Increased bcl-2/bax ratio in B-cell chronic lymphocytic leukemia is associated with a progressive pattern of disease

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In order to evaluate clinical implications of altered expression of bcl-2 and bax proteins in B-cell chronic lymphocytic leukemia (CLL) we studied 27 patients with this disease. Cytofluorometric levels of bcl-2 did not reflect the status of disease. In contrast bax expression was lower in progressive than in non-progressive disease, therefore leading to a higher bcl-2/bax ratio in patients of the former group. If confirmed in longitudinal studies, quantitative cytofluorometric evaluation of bcl-2 and bax protein might help to identify patients with progressive disease who could possibly benefit from early therapy.

In B-cell chronic lymphocytic leukemia (CLL), analysis of bcl-2 and bax expression may help to understand molecular mechanisms underlying extended survival of leukemic cells.[1,2] bcl-2/bax ratio was significantly higher in patients whose disease progressed and who had a poor response to chemotherapy.[3,4]

In a series of 27 $CD5^+$ B-cell CLL patients (16 untreated and 11 treated) we tried to validate these findings by assessing quantitative levels of bcl-2 and bax by flow cytometry (Absolute Flow Cytometer, Ortho Diagnostic System). Results were compared with those obtained from 6 healthy donors. For the purpose of the present study special attention was paid to previously untreated patients; indeed, 8 patients were studied at the time of disease-progression (i.e., change of clinical stage from A to B or C) and 8 during the indolent clinical course of disease.[5]

The immunomarkers used included CD3, CD5, CD22, κ and λ light chain immunoglobulins (Ortho, Raritan, NY, USA); CD19, CD20, CD23, CD11c (Becton Dickinson, San José, CA, USA), and FMC7 (Immunotech, Marseille, France). A marker was considered positive when it was expressed in over 30% of the cells analyzed. In order to detect cytoplasmic bcl-2 and bax proteins in individual cells, the cells were fixed and permeabilized using a commercially available kit (Fix & Perm Permeabilization Kit, CALTAG, Burlingame, CA, USA). Indirect staining for bcl-2 was performed by incubating permeabilized cells with anti-bcl-2 Mo Ab (124 Clone: IgG1, κ isotype, DAKO, Copenhagen, Denmark) as previously reported.[6] For indirect bax staining the cells were incubated with anti-bax polyclonal rabbit Ab (PharMingen, San Diego, CA, USA) (1:20 dilution for 30 min at 20°C), washed and then incubated again, this time incubated with swine anti-rabbit-Ig-FITC (DAKO, Copenhagen, Denmark) (1:100 dilution for 45 min at 4°C). Negative controls were performed by incubating cells directly with anti-rabbit-Ig-FITC serum. In all experiments double staining immunofluorescence was used (i.e., CD19-PE/bcl-2-FITC or CD19-PE/bax-FITC). The Quantitative Immuno-Fluorescence Indirect assay (QIFI kit, DAKO, Copenhagen, Denmark), was used for assessing the amount of bcl-2 and antigen density was expressed as antibody binding capacity (ABC) molecules/cell.[6]

Quantitative evaluation of bax protein expression was assessed on the basis of the value of mean fluorescence intensity (MFI) of the mean of channel fluorescence for each positive sample (linear acquisition, 0-250 channels) after ensuring day-to-day reproducibility of the flow cytometry data (CaliBRITE, Becton-Dickinson, Mountain View, CA, USA).

Intracellular levels of bcl-2 oncoprotein were higher in leukemic cells of B-CLL patients (bcl-2 ABC, 12.2×10^3 molecules/cell; range, 7.6-14.3) than in B-cells of healthy controls (bcl-2 ABC 11.4×10^3 molecules/cell; range, 11.2-11.5)($p = 0.01$; Mann-Whitney test). The same did not apply for bax protein ($p = 0.907$). Data dealing with the amount of bcl-2 and bax protein of B-cell CLL patients stratified on the basis of disease-status are presented in Table 1. As shown, the amount of bcl-2 did not differ with respect to the CLL clinical group (Table 1). As far as bax expression is concerned, a significantly lower lev-

