Detection of gammopathy by serum protein electrophoresis for predicting and managing therapy of lymphoproliferative disorder in 911 recipients of liver transplants

Antoinette Lemoine, Patrick Pham, Daniel Azoulay, Faouzi Saliba, Jean-François Emile, Raphaël Saffroy, Philippe Broet, Henri Bismuth, Didier Samuel, and Brigitte Debuire

Monitoring of posttransplantation lymphoproliferative disorder (LPD) is usually based on imaging, which lacks sensitivity. A prospective study in 911 consecutive recipients of liver transplants was conducted to assess the value of gammopathy monitoring by serum protein electrophoresis (SPE) and to compare it with conventional follow-up methods. Patients systematically underwent SPE testing just before transplantation, at least twice during the first year after transplantation, and once a year thereafter. Patients with LPD underwent SPE testing every month. Immunofixation was done if abnormalities were detected by SPE. Gammopathy was observed in 114 patients, 18 of whom had onset of LPD. In 3 other patients, LPD developed, but no gammopathy was detected before onset of LPD or while LPD was present. Multivariate analyses showed gammopathy (relative risk [RR], 65.3), more than one transplantation (RR, 7.5), and viral cirrhosis (RR, 2.8) to be independent prognostic factors associated with occurrence of LPD. LPD was treated by reducing immunosuppression, with or without chemotherapy, administration of anti-CD20 monoclonal antibody, or surgery. The mortality rate was 24% (5 of 21 patients). Remission, which occurred in 13 patients, was associated with disappearance of gammopathy in 10 patients. In 5 patients, normalization of SPE results preceded the diagnosis of remission based on imaging, by a mean of 4 months. For diagnosis of LPD remission, the positive and negative predictive values of disappearance of gammopathy were 91% and 100%, respectively; and gammopathy monitoring was more sensitive than imaging (100% and 38%, respectively). Gammopathy monitoring is an inexpensive, noninvasive, sensitive way to detect LPD and assess the efficacy of treatment. It could be used routinely in follow-up of recipients of transplants. (Blood. 2001; 98:1332-1338)

Introduction

Lymphoproliferative disorder (LPD) is a serious complication of organ transplantation. Diagnosis of LPD after transplantation is generally based on clinical symptoms (asthenia, fever, abdominal pain, and lymphadenopathy), results of imaging studies (ultrasound analysis or computed tomography [CT] scanning), and pathological findings. Any organ may be involved, although in patients who have undergone liver transplantation, LPD is frequently restricted to the liver. But LPD may also occur in the lymph nodes or gastrointestinal tract.[1-3] LPD is usually treated by reducing immunosuppression, but multidrug combination chemotherapy designed for high-grade lymphoma is often required.[3,4] Treatment efficacy is usually monitored by imaging and histological studies. However, imaging does not detect small tumors or indicate the degree of necrosis of the tumor, and biopsy for histological analysis is not always technically possible and cannot be used routinely for follow-up. Thus, new tools for monitoring the treatment of LPD are needed.

We previously showed that serum gammopathy after liver transplantation may act as an early marker of LPD that could be of great value, making it possible to reduce immunosuppression and thereby decrease the risk of LPD.[5,6] We here describe a prospective study in 911 consecutive recipients of liver transplants to assess the value of gammopathy monitoring. The study used serial serum protein electrophoresis (SPE) evaluations as a marker of LPD activity. We compared our results with those of conventional imaging and examined the progression of gammopathy during LPD treatment.

Patients and methods

Patients

The study was conducted from January 1993 to December 1999 in recipients of liver transplants followed in our institution. The study population was divided into 2 groups: a group of 477 patients who underwent orthotopic liver transplantation (OLT) with 541 liver grafts between January 1993 and December 1998; and a group of 434 of the 763 patients who underwent transplantation between 1985 and 1992, were still alive in January 1993, and were followed in our institution as outpatients between January 1993 and December 1999. There was no statistical difference between the 2 groups with respect to age, sex, reason for transplantation, or cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection status. The indication for transplantation was viral cirrhosis in 318 patients (117 with hepatitis C virus (HCV); 47 with hepatitis B virus (HBV); 14 with HCV and HBV; 64 with HBV and hepatitis D virus (HDV),

From Service de Biochimie et Biologie Moléculaire, Centre Hépato-biliaire, Anatomie Pathologique, UPRES 1596 Virus Hépatotropes et Cancer, INSERM U492 Epidémiologie et Biostatistiques, Hôpital Paul Brousse, Faculté de Médecine Paris-Sud et Assistance Publique de Paris, Villejuif, France.

