinary ET-1 titers, and vWF plasma activity were titered in the two PXE patients and in their three clinically unaffected relatives using a commercial sandwich immunoassay technique (R&D Systems, Minneapolis, MN, USA),[5] and an enzyme-linked immunosorbent assay method, respectively (Boehringer-Mannheim Co., Milan, Italy).[4] The results we obtained (Table 1) showed a marked increased in ET-1 plasma and urinary titers and in vWF plasma activity not only in the affected individuals, but also in the patient's healthy son, despite his lack of clinical signs of PXE.

Although we cannot predict whether this boy will develop PXE during his lifetime, the increase in ET-1 and vWF titers might be the first biochemical fingerprint, of this still not clinically evident disease.

However, the central question still remains unanswered; as a matter of fact, if the patient does go on to develop PXE, we do not know whether the observed early increase in ET-1 and vWF is the primary insult leading to overt PXE or simply the first sign of a still subclinical disease.

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Severe immune thrombocytopenia during formestane treatment

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Formestane is a new aromatase inhibitor used as second-line endocrine treatment for postmenopausal women with advanced breast cancer. The most frequent side effects are local reactions. Here we report the development of immune thrombocytopenia coinciding with administration of this drug.

Formestane (4-hydroxyandrostenedione) is a new competitive, irreversible, steroidal aromatase inhibitor, 30 to 60 times more potent than aminoglutethimide.[1] Aromatase is the enzyme responsible for the conversion of non-aromatic androgens, particularly androstenedione and testosterone, to aromatic estrogens: estrone and estradiol. In postmenopausal women androstenedione is converted to estrogens by aromatase in the skin, muscles, liver and fat tissue. Aromatase is also present in breast tumor tissue. Thus, formestane decreases both circulating and tumour estrogen levels and is a successful second-line endocrine treatment for post-menopausal women with advanced breast cancer in whom previous therapy with tamoxifen has failed.[1-3]

The most frequent side effects are local and transient reactions at the site of injection (gluteal pain,

erythema, pruritus, burning, abscesses). Mild systemic effects usually include hot flushes, rash, lethargy, dizziness, emotional lability, facial swelling, alopecia, vaginal spotting, nausea, leukopenia, constipation or rarely anaphylaxis.[4-6]

To date, there have been no reported cases of formestane-induced thrombocytopenia. Here we describe a case of transient, severe, immune thrombocytopenia during formestane administration.

A 55-year-old woman was referred to our department for evaluation of severe thrombocytopenia. Four years previously, an infiltrating ductal breast carcinoma ($T_2N_{1b}M_0$) was diagnosed and treated with surgery (radical mastectomy), standard chemotherapy according to the CAF regimen (cyclophosphamide, doxorubicin and 5-fluorouracil) and radiotherapy to scar and nodal areas. Two years after diagnosis, due to skeletal metastases, the patient was placed on endocrine therapy with tamoxifen. Two years later, X-rays and gammagraphy showed progression of metastases (D10-L5), and palliative radiotherapy was planned. Tamoxifen was changed for formestane at 250 mg i.m. every second week. Platelet count was 189×10^9/L.

After 3 doses of formestane a full blood count revealed severe thrombocytopenia: 17×10^9/L. There was no evidence of a hemorrhagic diathesis. Peripheral blood smears showed normal morphology and a *true* thrombocytopenia was confirmed: there were no platelet aggregates. EDTA-dependent pseudothrombocytopenia was ruled out. A bone marrow biopsy showed normocellularity, with an increased number of megakaryocytes. No evidence of tumor infiltration was found. Viral serological studies were negative. Other tests such as detection of antiphospholipid antibodies were also negative. A serologic study of platelet antibodies with the platelet immunofluorescence test[7] revealed the presence of an IgG platelet autoantibody: positive direct test and eluate. Drug dependent antibodies (*immune complexes* and *adsorption* mechanisms) were also investigated according to the methodological procedures described by Mueller-Eckhardt *et al.*[8] The *immune complex* mechanism was investigated incubating formestane with the serum of the patient before adding the target platelets. The *adsorption* mechanism was studied preincubating platelets with the offending drug and washing them before the serum of the patient was added. Neither mechanism seemed to be involved in the platelet destruction. Taking into account the serological results a *drug-independent* mechanism for formestane-induced immune thrombocytopenia was considered.

