Acute myeloblastic leukemia with trilineage myelodysplasia and CD34+ blast cells with abnormal chromatin clumping

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Abnormal chromatin clumping can be observed in myelodysplastic syndromes, myeloproliferative syndromes, acute leukemias and the abnormal chromatin clumping syndrome.[1] It occurs mainly in mature and semimature blood cells. We report a case of acute myeloid leukemia with trilineage myelodysplasia (AML/TMDS) in which there was chromatin clumping in blast cells.

A 66-year-old woman was hospitalized with weakness, anorexia, dry cough and dyspnea. Physical examination showed small generalized lymphadenopathy without hepatosplenomagaly.

Hematological data were: leukocytes 50.7×10^9/L (7% lymphocytes, 3% monocytes, 12% neutrophils, 1% myelocytes and 77% blasts); Hb 107 g/L, and platelets 142×10^9/L. Mature neutrophils showed pseudo-Pelger abnormality. Blasts were medium size with a high N/C ratio, basophilic cytoplasm without granules and occasionally, contained Auer rods. They had a nucleus with 1-2 nucleoli and abnormal dense chromatin accumulations (Figure 1). Myeloperoxidase staining was positive in 58% of them. Immunophenotyping showed expression of CD13, CD33, CD34, CD15, HLA-DR, C-Kit, CD4, CD7, CD11b and cMPO. The LAP index was zero. Bone marrow was hypercellular with a 17:1 myeloid:erythroid ratio, and erythroid and megakaryocytic dysplasia. The cell-cycle-phase analysis of peripheral blood blasts showed that 1.4% of cells were in the S-phase. The clonogenic growth *in vitro* at day +14, was marked by high cluster and low colony formation (50 colonies and 563 clusters per 10^5 cells plated :clust/col ratio >3.5). Karyotype analysis evidenced del 7q in 40% of the metaphases. Ultrastructural study showed blasts with a round nucleus, a single nucleolus and an integral nuclear membrane. Large well-defined blocks of heterochromatin were attached to the nuclear membrane and to the nucleolus in 48% of blast cells and occasionally, Auer rods were detected (Figure 2). The final diagnosis was *acute myeloid leukemia with trinlinage myelodysplasia* (AML/TMDS). Hematological remission was obtained after two courses of idarubicin plus cytarabine. However, 13 months later the patient relapsed.

Abnormal chromatin clumping (ACC) is a sign of severe dysplasia described in the abnormal chormatin clumping syndrome (ACCS), a distinct entity that has both proliferative and trilineal or bilineal dysplastic features,[1,2] but it is also present in myelodysplastic syndromes (MDS), reactive dyshemopoiesis,[3] chronic myelogenous leukemia both Ph' positive[4] and negative,[5] and acute myeloid leukemias. Furthermore it has been described in the erythroid and megakaryocyte series, in eosinophils and basophils,[2] in neutrophils as far as the myelocyte stage[1] and in lymphocytes,[6] but to our knowledge, not in blast cells. It seems to reflect an altered euchromatin/heterochromatin ratio.[1] If ACC is a sign of dysplastic maturation, it is not surprising that it can be present in AML/TMDS, a *de novo* AML with dysplastic features in all three hematopoietic lineages, including the pseudo-Pelger anomaly.[7] As in the case reported by Vallejo *et al.*,[8] there is a low level of S-phase cell cycle cells in peripheral blood and a high and anomalous cell clonogenic pattern of myelodysplastic/myeloproliferative type.[8,9] ACCS is genetically heterogeneous,[1,2,5]

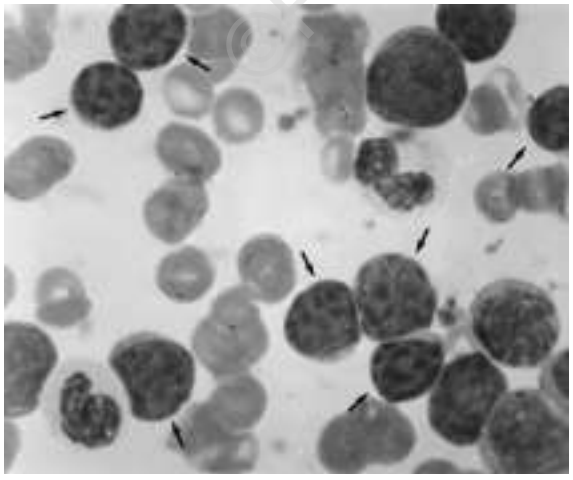

Figure 1. Numerous bone marrow blasts (arrows) presenting blocks of heterochromatin separated by clear zones and two mature granulocytes with pseudo-Pelger anomaly (May-Grünwald-Giemsa ×1,000).

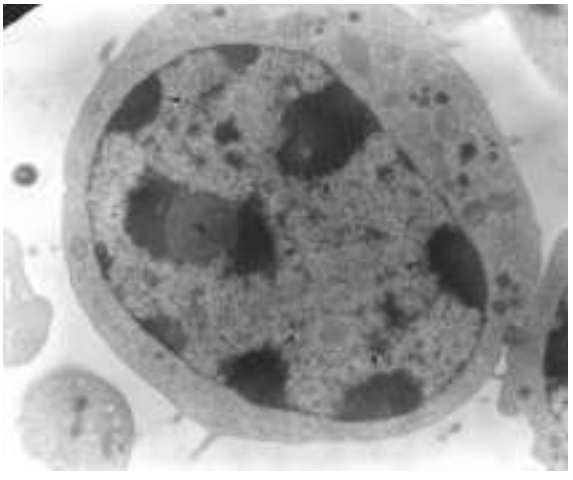

Figure 2. Blast with a single nucleous (n) and an integral nuclear membrane. Note the multiple large blocks of condensed chromatin (arrows) and the well preserved cytoplasmic organelles (Uranyl acetate-lead citrate, original magnification ×8,000).

but chromosome 7 abnormalities, which are frequently found in MDS and AML/TMDS,[7] have not been reported. Del 7q implies loss of genes, some of which play a major role in myeloid differentiation and have putative tumor suppressor function.[10] We did not find 17p aberrations in any metaphases.

In conclusion, this case shows that ACC is a sign of dysplastic maturation affecting not only mature and semimature cells of all hematopoietic lineages, but also stem cells.

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Key words

Chromatin clumping, acute myelogenous leukemia, trilineage leukemia

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Familial hairy cell leukemia: a HLA-linked disease or farmers-linked disease?

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In the 16 cases of familial HCL published, different HLA have been found. Although specific HLA antigens were found to have the same structure in some cases that suggests a genetic predisposition to HCL. Environmental factors, specifically farming labours, were implicated in too. We add two cases (father and son, both farmers), Their HLA haplotype has not been described, but the type A2Bw4Bw6 and Bw6, presented in father and/or son, have been.

A 70-year-old man was admitted to hospital for excessive sweating. The patient worked as a farmer. The physical examination revealed splenomegaly. The man's leukocyte count was 5.6×10^9/L, platelet count was 96×10^9/L, and he had a hemoglobin concentration of 11.8 g/L. The differential count showed 20% hairy cells CD19, CD20, CD25, CD11c. Bone marrow aspirate and biopsy were diagnostic of hairy cell leukemia (HCL). He was treated with α_2a-interferon, and remains in remission for five years since the diagnosis. Three years after his father's diagnosis, the 27-year-old son of the patient described above, was found to have thrombocytopenia. His physical examination revealed splenomegaly with bilateral axillary lymphadenopathy. The son was also a farmer. His leukocyte count was 10.3×10^9/L, with a platelet count of 83×10^9/L and a hemoglobin of 14 g/L. The differential count revealed 15% of hairy cells CD19, CD22, DR, CD20, CD23, CD25, CD11c, CD103 and FMC7 positive. Bone marrow aspirate and the biopsy were again diagnostis for HCL. The son was given 2-deoxycoformycin, and has now been in remission for two years since his diagnosis. HLA typing was carried out with standard procedures using a microcytotoxicity assay in both cases (Table 1).

Table 1. HLA typing.

Son	A80	Cw6	B45	Bw6	DR9	DR53	DQ2
	A32	C-	B67	Bw6	DR3	DR52	DQ2
Father	A80	Cw6	B45	Bw6	DR9	DR53	DQ2
	A2	C-	B51	Bw4	DR14	DR52	DQ5

Exposure to chemicals, occupational factors, ionizing radiation[1,2] and hereditability[3] have been reported

to be risk factors for HCL. Recently, an elevated risk for developing HCL has been reported in farmers.[1,2] Familial HCL, defined as the occurrence of HCL among numerous first degree family members, has previously been reported in only seven families.[4-10] Analyzing the HLA typing in these families, similarities has been found in the HLA types, raising the possibility that HCL is an HLA-linked disease. In contrast, no specific HLA antigens have been found in unrelated cases of HCL, but an increased frequency of B17 and DR11 antigens compared to those found in the normal Caucasian population, has been recorded.[11] In familial cases, there were different haplotypes reported to be specific for the disease: type A1 A3 B8 B149; type A1 B78; type A3 A9 B7 Cw610; type A3 B3 B7 DR2 7; type A2 Bw4 Bw62(15)Cw1DR4 DRw53 DQ33; and type A3 B7 or A2 Bw4 and Bw64. It is interesting that the type A3 B7 and A2 Bw4 Bw6 were reported in various cases and the type Bw6 was common in all the cases reported by two authors.[4,5] The hypothesis that HCL is an HLA-linked disease was, therefore, considered, but not proven.

