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Side Effects and Efficacy of Renal Sparing Immunosuppression in Pediatric Liver Transplantation - a Single Center Matched Cohort Study

Authors

Christoph Leiskau MD^{1,6}, , Eva-Doreen Pfister MD¹, Imeke Goldschmidt MD¹, Norman Junge MD¹, André Karch MD², Jeremy Rajanayagam MD³, Christian Lerch MD^{4,6}, Nicolas Richter MD⁵, Frank Lehner MD⁵, Harald Schrem MD^{5,6}, Ulrich Baumann MD^{1,7}

Departments of First Author and Corresponding Author:

¹Division of Pediatric Gastroenterology and Hepatology, Department of Pediatric Kidney, Liver und Metabolic Diseases, Hannover Medical School, Hannover, Germany;

⁶Core Facility Quality Management & Health Technology Assessment in Transplantation, Integrated Research and Treatment Center Transplantation (IFB-Tx), Hannover Medical School, Hannover, Germany.

⁷Birmingham Children's Hospital, Liver Unit and University of Birmingham, Institute of Immunology and Immunotherapy, UK

Running Head

Renal Sparing Immunosuppression in pLTx

Corresponding author

Prof. Dr. Ulrich Baumann

Division of Pediatric Gastroenterology and Hepatology

Department of Kidney, Liver und Metabolic Diseases,

Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.

T: +49 511 532 3233

Fax: +49 511 532 3294.

Email: Baumann.U@mh-hannover.de.

Authorship Statements

Authors

Christoph Leiskau MD^{1,6}, Eva-Doreen Pfister MD¹, Imeke Goldschmidt MD¹, Norman Junge MD¹, André Karch MD², Jeremy Rajanayagam MD³, Christian Lerch MD^{4,6}, Nicolas Richter MD⁵, Frank Lehner MD⁵, Harald Schrem MD^{5,6}, Ulrich Baumann MD¹

Affiliations

¹Division of Pediatric Gastroenterology and Hepatology, Department of Pediatric Kidney, Liver und Metabolic Diseases, Hannover Medical School, Hannover, Germany; ²Helmholtz Centre for Infection Research, Braunschweig, Germany; ³Department of Gastroenterology and Nutrition, Royal Children's Hospital, Melbourne, Australia ⁴Division of Pediatric Nephrology, Department of Pediatric Kidney-, Liver und Metabolic Diseases, Hannover Medical School, Hannover, Germany; ⁵General, Visceral and Transplant Surgery, Hannover Medical School, Hannover, Germany; ⁶Core Facility Quality Management & Health Technology Assessment in Transplantation, Integrated Research and Treatment Center Transplantation (IFB-Tx), Hannover Medical School, Hannover, Germany and ⁷Birmingham Children's Hospital, Liver Unit and University of Birmingham, Institute of Immunology and Immunotherapy, UK

ABSTRACT PAGE

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ABSTRACT:

OBJECTIVES: Immunosuppressive combination therapy with mycophenolate mofetil (MMF) can reduce calcineurin inhibitor (CNI) associated nephrotoxicity. We investigated effectiveness and safety of de novo MMF-Tacrolimus based immunosuppression after pediatric liver transplantation (pLTX).

METHODS: Patients after pLTX receiving immunosuppression with MMF/Tacrolimus (MMF/TAC) were compared to retrospectively selected age- and diagnosis-matched patients with Tacrolimus monotherapy (TAC) and Cyclosporine/Prednisolone therapy (CSA) (19 patients each, n=57).

Effectiveness, renal function and side effects were analysed for one year after pLTX.

RESULTS:

Tacrolimus reduction in combination therapy (0.7 µg/l over the year) was lower than aspired (2 µg/l).

Acute biopsy proven acute rejection occurred equally in MMF/TAC and TAC groups (31.6% each), being slightly higher in CSA group (42.1%;OR=1.5;95%CI=0.42-5.44;p=0.5).

