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ORIGINAL ARTICLE

Analysis of liver function in renal transplant recipients undergoing C2-monitoring for cyclosporine

Manfred Hecking,[1] Alexander Kainz,[1] Martin Schillinger,[2] Christian Posch,[3] Tudor Birsan,[3] Susanne Rasoul-Rockenschaub,[3] Georg A. Böhmig,[1] Sabine Schmaldienst,[1] Bruno Watschinger,[1] Walter H. Hörl,[1] Ferdinand Mühlbacher[3] and Marcus D. Säemann[1]

1 Department of Internal Medicine III, Clinical Division of Nephrology and Dialysis, Medical University of Vienna, Vienna, Austria
2 Department of Angiology, Medical University of Vienna, Vienna, Austria
3 Department of Surgery and Transplantation, Medical University of Vienna, Vienna, Austria

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Correspondence
Manfred Hecking MD, Medizinische Universität Wien, Vienna Competence Center, Lazarettgasse 19, A-1090 Vienna, Austria.
Tel.: +43 1 4016063005; fax: +43 1 40160963005; e-mail: manfred.hecking@meduniwien.ac.at

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Summary

There exists no systematic evaluation of liver function in renal allograft recipients undergoing C2-monitoring with Neoral® [cyclosporine A (CsA)-microemulsion]. In the present cohort analysis, we compared the hepatic profiles of C2-monitored ($n = 80$), C0-monitored ($n = 81$), and non-CsA-treated renal allograft recipients ($n = 29$), transplanted between 1/1999 and 2/2004 in our institution. While the C2-targets were set in accordance with ($n = 72$) or below ($n = 8$) the consensus on Neoral® (1500 ± 200 ng/ml), non-CsA-patients received FK506 ($n = 29$), partially in combination with rapamycin ($n = 13$) as primary immunosuppression. Analysis of maximum hepatic laboratory parameters and also repeated measures by ANOVA within 30 days post-transplant revealed highly significant elevations of direct, indirect and total bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and lactate dehydrogenase ($P < 0.001$) in the C2-group, in comparison with the C0- and the non-CsA-group. Bilirubin-levels were by far the most affected of all hepatic parameters, and correlated with C2-levels ($r^2 = 0.62$). Seventeen CsA-patients had excessive bilirubin-elevations (>4 mg/dl) and were therefore considered to be 'CsA-sensitive' [14 C2-patients (17% of all C2-patients), 3 C0-patients (4% of all C0-patients)]. Bilirubin- and the other parameter elevations in these patients were reversible upon withdrawal or lowering of CsA. Most 'CsA-sensitive' patients ($n = 12$, 70%) displayed pre-transplant hepatic impairment, indicating a pre-existing liver instability. Collectively, our data emphasize the need for increased awareness toward individual predispositions for CsA-sensitivity.

Introduction

Cyclosporine A (CsA)-monitoring using CsA-microemulsion formulation (Neoral®) and whole-blood concentration samples drawn 2 h after CsA-intake (C2) has been advocated to be more efficient than managing immunosuppression with previous CsA-formulations and/or CsA-trough (C0) levels [1–3]. C2-levels were not only shown to be the best single-point predictors of area under the time–concentration curve over the first four of the 12-h dosage interval [area under the curve (0–4 h)], and therefore of drug exposure [1,4], but also to correlate closely with the risk of acute graft rejection [2] (for review see Nashan *et al.* [5]) In *de novo* kidney transplant recipients, adjusting the patient to C2-targets of 1600–2000 ng/ml was shown to decrease the biopsy-proven rejection rates 3 months after transplantation to <12% [6]. Considering the potential for tailoring

immunosuppression individually, C2-monitoring was also proposed to reduce typical CsA-associated side-effects like nephrotoxicity and arterial hypertension [3,4,7–9].

Hepatotoxicity is another side-effect associated with the use of CsA [10,11]. Early studies in renal transplant patients reported not only alterations of several liver parameters in a considerable proportion of CsA-treated patients, but also rare, yet severe hepatobiliary complications, like biliary calculus disease [12,13]. These previous studies, however, examined patients treated with old CsA-formulations, and also high CsA-doses up to 17 mg/kg. Subsequent studies showed that CsA-treatment, also with lower CsA-doses, may lead not only to decreased hepatic excretory function as reflected by elevated serum bile acids and bilirubin [14], but also to distinct hepatocellular injury, as manifested by elevations in serum transaminases [15]. Because of further reduction of CsA-dosing along with decreased CsA target-levels, clinical reports of CsA-induced hepatotoxicity became rare after the early nineties [10].

C2-level monitoring was implemented as standard primary immunosuppressive regimen at our institution in January 2002, and almost entirely replaced C0-monitoring, while approximately 20% of patients were already treated with the newer immunosuppressive agents tacrolimus (FK506) and/or rapamycin. Recently, Birsan *et al.*, [16] from the same institution, showed that C2-patients received 1.7–2 times higher doses of CsA than C0-patients. We had observed a rise in several liver function parameters in some of these C2-patients, but realized that the only recent publication addressing this phenomenon is from Videla *et al.* [17] who reported a series of cases with severe, even 'life-threatening' hepatotoxicity associated with CsA-monitoring using C2-recommendations. These renal transplant recipients, however, had also received ketoconazole.

