ORIGINAL ARTICLE

Safety and efficacy of direct oral anticoagulants under long-term immunosuppressive therapy after liver, kidney and pancreas transplantation

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SUMMARY

The safety of direct oral anticoagulants (DOACs) in patients after solid organ transplantation (SOT) is not well defined. This study aimed at describing the safety and efficacy of DOACs in patients after SOT. Patients after kidney and/or liver transplantation under maintenance immunosuppression treated with rivaroxaban ($n = 26$), apixaban ($n = 20$) and edoxaban ($n = 1$) were included. Clinical data were collected retrospectively and using a questionnaire. DOAC plasma levels and thrombin generation (TG) were measured in patients after SOT and compared with nontransplanted controls receiving DOACs. DOACs were administered for 84.6 patient-years. Mean immunosuppressive trough levels after DOAC initiation increased from baseline by 18.8 ± 29.6% compared to 3.0 ± 16.5% in matched controls ($P = 0.004$), without significant differences in dose adjustments. No transplant rejection or significant change in liver or renal function was observed. There was one major bleeding after the observation period but no thromboembolic complication. DOAC plasma levels reached the expected range in all patients. The intrinsic hemostatic activity in transplanted patients was higher compared to nontransplant controls. Treatment with DOACs after SOT is safe and effective. Immunosuppressive trough levels should be monitored after DOAC initiation, particularly in the early phase after SOT. These data should be confirmed in a prospective study.

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Key words
direct oral anticoagulants, DOAC, drug interactions, immunosuppression, solid organ transplantation

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Introduction

Patients who underwent solid organ transplantation (SOT) are at higher risk of developing cardiovascular complications such as atrial fibrillation (AF) and venous thromboembolism (VTE) compared to the general population [1,2]. The cumulative incidence of new onset AF within 36 months after kidney transplantation is 7.3% [3]. With the increasing age of recipients of kidney and liver transplantation, even more patients will require anticoagulation for AF in the future. In addition, VTE is a common complication of the transplant procedure, frequent hospitalization and the prothrombotic state of transplant recipients [2].

DOACs are increasingly used for the treatment of VTE and AF in the general population because of their fixed dosing, a wider therapeutic window and a lower frequency of intracranial bleedings compared to vitamin K antagonists (VKA). As patients after SOT were not included into the DOAC approval trials, prospective data on their safety in this cohort are not available.

Tacrolimus and cyclosporine (CsA) are extensively metabolized via CYP3A4 and are substrates of permeability glycoprotein (P-gp). In addition, CsA is a potent inhibitor of P-gp and inhibits the intestinal and hepatic CYP3A4 [4]. As DOACs are dependent on the P-gp pathway and partially metabolized via the cytochrome P450-system, interactions between these drugs and immunosuppressive agents may occur. Currently available data on the use of DOACs in patients after SOT are mainly case reports and small case series primarily reporting patients with AF after heart and lung transplantation [5]. AF is less frequent in patients after nonthoracic SOT, but other risk factors like impaired renal and liver function are more common [6]. Caution is warranted when using DOAC in SOT recipients due the limited clinical data on interactions with the immunosuppressive agents, fluctuations in renal function and polypharmacy with concomitant P-gp-inhibitors [7]. Therefore, VKA are still widely used in patients after SOT due to safety concerns.

The aim of our study was to describe the real life experience on the use of DOACs after SOT, with special emphasis on in vivo drug–drug interactions with the immunosuppressive therapy, side effects and patients‘ convenience. In addition, DOAC plasma levels, D-Dimer and thrombin generation parameters were analyzed and compared with transplanted patients receiving VKA and nontransplant patients receiving DOACs.

Methods

This is a two-part noninterventional observational study of patients who underwent liver, kidney, pancreas or combined transplantation receiving oral anticoagulation. The retrospective part characterizes changes in the levels of immunosuppressive agents after the initiation of anticoagulation and describes side effects and thromboembolic complications, while DOAC plasma levels and hemostatic parameters were measured in the prospective part.

Retrospective part

For the retrospective part, all patients registered in the outpatient clinic of the University Transplant Centre Leipzig were screened and patients with documented use of DOACs between September 2015 and September 2018 were included. Data on acute or chronic transplant rejection, dose adjustments of immunosuppressive therapy after DOAC initiation, liver and renal parameters, thromboembolic and bleeding complications were collected from patients' records. Trough levels of cyclosporine, tacrolimus, everolimus and sirolimus before and after DOAC initiation were analyzed. Levels of mycophenolic acid compounds were not analyzed because they were not considered to be potentially influenced by DOAC therapy and the dosage is not level-dependent in our clinical practice. Changes in the trough levels in patients taking DOACs were compared with a cohort of transplanted patients 1:1 matched regarding sex, age (<35; 35–65; >65 years), time after transplantation (1–3; 3–12, 12–60 and > 60 months), immunosuppressive agent (tacrolimus, cyclosporine, everolimus, sirolimus) and time between the two measurements of the immunosuppressive trough levels (1, 3, 6 and > 9 months).

