Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Monoclonal gammopathy after liver transplantation: a risk factor for long-term medical complications other than malignancies

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Keywords
bacterial or viral infections, chronic kidney disease, cirrhosis, immunosuppression, monoclonal gammopathy, myeloma.

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Conflicts of Interest
The authors of this manuscript have no conflicts of interest to disclose.

Received: 24 May 2011
Revised requested: 22 June 2011
Accepted: 9 September 2011
Published online: 14 October 2011

doi:10.1111/j.1432-2277.2011.01362.x

Summary

The aims of the study were to evaluate (i) the prevalence of MGUS in patients after liver transplantation (LT), (ii) the role of MGUS as a risk factor for malignancy and other medical complications after LT. One hundred and fifty consecutive patients were included in the study and followed prospectively after LT for more than 18 months. Eighteen patients had MGUS before LT, whereas 49 patients developed MGUS after LT ('de novo' MGUS). Thirty-six of these patients showed a MGUS along all the follow up after LT ('permanent' MGUS). In 31 patients, MGUS disappeared after LT ('transient' MGUS). No patient with MGUS developed B-malignant lymphoproliferative disorder and only one patient developed a myeloma after LT. Comparing patients with 'permanent' MGUS to patients with 'transient' MGUS or without MGUS after LT, the former group showed a higher rate of serious infections (30% versus 13%, $P = 0.01$), chronic kidney disease (CKD) (75% versus 44%, $P = 0.001$) and mortality (33% versus 17%, $P = 0.04$). Permanent MGUS was confirmed as an independent risk factor for serious infections and CKD by multivariate analysis. Permanent MGUS after LT does not entail a significant risk of malignancy, but it is associated with a higher risk of serious infections and CKD.

Introduction

Monoclonal gammopathy (MG) of undetermined significance (MGUS) is the most common plasma cell-related disorder. MGUS is defined by a MG in serum ≤30 gr/l, a proportion of plasma cells in the bone marrow ≤10% and the absence of related symptoms [1]. In the general population, the prevalence of MGUS increases with age. It is about 3.2% in persons over 50 and 5.3% in those over 70, the prevalence being higher in men than in women [2]. Since patients with MGUS progress to myeloma multiple or B malignant lymphoproliferative disorders at a rate of 1% per year, approximately, it is not considered as 'benign' [3].

Several reports suggested the association of MGUS with a variety of diseases including: connective tissue disorders, neurological disorders, liver disease and solid organ transplantation. The association between solid organ transplantation and the development of MGUS is thought to be related to the immunosuppressive therapy and the consequent reduction of immunologic surveillance. The appearance of a MG has been already described after liver transplantation (LT). Badley *et al.* reported a prevalence of MGUS at 28% after LT [4]. Nevertheless, there are few data about the significance of MG after LT and on the risk of its progression to a malignancy. In addition, there is no evidence on the role of MGUS in the

pathogenesis of medical complications other than malignancy after LT.

The aim of this study was to define the prevalence of MG in cirrhotic patients and in liver transplant recipients, the rate of progression of MGUS to myeloma or B malignant lymphoproliferative disorders and the potential contribution of MGUS in the development of other medical complications after LT.

Patients and methods

A prospective review of all liver transplant recipients at our institution was carried out with the approval of our Institutional Ethical Committee. The only inclusion criterion was advanced liver cirrhosis as an indication for LT. The only exclusion criterion was a follow up after LT <18 months. The listing for LT was based on both the Italian Association for the Study of Liver and the American Association for the Study of Liver Diseases guidelines [5,6]. The timing for surgery was established on the basis of the Child-Turcotte-Pugh (CTP) score [7,8] up to 2003 and then on the basis of Mayo End Stage of Liver Disease (MELD) score [9,10]. After LT, patients were followed as outpatients monthly during the first year after LT and then every 6 months. Each visit included a clinical examination, blood sample examination and imaging procedures when required. Blood sample examination included a complete blood count, routine laboratory tests including liver and renal function, trough blood level of cyclosporine or tacrolimus, serum protein electrophoresis and routine urine analysis.