To acquire systematic knowledge about the incidence and nature of liver changes with Neoral® and C2-monitoring, we initiated the present single center analysis, where we consecutively analyzed renal allograft recipients transplanted during 1 year, and compared a cohort of C2-monitored patients to a cohort of C0-patients from 2 years before, and to non-CsA-patients.

Patients and methods

The hepatic and immunosuppressive profiles as well as several clinical characteristics of kidney-recipients transplanted at our institution were recorded in the present retrospective analysis. C2-patients treated with CsA-microemulsion formulation (Neoral®; Novartis, Basel, Switzerland) were recruited from the year 2/2003 to 2/2004 ($n = 80$). C0-patients were also treated with Neoral® and were recruited from 1/2001 to 1/2002 ($n = 81$), when C2-monitoring had not yet been implemented at our center. Non-CsA-patients were recruited from 1/1999 and 2/2004 ($n = 29$). A maximum of 30 postoperative days was analyzed.

Immunosuppressive protocols

The C2-level-targets in the C2-group were aimed at 1500 ± 200 ng/ml ($n = 72$), according to the consensus statement on Neoral® [18], or lower (1000 ± 150 ng/ml, $n = 8$). The C0-level-targets in the C0-group were aimed at 250 ± 50 ng/ml. Primary immunosuppression in the non-CsA-patients consisted of FK506 ($n = 29$), partially in combination with rapamycin ($n = 13$). As additional immunosuppression, all patients received mycophenolate mofetil (MMF), generally at a dose of 2 g/day. Intra-operative dexamethasone was tapered in all patients, from 40 to 4 mg/day within 6 days, thereafter the patients received prednisolone 20 mg/day. Thirty-five patients (12 in the C2-, 18 in the C0-, 5 in the non-CsA-group) were additionally treated with depleting antilymphocyte-antibodies (Thymoglobulin®; Sangstat, Fremont, CA, USA or ATG-Fresenius®; Fresenius, Bad Homburg, Germany) against acute rejection.

Renal function analysis

Daily creatinine levels and rejections were also recorded. As those findings had previously been published [16], the analysis of rejection was not the main purpose of our analysis. Therefore, all acute rejections, histologically classified according to Banff, during the first 12 months post-transplant, were only included in the treatment characteristics of patient groups, to determine if the treatment between both groups had been similar.

Statistical methods

Continuous data are reported as the median and interquartile range (IQR) and were compared by Kruskal–Wallis' test. Maximum values of laboratory test parameters were compared by ANOVA. Repeated measures by ANOVA were calculated using a mixed model. Non-normally distributed data were transformed by log-transformation before being entered into the model. Chi-squared test was used to determine significant differences of all other parameters. Correlation was determined by Pearson's correlation coefficient. All statistical analyses were performed using SAS for Windows 9.1. A P-value < 0.05 was considered statistically significant (marked as *). Highly significant P-values were reported as $P < 0.001$ (marked as **).

CsA-monitoring during follow-up

In the C2-group, while 50 patients were maintained on C2-monitoring during the entire observation period, 17 patients were switched from C2-monitoring to FK506, 12 patients from C2- to C0-monitoring and one patient to lower C2-levels. The reasons for terminating C2-monitoring were slow absorption ($n = 16$, unstable C2-levels), biopsy-proven rejection (Banff 1 or higher, $n = 3$), or biopsy-suspected rejection (Banff borderline, $n = 3$). C2-monitoring was also stopped in three patients with biopsy-proven and -suspected CsA-induced nephrotoxicity, as well as in the case of liver impairment ($n = 5$). In the C0-group, while 71 patients were maintained on CsA, 10 patients were switched to FK506 to optimize immunosuppression ($n = 9$) or because of neurological side-effects (tremor, $n = 1$).

Hepatic function analysis

All available test results of conjugated, unconjugated, and total bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma-glutamyl transferase (γ-GT), alkaline phosphatase (AP) and lactate dehydrogenase (LDH) were recorded. Statistical analysis was first performed with the maximum value of each patient reached by postoperative day 30. Secondly, repeated measures by ANOVA were calculated with the complete laboratory data found within baseline and 30 days. Thirdly, correlation analysis between bilirubin and CsA-doses as well as C2-levels was performed, within baseline and 30 days. Only total bilirubin was used for statistical calculations requiring more than just the maximum laboratory value.

Factors contributing to impaired hepatic function

As, this analysis being retrospective, the assignment of patients to their various immunosuppressive protocols had not been arbitrary, all available information on preoperative liver function was also recorded, including laboratory parameters, liver sonography, and hepatitis viral status. Along with the demographic findings, pretransplant hepatic data were evaluated to determine significant differences between both patient groups, in an attempt to rule out selection bias.