All treatment was discontinued. Regular follow-up was planned. The platelet count progressively recovered, returning to normal 4 months after the last dose of formestane. A new serologic study of platelet antibodies performed at this time was negative.

Although some drugs may induce thrombocytopenia by impairing megakaryocyte production (i.e. chemotherapeutic agents or thiazide diuretics), most drugs cause thrombocytopenia by eliciting an immune response.[9] Our patient developed severe thrombocytopenia (17×10^9/L) after 3 standard doses of formestane. As she was asymptomatic, formestane was stopped and no further treatment was required. Serological studies revealed the presence of a transient IgG platelet autoantibody. The serological findings, similar to those found in idiopathic autoimmune thrombocytopenia, and the clinical course were consistent with a *drug-independent* mechanism of formestane-induced thrombocytopenia. The list of drugs potentially capable of causing immune thrombocytopenia[10] is continuously growing.

Key words

Formestane, immune thrombocytopenia, side effects, platelet autoantibodies

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Study of hemostasis in pediatric patients with portal vein thrombosis

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We describe the behavior of hemostatic variables in children with portal vein thrombosis (PVT) and in a control pediatric population. Hereditary protein C (PC) or protein S (PS) deficiency was not a etiologic factor for PVT in children. Minor signs of comsumption of coagulation factors II, V, fibrinogen and hyperfibrinolysis were detected. One child had lupus anticoagulant (LA).

PVT is responsible for the majority of cases of portal hypertension and upper gastrointestinal tract hemorrhages in children.[1,2] PC and PS have not previously been investigated as an etiologic factor for PVT, but a hereditary tendency to thrombosis may lead to PVT. The hemostatic system may be altered due to a hepatic proteic synthesis insufficiency and/or disseminated intravascular coagulopathy due to thrombosis. As the main objective, we evaluated whether PC and PS deficiencies were etiologic factors for PVT. We also studied components of hemostasis in children with PVT in order to determine the mechanism involved in coagulation abnormalities, if present.

This study comprised all patients with PVT that were diagnosed by Pediatric Clinics at the University Hospital of Unicamp/Campinas, between 1987 and 1995. The patient group consisted of 20 children (8 males and 12 females) diagnosed as having PVT at the median age of 77 months (2→136). An age- and sex-matched control group comprised 20 children and another was composed of 61 normal children, 24 males and 37 females at the median age of 95 months (43→177). PC was measured by a coagulation method and total and free PS were determined by rocket immunoelectrophoresis.[3]

Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), coagulation factors II, V, VII, IX, X, XII and fibrinogen concentration were determined by coagulation methods. Euglobulin lysis time (ELT),[4] and lysis area on fibrin plate (LAFP) were performed by described procedures.[5] D-dimer was measured by latex method.[6] LA was screened by kaolin clotting time and dilute Russell viper venon time (dVVT) and confirmed by DVV confirm®, and frozen-thawed platelets.[7] For statistical analysis we used Student's *t* test.

There was no difference in PC and PS between patients and controls. None of the patients had a congenital deficiency of PC or PS. One patient demonstrated an acquired decreased PC level (62%); his parents were normal. One patient had LA which was reconfirmed later on. PT and TT were increased and coagulation factors II and V were decreased in patients when compared to the control group. Seven patients had D-dimer levels greater than 0.5 µg/mL. Prealbumin levels were decreased in patients.

Table 1.

	Patient group mean±SD	*Matched group mean±SD*	*Control group mean±SD*
APTT (R)	1.13±0.17	1.12±0.09	1.09±0.11
PT (R)	1.24±0.22°*	1.03±0.08*	1.04±0.08°
TT (R)	1.10±0.08*	1.02±0.07*	1.07±0.13
Factor II (%)	83.4±13.8°*	99.4±16.15*	97.0±14.57°
Factor V (%)	71.4±17.83°*	95.2±19.62*	94.4±22.28°
Factor VII (%)	106.95±35.57	106 ±19.59	97.4±26.10
Factor IX (%)	101.07±37.26	118.1±38.7	I107.2±35.24
Factor X (%)	88.82±19.65	91.3±15.29	85.8±12.66
Factor XII (%)	88.97±37.51	89.6±16.46	90.8±17.05
LAFP (mm^2)	240.7±79.43°*	75.9±38.02*	102.4±51.02°
ELT (min)	290±211.8°*	390±146.9	410±102.89°°
Fibrinogen (mg %)	200.7±61.97	245.5±69.21	280.9±66.31
Protein C (%)	100.8±31.04	101±34.93	109.2±21.48
Total protein S (%)	99.5±15.06	81.8±8.48	88.1±12.86
Free protein S (%)	94.76±11.83	86.1±11.95	100.6±21.77
Prealbumin (%)	66.4±21.5°*	78±19.32*	85.2±23.87°