We present two patients (father and son); HLA typing of our patients showed the haplotype A80 B45 Bw6 Cw6 DR9 DR53 DQ2. This haplotype has not previously been seen in reported cases of familial HCL. The father's HLA showed an interesting association of HLA type A2Bw4Bw6, previously reported.[4,5] We feel that our findings strengthen the possible association between these antigens and the development of HCL. However, environmental factors could play a role in the genesis of HCL within families. It has recently been published that the most frequent occupation among 48 Swedish men and women with HCL was farming or gardening (31%) (39% of the men).[2] Our two patients were farm workers exposed to suspected carcinogens. The existence of some HLA antigens in common suggests a familial predisposition to the disease,[3] however, familial HCL is not associated with a specific HLA haplotype; the role of an environmental risk factor, to which the affected members of the families were exposed, cannot be excluded either.

Key words

Familial hairy cell leukemia, hairy cells, HLA typing, HLA-linked disease

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Tumor burden and serum level of soluble CD25, CD8, CD23, CD54 and CD44 in non-Hodgkin's lymphoma

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We studied the value of soluble CD25, CD8, CD23, CD54 and CD44 serum levels as tumor burden markers in lymphoma. Soluble CD25 compared with the others sCD and the usual serum factors (albumin, lactate dehydrogenase, β_2-microglobulin, uric acid and C-reactive protein), showed the strongest correlation with the Ann Arbor stage and the number of affected localizations. sCD25 level is the most sensitive serum marker for tumor burden in lymphoma.

Tumor burden is an important prognostic factor in lymphoma.[1] Tumor burden is roughly approximated by physical examination, bone marrow biopsy and imaging techniques. Estimating tumor burden by a non-invasive method is an old interest in oncology. Over the last decade, the measurement of soluble receptors levels has been explored as an additional tool for the assessement of tumor burden and prognosis in patients with lymphoma.[2,3] Several investigations in different histologic subtypes of non-Hodgkin's lymphoma (NHL) have demonstrated the good association between soluble serum interleukin-

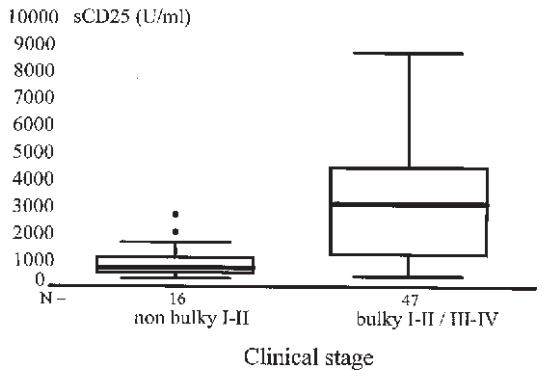

Figure 1. Serum sCD25 levels in relation to clinical stage. All patients with non bulky I-II stage had a serum sCD25 level < 3,000 U/mL. The highest value in the NHL group is not showed. The center line represents the median.

2 α receptor (sCD25) level and the tumor burden.[4,5] Soluble sCD25 was considered the most sensitive serum marker for tumor burden in lymphoma.[6] Others soluble receptors seem to be associated with tumor burden, e.g. sCD8,[7] sCD23,[8] sCD54[9] and sCD44,[10] and interesting biological-clinical correlations have been established in chronic lymphocytic leukemia.[11,12] The value of these new serum factors for estimating tumor burden had not been evaluated versus soluble CD25.

We measured serum levels of sCD25, sCD8, sCD23, sCD54 and sCD44s at diagnosis in 63 adults patients with NHL diagnosed from January 1991 to July 1994. Patients with clinical evidence of acute infection were not included in this study. The extent of disease, evaluated according to standard criteria, was expressed by the Ann Arbor stage system, and by the number of affected nodal or extranodal localizations. The histologic specimens were revised according to the updated Kiel classification. The series included 30 patients histologically classified as having low-grade NHL, and 33 patients classified as having high-grade NHL. Patients with chronic lymphocytic leukemia, hairy cell leukemia and Waldenström disease were excluded from analysis. Serum samples were also taken from 49 healthy adult persons (controls). The measurements were performed by enzyme-linked immunosorbent essay with commercially available kits: sCD25, sCD8 and sCD23 came from T Cell Science, sCD44 and sCD54 from Bender Medsystems.

Serum values of sCD25, sCD8, sCD54 and sCD44 were elevated in NHL in comparison with normal controls ($p < 0.0001$; see Table 1). Values of sCD25 were markedly increased, in contrast the median of sCD23 in NHL was not different from that in the control group. Of the sCD molecules, only sCD25 and sCD8 showed significant differences between stages I-II and III-IV (see Table 1). Nevertheless no cut-off for sCD25 or sCD8 could separate the patients well according to the stage. When we associated the stage with the presence of bulky disease, sCD25 showed some discriminant utility (see Figure 1): all patients with non bulky I-II stage (16 cases) had a sCD25 < 3000 U/mL. Nevertheless, 22 out of 47 with extended disease (bulky I-II and III-IV stages) also had a serum sCD25 < 3000 U/mL ($p = 0.0002$, chi-square test). Among sCD molecules, sCD25 showed the stronger correlation with the Ann Arbor stage, the number of affected localizations (nodal, extranodal and total), and

Table 2. Correlations between serum sCD levels and clinical parameters in NHL patients.

	sCD25 *p*	*sCD8* *p*	*sCD23* *p*	*sCD54* *p*	*sCD44* *p*	*LDH* *p*	*β_2m* *p*
High-grade NHL group							
Ann Arbor stage	**	ns	ns	*	*	**	*
Number localizations	**	*	ns	**	*	**	*
LDH	**	**	ns	**	**	-	**
β2-microglobulin	**	**	ns	**	**	**	-
Low-grade NHL group							
Ann Arbor stage	*	ns	ns	ns	ns	ns	ns
Number localizations	*	*	ns	ns	ns	*	*
LDH	**	ns	ns	**	*	-	*
β2-microglobulin	*	**	ns	ns	*	*	-

Spearman´s rank correlation is showed by the p value (two tailed).
**p ≤ 0.05; ** p≤ 0.01; ns: not significant (p > 0.05).*

Table 1. Serum sCD in NHL patients according to Ann Arbor stage.

	No. of cases	*sCD25 (U/mL)* *median*	*p*	*sCD8 (U/mL)* *median*	*p*	*sCD23 (U/mL)* *median*	*p*	*sCD54 (ng/mL)* *median*	*p*	*sCD44 (ng/mL)* *median*	*p*
Control vs NHL											
Control	49	385	< 0.0001	370	< 0.0001	140	ns	248	< 0.0001	502	< 0.0001
NHL	63	1,757		655		174		550		894	
Ann Arbor stage											
I-II	24	789	0.0006	544	0.0144	128	ns	502	ns	856	ns
III-IV	39	3,174		810		214		600		976	

Levels of sCD are shown as median. ns: not significant (p > 0.05).

the serum levels of lactate dehydrogenase (LDH) and β_2-microglobulin (β_2m), as shown in Table 2. Of all the serum factors studied, i.e. the sCD and the usual serum markers (LDH, β_2m, albumin, uric acid and C-reactive protein), sCD25 also showed the strongest correlation with tumor burden (data about albumin, uric acid and C-reactive protein are not shown in Table 2).

In conclusion, serum levels of sCD25, sCD8, sCD54 and sCD44 are roughly proportional to the burden of neoplasia, but sCD25 is clearly more sensitive as a marker of tumor burden than others sCD. sCD25 is also clearly a more sensitive marker of tumor burden than usual serum factors. Measurements of sCD25 can be indicated for stage assessment in all patients with NHL.

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Key words

Serum markers, tumor burden, sCD25, sCD8, sCD23, sCD54, sCD44, non- Hodgkin's lymphomas.

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Thyroid volume is progressively reduced as a sequela of neck irradiation for childhood Hodgkin's disease

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Thyroid volume reduction was observed, among 25 subjects off-therapy after Hodgkin's disease. The volume reduction was related to dose (p=0.014) and time from radiotherapy (p=0.01). The correlation was very specific since all patients with reduced volume had hypothyroidism, but not very sensitive since 25% of subjects with thyroid dysfunction had normal gland volume.