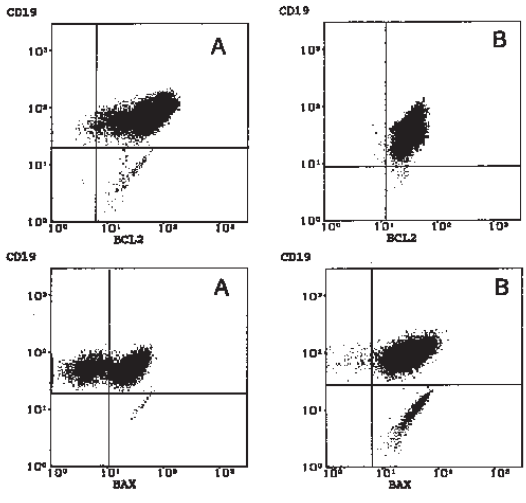

Figure 1. Relative levels of bcl-2 and bax protein expression in leukemic lymphocytes of two representative B-CLL patients. The pattern of bcl-2 expression (i.e., mean fluorescence intensity) was higher in patient A than in patient B. In contrast the pattern of bax expression was lower in patients A than in patient B. As a consequence bcl-2/bax ratios were as follows: patient A, 1.18; patient B, 1.04.

Table 1. Intracellular levels of bcl-2 and bax in CLL patients stratified on the basis of disease status.

	Untreated patients			*Treated patients*		
	Progressive (n = 8)	*Stable (n=8)*	*p-value*	*Responders (n = 7)*	*Resistant (n = 4)*	*p-value*
bcl-2 ABC (10^9 molecules/cell)	12.2 (10.4-13.8)	12.1 (11.7-13.4)	0.602	12 (7.6-14.2)	12.3 (9.1-12.9)	0.678
bax (MFI)	100.8 (87.6-118.2)	114.5 (104-138)	0.02	113 (101.6-126)	108 (103.6-119)	0.452
bcl-2/bax ratio	1.20 (1.10-1.41)	1.01 (0.87-1.18)	0.01	1.02 (0.85-1.16)	1.10 (0.92-1.20)	0.412

p-value = Mann-Whitney test; bcl-2/bax ratio refers to the ratio of mean fluorescence intensities (MFIs).

el of such a protein (bax MFI, 100.8; range, 87.6-118.2) could be observed in patients with progressive disease in comparison with those in whom CLL displayed an indolent clinical course (bax MFI, 114.5; range, 104-138; p = 0.02).

When dealing with bcl-2/bax ratios a higher value was found in patients who more frequently underwent disease-progression (bcl-2 MFI/bax MFI ratio, 1.20; range, 1.10-1.41) while a lower bcl-2 /bax ratio (1.01; range, 0.87-1.18) was consistent with stable disease (p = 0.01; Mann-Whitney test)(Figure 1). The small number of patients who received therapy (11 out of 27) prevented us from demonstrating any correlation between response to chemotherapy and bcl-2/bax ratio (p = 0.412).

Although bcl-2 is overexpressed in $CD5^+$ B-cell CLL, the clinical implications of such a finding are controversial. In a study involving 46 B-CLL patients, we failed to demonstrate any correlation between levels of bcl-2 oncoprotein and parameters of disease activity such as clinical stage, histopathologic pattern of bone marrow (BM) involvement or lymphocyte doubling time (LDT).[6] This observation apparently contrasts with the close relationship between high bcl-2 expression and reduced survival reported in two independent studies.[7,8] Different methods of bcl-2 detection (e.g., polymerase chain reaction [PCR] analysis of mRNA versus cytofluorometric protein detection) may account, at least in part, for these discrepancies. As far as bax expression is concerned, our results demonstrate that levels of such a protein are significantly higher in patients with stable disease thus providing clear evidence that bax acts as a tumor suppressor gene.[1] Furthermore, we explored clinical implications of combining information about bcl-2 and bax. To this purpose the bcl-2/bax ratio was correlated in each patient to the clinical outcome. Interestingly, a low bcl-2/bax ratio identified a subset of CLL patients with relatively indolent and stable disease thus confirming the results from Aguilar-Santelises *et al*.[3] We expect that future investigations will continue to evaluate either bcl-2 or bax expression using cytofluorometric assay since this is a reliable and not time-consuming procedure which is, therefore, useful in clinical practice.

Changes of bcl-2 and bax protein expression have also been shown to have a relevant impact on both *in vitro* and *in vivo* response to therapy with fludarabine.[10] Therefore, our results showing no correlation between bcl-2/bax ratio and response to chemotherapy, should be interpreted with caution because they are based on a small number of patients (i.e., 11 out of 27) mainly treated with chlorambucil.