Submitted August 21, 2000; accepted April 25, 2001.

Reprints: Antoinette Lemoine, Service de Biochimie et Biologie Moléculaire, Hôpital Paul Brousse, 14 Avenue Paul Vaillant Couturier, 94804 Villejuif Cédex, France; e-mail: antoinette.lemoine@pbr.ap-hop-paris.fr.

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21 with HBV, HCV, and HDV; and 55 with fulminant viral hepatitis) and a nonviral indication in 593 patients, including 34 with autoimmune disease. No informed consent was required because SPE was performed as part of routine practice.

All outpatients underwent serum gammopathy monitoring based on systematic SPE just before OLT (groups 1 and 2), at least twice in the first year after OLT (at 4 and 8 months after OLT in patients in group 1), and once a year thereafter if protein profiles were normal (groups 1 and 2). If abnormalities were observed on SPE, immunofixation was performed systematically and immunosuppression reduced, if possible, in association with serial SPE analyses (every month).

Assessment of serum gammopathy

From January 1993 to December 1995, serum proteins in blood samples were separated by zone electrophoresis on cellulose acetate (Helena, Saint-Leu La Forêt, France) and stained with Ponceau red. After January 1996, SPE was performed in agarose gels, with amidoblack staining (Sebia, Issy-les-Moulineaux, France). The protein bands were scanned with a densitometer (Sebia). Normal values for total immunoglobulin (Ig) concentration were 8 to 12 g/L. Immunofixation was done with Sebia agarose gel method by using mammalian antiserum against human IgG, IgA, and IgM and against human κ and λ light-chain determinants (free and bound). Igs were quantified with a nephelometer (Behring, Marburg, Germany); normal reference ranges were as follows: 6.7 to 12.5 g/L for IgG, 0.9 to 3.0 g/L for IgA, and 0.6 to 2.0 g/L for IgM. Gammopathy was defined as the occurrence of monoclonal or oligoclonal chains, with or without global hypergammaglobulinemia. Monoclonal gammopathy was defined according to standard criteria,[7] corresponding to the presence of a monoclonal Ig component in serum identified by immunofixation. Oligoclonal gammopathy was defined as the simultaneous presence of at least 2 subtypes of monoclonal protein.

Diagnosis of LPD

We searched actively for LPD by using imaging (ultrasound analysis and CT scanning) and SPE if clinical symptoms were observed (asthenia, fever, diffuse abdominal pain, and lymphadenopathy). If a mass was detected on imaging, percutaneous tumor ultrasonography or scan-guided biopsy was done to allow molecular and histological analysis and the tumor was then classified retrospectively according to the criteria proposed by the Society for Hematopathology workshop.[8] In all cases, LPD was diagnosed on the basis of pathological analysis of the tumor biopsy specimen. In situ hybridization analyses using κ and λ messenger RNA oligonucleotides and genotyping studies were done to determine the clonal nature of the tumor. Immunohistochemical detection with antilatent membrane protein 1 antibody and in situ hybridization using EBV early RNA (EBER) oligonucleotides were done separately to detect EBV in the proliferating lymphoid cells.[9]

Follow-up of patients with LPD

LPD treatment involved reducing immunosuppression, chemotherapy, administration of anti-CD20 monoclonal antibody (mAb; since October 1998), or a combination of these methods. Surgery or another transplantation was done if possible. LPD was monitored by using imaging (thoracic and abdominal CT scanning) and serial SPE (every month). LPD was considered to be cured when clinical symptoms resolved and no mass was detected on an imaging study.

Statistical analysis

Univariate logistic regression was used to identify factors predictive of gammopathy or LPD. The following factors were investigated: age at first treatment ($\leq$ 46 years versus $>$ 46 years), sex, CMV status (donor and recipient), CMV reactivation, viral cirrhosis, autoimmune cirrhosis, acute graft rejection, and use of antilymphocyte preparations or Orthoclone T3 (OKT3). These factors were then subjected to a multivariate analysis to investigate their prognostic role in the occurrence of gammopathy or LPD. For 2-dimensional contingency tables with small samples, we used the Fisher exact test, which does not require cell counts to be large. Two-tailed *P* values below .05 were considered to represent significance.