Classification of impaired hepatic function

The translation of hepatic laboratory parameters into a definition of liver impairment has resulted in the 'Bénichou-criteria' of drug-induced hepatotoxicity, which were decided upon at an international expert meeting [19]. This standardization demands a singular elevation of GPT or conjugated bilirubin to a value twice above the normal level, or a combined elevation of GOT, AP, and total bilirubin, given that at least one parameter is twice above the normal level. We determined the number of patients in each group meeting the Bénichou-criteria. Moreover, as bilirubin occupies a principal position within the definition of the Bénichou-criteria, we considered an elevation of total bilirubin over 4 mg/dl to be a clear sign of liver impairment. Thus, we predefined this limiting condition as an inclusion criterion into a patient subgroup which we termed 'CsA-sensitive'.

Results

Demographics and treatment characteristics

Patient demographics and treatment characteristics are provided in Table 1. Overall, the C2- and the C0-group were closely similar. The non-CsA-group, however, was smaller, although non-CsA-patients were recruited from a much longer period than CsA-patients (1/1999 to 2/2004). The small size of the non-CsA-group is not only a consequence of CsA being the standard calcineurin inhibitor at our institution, but it is also due to the circumstance that many non-CsA-patients received additional immunosuppression other than MMF and glucocorticoids, such as immunoabsorption or antibody induction. These patients were not included in the non-CsA-group, as they would not have matched the C2- and the C0-group.

Apart from the size, no major differences among the three patient groups were observed. First grafts were more frequently transplanted in the C2- than in the non-CsA-group ($P = 0.002$). This is due to the fact that in sensitized patients, FK506 is routinely employed as initial calcineurin inhibitor at our center. Regarding treatment characteristics, the median follow-up time was 7 days longer in the non-CsA-group than in the C0-group ($P = 0.049$). The incidence of acute rejections, graft losses, and patient deaths, however, were similar among C2-, C0-, and non-CsA-patients.

Comparison of maximum hepatic laboratory parameters

The distribution of the maximum hepatic laboratory parameters among C2-, C0- and non-CsA-patients is displayed in the box plots of Fig. 1. ANOVA of log-transformed values revealed that conjugated, unconjugated, and total bilirubin-levels, GOT, GPT and LDH were more frequently and more markedly elevated in C2-patients, when compared with C0- and non-CsA-patients. Interestingly, AP was decreased in the C2-group, when compared with the C0- and to the non-CsA-group.

Table 1. Patient demographics and treatment characteristics.

	Non-CsA (CsA) (n = 29)	C0 (n = 81)	C2 (n = 80)	P-value
Male/female	23/6	53/28	53/27	0.357
Median age (years) [interquartile range (IQR)]	50 (40–60)	51 (40–57)	49 (40–61)	0.981
First graft	23/6	80/1	74/6	**0.002**
Causes of renal failure				
Hypertension/nephrosclerosis	1	7	10	0.341
Diabetes	8	11	8	0.066
Glomerular disease/nephritis	7	21	28	0.357
Polycystic kidney disease	3	12	13	0.744
Shrunken kidney (unknown origin)	2	15	5	**0.036**
Other	8	15	16	0.577
Median follow-up (days) (IQR)	28 (24–29)	21 (17–28)	24 (18–32)	**0.049**
Immuosuppression (initial)				
CsA	0	81	80	**<0.001**
FK506	29	0	0	**<0.001**
Sirolimus	13	0	0	**<0.001**
Mycophenolate mofetil	21	81	80	**<0.001**
Glucocorticoids	29	81	80	NA
Antibody therapy (ATG)	5	18	12	0.490
Acute rejection†	6	24	18	0.481
Graft loss	4	6	7	0.584
Death of the patient	2	3	5	0.702

Significant values marked in bold. Kruskal–Wallis' test for comparison of median age and median follow-up. Chi-squared test for comparison of all other parameters. NA, not applicable; †histological Banff ASR at 12 month postoperatively; borderline-lesions not included.