GFR deteriorated significantly and comparably in all three groups ($p < 0.01$ each) without significant differences between the groups. Septicaemia was detected significantly more often in MMF/TAC (73.6%) than in TAC (31.6%) (OR 4.17; 1.07-16.27; $p = 0.04$). EBV reactivation occurred more often in CSA patients (84.2 %) while being similar in MMF/TAC (47.4%;OR 5.16;0.98-27.19; $p = 0.05$) and TAC (52.6%;8.16;1.48-44.89; $p = 0.02$) but occurred more often in CSA patients (84.2 % vs. 47.4% in MMF/TAC (OR 5.16;0.98-27.19; $p = 0.05$) and 52.6% in TAC group (8.16;1.48-44.89; $p = 0.02$); just as other viral infections (47.4% (CSA) vs. 15.8% (TAC);4.21;0.95-18.55; $p = 0.05$).

CONCLUSIONS: Our study does not provide additional evidence for a benefit of initial use of MMF/TAC over TAC regarding renal function, but raises concerns regarding a potentially increased risk of serious infections under MMF/TAC compared to TAC monotherapy at equivalent renal outcome but is limited by the minor CNI reduction in combination therapy.

Key words: Calcineurin Inhibitor; Tacrolimus; Mycophenolate Mofetil; Nephrotoxicity; Infections

INTRODUCTION

Calcineurin inhibition (CNI) has become the cornerstone of success in transplantation medicine (1, 2).

Today, Tacrolimus-based immunosuppressive protocols with or without steroids are most commonly used in pediatric liver transplantation (pLTx) worldwide (3). However, significant side effects have been reported, including nephrotoxicity, arterial hypertension, neurotoxicity, increased rate of infections due to over-immunosuppression, diabetes and cardiomyopathy.

Mycophenolate mofetil (MMF) was officially introduced in 2000 (approval of FDA) for liver transplantation as an adjuvant immunosuppression. The putative role of MMF is to support CNI minimisation while maintaining sufficient overall immunosuppression. Adult studies have demonstrated the benefits of de novo MMF-CNI protocols in reducing CNI side effects, in particular nephrotoxicity (4-7). Furthermore, CNI-related renal impairment has been shown to be reversible following the introduction of MMF with concurrent CNI dose reduction in pediatric (8-10) and adult (11-14) studies. Studies in adult medicine also describe a benefit in patient and graft survival after liver transplantation (15, 16). Conversion to MMF monotherapy has been used safely for adult LTx patients with chronic kidney disease (17).

Apart from its known hematological and gastrointestinal side effects (18), MMF might be associated with wound healing defects(19) and a higher rate of serious infections (20). Birkeland et al. described a lower rate of PTLT in adult kidney recipients when MMF was used (21).

Even though there are numerous studies comparing immunosuppressive regimens and their efficacy in adult liver transplant recipients, only few studies using MMF in pediatric transplantation have been published, with their focus on late introduction of MMF after renal impairment. To our knowledge there are no reports of the effectiveness of de novo MMF therapy in pLTx. Since there are no widely accepted standards for immunosuppression in pLTx, we aimed to systematically compare the safety and efficacy of combination Tacrolimus and MMF therapy (group MMF/TAC) to two historical age- and diagnosis-

matched control cohorts (group TAC: Tacrolimus monotherapy and group CSA: Cyclosporine A + steroids).

PATIENTS and METHODS

Patients

This is a single-center cohort study. In 2012, we modified our post-transplant protocol to include MMF with reduced Tacrolimus as one of our standard immunosuppression regimens. All transplant recipients received modified individualized immunosuppression based on previous history and individual risk profile. Out of 36 children who underwent orthotopic liver transplantation in 2012, 23 patients received a steroid free, CNI-reduced immunosuppression with Tacrolimus and Mycophenolate mofetil (group MMF/TAC) as standard immunosuppression. 13 patients were excluded as they received other immunosuppression according to their underlying diseases, mostly (n=10) CSA / Prednisolone. Four patients who started with MMF/TAC were excluded from this analysis due to discontinuation of MMF for longer than 4 weeks (n=3) or change of immunosuppression to Sirolimus (n=1). All 19 remaining patients who had continuous CNI reduced immunosuppression with MMF for at least one year after pLTx were included in this study.

For every MMF/TAC patient, we retrospectively selected two age- and diagnosis-matched patients for comparison, one of them having received Tacrolimus monotherapy (TAC group) and the other one with a combination of Cyclosporine A and steroids (CSA group). Matching by age was achieved by distributing the patients to four age groups at time of pLTx (see table 1); if there was more than one match for diagnosis and age, the closest age match was selected. All transplant procedures were performed between 2006 and 2015 at Hannover medical school. In the MMF/TAC group, pLTx was performed in 2012; the TAC group received their pLTx between 2013 and 2015. In the CSA group, pLTx was performed between 2006 and 2011.