LAFP-lysis area on fibrin plateELT-euglobulin lysis time;
R-relation of time obtained between patient and controls SD-standard deviation;
**p< 0.01; **p< 0.05; *between patient group and matched group;*
°p< 0.01; °°p< 0.05; ®between patient group and contrlos.

The importance of inherited prethrombotic states such deficiencies of antithrombin, PC, PS and factor V Leiden has been realized.[8] We had previously investigated factor V Leiden in these 20 children with PVT but none of them carried this mutation, suggesting it was not a risk factor for PVT in our children.[9] The analysis of coagulation inhibitors could reveal congenital disorders but also changes secondary to deficient liver synthesis resulting from the PVT. In this study, congenital deficiencies of coagulation inhibitors, potentially implicated in the etiology of PVT, were not found. Only one patient had low PC levels which were not congenital, since his parents had normal PC levels. This deficiency was, therefore, a consequence and not the cause of his PVT. His low factor V, prealbumin level, increase in TT and TP suggested a decrease in protein synthesis and/or intravascular coagulation. Our data suggest that intravascular coagulation had occurred, associated with decreased factor II, V and fibrinogen levels, hyperfibrinolysis and increased D-dimer. Changes in liver synthesis function cannot be ruled out, since prealbumin was diminished in these patients. Vitamin K

metabolism was unaffected since factors VII, IX, X, PC and PS were within the normal range. LA was found in one patient and may have been the cause of the PVT. Due to the importance of LA in thrombosis, this raises the question of whether LA could be investigated in children with PVT.

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Hyperammonemic encephalopathy in multiple myeloma

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We report two cases of hyperammonemic encephalopathy in patients with multiple myeloma. This rare complication, whose pathophysiology remains unknown, is associated with disease progression and so with a very bad prognosis. We believe that this complication should be included in the differential diagnosis of encephalopathy occurring in multiple myeloma.

Hyperammonemia is usually found in chronic liver diseases with portal-systemic shunts and acute fulminant hepatic failure.[1] It has also been described in hematologic malignancies such as acute leukemia,[2] following bone marrow transplantation[3] and in eleven patients with multiple myeloma (MM).[4-10] We report two new cases of hyperammonemic encephalopathy in MM.

Patient #1. IgG λ MM was diagnosed in a 56-year-old woman. Five courses of vincristine, adriamycin and dexamethasone (VAD) resulted in good partial remission. Three months later she presented with a one-week history of alternating lucidity and delirium, lethargy and inappropriate behavior. Serum electrolytes and creatinine were normal. Serum IgG λ spike amounted to 1920 mg/dL. A lumbar puncture and a computed axial tomography were unremarkable. The electroencephalogram presented changes compatible with metabolic encephalopathy. Plasma ammonium concentration was 170 mg/dL (normal < 82 mg/dL). Bilirubin, liver transaminases, coagulation tests, viral hepatitis serology, an abdominal ultrasound and a transjugular liver biopsy showed no alterations. Dietary nitrogen was eliminated and oral lactulose therapy was started, but mental status and plasma ammonium levels did not improve. A bone marrow (BM) aspirate showed 84% plasma cells. Three days after reinstituting chemotherapy (VAD), plasma ammonium decreased to normal and the patient became rapidly asymptomatic. Three months later the patient developed the same symptoms and died of disease progression.