In conclusion, further work is required to elucidate mechanisms leading to the up- or down-regulation of bcl-2 and bax in B-cell CLL and the clinical implications should be carefully analyzed in longitudinal studies. What emerges from the present study is that bcl-2 and bax, incorporating biological information important for the regulation of B-cell survival, are promising candidates for assessing the pace of disease. This is especially important for patients in early CLL for whom risk of disease progression cannot always be predicted by clinical parameters in current use.[9]

Key words

B-CLL, bcl-2, bax, disease progression

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Reversal of bone marrow fibrosis in idiopathic myelofibrosis after treatment with α-interferon

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A 14-year-old boy presenting with primary myelofibrosis (MMM) was treated with α-interferon (IFN). After 6 months his spleen was no longer palpable and blood counts had returned to normal. A reversal of bone marrow fibrosis was histologically documented. Although improvement in symptoms and blood counts has been reported, this is only the second description of reversal of bone marrow fibrosis in MMM after treatment.

In vitro studies have shown that IFN can suppress proliferation of hemopoietic precursor cells, especially those of the megakaryocytic cell line.[1,2] In addition, IFN can inhibit collagen synthesis by murine fibroblasts.[3] IFN has been used successfully in chronic myeloid leukemia (CML) and may reduce platelet number in essential thrombocythemia.[2] In MMM, however, only half of the patients gain a significant reduction in spleen size as well as in platelet numbers.[2] Except for one case[4] there is no reported evidence that IFN can reverse bone marrow fibrosis in MMM. We report the case of a young patient with MMM who got a clinical remission and normalization of reticulin fibers after IFN treatment.

Case report

A 14-year-old boy was referred to our hospital presenting with a 3-year history of transfusion-dependent anemia and hepatosplenomegaly. Liver 5 cm, spleen 13 cm; hemoglobin: 4.4 g, with anisocytosis, poikilocytosis and dacrocytes. Leukocytes: 4.8×10^9/L; metamyelocytes: 4%, band forms: 2%, segmented neutrophils 54%, eosinophils 2% and basophils 1%. Platelets: 134×10^9/L. Coombs' test was negative.

Bone marrow aspiration yielded a dry tap. A trephine biopsy (Figure 1) showed a marked diffuse increase in reticulin fibers. Megakaryocytes were atypical and increased in number. Granulocytic precursors and erythroblasts were present. A liver biopsy showed myeloid metaplasia in the sinusoids. Inherited red cell abnormalities, autoimmune diseases, nutritional deficiencies and hepatitis were excluded. The karyotype was normal. Due to the severity of the anemia in an adult form of MMM and the absence of autoimmune phenomena, treatment with IFN, 3×10^6 U s.c. three times per week (body surface = 1.17 m^2) was attempted. No significant side effects were observed. Six months later, neither liver nor spleen was palpable. Hemoglobin = 15.2 g, leukocytes 6.7×10^9/L; 1% bands, 41% neutrophils, 2% eosinophils and 0% basophils; platelets: 193×10^9/L. A new bone marrow biopsy (Figure 2) showed normocellular hemopoiesis. Megakaryocytes had a normal morphology. Reticulin fibers were only slightly increased. The dose of IFN was then reduced to 2×10^6 U twice a week. The patient remains well, having normal blood counts 24 months after the start of treatment.

MMM is more frequent after the age of 50 although it may be seen at any age. In children, myelofibrosis is often secondary to another condition (vitamin D deficiency or autoimmunity). The rare cases of idiopathic myelofibrosis usually have a more agressive form.[5-7] Our patient had clinical and hematologic features of adult MMM with a chronic evolution in spite of his young age. This has been described rarely.

The patient was treated with IFN because of the latter's antiproliferative activity in myeloproliferative disorders. IFN has been useful in controlling thrombocytosis and reducing spleen size in almost 70 patients reported in the literature. The criteria for using IFN therapy varied between authors, but it was used in patients with a more proliferative form of disease. The dose schedule was also varied. An optimal response has been achieved with $3\text{-}5\times10^6$ U three times per week for 4-6 months.[1,2,4] The dose used in our patient was in this range. After 6 months of treatment he had a complete clinical reponse and bone marrow biopsy showed an almost complete reversal of myelofibrosis. To our knowledge this is only the second case in which this event has been documented.[4] Interestingly, together with normalization of the peripheral blood counts, hemopoietic cell morphology and marrow fiber content returned to normal, showing that the neoplastic clone was suppressed by IFN. This phenomenon has also been recently described in CML.[8] The role of IFN in the treatment of bone marrow fibrosis in CML is controversial but it has recently been shown that after suppression of the Ph positive clone, the marrow con-