The following exclusion criteria were applied to all groups:

1. Combined transplantation of more than one organ
2. Primary malignant disease
3. Pre-existing renal disease (except for one patient with Alagille syndrome in each group)

4. Change of immunosuppressive regimen during observation period

Re-transplantation during observation period was not an exclusion criteria.

Patients were followed up for one year after transplantation. Laboratory data (Creatinine, urea, cystatin C, Cystatin-C-Clearance) were collected prior to transplantation and at 1-week, 2-weeks, 4-weeks, 3-months, 6-months and 12-months after transplantation (with trough levels for Tacrolimus/Cyclosporine A). EBV viral load in blood was monitored weekly during the inpatient stay after LTX, and thereafter every 3 months. Viral DNA-PCR of ≥ 3200 copies/ml or ≥ 1600 IU/ml was considered consistent with EBV reactivation. Patients were monitored for infection (fungal, bacterial and viral). Fungal (mycotic) and bacterial were determined based on appropriate clinical signs and culture from blood or wound specimens. Viral infections were confirmed in the context of appropriate symptoms and/or viral DNA/RNA PCR positive blood or other specimens. Wound healing defects were defined as tissue defects within in the abdominal scar requiring either another operational procedure or ongoing conservative treatment > 2 weeks after abdominal wall closure. The use of antihypertensive medication 3 months after pLTX was analysed, antihypertensive treatment was started with repeated blood pressures over 95th age- and length specific centile .

Histopathologic findings of liver biopsies were interpreted using Rejection Activity Index (RAI-Score); a score ≥ 3 was considered as biopsy proven acute graft rejection (BPAR). All histopathology slides were interpreted by the same two pathologists.

GFR was calculated by the new creatinine-GFR of Schwartz and his estimation considering Creatinine, Cystatin C, urea, patient height and sex (22).

Immunosuppressive therapy

All patients received corticosteroids (Soludecortin 300 mg/m² BSA, up to 500 mg IV) intraoperatively during the anhepatic phase, and Basiliximab (10 mg at weight < 35 kg, 20 mg at weight > 35 kg) at day 0 and day 4 after transplantation. Two patients in the TAC group received additional oral corticosteroids for underlying autoimmune disease.

Prednisolone dosing is shown in supporting information (SI 1). MMF was started 7 days post LTX and was dosed according to body weight with 10 mg/kg in 2 doses for two weeks gradually increasing to a maximum of 20 mg/kg/d (max. 2 x 750 mg) in two doses.

Tacrolimus was administered twice daily either as capsules or solution. None of the patients in this study were prescribed the extended release formula. Treatment was started within 12-hours post LTX with a loading dose of 0.1 mg/kg, then reduced to 0.05 mg/kg for the second dose and adjusted according to 12-hour-trough levels. All trough levels were assessed in the same laboratory. Target tacrolimus levels compared to actual average tacrolimus levels are shown for MMF/TAC group and TAC group in Figure 1. Tacrolimus dosages were adjusted by three experienced pediatric hepatologists.

In comparison to other studies, which described the aspired CNI reduction by using target levels(10), we aimed to verify the CNI reduction by comparison of actual CNI levels with target levels. Comparing CNI levels in sample controls (8, 9, 23) might lead to overestimation of temporary fluctuations. To achieve a more accurate approximation for the average tacrolimus exposition over the year, we included all 6 measured levels at the dates of our blood tests followed by linear interpolation as an approximation between two measuring points for the time in between dosages. An "area under the curve" for the trough levels over time – in the linear assumption - is calculated by $\frac{y_2-y_1}{2} \cdot x_2 - x_1$; with x being the time and y being the trough levels. Then the AUCs of every time interval were summed up and divided by the total time, having thus calculated an approximation for the average trough level over the year.

$$\frac{\sum_{n=1}^6 \left(\frac{y_n + y_{n+1}}{2} \cdot (x_{n+1} - x_n) \right)}{x_6 - x_1}$$

Data analysis

Conditional logistic regression was used for comparison of baseline characteristics, adverse drug effects and complications within the three groups. Repeated measures analysis of variants (ANOVA) was used to compare the three groups regarding Creatinine, GFR and Cystatin-C-GFR. Paired t-tests were used to

compare GFR before and 12 months after LTX within the groups. Missing data only existed in calculated GFR due to missing cystatin C values; these were handled by listwise deletion.

IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Figures were created using GraphPad Prism 7.02 (GraphPad software, San Diego, USA) and Excel (Microsoft Corp., Redmond, USA).

Ethics approval

This study was approved by the institutional ethics committee of Hannover Medical School (N. 3315 – 2016).

RESULTS

Baseline characteristics

Each group consisted of 19 patients, with no significant differences in baseline characteristics and perioperative data as shown in table 1.

All patients survived for the first year after LTX. Graft survival was 89.5% in MMF-TAC; 94.8% and 94.8% in CSA and TAC groups, respectively ($p=0.55$).

Tacrolimus trough level approximation by linear interpolation

Mean trough levels of Tacrolimus in the observational period were $5.9 \pm 0.9 \mu\text{g/l}$ (mean \pm SD) for the MMF/TAC group and $6.6 \pm 1.2 \mu\text{g/l}$ for the TAC group ($p=0.11$, 95%CI=-0.28–1.61). Mean difference to target level was $+0.8\mu\text{g/l}$ for MMF/TAC and $-0.5\mu\text{g/l}$ for TAC group (see figure 1).

Efficacy

In both the MMF/TAC and the TAC group, we observed biopsy proven acute rejection (RAI score ≥ 3) in 31.5% of cases during the first year after LTX. In the CSA group, 42.1 % of the patients suffered from BPAR (OR=1.5; 95%CI=0.42-5.44; $p=0.52$). No cases of chronic rejection were observed.

Adverse events

Renal function

All groups showed a significant deterioration in renal function post LTx with the nadir at three months, and stabilisation achieved by 12-months. Creatinine-GFR one year after LTx was significantly lower compared to the values prior to LTX in each group (MMF/TAC: mean difference ($\text{GFR}_{12\text{mo}} - \text{GFR}_0$) = $-51.2\text{ml/min/1.73m}^2$; 95%CI: -22.3 to -80.0; $p=0.002$; TAC: $-39.3\text{ ml/min/1.73m}^2$; 95%CI: -17.4 to -61.3; $p=0.001$, CSA: $-46.8\text{ml/min/1.73m}^2$; 95%CI: -15.4 to -78.1; $p=0.006$).

The same applies to estimated GFR values (Creatinine, Cystatin C, Urea) (see SI 2,3).

No significant differences in Creatinine GFR or estimated GFR between the three groups were seen at any time during the observation period (Figure 2, SI 2,3).

MMF-related adverse effects

Two patients in the MMF/TAC group had to discontinue MMF treatment for two and three weeks, respectively, due to gastrointestinal adverse events but could restart with a reduced dose of 10 mg/kg/d. Leukopenia occurred in 3 patients during co-medication with ganciclovir but improved after discontinuation of ganciclovir.

Infectious complications

The MMF/TAC group showed a significantly higher risk of septicemic bacterial or mycotic infections (68.4 %) compared to TAC (31.6 %; OR 4.17; 95%CI: 1.07–16.29; $p=0.04$); but not the CSA group (57.6 %; 95%CI: 0.75–10.40; OR 2.78; $p=0.13$). EBV reactivation was significantly lower in both groups using Tacrolimus (MMF/TAC: 47.4%; OR 0.12; 95%CI: 0.02–0.67; $p=0.02$; TAC: 52.6%, OR 0.19; 95%CI: 0.04–1.02; $p=0.05$) compared to the CSA group (84.2%).

The proportion of proven viral infections during the first year after LTX in MMF/TAC was more than double the TAC group (36.8% vs. 15.8%, OR = 2.77; 95%CI = 0.64 – 12.11; $p = 0.17$); however, this difference was not statistically significant. We found significantly less viral infections in the TAC group when compared to CSA (47.4%; OR 4.21; 95%CI= 0.95 – 18.55; $p=0.05$) (Figure 3). Most common non-EBV viral infections observed were caused by cytomegalovirus, adenovirus and enterovirus.