Diagnosis of MGUS

Serum protein electrophoresis was performed using capillary electrophoresis (Capillarys, Sebia, Paris, France). When a MG was detected by serum protein electrophoresis, the patients underwent serum immunosubtraction (Capillarys, Sebia) and/or immunofixation (Hydrasis, Sebia). Immunofixation of a 24 h urine specimen were performed to test for Bence Jones protein. The diagnostic criteria for MGUS were the following: (i) a serum MG level of less than 30 g/l, (ii) less than 10% plasma cells in the bone marrow and (iii) the absence of clinical evidence related to the proliferation of the monoclonal plasma cells (bone lesion, back pain, hypercalcemia). Bone marrow biopsy was performed in the presence of each of the following conditions: (i) MG higher than 15 g/l, (ii) an MG iso-type other than IgG and (iii) patients with unexplained anaemia, hypercalcemia or bone lesions. After LT, MGUS was defined permanent ('permanent' MGUS) when MG was confirmed at every control with electrophoresis and immunofixation. On the other site MGUS was defined transient ('transient' MGUS) if MG disappeared during follow up after LT.

Definitions and check up for medical complications after LT

Renal dysfunction was defined by a glomerular filtration rate ≤60 ml/min/1.73 m2 BSA at the time of LT [11,12]. Glomerular filtration rate was estimated by the four-variable formula used in the MDRD [13]. In the subsequent follow up, LT patients were checked for several medical complications. Chronic kidney disease (CKD) after LT was defined by a glomerular filtration rate ≤60 ml/min/1.73 m2 BSA for ≥3 months [11,12]. Clinically suspected acute rejection was confirmed by biopsy and graded according to Banff criteria [14]. During the first three months after LT, blood, urine and bile cultures, cytomegalovirus (CMV)-DNA, Epstein–Barr virus (EBV)-DNA were performed monthly. Then microbiological tests were performed only in the presence of clinical or laboratory evidence of an ongoing infection. For the purpose of the study, serious infections were defined by hospitalization *plus* one among the following: (i) sepsis or septic shock, (ii) infection-related graft failure, and (iii) infection-related other organ failure. HCV-related and HBV-related re-infections of the graft after LT were not considered in the computation of infectious complications after LT.

To detect possible 'de novo malignancy' after LT, patients were checked as follows: in all patients an annual dermatological examination was provided. A colonoscopy was provided every 5–10 years in subjects with no previous history of colon malignancy; every 1–2 years in subjects with previous history of malignancy; yearly in patients with ulcerative colitis, with random surveillance biopsies for dysplasia. In smokers or in patients transplanted for alcoholic cirrhosis a chest X-ray was carried out every 1–2 years. In these patients and in those with Barrett's oesophagus, oropharyngeal examination and upper gastrointestinal endoscopy were performed every 1–3 years. In males, a prostate digital examination and PSA were provided as for the general population. In females, a pelvic examination, a PAP smear and a mammography were provided as for the general population. For the purpose of the study skin cancer was included in the 'de novo malignancies' [15].

Irrespective of its cause (i.e. serious infections, recurrence of primitive hepatic disease, rejection, late surgical biliary or vascular complications), graft dysfunction was arbitrarily defined by the following criteria: prothrombin activity <50%, total serum bilirubin >3 mg/dl and albumin <3 g/dl.

Immunosuppression after LT

Patients were treated primarily with a calcineurin inhibitor (CNI) and steroid-based immunosuppression. The blood trough level of tacrolimus was maintained within 10–15 μg/l during the first month after LT and within 4–7 μg/l over the long term. The blood concentration of cyclosporine 2 h after oral ingestion of the drug was maintained within 800–1000 μg/l during the first month after LT and within 200–400 μg/l over the long term. Azathioprine (AZA) or mycophenolate mofetil (MMF) was introduced following either a biopsy proven-episode of acute rejection as an adjunctive therapy or a diagnosis of CKD to minimize the long-term dose of CNI. No patient received either AZA or MMF alone or AZA or MMF plus steroids. Therefore, for the purpose of the study, an immunosuppressive strategy with one agent means a CNI, with two agents it means a CNI plus long-term (>6 months) steroids or a CNI plus AZA or MMF, and with three agents it means a CNI plus long-term steroids plus AZA or MMF.

Statistical analysis

All data resulting from continuous variables were presented as medium ± standard error of the medium and/or as median and range; the data resulting from categorical variables were expressed as percentages. The differences between groups were determined by Wilcoxons test for continuous variables and the chi-square test was used to look for the differences in proportions. The Kaplan Meyer method was used to determine the probability to be free from CKD, serious infections and the probability of survival according to the presence of 'permanent' MGUS after LT.