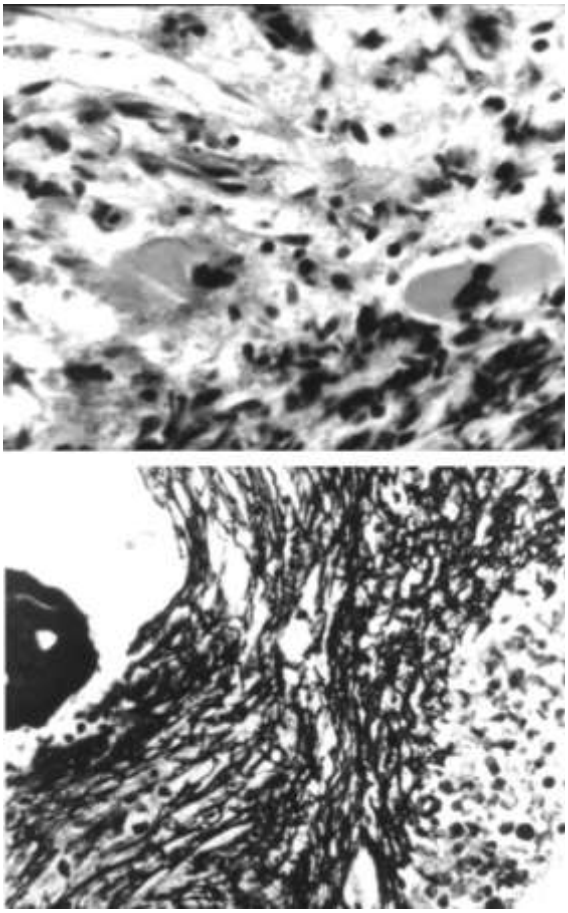

Figure 1. Bone marrow biopsy of the patient at diagnosis. Hemopoietic tissue with diffuse fibrosis (A). Two large atypic megakaryocytes can be seen. HE ×300. Marked increase in coarse reticulin fibers (B). Gomori ×300.

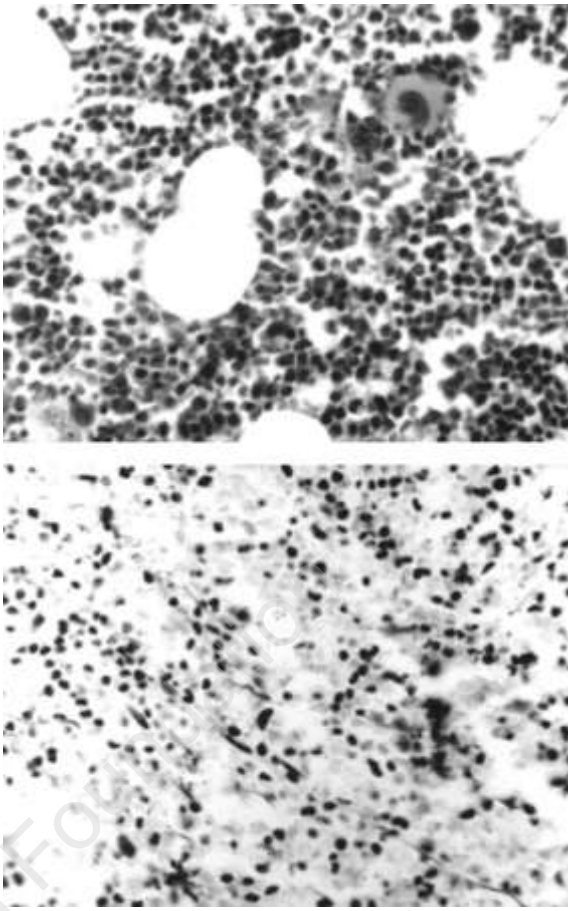

Figure 2. Bone marrow biopsy after 6 months treatment with IFN. Hemopoietic tissue with a normal architecture and presence of all 3 cell lines (A). Few reticulin fibers are present (B).

tent of fibers remains constant or is even reduced.[8,9] The good response observed in our case may be due to the different behavior of MMM in young patients, but documents that IFN is able to reverse bone marrow fibrosis even in more advanced forms of MMM.