Results

Incidence and characteristics of gammopathy during follow-up

Of the 911 patients followed after liver transplantation in our institution by using serial SPE and immunofixation, 114 presented with gammopathy leading to a reduction in the dose of immunosuppressant used. Initially, steroid doses were decreased by 30%. If there was no response, the dose of cyclosporine or tacrolimus was also reduced, by 50% to 70% of the initial dose, in accordance with liver enzyme levels and time since OLT. Patients underwent abdominal CT scanning, and additional scans were done more frequently than usual, particularly if patients had symptoms suggestive of LPD.

Risk factors for development of gammopathy were investigated (Table 1). In univariate logistic regression, sex, CMV status (donor and recipient), CMV reactivation indicated by viremia, viral cirrhosis (caused by HBV, HCV, and HDV), HCV infection, and autoimmune disease were not significant predictors of gammopathy. In contrast, patients older than 46 years (the median patient age) had a significantly higher risk of gammopathy (relative risk [RR], 1.82; 95% confidence interval [CI], 1.20-2.70). Graft rejection was also closely associated with a higher risk of gammopathy (RR, 1.54; 95% CI, 1.02-2.34). Ninety-five percent of the recipients of liver transplants were positive for EBV, 2 had EBV primary infection (associated with LPD), and 10 had EBV reactivation associated with symptomatic hepatitis after transplantation. Thus, the importance of these criteria as risk factors was not determined. Immunosuppressive treatments using antilymphocyte preparations or OKT3 did not influence development of gammopathy. In multivariate analysis, age was the only factor predictive of gammopathy; older patients had a risk of gammopathy that was 1.93 times higher (95% CI, 1.27-2.94) than that in younger patients.

Among the 114 patients in whom gammopathy developed after transplantation, 41 (36%) had gammopathy before the procedure. In 3 of these patients, LPD developed 2, 4, and 15 months, respectively, after OLT. The 41 patients were 9 women and 32 men with a mean age of 53 years (range, 21-69 years), and 26 of these

Table 1. Comparison of clinical characteristics of patients and occurrence of gammopathy or LPD

Characteristic	χ^2 test value for gammopathy (*P*)	χ^2 test value for LPD (*P*)
Age, y ($\leq$ 46, $>$ 46)*	7.79 (.005)	0.25 (.62)
CMV status of donor (+/−)	0.69 (.41)	1.71 (.19)
CMV status of recipient (+/−)	0.01 (.94)	1.19 (.27)
Sex (M/F)	2.45 (.12)	0.68 (.41)
CMV reactivation (+/−)	0.36 (.55)	0.63 (.43)
Acute graft rejection (+/−)	4.22 (.04)	0.04 (.85)
Viral cirrhosis (+/−)	0.61 (.43)	5.96 (.01)
HCV infection (+/−)	1.39 (.24)	1.10 (.29)
More than 1 transplantation (+/−)	1.74 (.19)	4.97 (.02)
Autoimmune disease (+/−)	1.18 (.28)	0.07 (.79)
Treatment		
SAL (+/−)	0.12 (.72)	0.10 (.75)
OKT3 (+/−)	1.23 (.27)	0.02 (.87)

SAL indicates serum antilymphocyte preparations.
*Median age of the 911 patients was 46 years.

patients underwent OLT because of viral cirrhosis. Gammopathy was monoclonal in 25 of these patients (22 had predominantly IgG type, and κ chains were observed in 16 of the 25). Of the 41 patients with gammopathy before transplantation, 19 had resolution of the condition in the first few months after transplantation, 3 had the same monoclonal abnormality for several years, and 19 had a new abnormality in the profile during posttransplantation follow-up (14 had various changes in the type or number of Igs, whereas 5 retained the same abnormality throughout follow-up). The median total γ-globulin concentration assessed by SPE and densitometry was 20.6 g/L (range, 6.9-39 g/L).

