TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Neurological complications after liver transplantation as a consequence of immunosuppression: univariate and multivariate analysis of risk factors

Gianluca Rompianesi,[1] Roberto Montalti,[1] Nicola Cautero,[1] Nicola De Ruvo,[1] Anthony Stafford,[2] Carolina Bronzoni,[3] Roberto Ballarin,[1] Lesley De Pietri,[4] Fabrizio Di Benedetto[1] and Giorgio E. Gerunda[3]

1 Liver Transplant Centre, Azienda Ospedaliero-Universitaria di Modena Policlinico, Modena, Italy
2 Institute of Liver Studies, King's College Hospital, London, UK
3 General Surgery, Azienda Ospedaliero-Universitaria di Modena Policlinico, Modena, Italy
4 Division of Anaesthesiology and Intensive Care Unit, Azienda Ospedaliero-Universitaria di Modena Policlinico, Modena, Italy

Keywords
calcineurin inhibitors, immunosuppression, liver transplantation, mTOR inhibitors, neurological complications.

Correspondence
Gianluca Rompianesi MD, Liver Transplant Centre, Azienda Ospedaliero-Universitaria di Modena Policlinico, via del Pozzo, 71, 41124 Modena, Italy.
Tel.: +39 059 4225265;
fax: +39 059 4223765;
e-mail: gianlucarompianesi@gmail.com

Conflicts of interest
The authors did not receive any conflict of interests that may bias the present study.

Received: 18 November 2014
Revision requested: 30 December 2014
Accepted: 15 March 2015
Published online: 6 April 2015

doi:10.1111/tri.12564

Summary

Neurological complications (NCs) can frequently and significantly affect morbidity and mortality of liver transplant (LT) recipients. We analysed incidence, risk factors, outcome and impact of the immunosuppressive therapy on NC development after LT. We analysed 478 LT in 440 patients, and 93 (19.5%) were followed by NCs. The average LOS was longer in patients experiencing NCs. The 1-, 3- and 5-year graft survival and patient survival were similar in patients with or without a NC. Multivariate analysis showed the following as independent risk factors for NC: a MELD score ≥20 (OR = 1.934, CI = 1.186–3.153) and an immunosuppressive regimen based on calcineurin inhibitors (CNIs) (OR = 1.669, CI = 1.009–2.760). Among patients receiving an everolimus-based immunosuppression, the 7.1% developed NCs, vs. the 16.9% in those receiving a CNI ($P = 0.039$). There was a 1-, 3- and 5-year NC-free survival of 81.7%, 81.1% and 77.7% in patients receiving a CNI-based regimen and 95.1%, 93.6% and 92.7% in those not receiving a CNI-based regimen ($P < 0.001$). In patients undergoing a LT and presenting with nonmodifiable risk factors for developing NCs, an immunosuppressive regimen based on CNIs is likely to result in a higher rate of NCs compared to mTOR inhibitors.

Introduction

Despite the constant advances in organ preservation, immunosuppression, surgical techniques and perioperative management, several complications may occur after liver transplantation (LT) and affect the recipient's postsurgical outcome. Neurologic complications (NC) can frequently and significantly affect morbidity and mortality and prolong the hospital length of stay (LOS) of LT recipients, therefore influencing the overall management costs. Such complications usually occur in the first weeks after LT [1], showing prevalence in the literature of 11 to >40% [1–4]. Several pre-LT, surgical and postoperative factors may contribute to the development of NCs. It is postulated that among these factors, the side effects of the immunosuppressive drugs may play a leading role [5–8]. The aim of this study was to retrospectively analyse prevalence, risk factors, outcome and the impact of the immunosuppressive therapy on NC development following LT in 440 recipients.

Materials and methods

From November 2000 to August 2012, we performed 478 LTs in 440 adult patients. The transplant was followed by a NC in 93 cases (19.5%). NCs were diagnosed by a consultant neurologist using clinical examination and if necessary additional investigations such as CT, MRI, EEG, EMG. All patients were included in the statistical analysis irrespective of length of follow-up. The 93 transplants whose recipient suffered from a NC made up the NC group, leaving the remaining 385 transplants as the control group. The number of transplants rather than patients was used for the analysis because 43 patients (9%) required more than one transplant. Requiring more than one transplant was included as a parameter in the analysis of risk factors. We considered in the analysis both major and minor NCs. Minor complications are usually self-limiting and do not require intervention or only a symptomatic treatment (tremors, mood alterations, sensory peripheral neuropathy, restless leg syndrome); all other NCs were considered as 'major': seizures, central pontine myelinolysis, flaccid paralysis, consciousness alterations, and toxic and metabolic encephalopathies [9,10]. Sensory peripheral neuropathy was defined as the presence of paraesthesia, dysaesthesia, allodynia or burning sensation in hands or feet, and an EMG was performed when indicated by the consultant neurologist.