A univariate logistic regression was used to identify factors predictive of complications and mortality after LT. Significant predictors at univariate analysis ($P < 0.20$) were then inserted in a multivariate logistic regression model. Statistical significance was established at a P-value < 0.05.

Results

The study included 150 consecutive patients (29 females and 121 males) who were followed up for more than 18 months after LT in our institution between 1995 and 2007. The medium age of patients at the time of LT was 50.80 ± 6.45 years. The medium duration of the follow up post-LT was 84.7 ± 5.75 months. The most common indications for LT were virus-related cirrhosis in 98 patients (65%), alcohol-related in 41 patients (27%), and the consequence of cholestatic liver disease in 11 patients (8%). Cirrhosis was HBV-related in 34 patients, HCV-related in 58 patients and HBV-HCV-related in six patients. Twelve patients with HCV-related cirrhosis and one patient with alcohol-related cirrhosis had a hepatocellular carcinoma within the Milan criteria at the time of LT.

Monoclonal gammopathy before LT

Monoclonal gammopathy was identified in 18 patients with cirrhosis (12%). The MG was already present in eight patients when they were referred to our Institution to start the evaluation for LT. In the remaining 10 patients, MG compared at a median time of 17 months (range 5–40 months) before LT. The medium value of MG was 3.33 ± 1.0 g/l. The MG was IgG in 72% of cases, IgA in 11% and IgM in 11% of cases. Bence Jones protein was detected in three patients. Comparing patients with cirrhosis with MG to those without MG no difference was found regarding gender, age, indication to LT, CTP, MELD scores and the presence of renal dysfunction at the time of LT (data not shown).

Monoclonal gammopathy after LT

After LT, the presence of a MG was confirmed in eight patients, while in 10 patients MG disappeared during the follow up (Fig. 1). Forty-nine patients developed a 'de novo' MG after LT (37%). Prevalence of MG was found to be higher after than before LT (37% vs. 12%, $P < 0.0001$). The 'de novo' MG was detected at 20.71 ± 5.32 months after LT. At the time of diagnosis the medium value of MG was 2.38 ± 0.98 g/l and Bence

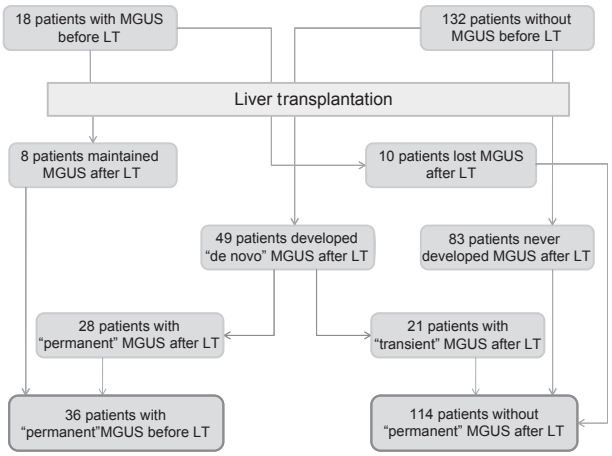

Figure 1 Flow chart indicating the presence or absence of monoclonal gammopathy of undetermined significance (MGUS) before liver transplantation (LT) and the appearance, disappearance or persistence of MGUS after LT.

Table 1. Demographic data, features of immunosuppressive therapy and main laboratory data according to the presence or absence of 'permanent' monoclonal gammopathy of undetermined significance (MGUS) 5 years after liver transplantation.

	Patients with permanent MGUS after LT (n = 30*)	Patients without MGUS after LT (n = 103†)	P
Gender: No. female/male: n	8/28	21/94	NS
Median age (range): years	62 (43–73)	56 (26–72)	NS
Immunosuppressive strategy: with one/two/three agents (%)	6/67/27	10/68/22	NS
Basal immunosuppressive agent: CsA/tacrolimus (%)	33/67	31/69	NS
Steroids: no/yes for <6 months/yes for >6 months (%)	14/19/67	16/12/72	NS
Median daily dose of CsA: mg (range)	125 (75–200)	125 (50–500)	NS
Median daily dose of tacrolimus: mg (range)	2.5 (0.5–6.0)	2.5 (0.5–8.0)	NS
Median trough blood level of CsA: μg/l (range)	135 (113–687)	174 (62–781)	NS
Median trough blood level of tacrolimus: μg/l (range)	5.5 (3.1–10.9)	6.4 (1.3–16)	NS
Total serum bilirubin (μmol/l)	15 (7.1–85)	14 (1.1–157)	NS
Serum albumin (g/l)	43 (30–53)	43 (27–61)	NS
Prothrombin activity (%)	88 (26–120)	82 (22–112)	NS
Serum urea (mmol/l)	10.5 (6,6–28)	8.8 (3.3–26)	**0.05**
Serum creatinine (μmol/l)	138 (63–368)	111 (38–492)	**0.005**

LT, liver transplantation.