After transplantation, we observed a total of 229 abnormal SPE profiles in the 114 patients. However, 43 of the patients (38%) had only one abnormal profile during follow-up, whereas the remaining patients had 2 (26 patients) or more (45 patients). Gammopathy was monoclonal in 44% of cases, of which 88% were IgG and 22% were IgM. We analyzed data for the 71 patients with at least 2 abnormal SPE after OLT and found that 26 (37%) had the same profile throughout follow-up, with 1 or 2 monoclonal Igs, and that LPD developed in 3 of these patients (patients 14, 18, and 20 in Table 2). The remaining patients had changes in the type or number of Igs, and LPD developed in 3 (patients 15, 19, and 21 in Table 2). Of the 26 patients with the same profile during the course of gammopathy, 60% had a monoclonal chain. The median total γ-globulin concentration was 10.6 g/L (range, 3.6-24.8 g/L)

We searched for risk factors for the development of diagnosed LPD in the 114 patients with positive results on gammopathy assessments. No differences were observed in age, sex, or reason for OLT in the 18 patients with and the 96 patients without LPD. In the patients in whom LPD developed, we observed fewer positive SPE profiles before OLT ($P = .07$), a lower mean total Ig concentration, as assessed by nephelometry (IgG and IgM and IgA; mean [SEM], 14.0 ± 1.9 versus 21 ± 1.3; $P = .02$) and fewer λ chains (1 λ and 9 κ versus 22 λ and 22 κ chains; $P = .03$). However, IgM was detected in 14 of 96 patients (14.5%) in whom LPD did not develop, whereas 7 of 18 patients (39%) with gammopathy had at least an IgM component at diagnosis of LPD ($P = .058$).

Incidence and characteristics of LPD during follow-up

LPD developed in 18 patients with gammopathy and in 3 patients (2.3%) who did not have gammopathy before the onset of LPD or while LPD was present. Table 3 shows characteristics of the 21 patients in whom LPD developed, and Table 1 shows results of an analysis comparing these patients with the control group of 890

Table 2. Results of gammopathy monitoring before onset, at onset, and during treatment of LPD

	Before onset of LPD		At onset of LPD			LPD treatment			Outcome of LPD treatment	
Patient	GP at OLT*	GP 4 mo after OLT*†	GP*	Mean total Ig (g/L)	GP components	Type of treatment	GP at end of treatment*	Imaging at end of treatment*	Outcome	Time after LPD (mo)
1	0	NA	0	17.2	0	IS, reOLT	0	0	Remission	1
2	0	NA	0	31.3	0	IS	0	0	Remission	4
3	0	NA	1	11.3	1 IgMλ + 2 IgMκ	IS	0	0	Remission	2
4	0	NA	1	10.8	1 IgMλ	IS	0	0	Remission	3
5	1	NA	1‡	10.9	2 IgGλ + 1 IgGκ	IS	0.5	0	Remission	6
6	0	NA	1	10.5	1 IgMλ + 3 IgGκ	IS, anti–IL-6, reOLT	0	0	Remission	10
7	0	NA	1	7.0	1 IgGλ + 3 IgGκ	IS, CT, anti-CD20	0	0	Remission	6
8	0	NA	1	7.7	2 IgGλ + 1 IgGκ + 1 IgMκ	IS, CT, anti-CD20	0	0	Remission	4
9	0	NA	1	12.0	3 IgGλ	IS, CT	1	1	Death	18
10	0	1	1	23.0	2 IgGκ	IS, RT	1	1	Death	8
11	0	1	1‡	9.3	2 IgGλ + 1 IgGκ	IS, CT, anti-CD20	1	1	No remission	8
12	0	1	1	11.3	1 IgMλ + 3 IgGκ	IS, anti-CD20	0.5	1	Death	4
13	1	0	0	9.6	0	IS, reOLT	0	0	Remission	1
14	0	1	1	9.8	1 IgMκ	IS, CT	0	0	Remission	7
15	0	1	1	14.5	1 IgGκ	IS, CT, surgery	0	0	Remission	8
16	0	0	1	3.5	Free λ	IS, CT	0	0	Remission	5
17	0	0	1	9.2	1 IgMκ	IS, CT	1	1	Death	7
18	0	1	1	12.5	1 IgGκ	IS, CT	0	0	Remission	5
19	0	1	1	15.6	1 IgGλ + 1 IgGκ	IS, anti-CD20	0	1	Improvement	3
20	0	1	1	13.0	2 IgGκ	IS, CT	1	1	Death	5
21	1	1	1‡	8.5	2 IgGκ	IS, anti-CD20	1	1	Improvement	8

GP indicates gammopathy; NA, nonapplicable; IS, reduction in immunosuppression 1; reOLT, more than 1 OLT; CT, chemotherapy; IL-6, interleukin-6; and RT, radiotherapy.

*0, normal results on serum protein electrophoresis or imaging; and 1, presence of GP and GP 4 represents the last SPE in these patients.