As the thyroid gland is frequently within the field of neck irradiation for Hodgkin's disease (HD), patients treated in this way may have an increased risk of secondary thyroid carcinoma.[1-3] It is, therefore, recommended that the follow-up of these patients includes thyroid ultrasound examination[4,5] and monitoring of thyroid hormones.[6] We followed-up 25 children who had been treated for Hodgkin's disease; 22 of them received neck irradiation, while performing thyroid ultrasound screening, we also measured the gland volume, and this information was compared with that of the thyroid function.

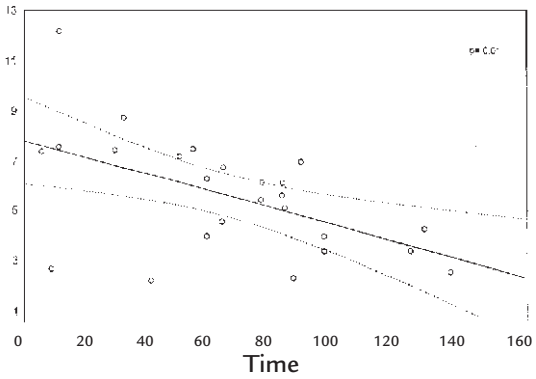

Figure 1. Regression line and 95% CI of the thyroid volume measured by us in patients evaluated at different times after completion of treatment for childhood Hodgkin's disease.

Table 1. Presenting features, treatment modalities and thyroid evaluation in 25 subjects treated for childhood Hodgkin's disease

Pts.	Sex/age	Chemotherapy	RT dose on neck	Laryngeal protection	Age at evaluation	FT4 (nv 7-19 pg/mL)	FT3 (nv 2.3-4 pg/mL	Basal TSH (nv 0.3-3.8)	Stim. TSH (peak)	Thyroid volume (mL) observed	Thyroid volume (mL) age-related mean±SD
1	F/8	MOPP x 8; ABVD x 3	22.5	no	21	3.1	0.6	3	17.5	2.9	6.3±1.5
2	M/7	MOPP x 3; ABVD x 3	25.5	yes	20	6.6*	2.5	6.1	26.9	4.6	6.3±1.5
3	M/11	MOPP x 3; ABVD x 3	26	no	22	6	2.9	3.7	18.7	3.7	6.3±1.5
4	M/13	MOPP x 3; ABVD x 3	42	yes	23	5.4	2.6	1.5	18.7	3.6	6.3±1.5
5	F/12	MOPP x 5; ABVD x 5	26	yes	20	8.1	1.8	4.7	22.3	5.6	6.3±1.5
6	M/6	MOPP x 3; ABVD x 3	25.5	yes	13	12.4	3.2	1.3	4.8	5.3	6.1±1.6
7	M/2	ABVD x 3	–	–	9	11.5	3.6	3.4	23.2	7.2	3.6±1.3
8	F/12	MOPP x 3; ABVD x 3	25	no	20	8.4	1.9	1.2	11.8	4.2	6.3±1.5
9	M/7	ABVD x 3	30	no	15	1*	2.6	15.2	100	2.5	6.3±1.5
10	M/11	MOPP x 3; ABVD x 3	–	–	18	9.9	4	2.1	10.2	6.3	6.3±1.5
11	M/7	MOPP x 3; ABVD x 3	26	yes	15	8.2	3.4	2.3	20	5.8	6.3±1.5
12	M/6	ABVD x 3	25.5	no	13	7.8	3.3	3.7	19.8	6.3	6.1±1.6
13	M/8	ABVD x 3; CEP x 11	25/37.6°	yes	15	6.2*	2.6	12.1	77.8	2.7	6.3±1.5
14	M/13	MOPP x 3; ABVD x 3	26	yes	19	9.9*	2.3	6.8	38.6	4.7	6.3±1.5
15	M/9	ABVD x 3	20	yes	14	5.6	2.4	3.6	12.8	6.9	6.3±1.5
16	M/6	MOPP x 1; ABVD x 2	20.8	yes	11	7.4	2.4	1.2	9.9	4.1	4.9±1.5
17	F/15	MOPP x 3; ABVD x 3	20	no	21	7.3	2.5	1.5	16.9	6.4	6.3±1.5
18	F/9	MOPP x 1; OPPA x 1; COPP x 2	–	–	14	6.6	3.3	2.6	41	7.6	6.3±1.5
19	M/12	MOPP x 3; ABVD x 3	20	yes	17	9.9*	2.3	4.2	35.5	7.3	6.3±1.5
20	F/12	MOPP x 3; ABVD x 3	20	yes	16	5.3*	3	11	103	2.3	6.3±1.5
21	F/14	ABVD x 3	20	no	17	7.5	2.2	1.8	13.8	8.8	6.3±1.5
22	M/11	MOPP x 3; ABVD x 3	20	no	15	5.8	2.9	9.5	29.1	7.5	6.3±1.5
23	F/13	ABVD x 3	20	yes	14	9.1	3	2.6	27.5	12.2	6.3±1.5
24	F/13	MOPP x 3; ABVD x 3	36	yes	15	9.1	3.6	2.4	14.8	7.6	6.3±1.5
25	F/8	MOPP x 3; ABVD x 3	23.6	yes	9	8.4	3.3	2.3	22	7.4	3.6±1.3

*ABVD = adriamycin + bleomycin + vinblastine + imidazole carboxamide; OPP = nitrogen mustard + vincristine + procarbazine + prednisone; OPPA = vincristine + procarbazine + prednisone + adriamycin; COPP = vincristine + procarbazine + prednisone + cyclophosphamide; CEP = CCNU + etoposide + prednisone; *on replacement therapy with thyroxine; °neck was irradiated twice, once during front-line mantle irradiation, and again after disease relapse.*

None of the patients developed symptoms related to thyroid dysfunction or overt thyroid enlargement; one patient had a single thyroid nodule and regional lymph node enlargement. Thyroid function (FT4, T4, FT3, T3, basal and stimulated TSH) was normal in 9 of the 25 patients (36%), including 6 of the 22 (27%) who had received neck irradiation and 3/3 patients not irradiated (Table 1); 8 patients (32%) had low FT3 and FT4 levels, with increased basal TSH. Increased TSH response to TRH was present in 7/8 subjects. In 6 cases, thyroid replacement therapy was given, another became euthyroid and the other is still under evaluation. The remaining 8 patients (32%) had low FT3 and FT4 levels, with normal basal TSH. After TRH stimulation, TSH response was raised in only two of these eight.

Ultrasound study. Sixteen patients (64%) had a normal thyroid, 9 had parenchymal cysts (n=4) or inhomogeneity (n=5); none of the patients showed parenchymal nodules. Thyroid volume was inferior to age-standardized volumes in 9/25 patients (36%) (Table 1). Thyroid volume was similar in male and female patients, and in patients older or younger than 15 years at the time of assessment. Although patients with low FT4 and high TSH values tended to have a lower thyroid volume, the difference was not statistically significant. Conversely, thyroid volume was significantly lower in patients with a radiotherapy dose >20 Gy and in patients off-therapy for >5 years. In the regression analysis none of the following were significantly associated with a lower thyroid volume: sex, radiotherapy site, radiotherapy dose (as a continuous variable), age at completion of treatment and age at current evaluation, levels of free T4 or of TSH, while time off-therapy, radiotherapy dose (cut-off 20 Gy), and, marginally, chemotherapy were significant. In the multivariate analysis only time off-therapy remained significantly associated with thyroid volume (p=0.01). This model showed that thyroid volume tended to decrease the longer the time since completion of treatment (Figure 1) and the more aggressive the chemotherapy and radiotherapy used.

In conclusion, thyroid ultrasound follow-up study for screening of thyroid nodules may provide additional information on thyroid volume, and this may be related to thyroid function. The thyroid volume was significantly inferior to age-standardized in 36% of the patients. Time elapsed from treatment completion was the only independent risk factor for this event.

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Chlorambucil synergizes with purine analogs in inducing *in vitro* cytotoxicity in B-cell chronic lymphocytic leukemia

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Combinations of different drug concentrations of CLB+FAMP and CLB+2-CDA were synergistic in, respectively, 42.9% and 34.8%. At leukemic cell survival ≤ 50%, 16.4% and 23.4% of all combinations were synergistic in the 2-CDA and FAMP groups, respectively. A significantly higher mean value of antagonistic interactions was observed in the 2-CDA group (p=0.037).

Fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CDA) and chlorambucil (CLB) induce apoptosis in chronic lymphocytic leukemia (CLL) B-cells.[1,2] In this study we examined whether CLB improved *in vitro* CLL cell chemosensitivity to either FAMP or 2-CDA. The results indicate that CLB synergizes *in vitro* with both purine analogs.