No patients were lost to follow-up, which generally consisted on a monthly ambulatory examination during the first year, four times during the second year, twice during the third year and annually thereafter. Factors analysed in the univariate analysis included patient's pre-LT characteristics such as patient age, gender, first/redo LT, indication, isolated/combined liver–kidney transplantation, presence of encephalopathy and model of end-stage liver disease (MELD) score; surgical variables such as graft ischaemia time, length of surgery and intraoperative hypotensive episodes; donor variables such as age, gender and percentage of steatosis; and finally the immunosuppressive regimen. We also analysed the overall hospital LOS and the intensive care unit LOS, the patient outcome after the NC and the graft and patient survival. The immunosuppressive therapy used during the first months after LT was based on calcineurin inhibitors (CNI) such as cyclosporine or tacrolimus, or mammalian target of rapamycin inhibitors (mTOR) such as rapamycin or everolimus. Trough level analysis compared the blood trough levels of the immunosuppressant drugs at the time of the NC diagnosis for patients in the NC group, with the average trough level for patients in the control group.

Limitations of this study are the retrospective analysis of data and that the patients were not randomized to receive the different immunosuppressive drugs.

In the analysis of risk factors, we considered the immunosuppressive regimen that the patients received before the development of the NC or in the first 3 months after the LT. The possible treatments given to patients with NCs were classified as follows: no treatment, immunosuppressive switch from CNI to mTOR inhibitors, adjustment of the daily dose of the immunosuppressive drugs to the lower limit of the therapeutic range or 'symptomatic treatment'. This was when additional medications were prescribed to treat the NCs such as antiepileptic drugs, antipsychotic drugs, benzodiazepine, *gamma*-aminobutyric acid (GABA) analogues and B-complex vitamins.

Statistical analysis

Continuous data were reported as mean ± standard deviation for the parameters with a normal distribution or median (range) for parameters without a normal distribution and were compared using the two-sided Student's *t*-test. Comparisons between groups for categorical variables were carried out using the chi-square test, with Yates' correction, or Fisher's exact test when appropriate. The optimal cut-off of continuous variables was obtained using the receiver operating characteristic (ROC) curve. Patient survival was evaluated using the Kaplan–Meier method and compared with the log-rank test. All variables with $P < 0.05$ in the univariate analysis were included in a multivariate logistic regression analysis by forward stepwise and backward stepwise method. The *P*-value of 0.05 or less was considered statistically significant. All analyses were carried out using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA).

Results

Mean follow-up for all patients was 53.4 ± 34.8 months, 48.9 ± 34.7 months in the NC group and 55.1 ± 34.8 in the control group ($P = 0.124$). The NCs occurred in a median time of 0.3 months (range 0–59.7 months) after LT. Among the 93 NCs observed, 49 of them (52.7%) were major, whilst 44 (47.3%) were minor (Table 1). In 30 patients (32.3%), the NCs have not been treated; in 23 patients (24.7%), an immunosuppressive switch from CNIs to mTOR inhibitors has been performed; in 11 patients (11.8%), the treatment consisted on an adjustment of the daily dose of the immunosuppressive drugs to the lower cut-off of the therapeutic range; and in 29 patients (31.2%), symptomatic treatment has been prescribed after assessment by a neurologist. Specifically, of the 50 major NCs, 27 cases (54%) had their immunosuppressive regimen modified and the remaining cases were treated symptomatically. Regarding the outcome of patients, 78 of the 93 patients who experienced a NC (83.9%) recovered fully from the NC, two patients died (2.2%) 4 and 11 days after

Table 1. Classification of the neurological complications.