†In patients 1 to 13, LPD developed in the first year after OLT; in patients 14 to 21, LPD developed more than 2 years after OLT or abnormal imaging.

‡Appearance of a new immunoglobulin component.

Table 3. Characteristics of the 21 patients in whom LPD developed

Patient	Age, y/sex	Reason for OLT	Immunosuppressive regimen	Acute graft rejection
1	34/F	Primary biliary cirrhosis	Steroids, Aza, ATG (10 days), tacrolimus	Yes
2	19/M	Glycogenosis	Steroids, tacrolimus	No
3	42/M	Fulminant hepatitis	Steroids, Aza, ATG (14 days), tacrolimus	Yes (OKT3)
4	45/M	Hemochromatosis	Steroids, Aza, tacrolimus	Yes
5	53/F	HBV + renal chronic rejection	Steroids, Aza, ATG (10 days), tacrolimus	No
6	50/F	HBV fulminant hepatitis	Steroids, tacrolimus	Yes
7	57/F	HCV	Steroids, Aza, CyA	No
8	46/M	HBV + renal chronic rejection	Steroids, Aza, ATG (10 days), tacrolimus	No
9	34/F	Amyloid polyneuropathy	Steroids, Aza, ATG 10 days, tacrolimus	No
10	29/M	HCV	Steroids, Aza, CyA	Yes
11	51/M	HBV	Steroids, tacrolimus	No
12	48/M	Alcohol abuse	Steroids, tacrolimus	No
13	38/M	HBV	Steroids, anti-CD25, CyA	Yes
14	44/F	HBV + HCV	Steroids, Aza, CyA	No
15	34/M	HCV + sclerosing cholangitis	Steroids, tacrolimus	No
16	42/M	HBV + HDV	Steroids, tacrolimus	Yes (OKT3, then long term)
17	54/M	HCV	Steroids, Aza, CyA	No
18	55/M	HCV	Steroids, tacrolimus	Yes
19	42/M	Alcohol abuse	Steroids, Aza, CyA	No
20	51/M	HBV + HDV	Steroids, Aza, CyA	No
21	39/M	HBV + HDV	Steroids, Aza, CyA	No

In patients 1 to 13, LPD developed in the first year after orthotopic liver transplantation; in patients 14 to 21, LPD developed more than 2 years after OLT.
Aza indicates azathioprine; ATG, antithymocyte globulins; and CyA, cyclosporine.

patients who did not have LPD (Table 1). Age at diagnosis, sex, CMV status (donor and recipient), CMV reactivation, autoimmune disease, and use of serum antilymphocyte preparations or OKT3 were not significant predictors of LPD on univariate analysis. In contrast, viral cirrhosis and more than one OLT were significantly associated with a higher risk of LPD ($P = .01$ and $P = .02$, respectively). However, gammopathy was a strong, significant predictor of LPD (RR, 49.0; 95% CI, 14-170). In multivariate analysis, gammopathy (RR, 65.3; 95% CI, 17.11-249.6), viral cirrhosis caused by infection with HBV, HCV, and HDV (RR, 2.77; 95% CI, 1.05-7.39), and more than one OLT (RR, 7.46; 95% CI, 2.03-27.3) were independent prognostic factors associated with an increase in the risk of LPD.

We compared patients with gammopathy in whom LPD did not develop with those in whom it did and found that viral cirrhosis (RR, 3.81, 95% CI, 1.33-11.55) was the only independent prognostic factor associated with LPD. No relation was found between the likelihood of developing LPD and the degree of gammopathy (monoclonal versus oligoclonal) or total serum Ig concentration above the normal range, as assessed by nephelometry.

Relation between gammopathy and characteristics of LPD

LPD was detected by imaging and SPE in all patients with clinical symptoms except 2, in whom LPD was detected by chance during a subsequent transplantation. Eighteen of the 21 patients with LPD (86%) had abnormal serum protein profiles (Table 2). In all cases, diagnosis of LPD was confirmed by histological analysis of tumor biopsy or surgical liver resection samples. Because the first systematic SPE assessment was done 4 months after OLT, the median time to appearance of gammopathy before onset of LPD was determined in patients in whom LPD developed more than 5 months after OLT (12 patients). The median time was 4 months (range, 1-90 months). If LPD occurred in the first year after transplantation, it was diagnosed rapidly after the appearance of gammopathy (mean time, 1.1 month). If LPD was diagnosed after the first year of transplantation, it was often associated with a long history of gammopathy despite a reduction in immunosuppression (to 90 months in patient 16). Six patients had one monoclonal component, 4 patients had 2, 4 had 3, and 4 had 4.