*6 of 36 patients with permanent MGUS were censored because they died within 5 years after LT; †11 of 144 patients without permanent MGUS were censored because eight of them died within 5 years after LT and three had follow up shorter than 5 years; statistically significant values were represented in bold characters.

Jones protein was present in three patients. MG was IgG in 73% of patients, IgA in 4.2% and IgM in 6.2%. In 57.1% of patients 'de novo' MG was permanent (Fig. 1) during the follow up with a medium value of MG about 2.34 ± 1.05 g/l. In 21 patients (42.9%) 'de novo' MG was transient (Fig. 1) and disappeared during the follow up after 51 ± 10.45 months from LT.

Considering patients with 'permanent' MGUS after LT (n = 36) as a whole, the medium value of MG at diagnosis was 2.56 ± 1.03 g/l and Bence Jones protein was present in only six patients. At 5 years after LT, patients with permanent MGUS after LT were compared to all the other patients (n = 114), no difference was found in features of immunosuppression (Table 1) whilst a higher median level of serum urea and creatinine was observed in the former. The same results were found when patients with 'permanent' MGUS were compared to patients who never developed MGUS after LT (data not shown). Well in keeping with this finding 75% of patients with 'permanent' MGUS developed CKD after LT while only 44% of the other patients developed this complication (P = 0.001) during the follow up (Table 2, Fig. 2). As regards other late medical complications after LT, the rate of 'de novo' malignancies was higher in the former group of patients, even if the difference was not statistically significant. Bone marrow biopsies were performed in five patients with permanent MGUS and in only one of them a smouldering myeloma was diagnosed. In this patient, the value of MG at the diagnosis of MGUS before LT was about 11 g/l, and after LT it progressively increased to 20 g/l. In addition, a new IgM monoclonal protein was detected 6 years after LT. The bone marrow biopsy at that time evidenced a per cent of plasma cells of 15%. No malignant lymphoproliferative disease was observed in patients with 'permanent' MGUS while five patients without 'permanent' MGUS developed a NH lymphoma during the follow up after LT. The rate of total bacterial or viral infections was similar in both groups, but patients with 'permanent' MGUS had more frequently serious infections (Table 2, Fig. 3). The aetiology and the site of infections as well as the rate of infection-related-sepsis or septic shock, infection related graft or other organ failure, were not different among the two groups. Nevertheless serious infections tended to be a more frequent cause of death in patients with permanent MGUS (Table 2).

Finally, the rate of mortality was significantly higher in patients with than in those without 'permanent' MGUS (Table 2, Fig. 4).

When 'permanent' MGUS was evaluated together with known risk factors for serious infections after LT, in univariate analysis only biopsy-proven acute rejection and 'permanent' MGUS after LT were confirmed as being significant (Table 3A). In multivariate analysis biopsy-proven acute rejection and 'permanent' MGUS were both confirmed as independent risk factors for serious infections after LT (Table 3B). Likewise 'permanent' MGUS was confirmed as an independent risk factor for CKD after LT at univariate (Table 4A) and multivariate analysis (Table 4B), together with the presence of a renal dysfunction before LT and the use of cyclosporine rather

Table 2. Complications after liver transplantation according to the presence (+ve) or absence (–ve) of 'permanent' monoclonal gammopathy of undetermined significance (MGUS).

