sis. Biologically, abrogation of multiple cell cycle control pathways provides growth advantage in MCL cell lines.[10] Hence, curative attempts for MCL must be deployed early, before secondary events accumulate.

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Comparative analysis of immunophenotypic methods for the assessment of minimal residual disease in hairy cell leukemia

Hairy cell leukemia patients in complete remission may have minimal residual disease (MRD). We performed flow cytometry (FCM) and immunohistochemistry (IHC) to detect MRD in 15 patients. FCM and IHC detected MRD+ in 64% and 46 % of the patients, respectively. MRD+ did not predict relapse.

Sir,

In hairy cell leukemia (HCL), minimal residual disease (MRD) is detected by immunohistochemistry (IHC) in 13% to 50% of cases in complete remission (CR).[1-4] It is worthy of note that flow cytometry (FCM) is barely used for this purpose.[5,6] The clinical meaning of MRD remains unclear; some researchers have associated it with relapse,[1] while others have not.[3] Until this controversy is clarified, we consider it important to establish the best technique to assess MRD. For this reason we comparatively analyzed MRD by FCM and IHC in 15 patients in CR after 2-chlorodeoxyadenosine (n=12) and α-interferon (n=3) treatments, and the correlation of MRD with relapse. CR was documented in all cases according to Spiers' criteria.[7] Median follow-up: 26 months (6 to 96).

IHC assessment: paraffin-embedded bone marrow (BM) biopsies analyzed by routine techniques (hematoxylin-eosin, Giemsa and reticulin staining) showed no HCL. Residual tricoleukocytes were identified using anti-CD20 (L-26) and

Table 1. MDR status by FCM and IHC of 11 patients.

Pt.	Therapy	CR	Follow-up (mos)	Flow cytometry PB	Flow cytometry BM	IHC (BM)	Cell. (%)	FG
1	2CDA	YES	14	–	ND	–	45	I
2	2CDA	YES	15	pos. 2/5%	ND	pos. 5% mix	50	I
3	IFN	YES	41	pos. 2%	pos. 0.34-2%	pos.5% mix	50	I
4	2CDA	YES	96	–	ND	–	40	I
5	2CDA	YES	51	pos. 2%	pos. 2.9%	pos. <5% mix	40	I
6	2CDA	YES	32	pos. 1%	ND	–	40	I
7	2CDA	YES	35	pos. 0.4%	pos. 1%	–	45	I
8	2CDA	YES	26	pos. 2%	pos. 2%	pos. 6/10% mix	60	I
9	2CDA	YES	6	–	–	–	60	II
10	2CDA	YES	6	–	pos. 0.50%	–	40	II
11	2CDA	YES	6	–	pos. 0.12%	–	35	0

FG: fibrosis grade; ND: not done. CR: complete remission. PB: peripheral blood. BM: bone marrow. IHC: immunohistochemistry. Fibrosis grade 0: absent; I: thin reticulin fibers; II: gross reticulin fibers. 2CDA: 2-chlorodeoxyadenosine. IFN: interferon.

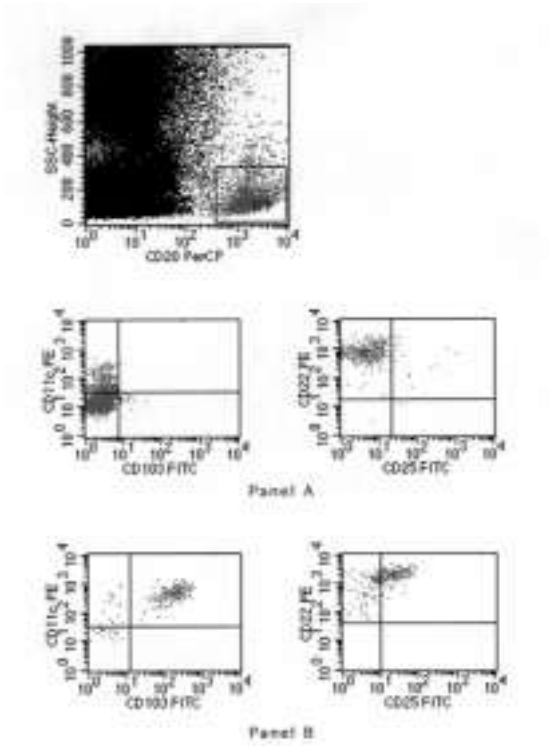

Figure 1. Panel 1: bone marrow parafin-embedded section (magnification 400x). Cluster pattern oresentation in MRD+ example with DBA44 staining and hairy cell morphology. Panel B: bone marrow paraffin-embedded section (magnification 400x). Scatter pattern with hairy cell morphology nd DBA44+ staining (arrow).

anti-DBA44 (Dako, USA). Based on Matutes' criteria,[3] BM sections were graded semiquantitatively, showing two infiltration patterns of CD20/DBA44$^+$ cells with tricoleukocyte morphology: clustered (minimum of 5 cells) and scattered, which were informed as percentages of total BM cellularity. Positive controls were active HCL by hematoxylin-eosin staining; >10% of infiltration was only evident with anti-CD20/DBA44. *MRD$^+$ definition*: 1-10% cells with tricoleukocyte morphology, CD20/DBA44$^+$, scattered or clustered. MRD$^+$ was present in 6/13 patients (46%), all in a mixed pattern. *BM cellularity*: normal in 9/13 patients, mildly decreased in 4/13. Grade I fibrosis, without a mesh-like pattern, was observed in 9/13.