Occurrence of gammopathy in patients in whom LPD developed was assessed according to the main clinicopathological characteristics of the tumor (Table 4). LPD that developed in the first year occurred mostly in the liver (10 patients) and hilum (3 patients). Patients in whom LPD occurred later presented with adenopathy (2 with colon, 1 with spleen, 2 with submaxillary, and 1 with retroperitoneal and cervical lymph node adenopathy) but also with LPD in the liver (2 patients).

Histological analysis showed a B phenotype for all the tumors, including 4 polymorphic (in patients 2, 3, 7, and 10) and 15 monomorphic cases of LPD, 1 plasmablastic lymphoma (in patient 15), and 1 follicular lymphoma (in patient 19). The 4 patients who presented with polymorphic LPD had oligoclonal gammopathy profiles; the 6 patients with a monoclonal Ig peak had monomorphic tumors. EBER was detected in 11 of 19 patients (58%) tested, 9 of whom had occurrence of LPD in the first 6 months after OLT. No correlation was found between gammopathy and the time to

Table 4. Relation between characteristics of LPD and gammopathy

Characteristic of LPD	Total no. of patients	No. of patients with gammopathy	No. of patients without gammopathy
Onset			
< 1 y after OLT	13	10	3
> 1 y after OLT	8	8	0
Site			
Liver or hilum	14	11	3
Lymph nodes	7	7	0
Histological findings			
Polymorphic	4	4	0
Monomorphic	15	12	3
Other	2	2	0
EBV early RNA			
Positive	11	9	2
Negative	8	7	1
Not determined	2	2	0

occurrence of LPD, the site of LPD, the histological features of LPD, or the presence of EBER in the tumor.

Gammopathy monitoring during treatment and follow-up of LPD

Posttransplantation LPD was treated by reducing immunosuppression in all patients who had initial blood concentrations of immunosuppressant high enough to make this possible. Such a reduction, without chemotherapy or immunotherapy, led to disappearance of LPD in 4 patients (patients 2-5) in a median time of 3.8 months. Four patients underwent another transplantation (patients 1, 6, and 13) or surgery (patient 15); one (patient 6) received anti–interleukin-6 therapy before the subsequent transplantation. Adjuvant chemotherapy using 6 courses of 6 days of administration of doxorubicin, vincristine, teniposide, and cyclophosphamide separated by 3-week intervals was required for 7 patients. One patient (patient 10) had radiotherapy. Immunotherapy with anti-CD20 mAb (rituximab; 1-2 courses of 4 injections of 375 mg/m^2 of body-surface area at 1-week intervals) was available beginning in 1998 and was used in 6 patients whose tumors had receptors for the anti-CD20 mAb detected by immunohistochemical analysis. Three of these patients also received chemotherapy (patients 7, 8, and 11). The decision to administer chemotherapy did not depend on the grade of lymphoma but on the response to immunosuppression reduction or treatment with anti-CD20 mAb.

Two patients (patients 10 and 17) died from LPD 8 and 7 months, respectively, after OLT, and 3 (patients 12, 20, and 9) died from infection resulting from neutropenia at 4, 5, and 18 months, respectively. Two of the patients who died from infection (patients 9 and 20) had partial remission just before death. All 5 patients had persistent gammopathy until death. The 6 patients treated with anti-CD20 mAb had a decrease in γ-globulinemia during and after treatment. The mean concentration of Igs after treatment was 7.4 g/L (range, 4.9-9.6 g/L; normal range, 6-12 g/L).

The tumors in 13 patients disappeared completely in a median time of 5 months (range, 1-10 months) of treatment, regardless of the type of treatment. Tumor disappearance was indicated by CT scanning and resolution of clinical signs. Three of the patients in whom tumors disappeared had no abnormal serum protein profiles and were excluded from the analysis. Gammopathy assessed by immunofixation disappeared in the remaining 10 patients with complete remission. In 5 of these patients (patients 3, 4, 7, 14, and 15), disappearance of gammopathy coincided with the diagnosis of remission based on CT scanning. In the other 5 patients (patients 5, 6, 8, 13, 16, and 18), normalization of the serum protein profile preceded the diagnosis of remission by a mean of 4 months (1.5-7 months). In patient 6, CT scanning done after the reduction in immunosuppression showed a 5-cm mass and liver biopsy showed only partial necrosis of the tumor, with gammopathy still evident. However, 7 months before another transplantation, serum gammopathy disappeared, although CT scanning done a few days before the transplantation was still suggestive of a persistent active tumor. Histological analysis of the explanted liver graft showed complete necrosis of the tumor.

