Impaired phagocytic function of polymorpho-nuclear neutrophils in B chronic lymphocytic leukemia.

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From 1996 to 1999 we analyzed 84 patients (48 male, 36 female, median age 62 years) with low grade B-NHL. According to the R.E.A.L. classification, 44 patients had follicular lymphoma (FL), 21 lymphocytic lymphoma (LL), 11 mantle-cell lymphoma (MCL), 5 hairy-cell leukemia (HCL) and 3 marginal zone B-cell lymphoma. The molecular analysis we used to evaluate MRD was based on the determination of IgH rearrangement through amplification by PCR. We chose the combination of FR2 and FR3A semi-nested methods, that, as has been demonstrated, reveals 85% of monoclonality. PCR analysis was performed on tissue, peripheral blood (PB) and bone marrow (BM) at diagnosis, and on only PB and BM after therapy and during follow-up. A patient is considered PCR positive when one of the samples results positive.

Results. Overall IgH+ rate was 83% (73% in FL and 93% in non-FL). We analyzed 71 patients at diagnosis: IgH rearrangement was identified in tissue, PB and BM samples in 87%, 60% and 72% of the cases, respectively. In FL (39 patients) IgH+ rates in tissue, PB and BM samples were 82%, 41% and 62% respectively, whereas in non-FL (32 patients) they were 95%, 83% and 90% respectively. Twenty-seven patients received conventional chemotherapy. IgH+ rates were 85% at diagnosis and 44% after therapy. Ten patients (6 FL, 3 LL and 1 MCL) underwent treatment with high-doses of cyclophosphamide followed by ABMT. IgH+ rate was 90% at diagnosis and 55% after ABMT.

In 1998 we started to search for Bcl-2 translocation as a molecular marker specific for FL. Twenty patients with FL were studied at diagnosis and 60% of them resulted Bcl-2+, whereas 12 patients were treated and 25% of them resulted Bcl-2+ after treatment.

Conclusions. Our results demonstrate that the IgH rearrangement is an extremely sensitive method in monitoring MRD in low grade B-NHL. In particular, it is more useful at diagnosis for non-FL, whereas it is advisable to use both IgH rearrangement and Bcl-2 translocation for FL. At diagnosis, the most significant results by using PCR analysis are obtained on tissue; however, it was important for us to perform it both on PB and BM because in some cases (11) we obtained PB+ and BM− after therapy. Conventional therapy and ABMT do not induce a significantly high molecular response. When a follow-up evaluation is performed there is a good correlation between molecular results and clinical outcome. It is then possible to associate the presence of monoclonality after ABMT with an increase of incidence of relapse. In conclusion, the detection of MRD by PCR of IgH rearrangement could have a prognostic value and a clinical significance, and most of all can be useful to assess the role of ABMT according to the results obtained.

**IMPAIRED PHAGOCYTIC FUNCTION OF POLYMORPHONUCLEAR NEUTROPHILS IN B CHRONIC LYMPHOCYTIC LEUKAEMIA**

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Introduction. The functional activity of polymorphonuclear neutrophils (PMNs) in patients with B-cell chronic lymphocytic leukemia (B-CLL) is still controversial due to the heterogeneity of the disease and individual variability, although normal absolute numbers of PMNs have been described. We have recently shown a significant (p<0.001) decrease in the ability to release reactive oxygen intermediates in the PM A-induced nitroblue tetrazolium test (NBT) by PMNs of B-CLL patients (37.9±19.0%, range 12-83%) compared with normal controls (81.5±2±7%, range 59-94%). Both, the number of NBT+ PMNs and the intensity of the formation of formazan crystals were decreased.

Methods. The detection of immature PMNs was measured by the activity of leucocyte alkaline phosphatase (LAP) using a Sigma LAP kit in 10 untreated Rai staged patients (age range 48-77) and 10 age-matched controls. PMN were analyzed morphologically to determine banding. A possible overall decrease in enzymes, related to oxygen-dependent bactericidal function was evaluated by the expression of myeloperoxidase (MPO) in PMNs using a cytochemical assay (Sigma). The ability of PMNs to attach and engulf opsonized target cells was also tested. For this purpose, Staphylococcus aureus were opsonized with specific rabbit polyclonal IgG antibodies (Molecular Probes) and added to PMNs isolated on double Ficoll gradients. Cells were incubated for 30 minutes at 37°C, washed and stained with Giemsa. The numbers of PMNs with internalized and/or attached opsonized bacteria were evaluated per 200 PMNs.