Key words

Myelofibrosis, children, interferon, treatment

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Moderate hyperhomocysteinemia is a highly prevalent defect in Spanish patients with venous thromboembolic disease

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Recent studies suggest that mild hyperhomocysteinemia may be a risk factor for venous thromboembolic disease (VTED). In this work we evaluated the prevalence of moderate hyperhomocysteinemia in patients with VTED in our area. We found hyperhomocysteinemia in 23.4% of 64 patients studied compared with 7.35% of 68 healthy controls (p=0.014). Our results suggest that moderate hyperhomocysteinemia is one of the most prevalent abnormalities associated with VTED.

Several studies have concluded that moderate hyperhomocysteinemia is an independent risk factor for atherosclerosis and arterial occlusive diseases in the general population.[1,2] Recent studies suggest that mild hyperhomocysteinemia may also be a risk factor for venous thromboembolic disease (VTED) and its recurrence.[3-6] The objective of this study was to evaluate whether VTED is associated with an increased prevalence of hyperhomocysteinemia in our area. Sixty-four consecutive unrelated Spanish patients with objectively diagnosed VTED (31 females and 33 males, mean age 52.16±15.70) and sixty-eight healthy controls (41 females and 27 males, mean age 46.6±10) were studied in our Institution, between January 1996 and December 1996. The assessment of hyperhomocysteinemia was performed by measuring the concentration of fasting plasma homocysteine and its increase 6 hours after oral methionine loading (PML) (0.1 g L-methionine/kg body weight). Concentrations of plasma homocysteine were determined by high-perfomance liquid chromatography and fluorescence detection.[7] In order to investigate other biological abnormalities causing thrombophilia, we also determined: antithrombin, plasminogen and amidolytic protein C by chromogenic substrates; anticoagulant activity of protein C; total protein S and free protein S by the ELISA method; antiphospholipid antibodies by ELISA; and the factor V Leiden mutation by standardized methods. Hyperhomocysteinemia was defined as fasting plasma homocysteine levels and/or PML absolute increments above the 95th percentile of the level in the control group (respectively 11.43 µmol/L and 28.72 µmol/L).

Hyperhomocysteinemia was detected in 15 patients (23.4%, IC 95% 13.0-33.8), eight females and seven males (mean age 63.18±8.65 yrs) and 5 subjects in the control group (7.35%) (p=0.014). Malignancies were 13 times more frequent in patients with hyperhomocysteinemia than in patients without it. Although the mechanisms underlying this association are unclear, higher plasma homocysteine in patients with cancer has been noted before.[8] It would be interesting to perform more studies to clarify the association between hyperhomocysteinemia and VTED in cancer patients.

Table 1. Patient characteristics.

	Total patients	*No HH*	*HH*
Number of patients	64	49	15
Mean age ±SD	52.1±15.70	49.64 ±15.91	63.18 ±8.65 (p<0.05)
Female:male ratio	33/31	25/24	8/7 (n.s)
Family history of VTED	25 (39.06%)	19 (38.77%)	8 (53.33%) (n.s)
Recurrent VTED	32 (50%)	25 (51.02%)	10 (66.66%) (n.s)
Mean age at first event	44.50	42.08	52.46 (p<0.05)
Malignant disease*	5 (7.81)	1 (2.04)	4 (26.66)
Other defects	5 (7.81%)	3 (6.12%)	2 (13.33%)
Oral contraceptives°	9 (14.06%)	7 (14.28%)	2 (13.33%)

*HH: hyperhomocysteinemia; VTD: venous thromboembolic disease; *when cancer patients are excluded from the analysis, the patients with VTD show a tendency toward higher plasma homocysteine than control group (p=0.06); °only women considered, n.s: non significant. Fisher's exact test.*

Within the group of patients who had had at least one objectively diagnosed VTED, the age at first event was lower in patients without hyperhomocysteinemia than in patients with hyperhomocysteinemia (42.08± 15.41 years compared with 52.46±8.13 years; p<0.05). Recurrences and family history of VTED were more frequent in patients with hyperhomocysteinemia than in patients without hyperhomocysteinemia, but differences were not significant. As for other deficiencies, two patients of the hyperhomocysteinemia group had antiphospholipid antibodies, whereas two patients of the non-hyperhomocysteinemic group had factor V Leiden mutation while another had activated protein C resistance without factor V Leiden mutation. Hyperhomocysteinemia did not seem to add to the thrombotic risk of oral contraceptives (Table 1).