Further adverse effects

Five patients (27.8 %) in the MMF/TAC group suffered from wound healing defects that required conservative treatment, with one patient needing surgery. Wound healing defects were observed more commonly in the MMF/TAC group than in the TAC (5.2 %; OR 6.65; 95%CI=0.65 – 68.26; $p=0.11$) and CSA groups (10.5 %, OR 3.17; 95%CI = 0.49 – 20.56; $p=0.23$).

There was a higher rate of hypertension requiring treatment in the MMF/TAC group (68.4%) versus 42.1 % in TAC and 42.1% in the CSA group (OR 3.06; 95%CI = 0.77–12.25; $p=0.11$).

31.6% of the patients in MMF/TAC group needed ongoing therapy with diuretics post discharge from the hospital; compared to 5.2 % in TAC (OR 0.14; 95%CI = 0.014 – 1.25; $p=0.08$) and 15.8 % in CSA (OR 0.43; 95%CI = 0.09 – 2.02; $p=0.29$). (Figure 3)

One case of PTLD was diagnosed in the TAC group 5 months after LTX, two patients in TAC group developed new onset of insulin dependent diabetes after LTX. Conditional logistic regression could not be performed due to low case numbers.

DISCUSSION

Our study is the first to compare de novo CNI-reduced immunosuppression combined with MMF to other immunosuppressive regimes after pediatric liver transplantation in an age- and diagnosis-matched cohort study. There are only few pediatric studies comparing different immunosuppressive regimens most of which have not verified the CNI reduction thoroughly. The most important limitation of our study is the minor reduction of Tacrolimus in combination therapy with MMF, so that a mean level reduction of 0.7 µg/l instead of aspired 2.0 µg/l was achieved. With this kept in mind, we see a tendency towards Tacrolimus monotherapy with respect to safety especially regarding infectious complications without a disadvantage in renal function over the first year. so Our results support our clinical observations and should serve as groundwork to plan further prospective trials.

We saw no significant differences in patient and graft survival between the three groups.

Graft loss occurred secondary to vascular complications (2 hepatic artery thromboses and 2 portal vein thromboses) within two weeks of the initial transplant and were therefore considered unrelated to immunosuppressive regimen.

The lower rate of BPAR in the two groups using Tacrolimus compared to CSA is consistent with the current literature in pediatric and adult cohorts (24, 25). The overall rate of BPAR during the first year after LTX was rather low compared to other studies (24, 26), which may be attributable to the use of Basiliximab as part of our induction protocol.

The observed deterioration of renal function with subsequent stabilisation towards the end of our observation period is consistent with other studies (27). In addition to the nephrotoxicity associated with CNI usage, the initial decline in renal function post transplant may be contributed to by the use of diuretic agents (e.g., Furosemide), antibiotics such as Vancomycin and antiviral treatment with Ganciclovir. Regarding these drugs, the same protocol was used in all groups. Furthermore, it must be taken into account that low creatinine in children with chronic liver disease often reflects poor nutritional and muscular status rather than good kidney function. Thus, a reduction in Creatinine-GFR

may be contributed to by the increased muscle mass and subsequent rise of Creatinine after pediatric LTX. This also explains higher initial values for GFR calculated by creatinine compared to the calculation that also takes BUN and Cystatin C into account.

The three groups took a similar course over time without significant differences, which might be explained by an insufficient reduction of Tacrolimus in combination therapy and a comparably short observation period. However, Becker et al.(23) also did not find any difference in renal function between Tacrolimus monotherapy and TAC/MMF therapy in adult LTX patients. Our results stand in contrast to the observations of Tannuri (8) and Evans (10) who saw an improvement of renal function shortly following the commencement of MMF treatment post LTx. Notably, most of these patients received MMF monotherapy. Other studies documenting better renal function observed an improvement only after several years. The reduction of Tacrolimus might not be sufficient to notice a renal benefit; however, it is important to note that in all previous pediatric studies MMF was introduced in patients with CNI-induced renal dysfunction (8-10, 28, 29), whereas our patients received de novo MMF therapy.