Patient #2. IgA κ MM was diagnosed in a 51-year-old man. Chemotherapy (VAD) and local radiotherapy to the ribs and lumbar spine were started. After 6 courses he presented with disorientation, bradypsychia and myoclonus. Neurological examination and a lumbar puncture were normal. A magnetic resonance imaging scan showed diffuse edema in the brain. The electroencephalogram recorded triphasic waves. Serum electrolytes and renal and liver function were normal. Plasma ammonia level was 233 mg/dL. Serum IgA κ spike amounted to 4000 mg/dL. There were progressive osteolytic lesions and 100% plasma cells in BM aspirate were demonstrated. The clinical manifestations were ascribed to hyperammonemic encephalopathy and treatment with dexamethasone (8 mg/day) was started. Hyperammonemia and the neurologic alterations improved immediately after chemotherapy (VAD) was instituted. One month later the patient again presented with hyperammonemic encephalopathy and died of dis-

ease progression.

Hyperviscosity and hypercalcemia are the usual causes of encephalopathy in MM. Hyperammonaemic encephalopathy is usually described in serious liver dysfunction and is characterized by lethargy, confusion and asterixis, which can progress to coma and death.[1]

Mitchell *et al*.[3] identified this complication in eight of 460 patients who had leukemia or had had a bone marrow transplantation. A few cases have also been reported in MM.[4-10] The etiology of this syndrome has yet to be determined. Matsuzaki *et al.*[6] found that the myeloma cells from a patient with hyperammonaemic encephalopathy secreted ammonia at a high level into the culture medium.

Different treatments (protein restriction, lactulose, neomycin, plasmapheresis, hemodialysis...) have been tried in this syndrome,[4-10] but only chemotherapy has been successful. Nevertheless this complication is associated with disease progression and so with a very bad prognosis. We suggest that hyperammonaemic encephalopathy should be included in the differential diagnosis of disturbances of consciousness in MM.

Keywords

Multiple myeloma, encephalopathy, hyperammonemia

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High prevalence of anti-HGV/E2 antibodies in HCV-positive patients with non Hodgkin's lymphoma

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We evaluated in a series of 33 HCV positive (both RT-PCR and HCV RIBA 2 assays) B cell non-Hodgkin's lymphomas (NHL) patients the prevalence of active and inactive HGV infection by HGV RNA assays (RT-PCR) and anti HGV antibodies directed against E2 structural protein (immunoenzimatic method), a reliable serologic marker of past HGV infection followed by viral clearance. We found only one patient with HGV positivity at RT-PCR (3%). Twenty-six of 33 patients were positive for anti HGV/E2 antibodies (78.8%) suggesting past infection. If confirmed, our preliminary data seem to suggest a higher incidence of HGV past infection in our group of HCV postive patients with B cell NHL.

A possibile etiologic correlation between hepatits C virus (HCV) and B cell NHL was recently suggested by some authors.[1-5] and the ability of HCV to infect lymphocytes and to determine clonal expansion of such cells has been clearly documented. Another flaviviridae agent, GBV-C/HGV, was recently isolated and described as a possible etiologic agent of non A–E hepatitis. There is a close molecular correlation between HCV and HGV and co-infection with HGV is frequent in HCV positive patients.

The prevalence of HGV infection in B cell lymphomas is not known, although if there are some preliminary data from Italy[6] and Japan.[7] Zignego *et al.*[6] reported a 6% of prevalence of HGV RNA in a series of 150 B cell NHLs: no significant differences in HGV prevalence was found between HCV positive (n = 37) and negative (n = 113) cases. The HGV prevalence in Italian NHL patients was similar to that observed in non A-E hepatitis patients but significantly higher than that in healthy subjects. On the other hand, Nakamura *et al.*[7] found 4 HCV RNA positive cases and one HGV RNA/HCV RNA positive case in a series of 51 B cell NHL patients (2% of prevalence for HGV RNA).

We, therefore, evaluated, in a series of 33 HCV positive (both RT-PCR and HCV RIBA 2 assays) B cell NHL patients, the prevalence of active and inactive

Table 1.

Subjects	*HGV RNA+*	*Anti HGV/E2+*
Controls	7/249 (2.8%)	177/506 (35%)
HCV+ B–NHL (n=33)	1/33 (3%)	26/33 (78.8%)

HGV infection by HGV RNA assays (RT-PCR) and anti-HGV antibodies directed against the E2 structural protein (immunoenzymatic method), a reliable serologic marker of past HGV infection followed by viral clearance.[8] Serum specimens were taken at the onset of the disease and stored at –20°C; we found only one patient with HGV positivity by RT-PCR (3%). Twenty-six of 33 patients were positive for anti HGV/E2 antibodies (78.8%) suggesting past infection.