This study is the first report on the prevalence of hyperhomocysteinemia in a Spanish population with VTED. It was present in about 23% of patients with VTED and our results suggest that moderate hyperhomocysteinemia is a common biologic abnormality in these individuals.[9] We are, therefore, of the opinion that homocysteine assessment should be included in the laboratory evaluation of patients with VTED. Measurements of fasting plasma homocysteine and post-methionine levels should be performed because the detection of hyperhomocys-

teinemia is considerably increased by using the latter test. After confirmation of the existence of hyperhomocysteinemia, other tests to study its possible origin (such as folate and vitamin B6 and B12, and investigation of renal function) as well as its treatment should be considered.

Funding

AC was supported by the Fundació per a la Bioquímica Clínica i Patologia Molecular.

Key words

Homocysteine, venous thrombosis, cardiovascular disease

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Percutaneous umbilical blood sampling in the management of immune thrombocytopenic purpura during pregnancy

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Severe neonatal thrombocytopenia occurs in about 15% of deliveries from women with immune thrombocytopenic purpura (ITP). Conflicting data exist about the real usefulness of percutaneous umbilical blood sampling (PUBS) in evaluating the fetal platelet count. We report successful experience, using PUBS, in the management of 12 pregnant women with ITP.

Immune thrombocytopenic purpura (ITP) is a common autoimmune disorder of young women, accounting for 3% of all cases of thrombocytopenia at the time of delivery.[1] ITP in pregnancy can cause an impairment of maternal, fetal or neonatal hemostasis. A maternal platelet count of $>30\times10^9$/L is only rarely associated with severe hemorrhage in pregnancy, during vaginal delivery or Cæsarean section.[2] There is some debate as to the real risk to the fetus and neonate, regardless of maternal or fetal platelet count or the route of delivery.[2-4] Reported data show a 15% incidence of severe neonatal thrombocytopenia (platelet count $<50\times10^9$/L), and a 1.5% incidence of intracranial hemorrhage (ICH).[5] However, other authors have documented a lower incidence of severe neonatal thrombocytopenia without any hemorrhagic complications.[1] Although some clinical and laboratory parameters have been proposed as being helpful in the identification of those pregnant women with ITP at risk of giving birth to severely thrombocytopenic neonates,[6] conclusive data are lacking.

Scioscia *et al.*[7] demonstrated the usefulness of percutaneous umbilical blood sampling (PUBS) in predicting fetal platelet count. PUBS may guide the mode of delivery and obviate unnecessary Cæsarean sections when fetal platelet count is $\geq 50\times10^9$/L. However, PUBS carries a risk of 1-2% of causing intrauterine fetal death or the need for urgent delivery.[7,8]

Our experience concerns 12 pregnant women (median age 30 yrs, range 21-39 yrs) submitted to PUBS. None had hepatitis B, C or HIV. Seven patients had a previous diagnosis of chronic ITP, whereas the other 5 were diagnosed during pregnancy (median time of diagnosis 18th week, range 8th-31st week) according to McMillan's criteria.[9] Six patients were primigravida and 6 multipara, 3 of whom had previously delivered a thrombocytopenic neonate. Patients in whom PUBS showed a fetal platelet count $< 50\times10^9$/L were submitted to Cæsarean section. PUBS was most often performed during the 38th-39th week of pregnancy (Table 1) with a 20 gauge needle.

Fetal blood sampling was successfully achieved in all 12 patients without any complications. Three fetuses with a platelet count $< 50\times10^9$/L were delivered by Cæsarean section. Spontaneous vaginal delivery was allowed to occur in all the other cases. Fetal and neonatal platelet counts always correlated. The interval between PUBS and delivery ranged from 0-7

Table 1. Clinical and laboratory data of 12 pregnant women with ITP and those of fetuses / neonates monitored by PUBS.

Pt.	PUBS (week)	Maternal Plt count (10^9/L)	Fetal Plt count (10^9/L)	Neonatal Plt count (10^9/L)	Maternal therapy	Mode of delivery	Bone aspirate	Anti-Plt Ab PaIgG/ SBIgG
1	39	83	210	238	C	Spon	Yes	-/-
2	36	20	15	54	C, Ig	Cs 37	Yes	+/+
3	38	88	48	33	/	Cs 38	Yes	+/+
4	39	73	376	300	/	Spon	Yes	-/+
5	38	56	60	100	C	Spon	Yes	-/+
6	38	81	282	243	C	Spon	Yes	-/+
7	38	80	268	231	C	Spon	Yes	-/+
8	39	66	203	257	C, Ig	Spon	Yes	+/+
9	38	76	47	52	C	Cs 39	Yes	+/+
10	35	29	245	210	C	Spon	Yes	+/+
11	39	44	230	205	C	Spon	Yes	+/+
12	38	45	130	110	C	Spon	Yes	-/-
Median		69.5	206	207				
Range		20-88	15-376	33-300				

