both groups, reaching median values of 1×10^9/L on day + 90 without statistically significant differences (Figure 1A). The CD3$^+$ subset recovered slowly in both groups during the study period achieving median values with the normal range at 1 year only in the NHL group (Figure 1B).[7] The number of CD4$^+$ T-lymphocytes was stable and $<200\times10^6$ cells/L during the first four months after transplant (except on day +60 in the lymphoma group). Thereafter, a slow recovery of the T helper subset was observed in both groups up to 1 year (p = ns) (Figure 1C). In both groups the CD8$^+$ subset increased rapidly starting from day +15, reaching median values above the normal range from day +60 (p = 0.04) in the lymphoma group and normal values from day +90 in the CLL group (Figure 1D). After day +30 the CD4/CD8$^+$ ratio remained constantly below 0.6 in both groups (p = ns) (Figure 1E). There was a marked deficiency of CD4$^+$ T cells during the study period, according with prior observations that CD3$^+$ and CD8$^+$ subsets recovered promptly while CD4$^+$ abnormalities persisted for several year after transplantation.[8-10] Starting from day +30 the numbers of CD19$^+$ B-lymphocytes tended to normalize, reaching median values above the normal range only at 1 year, in both groups (Figure 1F).

During the study period, the values of CD16/56$^+$ NK cells recovered to normal in the CLL group starting from day +30 while they were constantly above the normal range in the lymphoma group, this difference being statistically significant on day +30 and +60 (Figure 1G).

In conclusion, the immunological recovery after PBSCT was similar in the two groups of patients with different lymphoproliferative disorders and seemed not to be influenced by the disease itself or by the concomitant T-cell dysfunction typical of CLL. However, given that functional studies of T cells were not done and our sample size was small, these conclusions need confirmation.

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Stem Cell Transplantation

Hematopoietic abnormalities persist for more than six years after autologous peripheral blood stem cell transplantation in patients with non-Hodgkin's lymphoma

We used cell culture assays in 22 lymphoma patients who underwent autologous peripheral-blood stem-cell transplantation (PBSCT) in order to assess whether hematopoietic damage persists during a long-term follow-up. We found that hematopoietic and stromal cell compartments are impaired even six years after transplantation, although some parameters (CFU-E, CFU-GM, hemoglobin and white cell counts) seem to improve from the fourth year.

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Abnormalities in both hematopoietic and stromal bone marrow compartments have been described after allogeneic and autologous stem cell transplantation.[1-6] In the present study we used cell culture assays in order to ascertain whether this damage persists 1-6 years after PBSCT, to establish at what level it occurs and to measure its evolution over time.

Twenty-two patients with non-Hodgkin's lymphoma (NHL) who had received a peripheral blood stem cell transplant (PBSCT) were included in this study. Their median age was 43 (16-63) years, with a male/female ratio of 9/13. The median dose of progenitor cells infused was 3.3 (1.2-

16)×10⁶/kg CD34⁺ cells and 5.2 (0.02-20.3)×10⁸/kg mononuclear cells. Twenty-four normal subjects were used as controls. The median age of these controls was 35 (20-59) years and the male/female ratio was 6/18. Chemotherapy regimens received by patients before PBSCT included CHOP or Mega CHOP as first-line treatment and ESHAP as salvage therapy. Seventeen patients were mobilized using granulocyte colony-stimulating factor (G-CSF) alone and five patients with cyclophosphamide plus G-CSF. The conditioning regimen was BEAM in 19 cases or cyclophosphamide plus total body irradiation (TBI) in 3 patients. The median time to reach more than 0.5×10⁹/L granulocytes and more than 20×10⁹/L platelets was 12 (10-23) and 13 (9-30) days, respectively.

Clonogenic assays, plastic adherent Δ (PΔ) and long-term bone marrow culture (LTBMC) assays were performed as previously described.[5,7,8,9] Statistical analysis was carried out using the SPSS statistical software. The following non-parametric tests were used: Mann-Whitney's U test and Pearson's or Spearman's test for quantitative correlation.

As shown in Table 1, the number of committed progenitor cells and the colony-forming units granulocyte-monocyte (CFU-GM) production in LTBMC were significantly lower in the group of patients than in the control group (p <0.05 or p <0.01). The stromal layer confluence was also reduced in the patients, but this difference was not statistically significant. We also observed that the number of immature progenitors (day 21) determined by PΔ assays was lower in the post-PBSCT group than in the controls (15 [5-21] vs 39 [6-56]) but, again, this difference did not reach statistical significance.