This prevalence of HGV RNA was similar to that found in a a population of healthy subjects matched for age and sex, tested as controls (Chi-square test: NS). In contrast, the prevalence of HGV antibodies was significantly higher in the NHL population than in the controls (Chi square test: p< 0.0001: see Table 1).

Even if we compare our findings with the prevalence of anti HGV/E2 reported in healthy subjects, drug abusers and blood donors by Tacke *et al*.,[9] the detection of antibodies against HGV/E2 protein is much higher in our HCV postive NHL patients. It must be stressed that the immunoenzymatic method we used for anti HGV/E2 antibodies was tested as highly specific: therefore, cross reaction with anti-HCV-E2 antibodies is unlikely.

If confirmed, our preliminary data seem to suggest a higher incidence of past HGV infection in our group of HCV postive patients with B cell NHL. Whether or not this has any role (together with HCV) in the etiology or evolution of the neoplastic disease needs to be elucidated.

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Serum transferrin receptor in polycythemia

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We measured serum transferrin receptor (sTfR) levels in 22 patients with polycythemia vera and in 26 cases of secondary polycythemia. In our study, raised sTfR levels in both polycythemia groups were related to iron deficiency.

In normal adults a strong correlation has been shown to exist between serum transferrin receptor (sTfR) and standard ferrokinetic measurements of erythropoiesis[1] with the result that the level of erythropoietic activity is the most important determinant of sTfR. Increased expression of transferrin receptor (TfR) has also been documented on the surface of malignant tumor cells such as erythroleukemic cells.[2] Whether the shedding of these receptors can contribute to sTfR levels is still unclear. We measured sTfR in polycythemia patients to investigate its potential clinical usefulness. The sTfR was measured using a commercial immunoassay (ELISA) (Quantikine™, transferrin receptor EIA kit, R&D Systems, Minneapolis, USA) in 26 patients with secondary polycythemia (SP), 22 patients with polycythemia vera (PV) and 63 normal controls (REF group).

The sTfR levels (mean±SD) were 2.6±0.5 mg/L in the REF group, 5.9±3.9 mg/L in PV group and 4.7±2.9 mg/L in the SP group; sTfR was higher in both polycythemia groups than in the REF group (ANOVA, p<0.0001). After excluding patients with iron deficiency, however, the sTfR levels in both polycythemia groups were 2.8±0.9 mg/L in the SP group (n=15), and 2.9±0.8 mg/L in the PV group (n=8). Moreover, there was no difference with respect to

sTfR levels in the REF group (ANOVA, p=0.96).

Raised sTfR levels have been reported in cases with polycythemia[3,4] although it is unclear whether this increase is related to red cell mass, disease activity or iron status. Iron deficiency is closely associated with high sTfR values and sTfR levels progressively increase in parallel with the different iron deficiency stages, from the earliest stages with only low ferritin values to fully expressed iron deficiency anemia.[5] In our study, the difference in sTfR levels between polycythemia and REF groups was probably due to iron status. Thus, at variance with serum erythropoietin,[6] the possible role of sTfR in evaluating erythroblastic mass in polycythemia remains unresolved.

Key words

Polycythemia, serum transferrin receptor.

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Elective splenectomy in relapsing thrombotic thrombocytopenic purpura

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Between 20 and 40% of surviving patients with thrombotic thrombocytopenic purpura (TTP) have relapses. Plasma exchange therapy is usually effective in treating relapses, but this treatment does not prevent TTP recurrence. The role of splenectomy in relapsing TTP is still controversial. We describe a patient with multiple relapses of TTP who was successfully treated with elective splenectomy.

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening disorder of unknown pathophysiology. Without treatment, TTP is a rapidly progressive and fatal disease, with 90% of patients surviving less than 3 months. Treatment with plasma exchange, often used in combination with corticosteroids, antiplatelet agents, splenectomy and vincristine has reduced mortality to 20%.[1-3] However, between 20 and 40% of surviving patients have relapses. Relapses can occur as early as a few weeks after recovery, but also after an interval of many years.[4,5] Plasma exchange therapy is usually effective in treating relapses, but this treatment exposes the patient to blood products from numerous donors and does not reduce the relapse rate. Various interventions, including antiplatelet agents, corticosteroids and splenectomy have been used to prevent relapses.[2] Some studies have suggested that splenectomy has a role in the management of relapsing TTP, since it seems to reduce the frequency of relapse.[6-8] We describe a patient with multiple relapses of TTP who was successfully treated with elective splenectomy during remission.