	Patients with 'permanent' MGUS after LT (*n* 36)	All other patients (*n* = 114)	*P*
Biopsy-proven acute rejection: *n* (%)	8 (22)	20 (18)	NS
Recurrence of primitive liver disease: *n* (%)	23 (63)	57 (50)	NS
Graft dysfunction: *n* (%)	4 (11)	14 (12)	NS
Total bacterial and viral infections: *n* (%)*	13 (53)	50 (43)	NS
Serious bacterial or viral infections: *n* (%)	11 (85)	15 (30)	**0.01**
Cardiovascular complications : *n* (%)	18 (49)	71 (62)	NS
'*De novo*' malignancy : *n* (%)	9 (25)	16 (14)	NS
Hematological malignancy	1 (11)	5 (31)	NS
Non-hematological malignancy	8 (89)	11 (69)	NS
• Skin malignancy	2 (25)	4 (36)	NS
• Other malignancy	5 (75)	7 (64)	NS
CKD : *n* (%)	75	44	**0.001**
Mortality : *n* (%)	12 (33)	20 (17)	**0.04**
Due to graft failure: *n* (%)	2 (17)	5 (25)	NS
Due to malignancy: *n* (%)	2 (17)	5 (25)	NS
Due to infections: *n* (%)	4 (33)	3 (15)	NS
Due to cardiovascular diseases: *n* (%)	1 (8)	3 (15)	NS
Due to renal failure: *n* (%)	3 (25)	2 (10)	NS
Due to other causes: *n* (%)	0 (0)	2 (10)	NS

LT, liver transplantation; graft dysfunction was defined by the following criteria: (i) PT < 50%, total serum bilirubin > 3 mg/dl, and albumin < 3 g/dl; * = HCV or HVB recurrent infections after LT were not considered in this computation; CKD = chronic kidney disease which was defined as a glomerular filtration rate, estimated by MDRD formula, ≤60 ml/min 1.73 m2 BSA for more than 3 months; statistically significant values were represented in bold characters.

than tacrolimus as basal immunosuppressive agent. Finally, among the potential risk factors for mortality graft dysfunction, serious infections, 'de novo' malignancies and CKD were significantly associated with mortality in univariate (Table 5A) and multivariate analysis (Table 5B).

Patients who developed 'de novo' MGUS after LT were older than those patients who did not (median 58, range 38–67 years vs. 50, range 21–66 years, $P < 0.05$). Risk factors for the development of MGUS were investigated in these patients. In univariate as well as in multivariate logistic regression, among the potential predictive factors of 'de novo' MGUS after LT, only age was found to be significant (Table 6).

Discussion

The prevalence of MGUS in liver transplant recipients was 12%, representing a significantly higher value than

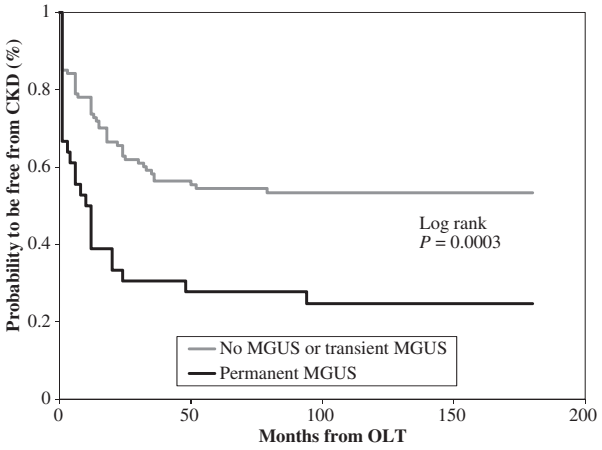

Figure 2 Kaplan–Meier plot of probability to be free from chronic kidney disease up to the end of the follow up according to the presence of a 'permanent' monoclonal gammopathy of undetermined significance (MGUS) after liver transplantation (LT). Black line indicates patients with 'permanent' MGUS and grey line indicate patients without 'permanent' MGUS after LT. Only patients with a follow up after LT ≥18 months were included into the study.

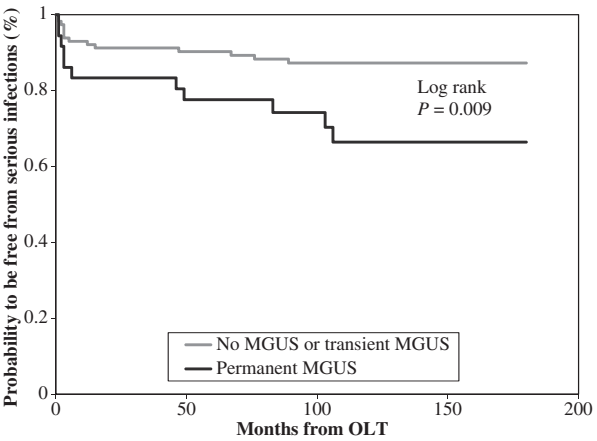

Figure 3 Kaplan–Meier plot of probability to be free from serious infections up to the end of the follow up according to the presence of a 'permanent' monoclonal gammopathy of undetermined significance (MGUS) after liver transplantation (LT). Black line indicates patients with 'permanent' MGUS and grey line indicate patients without 'permanent' MGUS after LT. Only patients with a follow up after LT ≥18 months were included into the study.