The remaining 3 patients (patients 11, 19, and 21) have so far had no remission, with a median follow-up of 8.5 months (range, 5-14 months). In one patient, LPD became more acute, with persistent gammopathy, whereas the other 2 patients had a distinct improvement in clinical signs and a decrease in tumor volume as assessed by CT scanning, with complete disappearance of serum gammopathy in one patient (patient 19).

Thirteen patients had remission of LPD; thus, the positive predictive value of disappearance of gammopathy for LPD remission was 91%. The negative predictive value was 100%, and the specificity and sensitivity were 87% and 100%, respectively. The positive and negative predictive values for imaging were 100% and 62%, respectively, and the specificity and sensitivity were 100% and 38%, respectively.

Discussion

This study in a series of 911 consecutive recipients of liver transplants demonstrates the value of SPE and immunofixation screening after transplantation as indicators of LPD activity. LPD occurred in 21 of the 911 patients and was associated with serum gammopathy in 18 of the 21 (86%). Remission with the disappearance of abnormalities of serum Ig component was in some cases identified by these techniques before its detection by imaging. Thus, SPE screening can be used to monitor LPD prevention and follow-up.

LPD affects 2% to 6% of patients with immunosuppression.[1-3,10] In our study, it affected 2.3%, a percentage similar to those in other series of liver-transplant recipients.[3,5,6,11] The tools used in follow-up of transplant recipients with LPD are limited and generally based on the use of minimal residual doses of immunosuppressive drugs, with monitoring by determination of whole-blood concentrations of these agents, sequential ultrasound imaging, and serologic tests for EBV.[12,13] However, monitoring of treatment with these means lacks the sensitivity required to assess residual disease. Imaging does not detect small tumors or indicate the degree of necrosis of the tumor. New tools are required for monitoring of LPD during follow-up. In particular, SPE seems to be a useful indicator of LPD activity. Indeed, we and others previously showed that SPE has a high predictive value for occurrence of LPD,[5,6] and we confirmed this result in the larger series described here. Serial analyses of serum protein profiles are done routinely in our institution, and immunosuppression is reduced if abnormalities are observed. This may result in disappearance of microscopic LPD and low rates of LPD and gammopathy. However, decreasing immunosuppression did not prevent onset of LPD in 18 patients with abnormal SPE profiles at diagnosis of LPD.

Positive results were obtained on SPE screening in 114 of the 911 patients (12.5%), including the patients in whom LPD developed. This prevalence is lower than the 32%[14] and 44%[11] previously reported after liver transplantation and transplantation of other solid organs, although similar analytical methods were used. The difference may be due to use of lower doses of immunosuppressive drugs, particularly antithymocyte globulins; or the timing of SPE and immunofixation screening, which was based on a 4-month sampling period during the first year after OLT, excluding the transient monoclonal peak, with a time to normalization of 2 or 3 months.[11] Unlike Pageaux et al,[11] we found that 36% of our patients (41 patients) had gammopathy before transplantation that was not associated with a risk of development of LPD. The mean age of these patients was 53 years, and most had a viral indication for OLT. However, all but 3 had a complete regression or change to production of new Ig chains within a few months after OLT. After OLT, gammopathy was monoclonal in 44% of cases and most of these involved the IgG class. Thirty-eight percent of patients had transient gammopathy, with only one abnormal SPE profile, a finding consistent with previous results.[11] In the remaining patients, the main characteristic of the gammopathy was a change in the type

of Ig or the number of clonal proliferations; only 26 patients had the same abnormality after OLT.