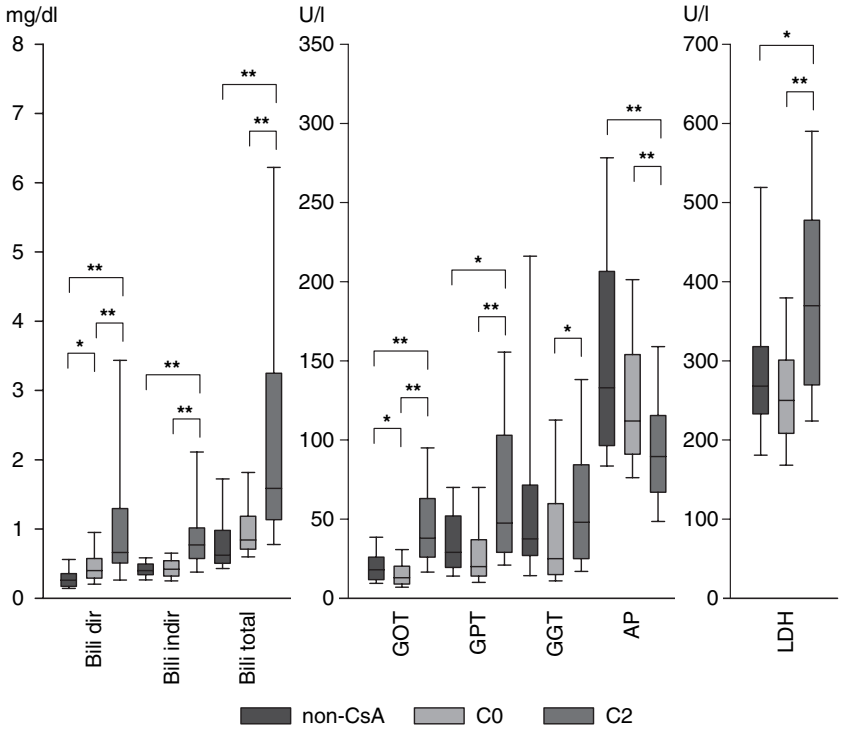

Figure 1 Comparison of maximum hepatic laboratory parameters (box plot). Maximum values, reached within 30 days after transplantation, were compared among C2, C0, and non-CsA-patients. Significant differences, as determined by ANOVA (log-transformed values) enclosed by brackets. *$P < 0.05$ **$P < 0.001$.

There were 59/80 patients in the C2-group (73.8%), versus 30/81 in the C0-group (37.0%), and 5/29 in the non-CsA-group (17.2%) meeting the 'Bénichou-criteria' of drug-induced hepatotoxicity [19] ($P < 0.001$). This excessive number in the C2-group resulted from 47/63 C2-patients with elevations of conjugated bilirubin more than twice the upper limit of normal (74.6%).

Development of hepatic laboratory parameters

All hepatic laboratory parameters were also analyzed at baseline and within 30 days by ANOVA of repeated measures (Fig. 2). [Correction added after online publication 8th October 2007: The words 'by ANOVA' were moved to their current position in the text]. Statistically, we found a highly significant elevation of bilirubin, GOT, GPT, and LDH in the C2-group, compared to the C0- and also to the non-CsA-control group. The daily values of γ-GT in the C2-group were found to be located between the C0- and the non-CsA-group. AP in the C2-group was consistently below the C0- and the non-CsA-group. Of the maximal hepatic laboratory parameter elevations in the C0-group in comparison to the non-CsA-group, only conjugated bilirubin and GOT were significant.

CsA-doses, CsA-levels, and bilirubin

Birsan *et al.* [16] have previously shown that, at our institution, the attempt to perform C2-monitoring according to the given recommendations led to 1.7–2 times higher CsA-doses in comparison to C0-monitoring. The C0- and the C2-cohorts evaluated in this analysis partially differed from Birsan's cohorts; therefore, we examined CsA-doses and levels at certain time-points and are providing this information in Table 2. Our data confirm that C2-patients received much higher CsA-doses than C0-patients. While