Data from patients who died between 2015 and 2018 were screened for the use of DOACs and analyzed for complications related to DOAC therapy including the cause of death.

Prospective part

Out of the total of 1138 patients after SOT since 1996 who were actively followed up between September 2015 and January 2018, 47 patients receiving DOACs were identified. During the same period 54 patients were treated with VKA. Among those 47 DOAC patients, 45 were scheduled for an upcoming visit from January until September 2018 and were eligible for the

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prospective part of this study. Patients were asked to complete a questionnaire on thromboembolic and bleeding complications. DOAC plasma levels, D-Dimer and thrombin generation (TG) parameters were measured in patients receiving DOACs, while the international normalized ratio (INR) and D-Dimer were measured in patients on VKA. Apixaban trough levels were drawn 10–14 h, rivaroxaban trough levels 22–26 h and peak plasma levels 3–4 h after DOAC intake. DOAC plasma levels and TG parameters in patients after SOT receiving 1 × 20 mg rivaroxaban, 2 × 2.5 mg apixaban or 2 × 5 mg apixaban were compared with data from nontransplant patients from the hemostasis outpatient clinic of the University Hospital of Leipzig receiving the same dose. The control group consisted of patients on stable anticoagulation for either VTE or AF coming for a routine check-up between August 2017 and January 2019. According to our institutional protocol, DOAC peak and trough plasma levels were measured once in all patients under long-term treatment with DOAC.

The study design is shown in Fig. 1.

Questionnaire

This questionnaire (Table S1) was completed by the patients together with the hemostaseologist to ensure accuracy. To limit recall bias, the questionnaire included only data on bleeding and thrombotic complications occurring within the last 6 months. Major bleedings were defined according to the ISTH criteria [8], while clinically relevant no major bleedings (CRNMB) were defined as bleedings leading to surgical intervention and / or hospitalization. All other bleedings were counted as minor bleedings. Patients were asked about invasive procedures, bridging with low molecular weight heparin (LMWH), postoperative bleedings and wound healing complications.

Laboratory measurement

Blood samples were collected in citrated vacuum containers via venipuncture. No indwelling catheters were used. Blood was centrifuged at 4000 rpm (1800 *g*) for 20 min to prepare platelet-poor plasma (PPP) and stored at −80°C until analysis. DOAC plasma levels were measured using the Innovance anti-Xa assay (Siemens Healthineers, Germany) and calibrated against apixaban and rivaroxaban with STA Apixaban and STA Rivaroxaban calibrators (Diagnostica Stago, France). D-dimer levels were determined by the particle-enhanced immunoturbidimetric assay Innovance D-Dimer (Siemens Medical Solutions) on the Behring Coagulation System analyzer. TG was assessed using the Calibrated Automated Thrombogram (CAT) (Diagnostica Stago, France) with commercially available test kits according to the manufacturer's instructions on a Fluoroskan Ascent (ThermoLabsystems OY, Helsinki, Finland) at 360/460 nm wavelength. CAT was carried out using 5 pM tissue factor (TF) as a final concentration. The thrombin generation parameters lag time (LT), endogenous thrombin potential (ETP), peak thrombin and