FCM study: peripheral blood (PB) and BM samples were incubated with fluorochrome-conjugated antibodies (CD20, CD22, CD25, SIg, CD11c and CD103 (Bly 7 clone) and run in a flow cytometer. A live-gate was established over CD20$^+$ cells. *MRD$^+$ definition*: B-cells with high expression of CD11c/CD25/CD103 in >0.3% in PB or above 0 in BM. Normal BM (n=5) and PB (n=7) contained either no CD103$^+$ cells or ≤ 0.3%, respectively. MRD$^+$ was detected in 9/14 cases (64%); mean percentages: 2.78±3.2 of total BM cellularity and 1.63±2.13 of PB leukocytes. MRD was detected in 6/7 BM but only 4/7 PB simultaneously performed. All patients were in CR and had normal PB morphology. Figure 1 shows the FCM study.

FCM and IHC were simultaneously performed in 11/15 patients: discordance was found in 4 MRD- cases by IHC but MRD+ by FCM, suggesting that FCM was more sensitive, though not statistically significantly so, presumably due to the small cohort of patients (Table 1). One MRD+ patient relapsed 36 months later. The difficulty of assessing MRD using IHC is based mainly on the lack of consensus over defining a positive result.[1-4,8] Since BM biopsy provides very important data, it should not be avoided. From our experience we want to highlight: FCM easily detects residual tricoleukocytes with high specificity; a negative PB does not exclude MRD in BM and finally, MRD+ does not predict relapse, although this is a matter requiring longer follow-up. We conclude that FCM is a reliable alternative method for assessing MRD in HCL.

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Oral glutamine supplements in autologous hematopoietic transplant: impact on gastrointestinal toxicity and plasma protein levels

Tumor cells are major glutamine (Gln) consumers and can compete with host cells for circulating Gln. Radio- and chemotherapy increase Gln depletion. Gln supplementation could reduce mucosal injury secondary to chemotherapy in autologous hematopoietic transplantation. However, the efficacy of oral Gln is still controversial.[1-6]

Sir,

In a prospective, controlled, randomized and double-blind study, we evaluated the tolerance to and efficacy of oral Gln in decreasing gastrointestinal (GI) toxicity; and the modifications of protein and Gln plasma concentrations in patients undergoing high dose chemotherapy and autologous hematopoietic transplantation. The patients were divided into 3 groups. They received 20 g/day of a) glutamine (Gln), (Adamin Glu, SHS, Barcelona, Spain), b) whole protein (WP), (Maxipro, SHS), or c) dextrinomaltose (DXM), (Pentamalt, Nutricia, Madrid, Spain). The daily dose was dissolved in 100 mL of milk, fruit juice or water. There were no differences between groups in demographic characteristics, primary hematologic disease, initial nutritional assessment, chemotherapy (including melphalan) or infectious prophylaxis. Institutional review board approval and written informed consent to the study protocol was obtained. The study ended when neutrophil count was > 500 cells/mm^3 or when total parenteral nutrition (TPN) was required due to GI toxicity. Blood samples were obtained on the day of admission and every 7 days to measure serum concentrations of albumin, retinol-binding protein, prealbumin, transferrin (Behring Nephelometer Analyzer II) and Gln (ion-exchange chromatography method, Beckman amino acid analyzer).

Table 1. Symptom scores according to NCOG criteria for the patients included in the three groups (median; range).

	GLN	*WP*	*DXM*
Diarrhea			
Duration (days)	4;0-22	3; 0-11	4; 0-11
Patients with diarrhea %	70. 6 %	70.6 %	45.5 %
Diarrhea score (day 1-14)	3; 0-21	3; 0-23	0; 0-13
Stomatitis			
Duration (days)	4; 0-18	4; 0-12	4.5; 0-14
Patients with stomatitis	70.6 %	64.7 %	72.7 %
Stomatitis score (day 1-14)	2; 0-21	1; 0-20	4; 0-17
Vomiting			
Duration (days)	4;0-12	0;0-10	6;0-13
Patients with vomiting %	88.2 %	76.5 %	90.9 %
Vomiting score (day 1-14)	8; 0-17	4; 0-20	4; 2-22

Table 2. Evolution of plasma proteins during the study.

	GLN	*WP*	*DXM*
Albumin (g/dL)			
Day 1	4.2±0.6	4.2±0.6	4.3±0.5
Day 7	3.8±0.4	3.9±0.5	3.4±1.1
Day 14	3.7±0.5	3.8±0.4	3.6±0.6
Transferrin (mg/dL)			
Day 1	213±48	207±46	209±46
Day 7	171±62	155±71	174±34
Day 14	164 ±15	171±41	143±23
Prealbumin (mg/dL)			
Day 1	25±8	23±5	26±6
Day 7	24±6	21±5	23±6
Day 14	16±2	18±7	19±6
RBP (mg/dL)			
Day 1	4.2±1.2	3.9±1.7	4.6±1.4
Day 7	4.0±1.4	4.3±2.0	4.0±1.5
Day 14	2.9±1.0	3.5± 0.9	3.2±1.3

RBP = retinol binding protein.

Primary endpoints were GI toxicity classified according to the NCOG criteria.[7] Secondary endpoints were serum Gln and protein concentrations, and the number of patients who required TPN. Data are expressed as X±SD. One-way analysis of variance, followed by the Tukey method of multiple comparisons, was used to compare group means. A 0.05 significance level was used.

In our study, all the patients received at least 90% of the prescribed oral supplements which were well tolerated. Fifty-nine, 41 and 64% of the patients in the GLN, WP and DXM groups, needed TPN due to GI adverse events secondary