Results: The intracellular expression of LAP by blood PMNs in B-CLL patients (67.7±13.2%, range 52-81%) did not differ from that in healthy controls (71.7±15.2, range 50-85%), excluding immaturity of the PMNs as a factor. Morphologic analysis confirmed that there were very few band-type immature PMNs in the blood of B-CLL patients. The expression of MPO by B-CLL PMNs (94.3±4±5%) was no different from that of normal controls (92.7±1±9%), suggesting that the oxygen-dependent peroxidase system was not impaired. Although attachment of the opsonized particles was similar to that in controls, there was, nevertheless, a significant decrease in internalization by B-CLL PMNs (Table 1).
received mitoxantrone (10 mg/m² given over 15 min. days for a maximum of 6 cycles; 31 patients also (25 mg/m²) in 30 min. infusion days 1-3, every 28 (300 mg/m² i.v. days 1-3) followed by fludarabine Twenty-two patients received cyclophosphamide in patients with recurrent low-grade lymphoma (LGL). Fifty-three patients entered the study. cyclophosphamide or with mitoxantrone and cyclophos- safety of fludarabine in combination with cyclophos- phagocytic function of PMNs of B-CLL patients Department of Hematology, San Martino Hospital, Genoa, Italy

**EXPERIENCE**

**TIVE TREATMENT FOR RELAPSED OR REFRACTORY LOW-FLUDARABINE IN COMBINATION THERAPY IS AN EFFEC-

The aim of our study was evaluate the efficacy and safety of fludarabine in combination with cyclophosphamide or with mitoxantrone and cyclophosphamide in patients with recurrent low-grade lymphoma (LGL). Fifty-three patients entered the study. Twenty-two patients received cyclophosphamide (300 mg/m² i.v. days 1-3) followed by fludarabine (25 mg/m²) in 30 min. infusion days 1-3, every 28 days for a maximum of 6 cycles; 31 patients also received mitoxantrone (10 mg/m² given over 15 min. infusion on day 1. All patients received antibiotic prophylaxis and growth factors (G-CSF) if grade III granulocytopenia (WHO) occurred. All patients had failed a median number of 2 (range 1 to 5) previous chemotherapy regimens containing either doxorubicin or mitoxantrone. Mean age was 56 years (35-75). According to the REAL classification, 12 patients had small B lymphocytic, 34 patients follicular, 5 mantle cell and 2 marginal zone NHL. The overall response rate in all patients was 88% (58% with CR). In FLU/CY it was 95% compared with 84% in FLU/CY/MITO. After 3 courses, 58% of overall CR was achieved with FLU/CY treatment and 90% with FLU/CY/MITO (p<0.02). There was no statistical difference in response rate between the treatments. Median time to disease progression was 6 months. One patients died with fever of unknown origin 3 months after 6 courses of FLU/CY while in CR. Both therapies were well tolerated. Grade 3 or 4 neutropenia was observed in 39 courses, and had a similar distribution between the two groups. Non-hematologic toxicity was very mild in both arms and represented by grade 1 nausea and vomiting in two patients. No other toxicity was observed. Both combination therapies were seen to be effective in treating recurrent low-grade lymphoma in patients previously treated with regimens containing doxorubicin or mitoxantrone, but fewer patients relapsed with FLU/CY/MITO and the fast activity of this treatment suggests it to be more useful. Overall results were similar, consequently it is difficult to draw sure indication about the opportuness of choosing one or the other treatment.

**CIS-PLATINUM, IDARUBICIN, PREDNISONE AS CONSOLIDATION THERAPY AFTER P-VABEC CHEMOTHERAPY FOR ELDERLY PATIENTS WITH DIFFUSE LARGE LYMPHOMAS. AN ITALIAN MULTICENTER RANDOMIZED STUDY**


Background. In a phase II study the P-VABEC regimen resulted to be an active and well tolerated therapy for elderly patients with diffuse large cell lymphomas (M artelli, J Clin Oncol 11:2363; 1993). Further studies reported on a larger series of patients treated with P-VABEC demonstrated that in spite of a high rate of complete response (CR) the event-free survival (EFS) rapidly decreased with a high incidence of early relapse. Moreover a significantly worse EFS was shown for patients with a high-risk IPI score compared to those with a low-risk IPI (M artelli, Ann Oncol 1999; 10 suppl. 3, 55). A phase II study reported by Caracciolo (Leuk Lymphoma 1997; 24:335) demonstrated an improvement of survival after cisplatin, idarubicin, prednisone (CIP) as consolidation chemotherapy in patients responsive to P-VABEC.

Purpose. To evaluate the activity and toxicity of CIP consolidation therapy after P-VABEC versus the standard P-VABEC regimen in a prospective, randomized, phase III study. Patients and methods. From October 1995 to April 2000 we enrolled 198 previously untreated patients with diffuse large cell lymphomas (according to the REAL classification), median age 70 years (range 60-85), stage II-IV. All eligible patients were randomized at diagnosis to receive P-VABEC (group 1) or P-VABEC-CIP (group 2). The P-VABEC-CIP is an 8 weekly regimen delivered on a out-patient basis: doxorubicin 30 mg/m², etoposide 100 mg/m², cyclophosphamide 350 mg/m² given in weeks 1, 3, 5, 7 and vincristine 1.4 mg/m², bleomycin 15 mg/td in weeks 2, 4, 6, 8. A daily prednisone dose of 50 mg is given orally during the entire regimen. The CIP consolidation therapy started 21 days after the last P-VABEC cycle, in patients who obtained a response (complete or partial). Patients with minimal response or progressive disease after P-VABEC were excluded from consolidation therapy. The CIP schedule consisted of cis-

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*Mann-Whitney test (the data represent mean ± standard deviation).