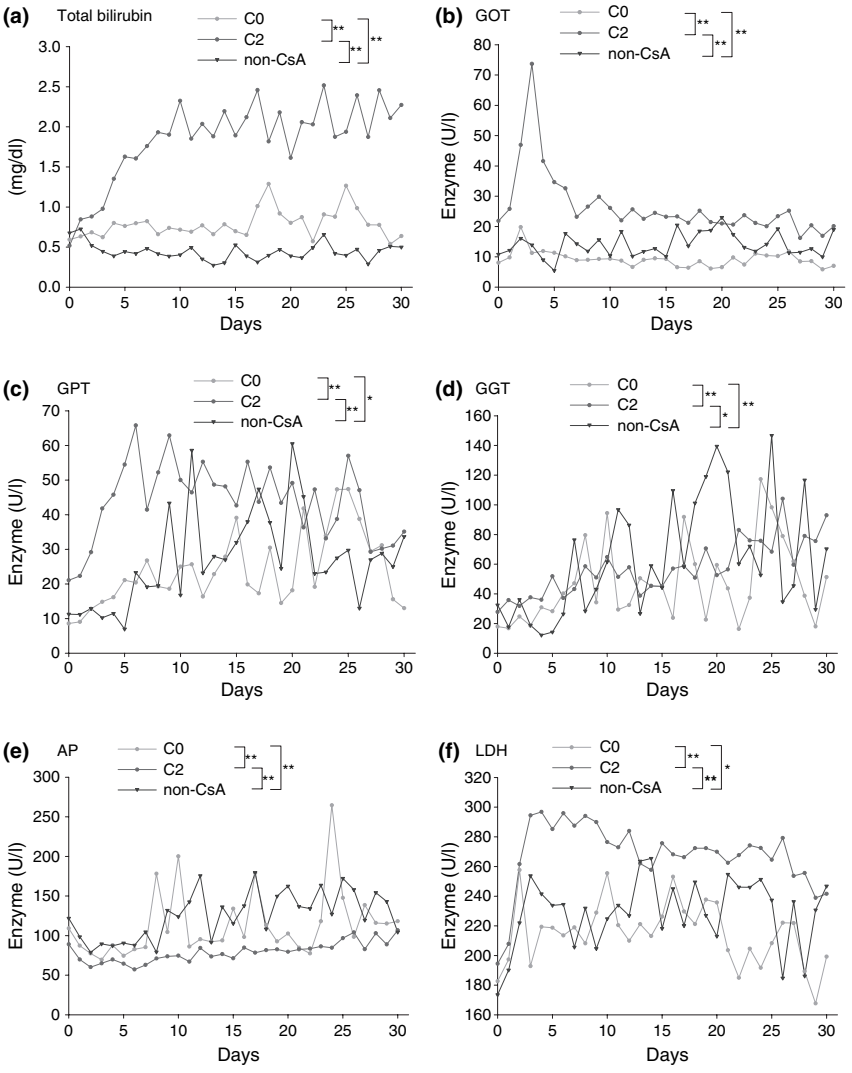

Figure 2 Development of hepatic laboratory parameters. Significant differences among C2, C0, and non-CsA-patients were determined by ANOVA of repeated measures (log-transformed, mean hepatic laboratory parameters) and are enclosed by brackets. $*P < 0.05$, $**P < 0.001$.

Table 2. Median cyclosporine A (CsA)-doses and CsA-levels during the first month post-transplant.

	C0-group		C2-group	
Day	Median C0-level [interquartile range (IQR)] (ng/ml)	Median CsA-dose (IQR) (mg/kg/day)	Median C2-level (IQR) (ng/ml)	Median CsA-dose (IQR) (mg/kg/day)
3	264.0 (190.0–349.0)	7.16 (5.88–8.85)	1490.0 (1087.0–1810.0)	11.05 (10.00–14.20)
5	278.0 (211.0–314.0)	5.63* (4.10–7.50)	1622.0 (1396.0–1940.0)	10.20 (8.00–13.44)
8	234.0 (202.0–282.0)	4.99** (3.31–5.84)	1555.0 (1256.0–1880.0)	8.96** (6.93–12.73)
10	231.0 (197.0–272.5)	4.46** (2.74–5.43)	1790.0 (1480.0–1900.0)	8.23** (6.88–10.63)
14	225.0 (184.0–48.0)	4.17* (3.45–5.15)	1657.5 (1206.5–1954.5)	7.74** (6.70–9.60)
21	232.0 (204.0–255.0)	3.94** (3.31–5.48)	1793.0 (1410.0–2189.5)	9.15** (7.14–9.89)
28	223.5 (207.5–248.5)	3.48** (2.94–6.86)	1910.0 (1200.0–2330.0)	9.05* (6.10–10.00)

*$P < 0.05$, **$P < 0.001$ compared with day 3.

the median CsA-dose in the C2-group was 1.5 times higher than in the C0-group on post-transplant day 3, it became 2.6 times higher on post-transplant day 28, thereby surpassing the ratio observed by Birsan *et al.* However, the number of C2-levels recorded at that time-point was small ($n = 8$), and smaller than the number of C0-levels recorded in the C0-group ($n = 16$), because of the discharge and the switching of C2-patients.

To examine the effect of CsA-administration on bilirubin-levels, daily median CsA-dose, CsA-level, and bilirubin were plotted against time, as depicted in Fig. 3a. The graphs demonstrate that bilirubin increased, as soon as