Neurological complications (n = 93)	No. (%)
Major complications	49 (52.7)
Seizures*	17 (18.3)
Consciousness alterations	13 (14)
Toxic/metabolic encephalopathy	12 (12.9)
Central pontine myelinolysis	6 (6.5)
Flaccid paralysis	1 (1.1)
Minor complications	44 (47.3)
Sensory peripheral neuropathy	30 (32.3)
Tremors	9 (9.7)
Mood alterations	4 (4.3)
Restless leg syndrome	1 (1.1)

*In one patient, the seizures were a consequence of disseminated aspergillosis with brain abscesses.

the diagnosis of the NC due to multiple organ failure and sepsis, respectively. The average hospital LOS of those who experienced a NC was significantly longer (26.2 ± 16.1 days vs. 18.1 ± 16.1 days; $P < 0.001$), especially in patients developing a major NC: 31.5 ± 28.5 days vs. 20.6 ± 10.1 days in patients developing a minor NC. The 1-, 3- and 5-year patient survival of patients who developed a NC was 78.2%, 70.5% and 70.5% vs. 83.4%, 75.0% and 69.1% ($P = 0.662$, Fig. 1). The 1-, 3- and 5-year graft survival of patients who developed a NC was 77.1%, 70.6% and 70.6% vs. 78.2%, 69.4% and 64.1% ($P = 0.443$, Fig. 1).

The 1-, 3- and 5-year patient survival of patients who developed a major NC was 72.8%, 67.2% and 67.2% vs. 84.0%, 74.1% and 74.1% in patients who developed a minor NC ($P = 0.626$).

The 1-, 3- and 5-year graft survival of patients who developed a major NC was 72.9%, 70.1% and 67.0% vs. 81.7%, 74.2% and 74.2% in patients who developed a minor NC ($P = 0.724$).

Among the risk factors analysed in 93 transplants, a MELD score ≥20 ($P = 0.012$), the presence of pre-LT encephalopathy ($P = 0.009$) and a CNI-based immunosuppression therapy ($P = 0.024$) were independent variables that help identify those at risk of developing NCs. Analysing separately the variables of the MELD score (serum total bilirubin, serum creatinine and INR), only a serum bilirubin level ≥4 mg/dl (68.4 μmol/l) was related to an higher incidence of NC ($P < 0.001$). All the factors analysed are summarized in Table 2. Multivariate logistic regression analysis showed that the variables independently associated with an increased risk of developing a NC were a MELD score ≥20 (OR = 1.934, CI = 1.186–3.153) and a CNI-based immunosuppression regimen (OR = 1.669, CI = 1.009–2.760). Of those who received everolimus immunosuppression, 7.1% of them developed a NC, compared with 21.7% of patients who received a CNI-based immunosuppression ($P = 0.06$). All the NCs of the five patients receiving everolimus were minor: two sensory peripheral neuropathy, two tremors and one mood alterations.

There was no significant difference in the average immunosuppressive drug blood trough levels between the two groups. Specifically, the average blood trough levels were as follows: 9.2 ± 3.4 vs. 9.3 ± 2.6 ng/ml ($P = 0.936$) for everolimus, 8.7 ± 3.6 vs. 8.4 ± 2.9 ng/ml ($P = 0.856$) for rapamycin, 9.2 ± 3.7 vs. 8.4 ± 2.8 ng/ml ($P = 0.207$) for tacrolimus and 234 ± 42 vs. 225 ± 34 ng/ml ($P = 0.109$) for CsA in patients in the NC group and control group, respectively.

The 1-, 3- and 5-year NC-free survival was 81.7%, 81.1% and 77.7% in patients receiving a CNI-based immunosuppression and 95.1%, 93.6% and 92.7% in patients receiving a CNI-free immunosuppression ($P < 0.001$, Fig. 2).

Discussion

Patient survival after LT dramatically improved since 1963 when Thomas Starzl performed the first LT in humans

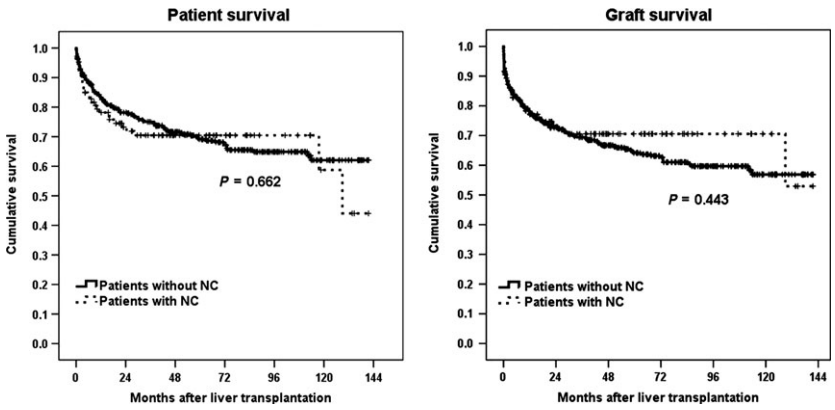

Figure 1 Overall patient and graft survival after LT in patients with and without neurological complications (NC).

