Cellular drug resistance is an important cause of failure of chemotherapy.[9] It has been reported that prior corticosteroid therapy could help identify a subset of Ph+ ALL patients who may be cured even if allogeneic bone marrow transplantation cannot be performed.[10]

Although our results showed no difference in remission induction rate or the number of deaths during remission therapy, the administration of IV dexamethasone for 4 days before starting chemotherapy did significantly decrease the bone marrow blast percentage at day +14, and the improved disease-free survival approached statistical significance. It is possible that dexamethasone increases the speed at which blasts are destroyed, but probably does not have any effect on blasts which are resistant to chemotherapy. Mid-term and long-term side effects, such as aseptic necrosis of the bone, will be assessed in a longer follow-up.

Our small number of patients precludes us from carrying out multivariate analysis, but we believe that, with more patients and a longer mean follow-up, this strategy will probably result in a significantly better disease-free survival than that achieved by the same chemotherapy regimen without prior administration of dexamethasone.

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Lymphoproliferative Disorders

Quantification of Epstein-Barr viral load and determination of a cut-off value to predict the risk of post-transplant lymphoproliferative disease in a renal transplant cohort

Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening Epstein-Barr virus (EBV)-driven B-cell malignancy occurring in 1 to 3% of renal transplant patients.[1,2] Recently, EBV DNA quantification has become a useful tool for identifying patients at risk of developing PTLD.[3-5] However, studies on EBV load differ in design, methodology and type of patients.

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We conducted a nested case-control study[6] sampled in a cohort of 1,800 renal transplant recipients to estimate the threshold value of EBV load predictive of PTLD development. Eleven PTLD patients were enrolled between January 2000 and December 2002 from a single Institution. The diagnosis of PTLD was established according to the WHO classification[7] and confirmed by *in situ* hybridization for EBV, using an EBV-encoded RNA (EBER) detection kit (Dako, Glostrup, Denmark). Fifty-five controls (5 per case) matched by age, date of transplant, type and dosage of immunosuppression and history of rejection were selected from the cohort (Table 1). Using real-time polymerase chain reaction (PCR) (LightCycler System, Roche, USA)[8] and primers for the EBNA-1 gene of EBV,[9] we quantified the EBV load in whole blood of cases and controls (1 sample/individual). Each sample was analyzed in duplicate. Each run included two negative (no DNA) and two positive (DNA from Daudi EBV-positive cell line, ATCC CCI-213, Rockville, MD, USA) controls. Albumin RQ-PCR amplification was used as tthe internal control.[8] The sample quantification is based on a calibration curve, prepared with a known concentration of a purified PCR product (Daudi cell line). The DNA concentration of the PCR product is estimated by optical density at 260 nm, and the copy number/mL is calculated by the formula: copies/mL $=(6.023\times10^{23}\times C\times OD_{260})/$ MWt, where C= 5×10^{-5} g/mL and MWt is the molecular weight of the base pairs of the PCR product $\times$ 6.58 $\times$ 102g.[10] In order to validate EBV quantification, five comparative groups were used: 10 healthy blood donors, 10 individuals with AIDS (CD4 $\leq$ 100 cells/mm^3), 10 patients with untreated Hodgkin's lymphoma (HL), 10 individuals with infectious mononucleosis (IM) and 5 children aged from 6 to 18 months. All PTLD cases and controls presented positive EBV-IgG and negative IgM. All blood donors, all AIDS patients and 9 HL patients were IgG-positive (no signs of active infection). All children were EBV-negative and all IM patients displayed serological evidence of active infection. The median time from renal transplantation to PTLD onset was 36 months. All cases showed elevated EBV load at

Table 1. Baseline characteristics of the study participants.

Characteristics	*Cases (n=11)*	*Controls (n=55)*	*p value*
Age (years)			
Median	38	38	1.00
Interquartile range	26-46	24-46	
Female sex (%)	3 (27.2)	15 (27.2)	1.00
Race or ethnic group (%)			
White	8 (72.7)	33 (60.0)	0.51
Renal failure cause			
Glomerulonephritis, n (%)	2 (18.1)	8 (14.5)	0.67
Systemic arterial hypertension, n (%)	5 (45.4)	27(49.0)	0.91
Pyelonephritis, n (%)	0	2 (3.6)	1.00
Diabetes, n (%)	4 (36.3)	15 (27.2)	0.72
Other, n (%)	0	3 (5.4)	1.00
Previous renal transplantation, n (%)	0	3 (5.4)	1.00
Renal donor			
Cadaveric, n (%)	7 (63.6)	40 (72.7)	0.72
Alive, n (%)	4 (36.4)	15 (27.3)	0.72
Rejection episodes, n (%)	2 (18.2)	12 (21.4)	1.00
Positive CMV antigenemia (in last 12 months)	0	0	1.00
Symptoms			
Renal function deterioration, n (%)	4 (36.4)	0	–
Fever, n (%)	9 (81.8)	0	–
Weight loss (>10%), n (%)	8 (72.7)	0	–
Night sweats, n (%)	9 (81.8)	0	–
Generalized lymphadenopathy, n (%)	9 (81.8)	0	–
Hepato/splenomegaly, n (%)	5 (45.4)	0	–