In order to assess whether hematopoietic abnormalities improved over time, patients were divided into two groups according to the time elapsed from the transplantation: less or more than 4 years (early and late groups, respectively). As shown in Table 2, we observed that the numbers of all progenitors were lower in the early group than in the late group, especially erythroid progenitors (p <0.05). Hemoglobin and WBC counts were also significantly lower in the early group (Table 2). When the two groups of patients were compared with controls, we observed that the number of CFU-GM was significantly lower in both (p = 0.002 and 0.04, respectively). However, the number of colony-forming units-erythroid CFU-E was significantly decreased only in the early group of patients (p = 0.04) (Table 2). When LTBMC were analyzed in the two groups, lower stromal layer confluence and fewer cell types were observed at 5 weeks of culture in the early post-transplant group than in the second group, although only the difference in cell types was statistically significant (p <0.05).

Table 1. Clonal and LTBMC 1-6 years after PBSCT.

	NHL Group (n=22)	*Control group (n=24)*	*p*
CFU-GM/10⁵ cells plated	38±29 32 (2-106)	78±71 60 (0-318)	0.048
CFU-E/10⁵ cells plated	30±31 20 (0-110)	55±46 46 (0-161)	0.09
CFU-MK/10⁵ cells plated	13±11 12 (0-31)	23±32 14 (0-126)	0.6
Stromal layer (%)	45±25 40 (5-100)	58±30 65 (10-100)	0.2
LTBMC/CFU-GM	532±357 460 (27-1,120)	4,818±4,773 3809 (177-15,245)	0.009

Results are expressed as mean±s.d. and median (range). LTBMC/CFU-GM: progenitors obtained by adding together the CFU-GM produced in long-term bone marrow cultures. Results obtained from 1×10⁵ cells plated. (%) Percentage of flask surface covered by the stromal layer.

We also performed a correlation study between *in vitro* data and other clinical variables. We found a strong negative correlation between the number of chemotherapy courses received and the immature progenitors obtained in Delta assays (p = 0.005; r=-0.94) as well as erythroid progenitors (p = 0.036, r=-0.5). The percentage of stromal layer confluence was negatively correlated with the patients' age (p = 0.02; r=-0.6).

The presence of a reduced number of committed progenitor cells and long-term culture-initiating cells (LTC-IC), despite normal peripheral blood cell counts, has been described after both allogeneic and autologous bone marrow transplantation.[1-6] Our results are similar to those previously reported, but this is the first study showing that hematopoietic damage persists even 6 years after the transplantation.

Morphologic analysis of the bone marrow stroma showed that the layers in transplanted patients were qualitatively different from those in controls. It has been shown that a functional stromal layer must display all cell components (adipocytes, cobblestone areas, etc).[10] The absence of these

Table 2. Comparison of haematopoietic damage after transplant over time.

Time after PBSCT	*Hb (g/dL)*	*WBC (×10⁹/L)*	*CFU-E*	*CFU-GM*
Early (< 4 years) (n=12)	13±1 13 (11-15)[1,3]	5.0±1.5 5.2 (2.5-7.6)[1,3]	17±20 12 (0-64)[1,3]	402±360 380 (27-1120)[1]
Late (≥ 4 years) (n=10)	14±1 14 (11-15)[2]	7.0±2.2 6.4 (3.5-1.1)	41±32 32 (0-110)	674±312 712(309-961)[2]
Control (n=24)	15±1 15 (12-17)	6.8±1.4 6.6 (4.9-9.2)	55±46 46 (0-161)	4818±4773 3809 (177-15245)

Results are expressed as mean±s.d. and median (range). [1]Differences statistically significant (p<0.05) between the early group and the control group; [2]differences statistically significant (p<0.05) between the late group and the control group; [3]differences statistically significant (p<0.05) between the early and the late groups; CFU-E: erythroid progenitors obtained from 10⁵ cells plated; CFU-GM: granulo-monocyte progenitors obtained from 10⁵ cells plated.

cells in our patients clearly indicates a damaged bone marrow microenvironment after PBSCT, which improves from the fourth year after transplantation.

At present, it is already known that previous chemo/radiotherapy produces a depletion in the stem cell compartment.[11,12] In our study, we only found a negative correlation between previous chemotherapy and the immature progenitors, suggesting that chemotherapy preferentially affects immature progenitors rather than committed ones. Taken together, our data seem to indicate that the hematopoietic and stromal cell compartments are impaired after PBSCT for a long period of time (even 6 years). However, 4 years after PBSCT, some parameters (CFU-E, CFU-GM, hemoglobin and white cell counts) seemed to improve.

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