Samples from 23 CLL patients were tested. Lymphocytes were separated as previously described.[3-5] CLB (Sigma, St Louis, Mo, USA), FAMP (Fludara, Schering AR, Germany) and 2-CDA (Leustatin, Ortho Biotech, USA) were employed at described concentrations.[3-5] MTT assay was performed as previously described.[3-5] The lethal dose (LD)[50] values, leukemic cell survival (LCS) and drug interactions were calculated by home made software.[3-5]

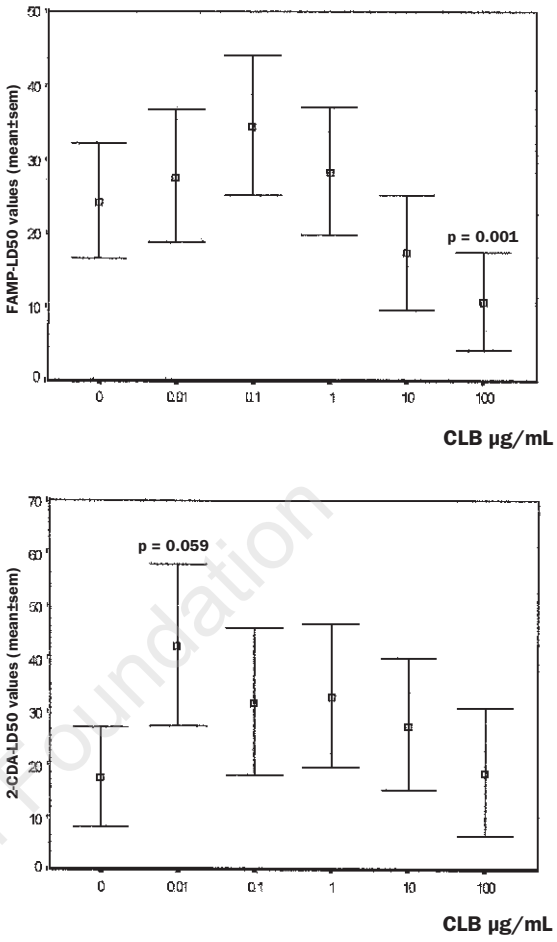

Figure 1. *In vitro* effect of several CLB concentrations on FAMP- (A) and 2-CDA- (B) LD[50] values at MTT assay. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test. The difference of the mean values was significant between CLB= 0 µg/mL and CLB = 100 µg/mL (p=0.001) in the FAMP group. A border line significance was observed in the 2-CDA group between CLB = 0 µg/mL and CLB=0.01 µg/mL (p=0.0592).

FAMP-LD50 values were significantly lower with 100 µg/mL concentrations of CLB; conversely higher 2-CDA-LD50 values were observed with 0.01 µg/mL of CLB (Figure 1). The interactions between CLB and either 2-CDA or FAMP, tested in respectively 420 and 525 combinations, were synergistic in 146 (34.8% of the total) and 225 (42.9% of the total) (Table 1). Similar percentages of additive interactions (15.7% and 18.8%) were detected with both purine analogs, while a higher percentage of antagonistic interactions was observed among the 2-CDA group. At LCS ≤ 50%, 23%

Table 1. ***In vitro*** **effect of drug interactions between CLB and FAMP in 21 CLL cases and between CLB and 2-CDA in 14. The mean values ± sem, the total number and the percentage of the total combinations in which each specific interaction was detected is indicated.**

	Sy interactions mean values±sem (total, %)	**p*	*Ad interactions mean values±sem (total, %)*	**p*	*An interactions mean values±sem (total, %)*	**p*
Any LCS						
FAMP	10.7±1.4 (225, 42.9%)	ns	4.71±0.95 (99, 18.8%)	ns	9.57±2.6 (201, 38.3%)	0.037
2-CDA	10.4±2.1 (146, 34.8%)		4.71±1.1 (66, 15.7%)		14.85±2.6 (208, 49.5%)	
LCS ≤ 50%						
FAMP	5.8±1.0 (123, 23.4%)	ns	3.6±0.8 (77, 14.7%)	ns	3.2±0.7 (69, 13.1%)	0.059
2-CDA	4.9±1.4 (69, 16.4%)		3.4±0.9 (48, 11.4)		6.2±1.7 (87, 20.7%)	

*Sy interactions = Synergistic interactions; Ad interactions = Additive interactions; An interactions = Antagonist interactions; LCS= Leukemic cell survival. *Wilcoxon rank-sum test. For CLB+2-CDA interactions, 30 different drug combinations were tested using 5 CLB (100, 10, 1, 0.1, 0.01 µg/mL) and 6 2-CDA (125, 12.5, 1.25, 0.125, 0.0125, 0.00125 µg/mL) concentrations. For CLB+FAMP interactions, 25 different drug combinations were tested using 5 CLB (100, 10, 1, 0.1, 0.01 µg/mL) and FAMP (100, 10, 1, 0.1, 0.01 µg/mL) concentrations.*

and 16.4% of the total drug combinations in the FAMP and 2-CDA group, respectively, interacted synergistically. A statistically significant higher mean value of antagonistic interactions was observed in the 2-CDA group for any LCS (p=0.037), while a border line significance was documented for LCS ≤ 50% (p=0.059).

Synergy was observed between CLB and 2'-deoxycoformycin in CLL cells,[6] while an additive effect was described for the combination CLB-FAMP.[7] We found additivity and synergism in 61.7% and 50.5% for CLB+FAMP and CLB+2-CDA, respectively. On the other hand, there was a significantly higher mean value of antagonistic interactions in the 2-CDA group. Furthermore, in our *in vitro* tests, synergism and additivity was found in 60.7% of the drug interactions at CLB ≤ 1 µg/mL and 2-CDA ≤ 0.0125 µg/mL, which closely represent the purine analog plasma concentration achieved in a clinical study of the 2-CDA-CLB drug combination.[8,9]

In conclusion, these results adds support to the notion that combination of an alkylating agent with a purine analog may be a practicable treatment for CLL.

Key words

CLL, MTT assay, fludarabine, chlorambucil, 2-chlorodeoxyadenosine, cytotoxicity, drug synergism

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Secondary acute myeloid leukemia following treatment with VP16-containing regimens for non-Hodgkin's lymphoma

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We report on two patients who developed a secondary acute myeloid leukemia (sAL) after treatment for non-Hodgkin's lymphoma (NHL) with regimens containing low to intermediate doses of VP16. Clinical and hematologic features in these two patients were consistent with epipodophyllotoxin-associated sAL. In one case, a rearrangement of chromosome band 11q23 was detected.

Etoposide or VP16 is an epipodophyllotoxin-derivate targeting the enzyme topoisomerase II. In the last few years, a number of reports have focused on the risk of secondary acute myeloid leukemia in patients treated with regimens including epipodophyllotoxins for prior independent malignancy.[1,2] The risk of sAL is related both to the cumulative dose and to the schedule of administration.[3,4]

Among 89 adult patients with NHL treated at our Institution with regimens containing low to intermediate cumulative doses of VP16, two cases developed acute myeloid leukemia.

Patient #1. A 41-year-old woman with diffuse large cell NHL stage IVA, received VACOP-B[5] for 12 weeks. Because of disease progression, she underwent salvage therapy with VIM-Ara-C (VP16 + ifosfamide + mitoxantrone + cytosine arabinoside) plus G-CSF. Seventeen months from diagnosis, the patient developed acute leukemia with central nervous system involvement. Bone marrow blasts showed a myelomonocytic morphology. Blast cells were positive for CD34, CD33, HLA-DR, and CD11c. Cytogenetic analysis on marrow cells revealed 46,XX, t(9;11)(p22;q23). The patient died after a salvage attempt high-dose Ara-C. The cumulative doses of drugs administered prior to leukemic evolution were: VP16 2,130 mg/m^2, doxorubicin 300 mg/m^2, mitoxantrone 40 mg/m^2.

Patient #2. A 67-year-old man with a peripheral T-cell non-Hodgkin's lymphoma, stage IV A (PCR assay positive for TCR-γ), was treated with P-VEBEC[6] plus G-CSF. Total exposure to VP16 was 400 mg/m^2 and to epirubicin 200 mg/m^2. At disease progression, he was treated with vincristine plus cyclophosphamide. Twelve months from diagnosis, he developed an acute monoblastic leukemia with skin and gum involvement. Marrow cells were positive for CD13, CD11c, and HLA-DR. PCR assay on cutaneous biopsy failed to show any rearrangement of TCRγ. The patient died one week after the diagnosis of leukemia.

The clinical and molecular findings in these two patients are consistent with epipodophyllotoxin-related sAL.[7,8] In both patients leukemia developed after a short latent period without a detectable preleukemic phase and the phenotype was monocytic. In patient #1 marrow blasts showed a t(9;11)(p22;q23) at cytogenetic analysis. The strong association between rearrangement at chromosome band 11q23 and previous therapy with topoisomerase II inhibitors, primarily epipodophyllotoxins, has been extensively elucidated at the molecular level.