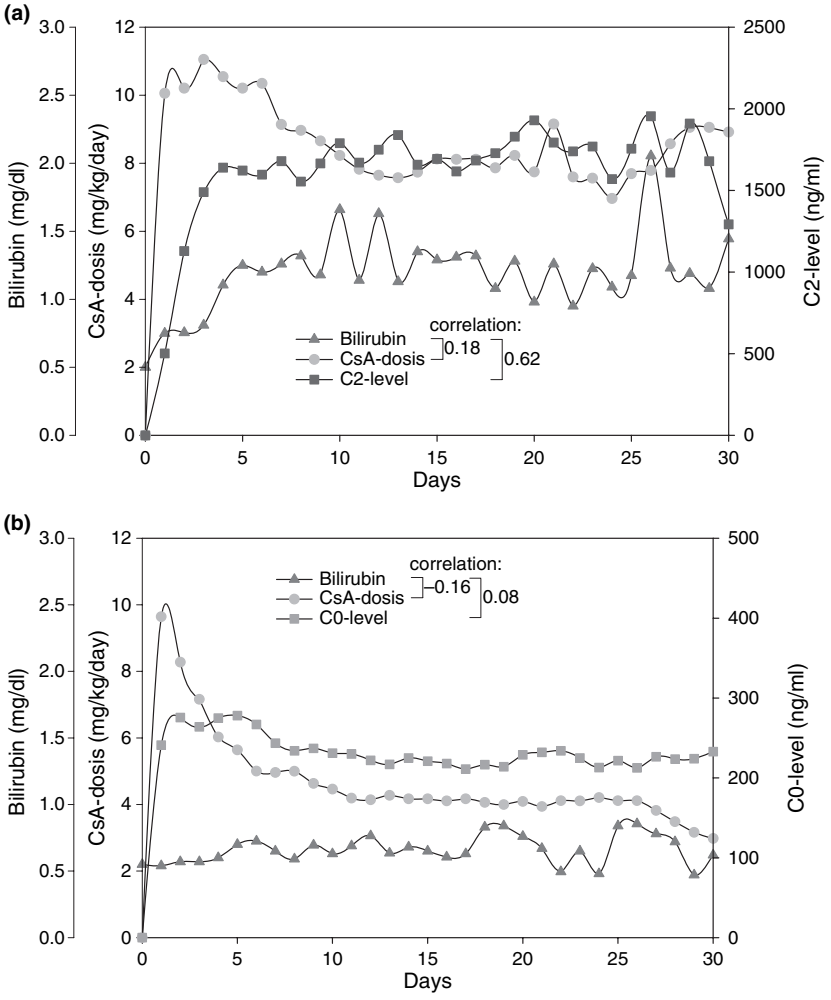

Figure 3 Relationship between CsA-dose, CsA-level, and bilirubin. (a) C2-patients (C2-level). (b) C0-patients (C0-level). Correlation was determined using Pearson's correlation coefficient.

CsA was administered. The overall median correlation between daily bilirubin values and C2-levels ($r^2 = 0.62$, $P < 0.001$) was higher than the same between bilirubin-levels and CsA-doses ($r^2 = 0.18$, $P = 0.335$). These r^2-values suggest that bilirubin-elevations can relate with C2-levels rather than CsA-doses and may determine the degree of a CsA-related hyperbilirubinemia. Thus, Fig. 3a suggests the presence of a biological gradient and reflects a mechanism of CsA-dependent bilirubin-transport inhibition inside the hepatocyte, which is consistent with *in vitro* findings [20–28] and will be discussed subsequently.

For the C0-group also, the daily median CsA-dose, CsA-(C0)-level, and bilirubin were plotted against time (Fig. 3b). The graphs demonstrate that bilirubin at baseline (0.55 mg/dl) in the C0-group is comparable to the C2-group (0.50 mg/dl), but thereafter does not increase in a manner similar to the bilirubin in the C2-group. Consistently, the overall median correlation between daily bilirubin values and C0-levels ($r^2 = 0.08$, $P = 0.672$) is much lower than the correlation between bilirubin and C2-levels.

'CsA-sensitive' patients

With hyperbilirubinemia being the primary change observed under C2-monitoring, 'CsA-sensitive' renal transplant patients were defined as CsA-treated individuals in whom we observed strong hyperbilirubinemia, with maximum bilirubin-levels of at least 4 mg/dl ($n = 17$, 14 C2-patients, 3 C0-patients). In contrast to the CsA-treated patients, none of the non-CsA-patients had bilirubin values above 2.92 mg/dl. Interestingly, hyperbilirubinemic 'CsA-sensitive' C2-patients displayed median C2-levels below the C2-levels of the whole C2-group (1540 ng/ml in comparison to 1604 ng/ml), but the median daily CsA-dose was higher than in the whole C2-group (10.20 mg/kg/day in comparison to 9.23 mg/kg/day).

The clinical course of the 17 hyperbilirubinemic patients is reported in Table 3. In five of these patients, 'hepatotoxicity' because of CsA was diagnosed, three patients were suspected or proven to suffer from renal toxicity, and one patient was reported to have unstable C2-levels. These nine patients, who belonged to the C2-group, were switched either to lower C2-levels, C0-monitoring or FK506. Except for one patient (patient C2-14, Table 3), who might have been switched too late, the bilirubin values of these patients decreased rapidly, mostly to a level around the normal range. The other liver parameters also decreased, although less rapidly.

The remaining eight hyperbilirubinemic CsA-patients (5 C2-, 3 C0-patients) were all maintained on CsA. The hepatic parameters in at least three of these patients remained high, and the diagnoses retrospectively retrieved in these patients were septic cholangitis, portal fibrosis, and carcinoma of extrahepatic bile duct. Four of the 17 hyperbilirubinemic patients died within 1 year, three in the C2-group and one in the C0-group (for details, see Table 3).

Investigation of the pretransplant findings demonstrated that 15 of the 17 hyperbilirubinemic 'CsA-sensitive' patients (88%) had either a hepatic diagnosis or a hepatic laboratory parameter elevation reported prior to transplantation. For example, pretransplant abdominal ultrasounds in the 'CsA-sensitive' patients revealed evidence of biliary calculus disease in four patients, cysts in two, hepatomegaly in one, amyloidosis in one, and hepatic hemangioma in another patient. Altogether, our pretransplant data suggest that the hepatic changes, observed in the 'CsA-sensitive' hyperbilirubinemic subgroup, did not occur *de novo*, but that C2-monitoring might have worsened a pre-existing form of liver impairment.