A 27-year-old female was diagnosed as having TTP in May 1992. Clinical manifestations in the initial episode of TTP were fatigue, headache, hematuria, petechiae and hematomas. At admission, biological findings were as follows: hemoglobin 8.2 g/dL, platelet count 15×10^9/L, LDH 897 U/L, bilirubin 1.8 mg/dL and serum creatinine 1 g/dL. The initial episode and two early recurrences through 1992 were successfully treated with fresh plasma transfusions, plasma exchange, corticosteroids and vincristine (Figure 1). The patient remained in remission until 1994. Between March 1994 and February 1996, this patient had five relapses (incidence of 2.5 relapses/year). The disease-free interval varied from 3 weeks to 9 months (Figure 1). Presenting signs and symptoms during relapses were similar but less severe than those observed in the first episode. The patient repeatedly responded to therapy with plasma infusions and prednisone. Splenectomy was performed 18 days after the last relapse when the platelet counts and LDH levels had returned to normal values with the treatment schedule mentioned before (Figure 1). One week before splenectomy pneumococcal vaccine was given. There were no perioperative complications. The spleen was not enlarged (weight 110 g). The histologic study showed a moderated hyperplasia of follicles in the white pulp. Microthrombi were absent. In the 2 years of follow-up since the splenectomy, the patient had no further relapses.

The pathophysiology of TTP is still poorly under-

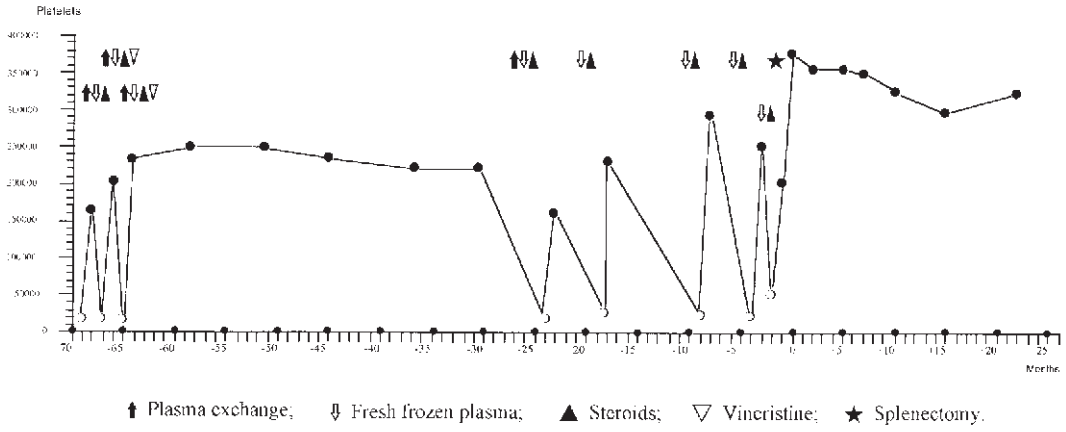

Figure 1. Platelet counts before and after treatment.

stood. The current view includes endothelial damage, presence of abnormally large factor VIII/von Willebrand multimeric structures and a platelet aggregating factor.[9] The contribution of the spleen in this process is unknown. Despite this fact, splenectomy remains an important part of multimodality therapy for TTP. Splenectomy has been used primarily in patients in whom plasma exchange failed to improve haematologic values, but it has been associated with a high mortality.[1,2] Much better results have been obtained when splenectomy was performed at the time of relapse in patients with relapsing TTP.[6,7] This seems to increase disease free intervals with less morbidity and mortality. Recently it has been suggested that splenectomy for relapsing TTP would be more convenient while in remission than splenectomy performed during the relapses. In a small series, splenectomy performed during remission at least postponed relapses in all cases with no surgery-related deaths or major complications.[8] Elective splenectomy was done in our patient after remission of the seventh relapse, and two years later the patient remains in remission. Our result supports evidence that elective splenectomy increases disease-free intervals in patients with relapsing TTP with a minimal risk of perioperative complications. Further studies are warranted to elucidate the role of elective splenectomy in relapsing TTP.

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