Thus far, no cases of sAL with features of topoisomerase-associated leukemia have been reported in NHL patients treated with chemotherapy containing standard doses of VP16. The development of VP16-related sAL has been recently observed following exposure to low doses of VP16 in one patient with Hodgkin's disease and one with virus-associated hemophagocytic syndrome.[9] In our patients, the leukemogenic potential of VP16 may have been enhanced by the concomitant use of other potentially leukemogenic agents such as anthracyclines and mitoxantrone (intercalative agents acting on topoisomerase II), and cyclophosphamide. On the other hand, anthracyclines are administered to relatively low cumulative doses because of their cardiac toxicity, and cyclophosphamide was associated with a nonsignificant increased risk of secondary leukemia in NHL patients.[10] In addition to antineoplastic drugs, our two patients received G-CSF. To date, it is unknown whether this drug may accelerate the development of acute leukemia after genetic damage of hemopoietic stem cells by epipodophyllotoxins.

In conclusion, our data show that VP16-containing regimens currently used in the treatment of NHL may carry a risk of secondary acute leukemia, even after relatively low cumulative exposure to VP16. Although sAL seems an infrequent event, this complication should be considered because of the increasing use of VP16 in the chemotherapy of NHL.

Key words

VP16, secondary leukemia

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Pulmonary multinodular relapse of non-Hodgkin's lymphoma

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We describe here a case of pulmonary multinodular relapse of non-Hodgkin's lymphoma following autologous stem cell transplantation.

A 44-year-old patient was admitted for autologous peripheral stem cell transplantation. His diagnosis was diffuse large cell B lymphoma, stage II with bulky disease. After an initial complete remission he had relapsed and a second partial remission was achieved with ESHAP chemotherapy.

Transplantation was performed without incidences; in computerized tomography (CT) revealed two small para-aortic lymph nodes, which were evaluated by gallium scan showing their residual nature, thus the patient was considerd to have achieved complete remission. Radiotherapy was administered to the bulky zone and a new CT showed no change in the size of residual nodes, but small nodular images appeared in the lung parenchyma. Chest radiography showed a pattern of small, ill-defined nodular images (Figure 1). At this point, six months after transplantation, the patient's only complaint was mild cough, with no dyspnea or fever. Physical examination yielded no significative findings. The platelet count was 35×10^9/L, attributed to delayed recovery of platelets after transplantation. Several tests were performed in order to determine the nature of the pulmonary disease.

Except for the platelet count, the rest of the blood count was within normal ranges as were the lactate dehydrogenase concentration and arterial O_2 saturation. Mantoux test and serology for *Aspergillus* were negative and so, too, was cytomegalovirus antigen detection. Fibrobronchoscopic findings were nonspecific; cytological analysis of bronchoalveolar lavage (BAL) specimens demonstrated a hemorrhagic background and the presence of hemosiderin-laden macrophages. Bacteriologic cultures and fluoroscopy for *Mycobacteria* were negative. This led to the diagnosis of alveolar hemorrhage, prompting an intensive schedule of platelet support in order to maintain the platelet count above 50×10^9/L.

Three weeks later, the patient's status remained unchanged, and a new radiograph showed the growth of nodules. In view of this progression, regardless of the patient's good status, an open lung biopsy was performed. Histopathologic findings led to the diagnosis of lung infiltration by lymphoma, with a nodu-

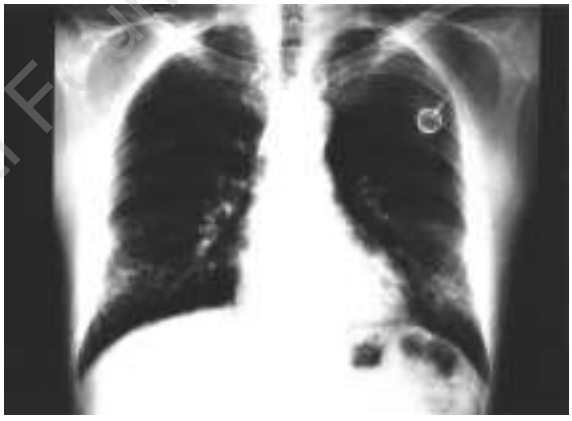

Figure 1. Chest radiography: nodular opacities, predominantly in basal zones.

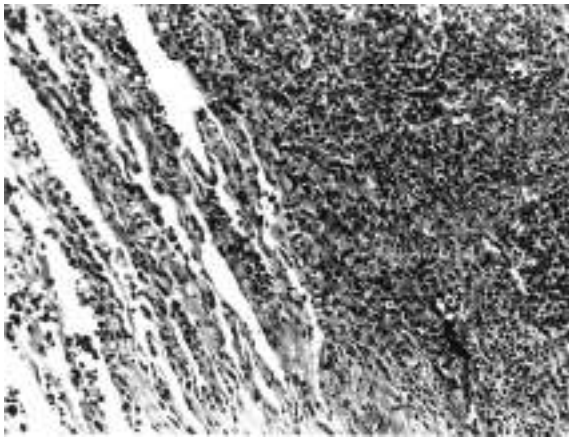

Figure 2. Lung biopsy: diffuse large cell B lymphoma, nodular infiltration (200 x).

lar pattern (Figure 2). The final diagnosis was pulmonary relapse of lymphoma, since no nodal involvement was assessed.

After bone marrow transplantation, pulmonary complications occur in 40% to 60% of patients.[1,2] Most of these have nonspecific radiologic features, requiring additional diagnostic procedures. Among those occurring in the late post-transplant period, idiopathic interstitial pneumonia, cryptogenic organizing pneumonia, restrictive and obstructive diseases as well as infectious complications usually have vague radiologic features in common which do not permit a diagnosis. Although CT may be able to provide more specific diagnoses with greater confidence, these complications can not be easily characterized by x-ray imaging.[3] Nodular low-density, ill-defined opacities have been described in lung involvement of lymphomatous relapse, but several other patterns have been found: alveolar infiltrates, interstitial infiltrates and combinations of these; thus, no specific pattern can be considered as diagnostic.[4] On radiological grounds, diffuse alveolar hemorrhage (DAH) and lymphomatous relapse are indistinguishable. Clinical signs and symptoms are nonspecific, but DAH is usually found in the immediate post-transplant period, with a rapidly fatal clinical course,[5] while relapse usually progresses more slowly, depending on the growth rate of the tumour. On the other hand, BAL from patients with lymphoma may yield bloody returns with hemosiderin-laden macrophages, which has been considered the hallmark of DAH. Thus, radiologic evolution with growing nodular lesions was an important clue leading to the diagnosis, although in order to make the diagnosis an open lung bopsy was required. The possibility of relapse must be borne in mind when approaching a differential diagnosis of pulmonary complications after bone marrow transplantation.

Key words

Non-Hodgkin's lymphoma, pulmonary relapse

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Pathologic rupture of the spleen as the initial manifestation in acute lymphoblastic leukemia

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Pathologic splenic rupture is a rare and life-threatening complication of acute leukemia. It is even more uncommon as the initial manifestation, and only a few cases has been reported in the literature. Early recognition of this complication is vital because the prognosis is fatal without immediate treatment by splenectomy. We report the case of a spontaneous spleen rupture irreversibly complicating the onset of acute lymphoblastic leukemia in a 19-year-old man, in spite of splenectomy. In our case abdominal ultrasound was a good, non-invasive diagnostic test. Therefore, we believe that the course of the underlying disease and the physical condition of the patient dramatically influenced the disease evolution.

Spontaneous rupture of the spleen has been reported in many diseases associated with splenomegaly, e.g. infectious diseases, inflamatory diseases, and hematological malignancies.[1-4] Non traumatic rupture of the spleen is a rare and life-threatening complication of acute leukemia.[1-10] However, the splenic rupture as the initial symptom of acute leukemia is extremely unusual and only a few cases are reported in the literature.[5-10]

We report the fatal course of a patient with acute lymphoblastic leukemia (ALL) in which pathologic rupture of spleen was the initial manifestation of the disease.

A 19-year-old man was admitted to hospital with a two week history of weakness, nausea, vomiting and epigastric pain. There was no history of fever or bleeding diathesis. On admission, he had a petechial rash and severe pain in the left upper quadrant of the abdomen radiating up to the left scapula. Physical examination showed an acutely ill patient with a petechial rash on his legs, thorax and abdomen, without lymphadenopathy and with painful abdominal distension. He was pale, tachycardic and had a blood pressure of 60/40 mm Hg. The peripheral blood count showed hemoglobin 71 g/L, leukocyte count 640×10^9/L with 100% lymphoblastic cells and platelet count 68×10^9/L, fibrinogen 103 mg/dL, prothrombin time 25%, partial thromboplastin time 50". Additional results of laboratory tests were AST 692

U/L, ALT 163 U/L, serum amylase 1,402 U/L, uric acid 15 mg/dL, LDH 9,030 U/L, blood urea and creatinine concentrations within the normal limits. An abdominal ultrasound revealed free peritoneal fluid and an enlarged spleen. An emergency laparotomy and splenectomy were performed and the patient was given supportive therapy with blood, platelets, fresh frozen plasma and fibrinogen. Operative findings were two and a half liters of intraperitoneal blood, with marked splenomegaly (size 17×16×8 cm, weight 841 g). Four lacerations were found with an active bleeding. Microscopic examination of the spleen revealed diffuse infiltration with leukemic cells and multiple small hemorrhagic foci in the parenchyma with a subcapsular hematoma.