CMV: cytomegalovirus. There was no statistical difference between the characteristics of cases and controls .

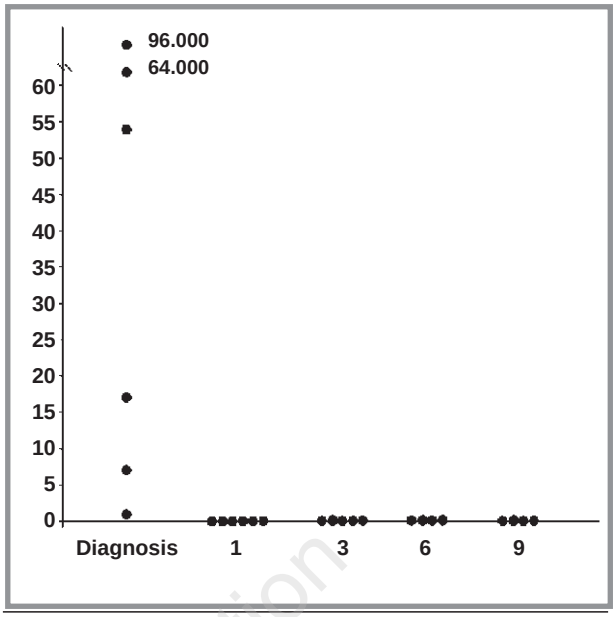

Figure 1. Dynamics of viral load in PTLD cases at diagnosis and after treatment. X: diagnosis and months after treatment. Y: copies/10^6 peripheral blood mononuclear cells (PBMC).

diagnosis, with a median of 17,400 copies/10^6 peripheral blood mononuclear cells (PBMC) (range 1,000 to 227,590), and this load was statistically different from that found in controls [median 49.5 copies/106 PBMC (range 49.5 to 11,500), p = 0.001]. To confirm this result, the EBV load of each case was compared to those of the five controls for that case: we found a statistically significant difference for this comparison, too (p = 0.008). All children, blood donors and HL patients had either low or undetectable EBV levels, these levels being statistically different from those recorded in the cases ($p \leq 0.001$). In contrast, HIV-positive and IM patients presented high EBV loads, resembling those in PTLD cases (p = 0.202 and 0.360, respectively). If a viral load greater than 1,000 copies/10^6 PBMC was taken to diagnose PTLD, the sensitivity of the test was 1.00, specificity 0.60, positive predictive value (PPV) 0.30 and negative predictive value (NPV) 1.00. Taking a cut-off value of 5,000 copies/10^6 PBMC, the sensitivity was 0.73, specificity 0.82, PPV 0.44 and NPV 0.94. Increasing the specificity and PPV at the expense of decreasing sensitivity would put less healthy immunosuppressed patients at risk of any therapeutic intervention (10/55) and engraftment loss, but would probably miss some early PTLD diagnoses (3/11). All patients underwent reduction of immunosuppression and chemotherapy or rituximab or radiotherapy. All patients who achieved complete remission cleared their viral load within 1 month after starting therapy (Figure 1).

In summary, establishing a cut-off value is more a management decision than a statistical one, and, although attractive, is hampered by clinical difficulties in the face of appropriate care for transplanted patients. Furthermore, EBV load is an essential component of predicting a diagnosis of PTLD, but it is not the only one. In order to facilitate a physician's decision (reduction of immunosuppression or use of pre-emptive therapy with rituximab), monitoring EBV levels can probably be limited to high-risk periods, such as during a switch of immunosuppression and/or rejection episodes, when increased immunosuppression is needed.

Our data demonstrate that clearance of EBV load occurs early after therapy and its maintenance depends on the restoration of T-cell defenses after reduction of immunosuppression. We believe that our results could be useful in the setting of renal transplantation, but should not be extrapolated to patients with other organ transplants. Prospective multiinstitutional studies with longer follow-up, using standardized methodology to examine EBV load and specific T-cell immunity are urgently needed in different types of transplant.