that described in the general population older than 50 years (3.2%) [2]. It can be hypothesized that this finding may depend on the fact that 65% of patients included in the study had viral-related cirrhosis, since HBV and HCV infection are correlated with the developments of B-cell clones [16]. However, in our series no difference was found in the aetiology of the liver disease between patients with MGUS and those without MGUS

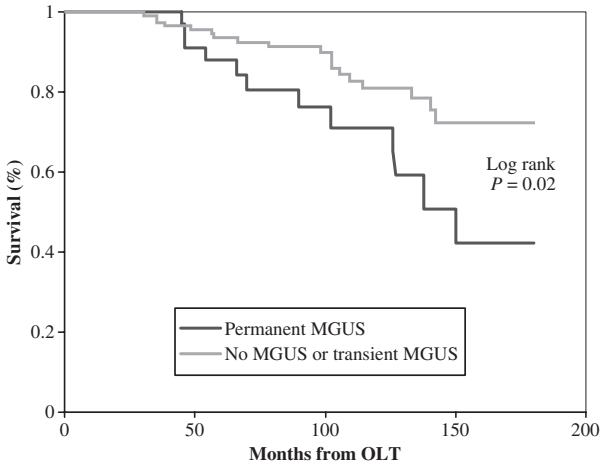

Figure 4 Kaplan–Meier plot of overall survival up to the end of the follow up according to the presence of a 'permanent' monoclonal gammopathy of undetermined significance (MGUS) after liver transplantation (LT). Black line indicates patients with 'permanent' MGUS and grey line indicate patients without 'permanent' MGUS after LT. Only patients with a follow up after LT ≥18 months were included into the study.

Table 3. (A) Univariate analysis of potential predictive factors for serious infections. (B) Multivariate analysis of potential predictive factors for serious infection.

(A)	OR	CI	*P*
Biopsy-proven acute rejection	4.52	1.78–11.50	0.002
'Permanent' MGUS after LT	2.90	1.19–7.09	0.019
Diabetes	2.05	0.84–5.00	0.116
Immunosuppressive strategy with one vs. two or three agents	1.21	0.46–3.16	0.701
Steroids > than 6 months	1.53	0.57–4.11	0.399
(B)			
Biopsy-proven acute rejection	3.90	1.45–10.50	0.007
'Permanent' MGUS after LT	3.31	1.24–8.83	0.016

MGUS, monoclonal gammopathy of undetermined significance; LT, liver transplantation.

Table 4. (A) Univariate analysis of potential predictive factors for CKD. (B) Multivariate analysis of potential predictive factors for CKD.

(A)	OR	CI	*P*
Pre-LT renal dysfunction	5.43	1.97–14.93	0.001
CsA vs. FK 506 as basal immunosuppressive agent	3.96	1.84–8.49	0.0004
'Permanent' MGUS after LT	4.57	2.05–10.21	0.0002
Aetiology of the primitive liver disease:			
Alcohol-related vs. viral-related	1.55	0.70–3.43	0.424
Other vs. viral-related	0.64	0.13–3.17	
Diabetes	1.04	0.46–2.34	0.935
(B)			
Pre-LT renal dysfunction	7.31	2.21–24.21	0.001
CsA vs. FK 506 as basal immunosuppressive agent	7.34	2.29–23.46	0.0008
'Permanent' MGUS after LT	3.675	1.970–7.374	0.001

LT, liver transplantation; CsA, cyclosporine.
Pre-renal dysfunction was defined by a glomerular filtration rate, estimated by MDRD formula, ≤60 ml/min 1.73 m2 BSA at the time of LT; CKD = chronic kidney disease which was defined as a glomerular filtration rate, estimated by MDRD formula, ≤60 ml/min 1.73 m2 BSA for more than 3 months.

Table 5. (A) Univariate analysis of potential predictive factors for mortality. (B) Multivariate analysis of potential predictive factors for mortality.