The occurrence of gammopathy and its variability after transplantation may result from repeated antigenic stimulation that triggers the outgrowth of a specific B-cell clone in the presence of immunosuppression, either directly or by means of activation of cytokines such as interleukin-10.[15,16] Similar observations were made regarding the occurrence of gammopathy in patients in whom LPD develops after heart or kidney transplantation.[5,6,11,17] The discrepancy observed between the number and types of Ig implicated in the gammopathy and the predominant histological characteristics of the tumor may be due to the emergence of clones producing different types of Ig during tumor progression. We also found plasma cells, the principal Ig-producing cells, in polymorphic but not in monomorphic LPD, except for plasmablastoma. Clinically, the presence of serum IgM seems to be preferentially associated with LPD.[17]

Pageaux et al[11] reported an absence of correlation between the appearance of gammopathy after transplantation and the indication for transplantation, except in cases of viral infection. In our study, 2 risk factors were significantly associated with the occurrence of gammopathy: age above 46 years and an episode of acute graft rejection. However, in multivariate analysis, age was the only predictive factor for gammopathy. Peest et al[14] had similar results, but no similar findings were reported by Pageaux et al.[11] We observed no correlation with underlying diseases such as autoimmune disorders. This may have been because of the small number of patients with autoimmune disease (34) in our cohort of 911 patients.

When we analyzed the risk factors for occurrence of LPD, we found underlying viral cirrhosis, more than one transplantation, and gammopathy to be independent prognostic factors. The RR of developing LPD if gammopathy was detected was 65, with a 95% CI of 17 to 250 (because of the small number of patients in whom LPD developed). The only prognostic factor for development of LPD in the presence of gammopathy was underlying viral cirrhosis before OLT (RR, 4). Previous studies showed that both use of OKT3 and CMV infection and CMV disease may increase the risk of LPD.[18-22] In our series of patients older than 18 years, this was not observed. This may have been because of the limited use of OKT3 since tacrolimus became available in the early 1990s and the administration of 3 months of prophylactic therapy with ganciclovir and acyclovir in high-risk patients (CMV-seronegative recipient and CMV-seropositive donor) and CMV-seropositive patients, respectively.

Previous studies suggested that reducing immunosuppression is the best initial therapy for patients with early LPD, although even complete discontinuation of immunosuppressive measures appears to be insufficient for those with widespread disease.[23] In our study, LPD developed in 13 patients (62%) during the first year after OLT and reducing immunosuppression was effective in 4 of them. Among the 8 patients in whom LPD developed after 2 years, all but 2 underwent first-line chemotherapy. Complete remission was observed in 4 of the 8 patients. Thus, the efficacy of treatment did not depend on the time to occurrence of LPD or its histological grade.

The mortality rate among patients with LPD and chemotherapy complications was 24% (5 of 21 patients), and 4 of the 5 patients who died had monoclonal tumors. This proportion is similar to the 25% rate reported for treatment of LPD after heart transplantation by using a different chemotherapy combination (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide-cytarabine, bleomycin, vincristine, and methotrexate)[24] and is lower than the 52% rate in 4 major series of posttransplantation clonal lymphomas.[21,22,25] In our series, 2 patients died from infection but had partial remission of the tumor. Gammopathy monitoring could have been used to decrease or stop chemotherapy.

Complete remission as assessed by imaging and resolution of clinical symptoms was observed in 13 patients. However, in 5 patients, the gammopathy disappeared although imaging indicated the presence of a mass 1.5 to 7 months earlier. At that time, the positive and negative predictive values of disappearance of gammopathy for complete remission of LPD were 91% and 100%, respectively, and the sensitivity and specificity were 100% and 87%, respectively. Imaging had higher specificity and positive predictive values (100%) because one patient was considered not to have remission on imaging assessment despite the disappearance of gammopathy. The sensitivity and negative predictive values of imaging for detection of remission (38% and 62%, respectively) were lower than those for gammopathy monitoring.

Therefore, monitoring of gammopathy during treatment of LPD after liver transplantation is a useful way to assess efficacy of the treatment. It may also be used in patients in whom LPD develops after heart or kidney transplantation.[5,6,11,17] Samples could be obtained every month for the first 6 months after transplantation to screen for early development of LPD. Subsequently, samples could be obtained at 9 months, 1 year, and once a year thereafter. This recommendation is based on our finding that 62% of cases of LPD developed in the first year and 43% in the first 4 months after OLT. This type of monitoring requires only a blood sample, is less risky than biopsy, and reflects the functional activity of the tumor, unlike ultrasonography or CT scanning. SPE is inexpensive, and immunofixation is done only if the serum protein profile has abnormalities (in our series, this occurred in 114 of 911 patients [12.5%]).

In conclusion, we suggest monitoring the prevention and treatment of LPD in patients who have undergone transplantation by using SPE combined with periodic imaging, with adjustment of immunosuppression and chemotherapy or immunotherapy, until the disappearance of gammopathy or remission of LPD.

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