Abbreviations: C = corticosteroids; Ig = high dose immunoglobulin; Cs = Cæsarean section; Spon = spontaneous full-term delivery.

days. The three severely thrombocytopenic neonates did not manifest a hemorrhagic syndrome and spontaneously recovered a normal platelet count within 2 weeks. Occasional fetal morbidity or mortality from hemorrhagic complications of ITP during pregnancy encourage some authors to favor the use of PUBS.[7, 10] Other authors argue that the risks associated with PUBS are greater[2,4] and recommend determining the route of delivery by maternal obstetric indications.

Our encouraging experience provides further evidence that in skilled hands PUBS may be useful in the management of pregnant women with ITP, providing a safe way to guide the mode, site and time of delivery.

Key words

Immune thrombocytopenic purpura, pregnancy, percutaneous umbilical blood sampling

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Adenovirus pneumonitis successfully treated with intravenous ribavirin

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Adenovirus infections are a frequent cause of severe complications in the post allogeneic bone marrow transplantation period, and to date, no established form of treatment exists. We report the case of an autologous bone marrow transplant recipient who developed adenovirus pneumonitis which was successfully treated with intravenous ribavirin.

Since conditioning regimens largely ablate virus specific immunity, there may be a reactivation of latent viruses such as adenovirus. The incidence of adenovirus infection in BMT recipients, according to the largest published review was 5%[1] although it may be as high as 18 % in the pediatric population, in second place after herpes simplex.[2] When disseminated adenovirus infection occurs, it mainly affects the urinary tract, liver, gut and lungs, and can prove fatal in half the cases.

Adenovirus is more common after an allogeneic transplant, and a significant relationship between post-transplant adenovirus infection and the occurrence of acute graft-versus-host disease has been described.[1] We present the case of a patient who developed adenovirus pneumonitis after undergoing an autologous BMT, and who was successfully treated with intravenous ribavirin.

A 43-year-old man with acute myeloid leukemia in first remission underwent autologous BMT using TBI (13.2 Gy) and CY 60 mg/kg two day conditioning. On day 0, 300 cc of autologous bone marrow was infused with CMN 2.17×10^8/kg and CFU-GM 4.34×10^4/kg. On day +20, after persistent fever without an identifiable focus treated with imipenem-teicoplanine-amphotericin B, he developed a persistent non-productive cough, dyspnea, hypoxemia, a

worsening in his general condition, as well as painful hepatomegaly. Analysis showed bilirubin 3.7 mg/dL (normal values up to 1) LDH 638 U/L (normal values up to 460); chest X-ray revealed diffuse alveolar-interstitial infiltrates. The BAL performed ruled out *Pneumocystis carinii*, HSV, RSV, CMV, Legionella, BARR or fungal infection. The echocardiogram showed no abnormalities. Saline restriction measures were taken and diuresis was stimulated but the patient's condition did not improve.

On day +28 he was transferred to the intensive care unit. One day later, adenovirus was isolated in BAL, so i.v ribavirin was administered along with assisted ventilation; 48 hours later the fever disappeared and a marked improvement was observed in breathing and liver function. Total resolution occurred on day +37. The ribavirin dosage administered was 15 mg/Kg every 6 hours for 8 days. A further BAL was carried out on day +46 which was negative for adenovirus. The leukocytic graft reached 1,000 leukocytes with 500 granulocytes on day +29, fell to 200 on day +36 which required G-CSF and remained at < 500 granulocytes up to day +48.

Although adenovirus may remain present in tonsillar and other lymphoid tissue for prolonged periods, if isolated from a BAL done under optimal conditions in which no other pathogens can be found, this can be considered diagnostic of acute adenovirus. To date, the efficacy of intravenous ribavirin has been demonstrated in adenovirus infections such as cystitis,[3-5] nephritis,[6] gastroenteritis,[7] pneumonitis,[8] and disseminated adenovirus infection.[9] The dosage employed by most authors varied between 15 and 30 mg/kg/d divided in three doses. In our case, following Wulffraat *et al.*,[8] we administered a dosage of 15 mg/kg/6 h (a total dosage of 60 mg/kg/d), which led to rapid clinical improvement and clearance of adenovirus infection. This did, however, have a negative affect on the leukocytic graft which, fortunately, was reversible. The hematologic effects of ribavirin have been investigated in Rhesus monkeys. Mild normocytic anemia or severe anemia occurred when ribavirin was administered at dosages of up to 30 or 50 mg/kg/day, respectively; however, no significant effects were observed on white blood cells.[10]

Like other authors, we consider that intravenous ribavirin is an effective treatment for adenovirus infection, but believe it is necessary to determine the exact dosage at which toxic effects are avoided but efficacy is maintained.