Table 2. Univariate analysis of risk factors for developing a neurological complication (NC). Statistically significant values ($p < 0.05$) highlighted in bold.

		NC group (93 transplants)	Control group (385 transplants)	*P*-value
Recipient age	Years	51.8 ± 11.1	52.2 ± 11.1	0.755
Gender	M/F	65 (18.5%)/28 (22.0%)	286 (81.5%)/99 (78.0%)	0.465
Pre-LT encephalopathy	Yes/no	53 (25.0%)/40 (15.0%)	159 (75.0%)/226 (85.0%)	**0.009**
Primary transplant	Yes/no	80 (18.8%)/13 (24.5%)	345 (81.2%)/40 (75.5%)	0.421
Combined transplant	Yes/no	6 (25.0%)/87 (19.2%)	18 (75.0%)/367 (80.8%)	0.660
Living donor LT	Yes/no	8 (21.6%)/85 (19.3%)	29 (79.4%)/356 (80.7%)	0.896
UNOS status 1	Yes/no	10 (20.0%)/83 (19.4%)	40 (80.0%)/345 (80.6%)	0.931
Indication	Alcohol	12 (23.1%)/81 (19.0%)	40 (77.9%)/345 (81.0%)	0.608
	HBV/HCV	61 (19.5%)/32 (18.7%)	246 (80.5%)/139 (81.3%)	0.853
	Cholestatic	15 (13.0%)/78 (17.2%)	110 (87.0%)/375 (82.8%)	0.205
HCC	Yes/no	35 (17.6%)/58 (20.8%)	164 (82.4%)/221 (79.2%)	0.451
Cold ischaemia time	Minutes	344 ± 117	345 ± 118	0.941
Length of surgery	Minutes	509 ± 170	492 ± 120	0.263
Hypotensive episodes	Yes/no	5 (20.1%)/88 (19.4%)	19 (79.9%)/366 (80.6%)	0.929
Donor age	Years	53 ± 19	56 ± 19	0.0172
MELD score	Value	22.3 ± 9.4	18.6 ± 9.2	**<0.001**
	Bilirubin (mg/dl)	11.2 ± 11.5	6.6 ± 11.5	**<0.001**
	Creatinine (mg/dl)	1.4 ± 1.1	1.2 ± 1.1	0.116
	INR (value)	2.0 ± 7.2	2.1 ± 7.2	0.904
MELD score ≥20	Yes/no	44 (25.9%)/49 (15.9%)	126 (74.1%)/259 (84.1%)	**0.012**
Serum bilirubin ≥4 mg/dl	Yes/no	42 (25%)/51 (16.5%)	126 (75%)/259 (83.5%)	**0.033**
Administration of CNI	Yes/no	82 (21.7%)/11 (11.0%)	296 (78.3%)/89 (89.0%)	**0.024**

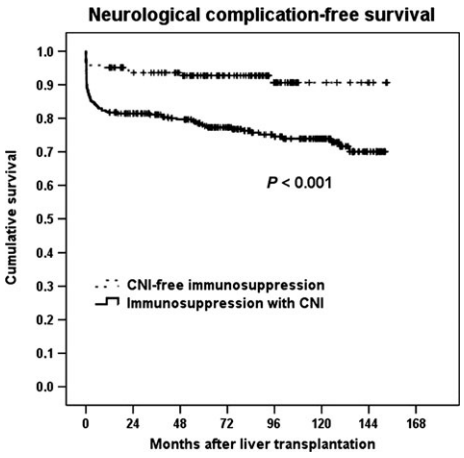

Figure 2 NC-free survival in patients receiving and not receiving an immunosuppression regimen based on calcineurin inhibitors (CNI).