On the basis of the morphologic characteristics of the peripheral blood and a bone marrow aspirate, cytochemical staining and immunophenotyping of the blast cells, the diagnosis of T-cell ALL was established. The immunophenotype revealed that the blasts were positive for CD1, CD7, CD2, CD5, CD8, CD34, CD38 and TdT, and negative for CD3, CD4, CD19, CD20, CD10, DR, CD14, CD13 and CD33. Immediately after the splenectomy a cytoreductor treatment was initiated with prednisone, vincristine and daunorubicin with intensive prophylaxis of lysis tumour syndrome.

The patient continued to be hemodynamically unstable, in renal failure and have hemorrhagic episodes with disseminated intravascular coagulation and hyperfibrinolysis refractory to supportive therapy. He died 48 hours after arriving at hospital. Permission to carry out a *post-mortem* examination was denied.

The diagnosis of splenic rupture must be considered in all patients with hematologic malignancies and a new abdominal pain, acute or subacute, hypotension and sudden anemia, even more so if there is not previous history of trauma. Diagnosis is based on clinical signs (abdominal pain, splenomegaly, hypotension, tachycardia, etc.) and confirmatory diagnostic tests. Although some authors have reported paracentesis to be the most effective diagnostic procedure,[4,9] we have found that abdominal ultrasound can be a good, non-invasive technique without risk to patients who are hemodynamically unstable . In our case, the abdominal ultrasound was diagnostic and the splenectomy was performed immediately.

The prognosis in splenic rupture is poor; in the non-operative cases reviewed the mortality was 100%. The survival of patients following splenectomy is probably well correlated with the course of the underlying disease. Aggressive management with early surgical intervention and appropriate hemoderivative support is important.

Key words

Spleen, pathological rupture, ALL, initial manifestation

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Interferon-α2b is not effective in the treatment of refractory immune thrombocytopenic purpura

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About 25-30% of patients with immune thrombocytopenic purpura (ITP) are refractory to corticosteroids, splenectomy and other treatments. It has been suggested that interferon-α2b (IFN-α2b) may be useful in the treatment of chronic refractory ITP patients. We treated 9 chronic refractory ITP patients with IFN-α2b: the results were poor.

Immune thrombocytopenic purpura (ITP) is an autoimmune disease mediated by antiplatelet antibodies. Corticosteroids and splenectomy are effective treatment in the majority of patients. However, 25-30% of patients are refractory to these treatments, thus, morbidity increases, and the mortality rate rises to about 16%.[1] It has been suggested that inter-

Table 1. Patients' clinical and laboratory characteristics and response to IFN-α2b therapy.

Patient	Plts ($x10^9$/L) PRE	Plts ($x10^9$/L) POST	Hb (g/dL) PRE	Hb (g/dL) POST	WBC ($x10^6$/L) PRE	WBC ($x10^6$/L) POST	Hemorrhage PRE	Hemorrhage POST	Anti-platelet autoantibodies PRE	Anti-platelet autoantibodies POST	Previous treatments
1	33	39	14.9	14	7.9	4.8	–	–	-----	PaIgG + SBIgG	C
2	14	14	14	14	8.1	7.6	–	–	–	PaIgG	S, C, A
3*	36	18	10.3		4.5		–	+	PaIgG	PaIgG + SBIgG	C, A
4*	7	1	13.6	13.9	6.2	4.4	–	+	PaIgG + SBIgG	N.E.°	C,A, S, Ig
5*	30	2	12.9		13.3		–	+	-	N.E.°	C, S, A
6	30	28	13.2	13.7	3.9	4.3	–	-	PaIgG + SBIgG	PaIgG + SBIgG	C, A, Ig
7*	14	2	11.6	11.9	4.2	5.8	–	+	PaIgG + SBIgG	PaIgG + SBIgG	C, S, A
8	45	46	14.6	13.7	4.5	3.8	–	–	PaIgG	PaIgG	C, A
9	21	106	14.9	14.2	9.1	4.9	–	–	PaIgG	PaIgG	C, A

**Therapy was withdrawn due to hemorrhage and/or worsening of thrombocytopenia; °N.E.= Not evaluated. C = Corticosteroids; A = Azathioprine; S = splenectomy; Ig = Immunoglobulins.*

Table 2. Phenotype of peripheral blood lymphocytes in ITP patients treated with IFN-α2b. Values are expressed as percentages.

	CD3 PRE	CD3 POST	CD4 PRE	CD4 POST	CD8 PRE	CD8 POST	CD57 PRE	CD57 POST	CD20 PRE	CD20 POST
1	52	64	34	31	25	32	12	18	7	11
2	71	68	42	45	28	28	7	9	10	12
3	57	61	23	33	30	30	16	20	9	11
4	62	/	40	/	28	/	11	/	5	/
5	43	/	39	/	11	/	7	/	4	/
6	59	65	22	31	40	39	17	22	9	13
7	44	56	29	31	20	22	6	9	5	8
8	79	81	55	59	26	29	9	12	6	6
9	67	66	50	45	14	24	5	12	5	9

PRE= before α-IFN treatment. POST= after α-IFN treatment.

feron-α (IFN-α) may be beneficial because of its immunomodulant activity,[2,3] but the data in the literature are not concordant.[4,5]

We used IFN-α2b to treat 9 refractory ITP patients (3 males, 6 females; median age 55 yrs, range 37-70 yrs) with a diagnosis of chronic ITP made according to the criteria of McMillan,[6] and who were negative for hepatitis B and C and HIV. The patients had a median duration of disease of 37 months (range 21-357), a platelet count <50×10^9/L, and had been off-therapy for at least one month. IFN-α2b was administered alone at a dosage of 3 MU s.c. × 3/week for five weeks. Antiplatelet autoantibodies (PaIgG and SBIgG) were detected by the standard immunofluorescence method and flow cytometer analysis (Table 1), as already described.[7] A study of main lymphocyte subsets was also performed (Table 2). Clinical examination and platelet count were evaluated weekly.

Therapy was well tolerated, but 4/9 patients withdrew from treatment owing to worsening of the thrombocytopenia and/or appearance of a hemorrhage syndrome. Two patients needed hospitalization and platelet transfusions. Autoimmunity increased in 3 patients. Only one patient (#9) developed a significant increase in the platelet count (21 →106×10^9/L) after administration of IFN-α2b (Table 1), but she lost the response 2 months later.

As regards the lymphocyte subset study, no true differences were seen between before and after treatment with IFN-α2b. The CD3 and CD20 levels remained unchanged throughout the treatment, while CD4/8 ratio was highly variable (Table 2).

Our short series of chronic refractory ITP patients showed a remarkably poor response to IFN-α2b therapy. It is possible that our series largely comprised a subset of patients in whom more aggressive disease and/or prolonged immunosuppressive therapy were associated with a different pathogenetic pathway that obviated the action of IFN-α2b.

IFN-α2b is known to have an antiproliferative action,[8] and can induce the appearance of autoimmune thrombocytopenia[9] or autoimmune disorders.[10] Data from the literature suggests that IFN-α2b is capable of modifying immunologic response by enhancing NK response and by leading some T cells to differentiate into the Th1 subset that secretes IL2 and IFNγ. It has recently been reported that ITP patients who respond to IFN show an increase of IL2 and IFNγ production, accompanied by a decrease in IL4 production.[5] A likely explanation for the therapeutic effect of IFN-α is that by inducing T cells to differentiate into the Th1 subset it indirectly exerts a cytotoxic action on autoreactive cell clones. Non-responder patients might be not capable of producing IL2 and IFNγ in response to IFN-α2b.

In view of this, we think that due to its unforeseeable effects on autoantibody production IFN-α2b should not be considered a safe or satisfactory treatment for refractory ITP patients.

Key words

Chronic refractory ITP, IFN-α2b, immune system

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Long-term disappearance of previous chromosomal abnormalities in myelodysplastic syndromes treated with low dose cytosine arabinoside and granulocyte/macrophage-colony stimulating factor

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Most therapies for elderly patients with myelodysplastic syndromes offer few short responses and little improvement in survival. We describe two patients who, after several cycles of low dose cytosine arabinoside and GM-CSF, achieved and maintained complete remission and became tranfusion independent. Previous chromosomal abnormalities also disappeared and karyotype remains normal.

No uniformly accepted treatment is available for elderly patients with myelodysplastic syndromes (MDS).[1] We present two MDS patients treated with combined low-dose araC and GM-CSF who achieved a complete (CR) clinical, hematological and cytogenetic response.