Key words

Autologous bone marrow transplantation, adenovirus pneumonitis, ribavirin

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Portal and mesenteric venous thrombosis in a patient heterozygous for the 20210 A allele of the prothrombin gene

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We give the first description of portal and mesenteric venous thrombosis associated with the 20210 A allele of the prothrombin gene in a 48-year-old woman after splenectomy.

Recently, the 20210 A mutation of the prothrombin gene has been described in patients who have had venous thromboses in unusual sites, such as the superior sagittal sinus and in the Budd-Chiari syndrome.[1,2] Mesenteric thrombosis in patients with idiopathic thrombocytopenic purpura (ITP) undergoing splenectomy is uncommon. The usually transient post-splenectomy thrombocytosis has a not well defined effect on the development of thromboembolism.

However, this condition can be a predisposing risk factor for thrombosis in chronic myeloproliferative disorders and hemolytic anemias.[3] Moreover, genetic abnormalities such as inherited deficiencies of antithrombin III, protein C and protein S and factor V Leiden, have been associated with portal thrombosis.[4,5] To our knowledge we give the first description of a patient with venous portal and mesenteric thrombosis who is heterozygous for the 20210 A allele of the prothrombin gene.

A forty-eight year old woman was admitted to our hospital on May 19, 1997, complaining of diffuse abdominal pain for ten days, and fever for two days prior to admission. She had no history of thrombotic events. The patient had been diagnosed in June 1996 as having ITP. She was refractory to steroids so she had undergone a splenectomy on April 23, 1997 without any acute complications.

The patient was conscious when admitted to hospital. Her temperature was 37ºC and blood pressure was 130/80 mmHg. Lungs and heart were normal. Abdominal examination revealed no remarkable abnormalities. Routine laboratory findings were normal, except for an increased platelet count (838,000/mm^3). Ultrasonography of the upper and lower abdomen showed an abnormal liquid collection (about 150 mL) in a perivesical location and the pouch of Douglas. A computed tomography scan showed abnormal hypodensity of the superior mesenteric vein with no contrast filling of mesenteric and portal vein districts. An arteriography was also performed which revealed mild stenosis of the superior mesenteric artery, 2-3 centimeters from its origin with no contrast filling of the mesenteric vein. Furthermore, there was an apparent lack of contrast filling in the superior portal vein, supporting the possibility of another thrombus in this location.

The patient started anticoagulation therapy with non-fractionated heparin; her symptoms improved. Ten days after, an echo Doppler scan showed partial recovery of portal vein patency. The patient was discharged with optimal INR levels.

Factor V Leiden or deficiencies of antithrombin III, protein C or protein S were excluded. Antiphospholipid antibodies and lupus like anticoagulant were not observed. Analysis of the prothrombin gene was performed as described elsewhere,[6] showing the presence of heterozygosity for the 20210 A mutation. This is the first reported clinical observation of a patient with portal and mesenteric venous thrombosis associated with a 20210 A genotype of the prothrombin gene. The combined presence of two predisposing factors for the development of venous thrombosis (recent surgery and thrombocytosis) could increase thrombin formation which, for a subject with the 20210 A mutation (with higher plasma thrombin levels),[7] could trigger clot formation in unusual locations.

This mutation has been considered as a mild risk factor for venous thrombosis. Its prevalence is about 2% in healthy controls and 6% in unselected consecutive patients with venous thrombosis. However, our case and two recent reports of this mutation being found in patients with venous thrombosis in unusual locations, suggest that the 20210 A allele of the prothrombin gene could have a similar clinical penetrance to other inherited deficiencies. This seems to indicate the need for systematic screening for this mutation, as well as for other thrombosis risk factors in all cases of mesenteric and portal vein thrombosis.

Funding

The study has been supported by FIS 97/1150.

Key words

Prothrombin 20210 A, portal thrombosis, splenectomy.

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