[11]. These achievements are partially as a result of the advances and innovations in immunosuppression, in surgical techniques and in medical therapies that allow a better perioperative management of pathologies such as viral hepatitis and hepatocellular carcinoma. In particular, the introduction of cyclosporine A (CsA) as an immunosuppressant in 1980 produced a marked increase in survival rates [12]. Currently, in an era where the 1-year patient survival rates are generally set at 80–90%, a fundamental aspect is to identify the factors that may affect not only patient survival but also the quality of life of transplant recipients. The NCs may occur frequently after LT and may manifest as a life-threatening situation or as a mild disorder. Both can adversely affect the patient's activities of daily living and quality of life. LT recipients are more likely to develop a NC than other transplanted organ recipients (kidney, pancreas, heart, lung), with a prevalence that in some series is reported to be higher than 40% [5,10], this is partly due to the complexity, the length of the surgery and the general poor clinical condition of the patients undergoing LT. Our results and previous series suggest a link between the development of NCs and the immunosuppressive regimen [5–8]. CsA and tacrolimus are the most widely used immunosuppressive agents although their administration may be burdened by several side effects. Both have similar immunosuppressive activity and consequently similar neurologic side effects. Although the exact mechanism has not been clarified yet, NC development secondary to CNI use seems multifactorial: it may depend on the them binding to immunophilins, thereby blocking the protective role these proteins have on neuronal function [13,14] but also on the inhibition of both constitutive and inducible nitric oxide (NO) synthase activity and neuronal and endothelial NO expressions, resulting in a reduction in NO brain levels that leads to vasoconstriction [15]. Although only recently introduced into common practice post-LT, mTOR inhibitors in renal patients seem to have a low incidence of neurotoxicity [16,17]. mTOR inhibitors have a different side effect profile than CNIs. They bind to the same immunophilins as tacrolimus, known as the FKBP (FK506 binding

protein), but in the case of mTOR inhibitors, this binding results in the inhibition of mTOR-mediated signal transduction pathways and consequently the signal III pathway that determines the arrest of the cell cycle in G1 phase. CNIs when complexed with the immunophilins form a ternary complex with calcineurin, causing its inactivation that results in the inhibition of the signal I pathway of T-cell activation [18]. We therefore observed an incidence of 19.5% of NCs following LT, 52.7% of them classified as major NC, an incidence similar to the reports published in the literature [1–4]. In our study, the most common NC observed was sensory peripheral neuropathy (32.3%) followed by seizures (18.3%; Table 1). The univariate analysis of risk factors for NCs showed an increased incidence in those with a MELD score ≥20, pre-LT encephalopathy and a CNI-based immunosuppressive regimen (Table 2). Subanalysis of the MELD parameters showed that only the serum bilirubin level was statistically different between patients with and without a NC; in particular, patients with a bilirubin level ≥4 mg/dl (68.4 μmol/l) had an incidence of NC of 25% vs. 16.5% ($P = 0.033$). A MELD score-based organ allocation model is currently adopted worldwide; MELD score itself was found as an independent risk factor for the development of NC, resulting in the fact that the patients who are going to be transplanted are the ones with the higher MELD score and therefore the higher risk of developing NCs. Among the different combinations of immunosuppressive regimens: CNI, mTOR inhibitors, CNIs associated with mTOR inhibitors and no immunosuppression, patients who received CNIs alone had a significantly higher rate of NC: 21.7% ($P = 0.024$), with an odds ratio of 1.669 (C.I.: 1.009–2.760), also resulting in an inferior NC-free survival ($P < 0.001$). In our study, which is similar to previous studies, patients with NC have longer hospital LOS, but the overall survival rates were the same [19–23]. In one case only (0.2%), we observed a NC following a central nervous system infection: one patient presented with seizures as a consequence of disseminated aspergillosis with brain abscesses. This result is perhaps lower than would be expected from the literature, with incidences ranging from 1 to 10% [24]. Although the difference in average blood trough levels of the immunosuppressant drugs between the NC group and control group failed to reach statistical significance, patients in the NC group receiving CNIs had higher average trough levels compared with the control group ($P = 0.207$ and $P = 0.109$, respectively, for tacrolimus and CsA), suggesting a possible contribution of the higher CNI dosage in the NC development process. Our study did have a high incidence of NC in patients receiving mTOR inhibitors, as a result of preference in our institution for the primary use of such immunosuppressants in patients with severe pre-LT encephalopathy. In 34 of the 82 patients (41.5%) receiving CNIs, a switch to mTOR inhibitors or a reduction of the daily dose administered was performed (23 switched; 11 had their daily dose reduced). In 30 cases (88.2%), a complete neurological recovery was seen, demonstrating the impact CNI immunosuppressants have on NC development. A complete recovery was observed in 20 of 23 (86.9%) patients switched to mTOR inhibitors and in 10 of 11 (90.9%) patients in whom a dose reduction was performed.

The choice of the early immunosuppressive therapy should therefore consider the risk/benefits ratio of the early administration of mTOR inhibitors. In conclusion, in patients undergoing a LT and presenting nonmodifiable risk factors for developing NC (a MELD score ≥20) if possible, the administration of CNI should be avoided, preferring the utilization of mTOR inhibitors.