We found a significantly lower risk of bacterial and mycotic septic episodes in the Tacrolimus monotherapy group compared to MMF combination therapy. A possible explanation again is the increased overall immunosuppression caused by the higher than aspired levels of Tacrolimus in the MMF/TAC group. , but Loinaz and colleagues also found an increased risk for bacterial infections in small bowel transplantation when MMF is added(30). Other studies however describe no significant differences in infection rates when using combination therapy with MMF(31).

Interestingly, the rate of EBV-reactivation was significantly lower for the two groups using Tacrolimus compared to the CSA group, while the rate of other viral infections was lowest for Tacrolimus monotherapy. This stands in contrast to the study by Cox (32), who described a higher rate of EBV infection in patients receiving Tacrolimus therapy compared to CSA. Few patients within the CSA group historically received immunoglobulins instead of Ganciclovir as viral prophylaxis which may account for

these differences, but the results also question the theory of over immunosuppression in the TAC/MMF group.

The higher rate in wound healing defects in the MMF/TAC group supports the study of Lopau (19) and stands in contrast to the study of Flechner (33).

The higher rate of antihypertensive treatment in TAC/MMF group probably is related to the higher overall immunosuppression, however, hypertension also is described as a possible adverse event of MMF.

The case of PTLD in TAC group could fortunately be brought to remission by reduction of immunosuppression. Other studies also found an increased risk for PTLD in pediatric LTX patients treated with Tacrolimus compared to cyclosporin (34). The rate of PTLD observed for Tacrolimus monotherapy (5.3%) is consistent with the literature (Cao et al: 6.9% (35)).

Patients who developed post transplant diabetes in TAC group were additionally treated with longterm low-dose steroids for underlying autoimmune disease.

The most important limitation of our study was the inability to achieve the aspired Tacrolimus reduction in the MMF/TAC group. Insufficient trough level adaption remains a key problem for clinicians that is not even mentioned or examined in many studies and should strictly be monitored in future trials. Further on, our study uses historical controls, opening the opportunity for time-dependent confounding factors.

The benefit for Tacrolimus monotherapy due to high efficacy and significantly lower rates of bacterial and viral infections at comparable renal outcome and the higher risk for cytopenia and gastrointestinal side effects when MMF is added is put into perspective by the minor Tacrolimus reduction which can explain the missing renal benefit whilst contributing to infectious complications. Despite the limitations, our study provides important information regarding the difficulties and side effects of early CNI and MMF combination therapy after pLTx. Pediatric studies so far have only examined MMF introduction

after CNI-induced renal impairment, to our knowledge this is the first pediatric study to assess the efficacy and safety of de novo MMF and Tacrolimus in liver transplant recipients. A prospective comparison of Tacrolimus and de novo MMF/Tacrolimus therapy ensuring the achievement of the aspired reduced trough levels in a RCT regarding renal function and infectious complications is desirable.

AUTHOR CONTRIBUTIONS

Christoph Leiskau: Study concept and design, Data acquisition and quality assessment, statistical analysis, drafting of the manuscript

Eva-Doreen Pfister: Critical revision of the manuscript for intellectual content

Imeke Goldschmidt: Critical revision of the manuscript for intellectual content

Norman Junge: Critical revision of the manuscript for intellectual content

André Karch: Contribution to statistical analysis, critical revision of the manuscript for intellectual content

Jeremy Rajanayagam: Critical revision of the manuscript for intellectual content

Christian Lerch: Contribution to statistical analysis, critical revision of the manuscript for intellectual content

Nicolas Richter: Critical revision of the manuscript for intellectual content

Frank Lehner: Critical revision of the manuscript for intellectual content

Harald Schrem: Critical revision of the manuscript for intellectual content

Ulrich Baumann: Study concept and design, critical revision of the manuscript for intellectual content

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FIGURE LEGENDS

Figure 1: Achieved Tacrolimus levels in comparison to target levels (TAC; MMF/TAC)

Figure 2: Course of calculated Creatinine-GFR (Schwartz) [ml/min/1.73m²]

Figure 3: Infectious complications [%]

Bact./myc. Inf.: bacterial or mycotic septic episodes; EBV: EBV reactivations; Viral inf.: Viral infections other than EBV

Figure 4: Rejections and other complications [%]

Wound healing def.: wound healing defects; Antihypert. treatm.: Antihypertensive treatment; Diur. treatm.: Diuretic treatment.