Case #1. A 71-year-old-woman diagnosed in 1992 as having refractory anemia was referred in 1995 because of severe cytopenias and elevated transfusional requirements. Bone marrow (BM) aspirate was hypercellular with trilineal dysplasia and 12% myeloblasts. Cytogenetics: 46,XX (45% metaphases)/46, XX, t(5;13)(q13; q14) (35%)/47,XX,+8 (20%). She started low-dose ara-C (10 mg/m^2/d) and GM-CSF (150 mg/d), days 1 to 14, every month. After the fourth cycle she did not need further transfusions. Data in August 1996: normal karyotype; less than 1% of blasts in BM; WBC count, 3.3×10^9/L; hemoglobin (Hb), 143 g/L; 124×10^9 platelets/L. Side-effects were mild (except for flu-like syndrome related to GM-CSF), thus allowing us to administer up to 20 cycles of this protocol. The patient remains stable without complications 24 months after the onset of treatment (Figure 1).

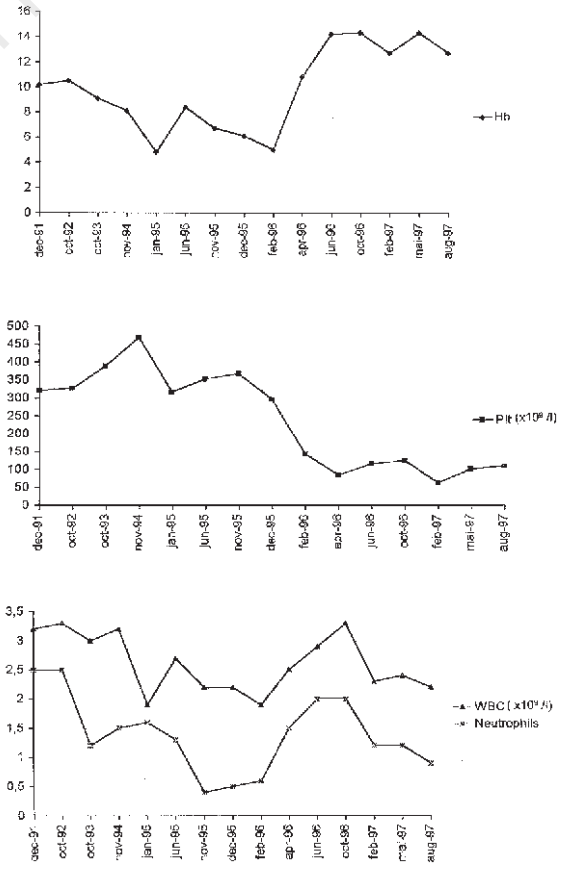

Figure 1. Case #1.

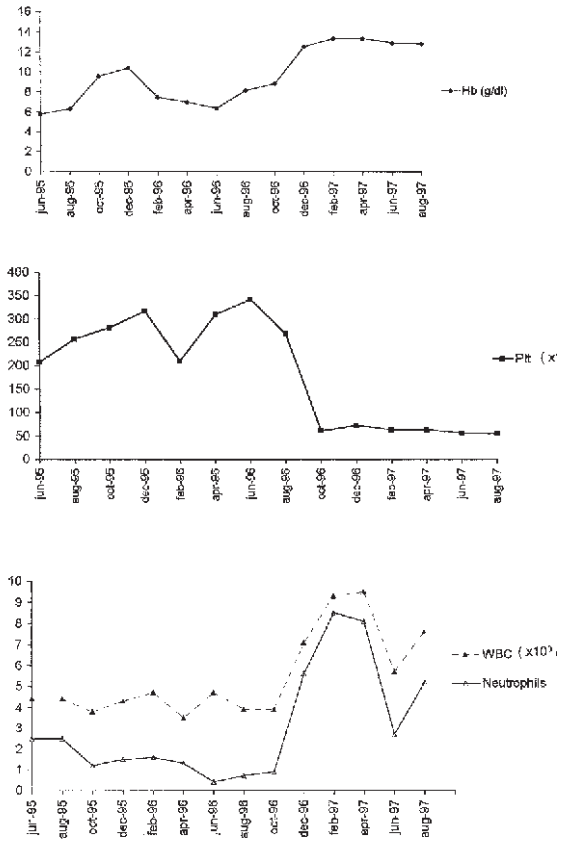

Figure 2. Case #2.

Case #2. A 68-year-old woman was diagnosed as having refractory anemia with ring sideroblasts . Folic acid, vitamin B6 and danazol did not prevent a progressive worsening in blood counts. By August 1996, she needed weekly transfusions and her neutrophil count was 0.7×10^9/L. BM aspirate revealed severe trilineal dysplasia, 4% myeloblasts, and occasional Auer rods; karyotype: 46, XX, del(5)(q13; q33). At this moment the protocol was initiated. Data after the fifth cycle: normal karyotype; less than 1% myeloblasts in BM; WBC 12.3×10^9/L (76% neutrophils); Hb 134 g/L without need for further transfusions. By the 17th month of treatment she maintains a complete response (Figure 2).

The outlook of MDS patients with excess of blasts, pancytopenia and chromosomal abnormalities is ominous.[2] Therapy in older individuals usually aims at merely prolonging survival. Intensive chemotherapy attains variable CR rates of short duration with important morbidity.[1,3] For these reasons milder therapies have been tried: results with low-dose ara-C are similar to intensive protocols, sharing their lack of effect on prolongation of survival;[4,5] GM-CSF increases neutrophil counts and decreases infection rate in these patients. Wadhan-Raj reported the suppression of the myelodysplastic clone and stimulation of polyclonal hematopoiesis after GM-CSF.[6] In a EORTC series of 82 patients given ara-C and GM-CSF, response rate was significant enough (63%) to suggest a role for this combination.[7]

We report two cases whose originality lies in the fact that previous chromosomal abnormalities disappeared under prolonged therapy. Up till now, it is not clear how many cycles should be delivered; besides, most available data come from assays with few courses. In contrast, our patients are kept indefinitely under treatment, provided that side effects are not unbearable or disease progresses overtly.[8] The uncertainty about the relationship between cytogenetic response and cure[9] sustains our long-term policy.

To sum up, we agree with other investigators that ara-C and GM-CSF[10] therapy is useful in selected MDS patients.[7] The dosage and minimum number of cycles remain unclear.

Key words

Myelodysplastic syndromes, cytosine arabinoside, granulocyte/macrophage-colony stimulating factor, complete remission

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Alloimmunization against human platelet antigen 2 (HPA2) in a series of multitransfused β-thalassemia patients

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In our study we investigated the presence of anti-human platelet antigen (HPA) alloantibodies in a series of 10 β-thalassemia *major* patients submitted for more than 10 years to periodic blood transfusions (every 2-3 weeks). We found that 2 out of the 10 patients developed anti-HPA2a+HPA1b and anti-HPA2b antibodies. Our results highlight that HPA alloimmunization in multitransfused patients is a real possibility.

Patients affected by β-thalassemia *major* are usually submitted to many transfusions of packed red blood cells during their life. Multitransfused patients are exposed to different immunogens due to the presence of leukocytes and platelets in packed red blood cells.[1,2] Antibodies raised against the latter components are responsible for some of the febrile non-hemolytic transfusions reactions (FNHTR) and filtered packed red cells are then needed to avoid this kind of reactions. Little is known about possible alloimmunization against human platelets antigens (HPA); this is more difficult to study and to characterize than HLA alloimmunization.[3,4] In this context we analyzed our series of β-thalassemia patients to find alloimmunization against red blood cells, leukocyte and platelets.

Ten patients affected by β-thalassemia *major* (clinical characteristics summarized in Table 1) were submitted to periodic tests for the presence of alloantibodies. Tests were also performed in the case of transfusion reactions.

Antibodies against red cell antigens were detected by standard indirect tests using rabbit anti-human immunoglobulin antisera. Antibodies against HLA were analyzed by a standard cytotoxicity test on HLA-typed donors.

The search for alloantibodies against HPA was performed by a standard indirect immunofluorescence test on random and HPA-typed donor platelets.[5] The samples were also run on a monoclonal antibody-immobilized platelet antigen (MAIPA) test[6] to define the specificity of the recognized antigen. Patients found positive for anti-HPA antibodies were genotyped for HPA-1,-2-,3,-5 genes with SSP-PCR.

Clinical records (Table 1) showed that 6 patients had suffered from FNHTR, indicating a possible alloimmunization against platelets and/or leukocytes. Two patients showed alloimmunization against red blood cell (one anti-Kell and the other anti-Kp^a), while 5 out of 10 had HLA antibodies with a very wide specificity (>80% of positive donors). One patient (#4) had anti- HLA-B35+51 antibodies.

Concerning HPA alloantibodies, patient #1 had HPA2b alloantibodies and patient #2 HPA2a+HPA1b alloantibodies. It should be underlined that HLA antibodies with wide reactivity were found in sera from both patients, and patient #1 had also anti-Kell antibodies. HPA gene typing showed that patient n.1 (anti-HPA2b) was HPA1a/a, HPA2a/a, HPA3b/b, HPA5a/b. Patient #2 (anti-HPA2a) showed the following typing: HPA1a/a, HPA2b/b, HPA3a/a, HPA5a/a.