Discussion

Hepatic changes in renal allograft recipients undergoing C2-monitoring have not yet been systematically analyzed. By studying a cohort of C2-monitored patients treated according to an international consensus statement on Neoral® [18], we demonstrated characteristic changes of liver function parameters in comparison with C0- and with non-CsA patients. Conjugated, total, and unconjugated bilirubin as well as GOT, GPT and LDH maximum values reached high statistical significance. This finding was confirmed by daily ANOVA from baseline to 30 days. As bilirubin was correlated with C2-levels, which are the best single-point predictors of the area under the time–concentration curve and therefore of drug exposure [1,4], we judge bilirubin to be the most sensitive, and thus the primary clinical parameter enabling to determine the hepatic side-effects under CsA-treatment.

A conceptual framework for our observation is provided by findings from numerous *in vitro* studies, which demonstrated that CsA inhibits ATP-dependent transport of several substances, especially of conjugated bilirubin, across the hepatocyte canalicular membrane [20–31]. Apparently, the primary hepatic event after CsA-uptake is the blocking of the human bile salt export pump [24,32]. Recently, it was demonstrated that hydrophobic bile acids can cause hepatocellular necrosis and apoptosis via altered mitochondrial permeability and oxidative stress [33]. Therefore, generalized liver enzyme-elevations in CsA-treated patients seem to indicate a more severe degree of liver impairment than bilirubin-elevations alone.

In our opinion, the most interesting information of our analysis is provided by the subgroup of 17 'CsA-sensitive' patients with bilirubin values exceeding 4 mg/dl. In these patients, we observed not only clinical

Table 3. Clinical course and hepatic function in hyperbilirubinemic patients.

Patients' ID	Antibody treatment	Hepatic diagnosis known before transplantation	$Bili_{pre}$ (mg/dl)	$Other_{pre}$‡ (U/l)	Switch (reason)	$Bili_{3\ months}$† (mg/dl)	$Other_{3\ months}$†‡ (U/l)	Hepatic follow-up
C2-3	No	Hepatitis B core antibody positive	0.34	AP 213	Lower C2: day 34 (hepatotoxicity)	1.1	All normal	Inapparent hepatic follow-up
C2-11	No	Liver cysts, sludge (by ultrasound)	0.49	γ-GT 98	No	1.19	γ-GT 138	Inapparent hepatic follow-up
C2-12	No	Amyloidosis, cholecystectomy	0.3	All normal	C0: day 18 (hepatotoxicity)	0.97	All normal	Inapparent hepatic follow-up
C2-14	Yes	Hepatic hemangioma	1.0	All normal	C0: day 31 FK506: day 33 (hepatotoxicity)	4.18	GOT 192	Reversible liver insufficiency requiring MARS therapy from day 35 to 39 post-operatively
C2-15	Yes	Cholecystolithiasis	0.69	γ-GT 82 U/l	C0: day 14 FK506: day 22 (hepatotoxicity)	0.73	γ-GT 70	Inapparent hepatic follow-up
C2-24	No	Cholecystolithiasis, alcohol abuse 10 years ago	0.5	All normal	FK506: day 12 (kidney biopsy: CsA-toxicity)	(pat. died before)	(pat. died before)	Inapparent hepatic follow-up; death on day 61 because of several non-hepatic complications
C2-28	No	None	0.33	All normal	C0: day 11 (kidney biopsy: CsA-toxicity)	0.3	All normal	Inapparent hepatic follow-up
C2-38	No	Irregular antibodies against erythrocytes	0.36	AP 130	No	2.82	LDH 559	Septic cholangitis led to cholecystectomy on day 45; death on day 83 because of cardiorespiratory failure
C2-56	Yes	None	0.37	All normal	C0: day 9 FK506: day 30 (hepatotoxicity)	0.58	γ-GT 72	Inapparent hepatic follow-up
C2-60	No	Cholecystectomy 3 years before	0.55	All normal	No	Missing	Missing	Missing
C2-62	Yes	None	0.41	AP 166	C0: day 9 FK506: day 26 (kidney biopsy: CsA-toxicity)	0.34	All normal	Inapparent hepatic follow-up
C2-68	No	None	0.92	γ-GT 69	No	0.91	γ-GT 78	Inapparent hepatic follow-up
C2-70	Yes	Hepatomegaly	0.7	All normal	No	(pat. died before)	(pat. died before)	Hepatic failure with transaminases > 1000 U/L; death on day 12 because of cardiorespiratory failure
C2-74	No	None	0.3	AP 126	C0: day 18 (unstable C2-levels)	1.27	GPT 134	Inapparent hepatic follow-up
C0-46	No	Liver cirrhosis	1.15	γ-GT 420	No	2.99	γ-GT 518	Portal fibrosis, cholecystolithiasis
C0-76	Yes	Chronically active hepatitis C	1.46	γ-GT 77	No	3.08	γ-GT 203	Carcinoma of extrahepatic bile duct, death on day 267
C0-79	Yes	Hepatitis C, hepatic cysts	0.35	All normal	No	Missing	Missing	Missing

CsA, cyclosporine A; GPT, glutamic pyruvic transaminase; GOT, gamma glutamic oxaloacetic transaminase; γ-GT, gamma-glutamyl transferase; AP, alkaline phosphatase; LDH, lactate dehydrogenase; MARS, Molecular Adsorbents Recirculation System (liver dialysis device).