Authorship

GR and GEG: designed research. RM and LDP: analysed data. NC, FDB, NDR and RB: collected data. AS, CB and GR: wrote the manuscript.

Funding

The authors did not receive any funding or had any commercial associations that may bias the present study.

References

1. Bronster DJ, Emre S, Boccagni P, Sheiner PA, Schwartz ME, Miller CM. Central nervous system complications in liver transplant recipients–incidence, timing, and long-term follow-up. *Clin Transplant* 2000; **14**: 1.
2. Wijdicks EF, Wiesner RH, Krom RA. Neurotoxicity in liver transplant recipients with cyclosporine immunosuppression. *Neurology* 1995; **45**: 1962.
3. Yu J, Zheng SS, Liang TB, Shen Y, Wang WL, Ke QH. Possible causes of central pontine myelinolysis after liver transplantation. *World J Gastroenterol* 2004; **10**: 2540.
4. Saner F, Gu Y, Minouchehr S, *et al.* Neurological complications after cadaveric and living donor liver transplantation. *J Neurol* 2006; **253**: 612.
5. Senzolo M, Ferronato C, Burra P. Neurologic complications after solid organ transplantation. *Transpl Int* 2009; **22**: 269.
6. Wijdicks EF, Hocker SE. Neurologic complications of liver transplantation. *Handb Clin Neurol* 2014; **121**: 1257.
7. Zivković SA. Neurologic complications after liver transplantation. *World J Hepatol* 2013; **5**: 409.
8. Barbas AS, Rege AS, Castleberry AW, *et al.* Posterior reversible encephalopathy syndrome independently associated with tacrolimus and sirolimus after multivisceral transplantation. *Am J Transplant* 2013; **13**: 808.

9. Stracciari A, Guarino M. Neuropsychiatric complications of liver transplantation. *Metab Brain Dis* 2001; **16**: 3.
10. Amodio P, Biancardi A, Montagnese S, *et al.* Neurological complications after orthotopic liver transplantation. *Dig Liver Dis* 2007; **39**: 740.
11. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the liver in humans. *Surg Gynecol Obstet* 1963; **117**: 659.
12. Gordon RD, Shaw Jr BW, Iwatsuki S, Esquivel CO, Starzl TE. Indications for liver transplantation in the cyclosporine era. *Surg Clin North Am* 1986; **66**: 541.
13. Dawson TM. Immunosuppressants, immunophilins, and the nervous system. *Ann Neurol* 1996; **40**: 559.
14. Steiner JP, Dawson TM, Fotuhi M, *et al.* High brain densities of the immunophilin FKBP colocalized with calcineurin. *Nature* 1992; **358**: 584.
15. Diaz-Ruiz A, Vergara P, Perez-Severiano F, *et al.* Cyclosporin-A inhibits inducible nitric oxide synthase activity and expression after spinal cord injury in rats. *Neurosci Lett* 2004; **357**: 49.
16. Murgia MG, Jordan S, Kahan BD. The side effect profile of sirolimus: a phase I study in quiescent cyclosporine-prednisone-treated renal transplant patients. *Kidney Int* 1996; **49**: 209.
17. Kahan BD, Wong RL, Carter C, *et al.* A phase I study of a 4-week course of SDZ-RAD (RAD) quiescent cyclosporine-prednisone-treated renal transplant recipients. *Transplantation* 1999; **68**: 1100.
18. Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc* 2003; **35**(Suppl. 3): 7S.
19. Lewis MB, Howdle PD. Neurologic complications of liver transplantation in adults. *Neurology* 2003; **61**: 1174.
20. Pujol A, Graus F, Rimola A, *et al.* Predictive factors of in-hospital CNS complications following liver transplantation. *Neurology* 1994; **44**: 1226.
21. Kim BS, Lee SG, Hwang S, *et al.* Neurologic complications in adult living donor liver transplant recipients. *Clin Transplant* 2007; **21**: 544.
22. Dhar R, Young GB, Marotta P. Perioperative neurological complications after liver transplantation are best predicted by pre-transplant hepatic encephalopathy. *Neurocrit Care* 2008; **8**: 253.
23. Emiroglu R, Ayvaz I, Moray G, Karakayali H, Haberal M. Tacrolimus-related neurologic and renal complications in liver transplantation: a single-center experience. *Transplant Proc* 2006; **38**: 619.
24. Cohen BA, Stosor V. Opportunistic infections of the central nervous system in the transplant patient. *Curr Neurol Neurosci Rep* 2013; **13**: 376.