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Malignant Lymphomas

High incidences of malignant lymphoma in patients infected with hepatitis B or hepatitis C virus

It is still not clear whether there is an association between malignant lymphomas and hepatitis B or hepatitis C virus infections. Most studies from Italy and Japan support this association, but studies from other countries generally do not. We conducted a hospital-based case-control study to evaluate this association and show a significant association between the development of malignant lymphoma and infections by these viruses.

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There are conflicting data on an association between malignant lymphoma and hepatitis B virus (HBV) or hepatitis C virus (HCV) infections. Most studies from Italy[1,2] and Japan[3,4] support this association, but those from other countries[5,6] generally do not. We conducted a hospital-based case-control study in Japan to evaluate this association.

We selected 145 consecutive patients with malignant lymphoma who were admitted to Toranomon Hospital in Japan between 1995 and 2001. All the patients were first diagnosed with malignant lymphoma and had not received any chemotherapy. Controls were selected randomly from patients admitted to the orthopedics (n=290) and ear, nose and throat (ENT) (n=284) departments of the hospital. The case-patients were matched for age, sex and year of visit with the controls in a 1:2 ratio. We excluded control patients with a history of malignancy. Age was further adjusted as a continuous variable in conditional logistic regression analysis. The following data were extracted from the patients' medical records; age, sex, smoking habits, alcohol use, HBs antigen (HBs-Ag), anti-HCV antibody (HCV-Ab), anti-HTLV-1 antibody, anti-HIV antibody, and a history of malignancy. The definition of HBV and HCV infection was a positive result for HBs-Ag and HCV-Ab, respectively. The diagnoses of malignant lymphoma was confirmed by two hematopathologists who independently reviewed 126 available samples.

All statistical analyses were performed with STATA, ver.7 (software STATA Corp., College Station, TX, USA). Bivariate analyses were performed with the χ^2, Fisher's exact, or Wilcoxon signed-rank test. Attribution of HBV/HCV infection was evaluated in terms of odds ratios (OR) and 95% confidence intervals (CI). A conditional logistic regression model was applied to estimate ORs and 95% CI. Analyses were initially conducted separately for cases vs. orthopedic controls, and cases vs. ENT controls. However, since analyses for both control groups showed similar trends, these two subgroups of controls were combined and overall analysis was conducted. The *sampsi* procedure of STATA was used to calculate the statistical power necessary to detect a difference between observed percentages of subjects HBV/HCV infection among cases and controls with a type I error of 0.05. Estimated values were 60% for HBV in two-tailed analysis, when the prevalence of infection was 5% among cases and 1% among controls. The corresponding value for HCV was 55%, when its prevalence was 11% among cases and 5% among controls. The study protocol was approved by the institutional review board.

The patients' characteristics are shown in Table 1. Of the 145 cases, 120, 16, and 7 were diagnosed with B-cell, T/NK-cell neoplasms, and Hodgkin's lymphoma, respectively. Two other patients had malignant lymphoma but the histologic subtype could not be classified. Seven patients with HBV infection developed malignant lymphoma: the histologic subtypes were diffuse large B-cell (n=4), follicular B-cell (n=1), and adult T-cell lymphoma (n=1). The prevalence of HBV infection among cases was significantly higher than among orthopedic (OR: 4.67, $p = 0.026$), ENT (OR: 4.67, $p = 0.01$) or combined (OR: 4.67, $p = 0.006$) controls. Age-adjusted OR (aOR) between cases and orthopedic (aOR: 4.74, $p = 0.024$), ENT (aOR: 8.34, $p = 0.006$) or combined (aOR: 5.36, $p = 0.003$) controls were significant. Fifteen patients with HCV infection developed malignant lymphoma. Histologic subtypes were diffuse large B-cell (n=11), follicular B-cell (n=2), peripheral T-cell (n=1), and unclassified (n=1). The prevalence of HCV infection among cases was higher than among orthopedic (OR:3.83, $p = 0.002$), ENT (OR:2.06, $p=0.099$) or combined (OR:1.96, $p = 0.005$) controls. The aOR between cases and orthopedic (aOR:3.80, $p = 0.002$), ENT (aOR:1.86, $p = 0.086$) and combined (aOR:2.17, $p = 0.002$) controls were significant. Multivariate analysis showed significant associations between both HBV and HCV infection, and malignant lymphoma (Table 2).

Considering the incidence of malignant lymphoma, case-control studies are the most feasible method to evaluate associations between HBV/HCV and lymphoma. However, most previous case-control studies had some methodological limi-