Patients were included, if total bilirubin during first 30 days after transplantation exceeded 4 mg/dl at least once (C2: 14 patients, C0: 3 patients).

†Approximately 3 months. ‡Hepatic parameter with the strongest elevation other than bilirubin.

complications, but also a high percentage of pre-existing overall liver-abnormalities (88%). The observed liver changes under CsA-treatment were mostly reversible upon withdrawal or lowering of CsA-doses. Therefore, although the large randomized studies comparing FK506 or sirolimus to CsA have not detected a higher incidence of liver abnormalities in the CsA-group [34–38] (for review see [39]), we would like to emphasize that a particular subgroup of renal transplant patients, such as the one we detected, might also exist in other transplant centers. This subgroup of patients could potentially benefit from an increased awareness for, primarily, hyperbilirubinemia and subsequently transaminase-elevations under CsA-treatment.

Surprisingly, AP-levels were significantly decreased in the C2-group, in comparison with the C0- and in comparison with the non-CsA-group. At the moment, we do not have a definite explanation for the lowering of AP in the C2-group. As AP is made up of roughly 50% liver-specific and about 50% bone-specific AP, one might speculate that the lowering of AP in the entire C2-group could be due to an influence of CsA on bone metabolism. Indeed, a trend ($P = 0.08$) toward an increase of bone-specific AP has recently been described in kidney-transplanted CsA-patients in comparison with FK506-patients, suggesting that FK506 (but not CsA) may have a favorable bone effect [40]. Further studies determining the levels of liver- and bone-specific AP in CsA-treated patients might be able to provide additional information which could help explain the lowering of AP in the C2-group examined here above.

In a previous study, Einecke *et al.* [41] detected a rise in GOT three times above the upper limit of normal in 18/38 renal transplant recipients who were adjusted to a C2-target level of 1500 ng/ml (50%) and also received basiliximab. In the present analysis, eight C2-patients (10%), but no C0 and no non-CsA-patients reached or surpassed these GOT-levels. As the CsA-doses and C2-levels in Einecke's C2-group were generally lower than in this analysis, the clearly elevated number of GOT-elevations is surprising. A detailed comparison of the remaining liver parameters as well as evidence of potential pretransplant hepatic disorders in these patients would be necessary to account for the difference between these results.

Our analysis has several limitations, due to its retrospective nature. Hepatic laboratory parameters were not available on a daily basis in all patients, and the pretransplant data were not uniformly assessed at exactly the same time-point before the initiation of C2-monitoring. Moreover, the sample size for the non-CsA-group was limited, although the retrieval of non-CsA patients was extended to a period of 6 years, whereas the C2-patients were recruited from only 1 year. Therefore, the ability to draw a strong conclusion from the comparison between the C2-group and the non-CsA-group is hindered, and our retrospective analysis can only suggest, but not prove, that the hepatic changes in C2-patients are CsA-induced.

Aside from the comparison between C2- and non-CsA-patients, the analysis of C2- and C0-patients is showing clearly that bilirubin and several other liver parameters were elevated in the C2-group, and that there were more C2-patients with considerable hepatic impairment. Most probably, the higher dosage of CsA in the C2-group is inducing the hepatic changes presented here above. Nevertheless, as the CsA-treated patients with hyperbilirubinemia above 4 mg/dl seemed to have an *a priori* liver impairment, we would like to note that CsA, while it may induce these hepatic changes, is not primarily responsible for them. Most importantly, the hepatic changes we observed were reversible. Thus, while our analysis is not suited, nor should it attempt to prove that CsA is hepatotoxic *per se*, we find it essential to state that, according to our results, hyperbilirubinemia is the most sensitive tool permitting to distinguish among the many patients who have no apparent hepatic problem under CsA and those few who might benefit from lowering or withdrawal. Future studies are necessary to determine whether the exclusion of 'CsA-sensitive' patients from treatment with this immunosuppressive agent, along with an increased awareness of its differential influence on liver function parameters, may lower the incidence of hepatic changes under C2-monitoring.

Authorship

MH collected the data, planned the analysis and wrote the paper; AK performed the statistical analyses, made the figures and corrected the paper; MS planned the statistical analyses and corrected the paper; CP collected the data and discussed the results; TB contributed the C0-data. SRR contributed access to data; GB and BW corrected the paper; SS initiated the analysis; WH and FM initiated the analysis and discussed the results; MS initiated and planned the analyses, discussed the results and corrected the paper.

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