(A)	OR	CI	*P*
Graft dysfunction	12.95	3.73–45.04	0.0001
'De novo' malignancy	2.90	1.16–7.23	0.022
CKD	2.93	1.25–6.88	0.013
Serious infections	3.60	1.45–8.92	0.006
'*Permanent* 'MGUS after LT	2.35	1.01–5.46	0.047
Cardiovascular complications	1.41	0.61–3.24	0.424
Recurrent of primitive liver disease	1.91	0.84–4.31	0.119
(B)			
Graft dysfunction	17.52	4.13–74.26	0.0001
'De novo' malignancy	5.39	1.82–15.94	0.002
CKD	3.64	1.30–10.19	0.014
Serious infections	3.08	1.00–9.46	0.049

LT, liver transplantation.
Graft dysfunction was defined was defined by the following criteria: (i) PT < 50%, total serum bilirubin > 3 mg/dl, and albumin < 3 g/dl; CKD = chronic kidney injury which was defined as a glomerular filtration rate, estimated by MDRD formula, ≤60 ml/min 1.73 m2 BSA for more than 3 months.

before LT. Therefore, other factors may be involved in the development of MGUS in patients with end-stage liver disease, including the reduced immunologic surveillance caused by liver failure itself, which can favour the proliferative 'escape' of a B-cell clone, irrespective of the cause of the primitive liver disease [17]. This interpretation is in keeping with the second finding in our study that is the higher prevalence of MGUS after than before LT (37% vs. 12%, $P < 0.0001$) since the degree of immunodeficiency increases considerably after transplantation due to both the immunosuppressive regimen and viral infections. A prevalence of MGUS ranging from 10% to 25% and of 25% has already been described in renal transplant recipients [18,19] and in cardiac transplant recipients [20], respectively. After LT, the prevalence of MGUS reported so far ranged from 8.5% to 28% [4,21]. Risk factors for the development of MGUS

Table 6. (A) Univariate analysis of potential predictive factors for 'de novo' monoclonal gammopathy of undetermined significance (MGUS) after liver transplantation (LT). (B) Multivariate analysis of potential predictive factors for 'de novo' MGUS after liver transplantation.

(A)	RR	CI	*P*
Age	1.04	1.00–1.08	**0.003**
Gender	1.55	0.64–3.81	0.331
Aetiology of the liver disease			
Alcoholic vs. viral	1.33	0.60–2.96	0.711
Other vs. viral	1.24	0.33–4.75	0.912
CMV infection	0.55	0.22–1.38	0.204
EBV infection	1.66	0.17–16.48	0.664
Biopsy-proven acute rejection	0.59	0.24–1.42	0.239
Immunosuppressive strategy			
One or two agents vs. three agents	0.91	0.40–2.09	0.834
Basal immunosuppressive agent			
Tacrolimus vs. CsA	0.66	0.31–1.40	0.280
Steroids >6 month	1.01	0.47–2.22	0.966
Recurrence of primitive liver disease	0.92	0.45–1.87	0.816
(B)			
Age	1.06	1.01–1.10	0.009

CMV, cytomegalovirus; EBV, Epstein Barr virus; CsA, cyclosporine; statistically significance values were represented in bold characters.

after solid organ transplantation were found to be the age of the recipients, immunosuppressive therapy and the mismatch donor/recipients about CMV and HHV-8 [4,20,21]. As regards immunosuppressive therapy, Caforio ALP *et al.* reported an association between rejection, high dose of CsA and/or of steroids, and the development of 'de novo' MGUS in cardiac transplant recipients [20]. In their study, Regamey N. *et al.* [19] showed that the use of OKT3 after LT was a risk factor for the development of 'de novo' MGUS. The results of our study seem to confirm the importance of the recipient's age, but not that of rejection, immunosuppressive strategy, and CMV or EBV infections as predictive factors for the development of 'de novo' MGUS (Table 6). The lack of correlation between CMV or EBV infections and 'de novo' MGUS, together with the finding of no significant difference in the type and intensity of immunosuppressive strategy in patients with and without 'de novo' MGUS after LT (Table 6) make it difficult also to explain why MGUS disappeared in up to 46.2% of cases after LT in our study. Nevertheless, this is not the first observation of the potential reversibility of MGUS in liver transplant recipients, and it is well in keeping with that described by Pham H. *et al.* [22].