We analyzed sera from 10 multitransfused patients suffering from β-thalassemia major, looking for both HLA and HPA specificities. HLA antibodies were found in 6 out of the 10 patients. Our investigation also showed that HPA alloimmunization is a real possibility since two patients developed HPA antibodies (anti HPA2b and anti HPA2a+HPA1b).

In multitranfused patients alloimmunization is usually regarded as strictly related to the presence of HLA alloantibodies, since these are responsible for most of the FNHTRs. In addition HPA alloantibodies may be implicated. A retrospective analysis showed that patient #2 also suffered from FNHTR also when receiving blood from HLA-matched donors, positive for the HPA2a antigen. These results suggest that HPA may have been responsible for the FNHTRs.

Table 1. Characteristics of β-thalassemia patients.

N.	Pts.	Age/Sex	No. of transfused RBC units (non-filtered U until 1990)	No. of transfused RBC units (filtered U beginning from 1991)	Transfusion reactions
1	C.M.	26/M	464	215	chills-hyperthermia
2	C.A.	24/F	568	160	chills-hyperthermia
3	D.F.M.	34/F	760	212	chills-hyperthermia
4	D.L	21/F	423	202	chills-hyperthermia
5	F.L	31/M	804	240	chills-hyperthermia
6	F.A.	23/F	420	184	none
7	F.M.	19/M	260	162	none
8	I.I.	27/F	740	187	chills-hyperthermia
9	R.A.	21/F	324	164	none
10	S.F	26/M	580	215	none

M= male; F=female; U=units.

A few considerations should be highlighted: (i) in chronically multitransfused patients, HPA alloantibodies might be responsible for some of the FNHTRs; (ii) in our series rare HPA specificities were found, involving the HPA2 alloantigens. In this context, it could be hypothesized that the mechanisms of recognition in multitransfused patients might be different: alloantigens expressed on the CD42 protein (HPA2) might be more immunogenic in multitransfused patients than alloantigens expressed in the CD41/61 complex (HPA1 or HPA3) or in the CD49b-related antigen (HPA5), which are more frequently involved in neonatal alloimmune thrombocytopenia or in post-transfusional purpura.[7-10]

Key words

Alloimmunization, β-thalassemia, human platelet antigen (HPA), multitransfused patients

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Spontaneous decrease of spleen size in a patient with type 1 Gaucher's disease

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We present a patient with type 1 Gaucher's disease in whom the spleen size during 34 years of follow-up reached a maximum of 6 cm. below the costal margin, but in 1993 began to decrease spontaneously and presently can no longer be felt by abdominal palpation.

Gaucher's disease is an autosomal, recessive storage disease due to glucocerebrosidase deficiency; the spleen may increase to ten times the normal size.[1] We have treated more than 30 patients with this condition, but the patient presented here is the only one in our series in whom a spontaneous regression of the spleen size was noted.

A.M. is a 62-year-old nurse of Ashkenazi origin, a mother of two sons. In 1959, when she was 37 year-old, she was diagnosed as having Gaucher's disease following the appearance of mild purpura, petechiae and hepatosplenomegaly of 2 and 4 cm. below the costal margins. Blood examinations showed: hemoglobin 12.2 g/dL, white blood cells 4.7×10^9/L and platelets 330×10^9/L. Serum acid phosphatase 5.5 U (normal range 0-0.8 U). Bone marrow aspiration biopsy revealed Gaucher's cells. X-ray examination was remarkable for Erlenmeyer flask deformity of the femora.

One son is a heterozygous carrier of the disease.

During the years of follow-up the size of the spleen progressively increased, reaching a maximum of 6 cm. below the costal margin. Bone biopsies performed in 1966 and 1973 showed the presence of Gaucher's cells. However, beginning in 1993, the size of the spleen progressively decreased by about one cm per year until 1996, when the spleen could be not palpated at all. Two abdominal ultrasounds and a ^{99}Technetium sulfur colloidal scan, showed a spleen size of 10 cm. A Doppler examination of the spleen vessels was without pathological findings. Examination of her peripheral white blood cells showed that she is a homozygote for the 1226 G variant of Gaucher's disease.

The onset of the disease, the clinical and laboratory findings and the family history of the patient are consistent with the diagnosis of adult, type 1 Gaucher's disease. The long course and almost asymptomatic presentation of the illness, exclude the possibility of myeloproliferative disorders in which pseudo-Gaucher's cells may be found. Our patient should be distinguished from those with asplenomegalic (cryp-

tic) Gaucher's disease that is a rare form, of which only a few cases have been reported. Morrison *et al*.[2] described 2 patients with asplenomegalic Gaucher's disease in whom the presenting symptoms were purpura and anaemia respectively and the diagnosis was established only after bone marrow examination. The authors cited two more cases of Gaucher's disease without splenomegaly. In a series of 34 patients, Matoth *et al*.[3] treated only one 9-year-old patient in whom the spleen was not palpable. In our series of more than 30 cases with Gaucher's disease, we had one asplenomegalic patient. Since there is a correlation between the size of the spleen and the severity of the symptoms including the hematological findings, it is possible that some asymptomatic patients with Gaucher's disease remain undiagnosed. With the introduction of enzyme replacement therapy, a reduction of the spleen size has been reported in both type 1 and 2 Gaucher's disease.[4-7]

In our patient, causes of the decrease in spleen size, such as infarctions and obtrusion of the splenic blood vessels, were excluded on the basis of lack of clinical symptoms and normal ultrasound and isotope scan examinations. Absence of red cell pitting, Howell-Jolly and Heinz bodies excluded the possibility of hyposplenism in which the spleen function is impaired. On the contrary, although the spleen in our patient decreased to a normal size, its function remained increased, judging from the mild peripheral blood pancytopenia.

Key words

Gaucher's disease, spleen, splenomegaly

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Acute leukemias after treatment with radioiodine for thyroid cancer

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Leukemia is an uncommon complication of exposure to radioiodine (^{131}I), used in treatment of thyroid cancer, because low doses are now used. We report two cases of acute myelogenous leukemia developed after the treatment of a thyroid carcinoma with a small dose of ^{131}I.

Radioiodine (^{131}I) has been used in the treatment of thyroid cancer in order to eliminate residual thyroid tissue after thyroidectomy and to treat metastasic disease.[1,2] Leukemia is one of the most prominent late effects of exposure to ionising radiation,[3] but is an uncommon complication of exposure to ^{131}I.[4] Most cases reported in the literature, have occurred after cumulative dosages higher than 800 mCI, but we report two cases of acute myelogenous leukemia after a small dose of ^{131}I.

A 34-year-old woman was admitted at our hospital for anemia and a bone marrow aspirate revealed M2 acute myeloid leukemia. Chemotherapy was ordered and achieved complete remission. Three years later, the myeloid disorder relapsed and she received an autologous bone marrow, remaining in remission on date. Two years before of the leukemia, she was diagnosed papillary thyroid carcinoma, which was surgically intervened. A post-operative total body iodine scan showed cervical uptake, so she received a single dosage of 150 mCi ^{131}I. Eight months later she had a negative whole body ^{131}I scan. The patient' condition remained stable thereafter on a suppressive dose of thyroid hormone.

A 43-year-old female was admitted at our departament for thrombocytopenia. An acute promyelocytic leukemia was diagnosed and she was treated with ATRA (all-trans-retinoic acid) and chemotherapy. She remained in remission 2 years but died later during the relapse treatment. Five years before of the leukemia, she was diagnosed of a papillary thryroid carcinoma and a partial thyroidectomy was performed. A post-operative total body iodine scan showed residual thyroid activity but no metastasic lessions. She received a dose of 150 mCi ^{131}I and 3 subsequent follow-up scans which were normal.

It is believed that ionizing radiation can be leukemogenic. Acute leukemias have been reported after radioiodine therapy for thyroid cancer,[3,4] The bone marrow should not recieve a total dose which exceed 1000 mCi, and there should have an interval at least

one year between doses.[1] Almost all cases reported in the literature occurred after a total dose of more than 800 mCi and with an interval between doses of 2 and 6 months.[1,5]

Our patients received only a single dose of 150 mCi and, to our knowledge, the case previously reported of acute leukemia after a small dose of radioiodine, were a patient who received 2 doses of 150 mCi,[1] and 4 cases of chronic myeloid leukemia after a total dose less than 150 mCi.[4] On the other hand, no cases of leukemia was observed after a follow up of 10 years, in a long serie of 1771 patients treated for a thyroid cancer, with low total dosage.[6] Likewise the treatment or diagnosis of hyperthyroidism with very low doses (usually 4 to 30 mCi) has not been correlated to an increased incidence of acute leukemia.[3]

In conclusion, two cases of leukemia after a small dose of ^{131}I, developed in a period longer than two years, so perhaps they could be considered as a second neoplasia instead of a secondary effect of radioactive treatment.

Anyway, this low and perhaps unrelated risk of leukaemia should not be a contraindication to ^{131}I therapy.

Key words

Thyroid cancer, radioidine, myeloid leukemia

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