In the general population, there is strong evidence that patients with MGUS can progress to myeloma multiple or other B-cell malignant lymphoproliferative disorders and that this progression is time dependent. Conversely, there are conflicting data concerning the progression of MGUS into myeloma or B-cell malignant lymphoproliferative disease after solid organ transplantation. As far as myeloma, Rostaing L. *et al.* [23] described the development of smouldering myeloma in two of five patients with MGUS diagnosed before renal transplantation within 3–9 years after grafting, whereas Caforio ALP *et al.* [20] described no case in cardiac transplant recipients. To our knowledge, the observation of only one case of progression from MGUS to a smouldering myeloma in our series of liver transplant recipients is the first one which is based on a long-term follow up. As far as B-cell malignant lymphoproliferative disease, the prevalence of lymphoma (3.3%) in our study was within the range of variability observed previously (1.1–15%) in liver transplant recipients [21,22]. Nevertheless, in contrast with Lemoine A. *et al.* [21] we failed to show any correlation between MG and lymphoma after LT. The lack of a correlation between MGUS and either myeloma or lymphoma after LT, might suggest that monitoring MGUS after LT is not necessary. But, this conclusion stands in deep contrast with the main and most original observation of our study that is the finding of a correlation between MGUS and the development of important medical complications like serious infections and CKD after LT. Bacterial and viral infections are an important complication in solid organ transplant recipients as a consequence of immunosuppressive agents [23,24]. Few studies have reported an association between MGUS and viral or bacterial infections in general population. Gregersen *et al.* [25] described a correlation between bacteraemia and MGUS. In addition, Gregersen *et al.* showed also that the main causes of death in patients with MGUS are cardiovascular complications followed by infections [26]. More recently, the association between MGUS and bacterial infections was confirmed by Kristinsson *et al.* [27]. In our study, the presence of MGUS as well as the occurrence of biopsy-proven acute rejection was associated with a higher probability of developing serious infections even if a specific pattern of MGUS-related infection was not identified. To speculate on the underlying pathophysiological basis of the increased risk for serious bacterial infections in patients with MGUS, it may be assumed that MGUS has been associated with hypogammaglobulinemia of the non-monoclonal immunoglobulin heavy chain classes and that such low polyclonal immunoglobulin levels may be associated with impaired specific antibody production [28].

Chronic kidney disease is also a very common complication with a severe impact on the prognosis in liver transplant recipients [29]. Risk factors for the development of CKD are thought to be: age, gender, pre-LT renal disease, HCV hepatic disease and diabetes mellitus, and, overall, CNI-related nephrotoxicity [29–31]. In our study

the use of CsA, pre-LT renal disease, viral aetiology of the hepatic disease were confirmed as risk factors for CKD after LT. Because of the presence of these factors, the occurrence of MGUS in liver transplant recipients seems to have a more negative impact on renal function than in the general population [27]. In addition, a correlation was found for the first time between the presence of MGUS and the development of CKD also in patients with a very low serum level of MP and no Bence-Jones proteinuria. Several mechanisms other than monoclonal protein deposition can be involved in the pathogenesis of renal damage including: deposition of light chains in the renal glomeruli and tubules, light chain endocytosis with consequent abnormal recruitment of inflammation cytokines [32–34]. Finally, when the most common causes of mortality in LT recipients were considered [35], a permanent MGUS was found to be a risk factor for mortality after LT in the univariate logistic regression analysis even if this data was not confirmed in the multivariate analysis.

Our study has some limitations as far as the potential origin of 'de novo' MGUS after LT is concerned since the contribution of ensuing potential pathogenetic factors was not considered: (i) the cumulative dose of immunosuppressive agents, (ii) the match donor/recipient for CMV, (iii) the state of the recipient for HHV-8, and (iv) the limits of MDRD estimation of GFR [36]. Nevertheless, the study provides new important insights into the evolution and impact of MGUS after LT suggesting the opportunity to modulate immunosuppression regimens in those patients who have MGUS in order to mitigate the further risk of serious infections and/or CKD.

In conclusion, the prevalence of MGUS in patients with cirrhosis (12%) and in liver transplant recipients (37%) is significantly higher than in a general population matched for age. MGUS after LT disappeared during the follow up in 46.2% of patients. After LT the rate of progression of MGUS in myeloma or B-cell malignant lymphoproliferative disease is clinically irrelevant. Instead, the presence of MGUS is a risk factor for the development of serious infections and CKD.

Authorship

AG: designed research and wrote the paper. FM, MS: performed research. SR: performed research and collected data. SF, ACF, FA: analyzed data. MM, MC, GZ, AR: collected data. MP, AS: performed laboratory tests. UC: designed research. PA: designed and wrote the paper.

Funding

No funding supported the work submitted.

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