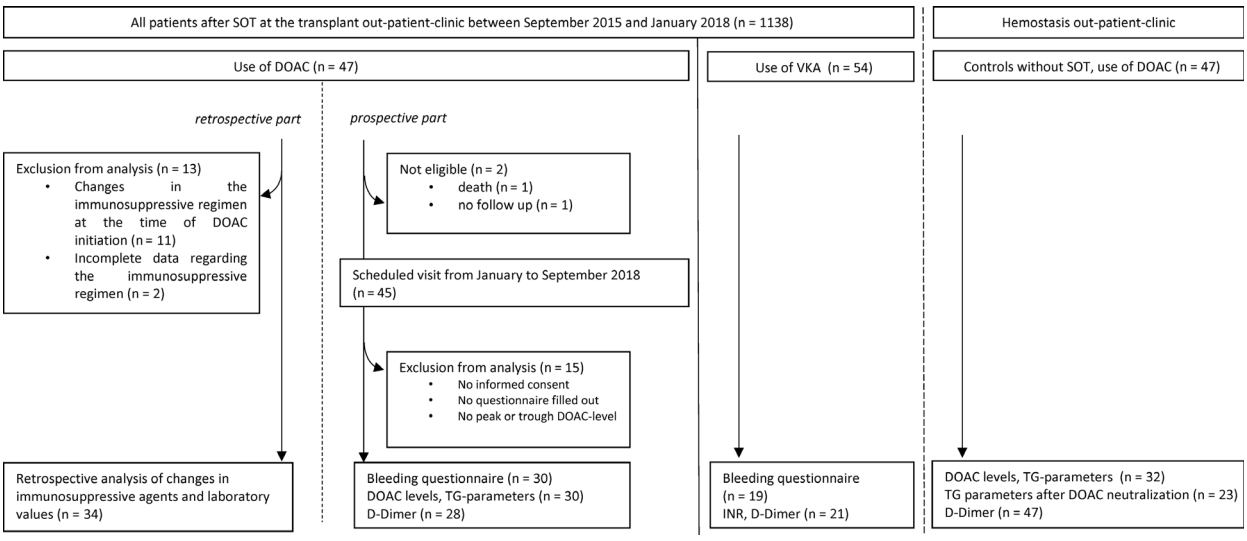

Figure 1 Study design. Abbreviations: DOAC, direct oral anticoagulant; VKA, vitamin K-antagonist; TG, thrombin generation; SOT, solid organ transplantation.

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time to peak (TTP) were measured and velocity index (VI) calculated. To investigate the intrinsic hemostatic activity, DOAC activity was neutralized ex vivo by incubating plasma samples with charcoal at a final concentration of 5 mg/ml.

Statistical analysis

Statistical analysis was performed using the software SPSS version 24 (SPSS Inc, Chicago, IL, USA). Continuous variables were displayed as mean ± standard deviation (SD) in case of normally distributed data or as median with range in brackets (simple range or interquartile range [IQR]) for not normally distributed data. Categorical variables are shown with frequency (%) and compared using Fischer´s exact test. Binary logistic regression was performed to test for factors associated with bleeding events. *P*-values < 0.05 are considered statistically significant.

Ethical considerations

The study was approved by the Ethics committee of the University of Leipzig (reference 163-17-ek) and conducted according to the declaration of Helsinki. Informed consent was obtained from all participants before inclusion into the prospective part of the study and from the nontransplant controls.

Results

Patient characteristics

Of the 47 patients on DOAC therapy, 23 had undergone liver transplantation, 19 kidney transplantation and five had a combined SOT. Median age in this cohort at the time of DOAC initiation was 65 (range 32–80) years. Most patients received a calcineurin inhibitor (CNI) containing immunosuppressive regimen (tacrolimus $n = 32$, cyclosporine $n = 9$), either as monotherapy ($n = 8$), or combined with mycophenolate mofetil ($n = 23$) or combined with inhibitor of mammalian target of rapamycin (mTORi, $n = 10$). Monotherapy with mTORi was administered in four patients, while mTORi was combined with mycophenolate mofetil in two patients.

The most frequent indications for DOAC therapy were AF ($n = 25$), VTE ($n = 16$) and portal vein thrombosis (PVT) ($n = 5$). DOACs were administered for a median of 17.3 months (84.6 patient-years).

A total of 26 patients were treated with rivaroxaban, 20 with apixaban and one patient with edoxaban. Rivaroxaban dose was reduced to 15 mg once daily in 16 (61.5%) patients due to impaired renal function. Apixaban dose was reduced to 2.5 mg twice-daily in 10 (50.0%) patients. Reasons for dose reduction of apixaban were renal impairment in 6 patients, extended prophylaxis after VTE in one patient and extended prophylaxis after PVT in three patients, with concomitant thrombocytopenia in two of them. All other patients were treated with standard therapeutic doses of DOAC. Standard therapeutic doses were 1 × 20 mg rivaroxaban, 2 × 5 mg apixaban and 1 × 60 mg edoxaban.

The mean glomerular filtration rate (GFR) was higher in patients after liver compared to kidney transplantation (57.0 ± 22.0 ml/min vs. 41.0 ± 17.8 ml/min, $P = 0.014$), but there was no difference in the proportion of patients with chronic kidney disease (CKD) stage 3 and 4 (liver 15/24, kidney 19/23, $P = 0.193$). Patient's characteristics are shown in Table 1.

Retrospective part

Safety

During the observation period from 2015 to 2018, 95 patients died after transplantation, of whom 7 (7.3%) patients were on oral anticoagulation (DOAC $n = 5$, phenprocoumon $n = 2$). The causes of death in these patients were not related to anticoagulation. No patient withdrew anticoagulation due to adverse events. No graft rejection or relevant increase of ALT was observed in any patient on DOAC therapy during the observation period. The median time interval between the last visit before and the first visit after DOAC initiation was 66 (IQR: 36–97) days. The mean change of ALT between these two visits was −18 ± 78 IU/l, while the mean change in GFR was −1.1 ± 6.7 ml/min in all patients and −2.2 ± 7.7 ml/min in the subgroup of patients after kidney transplantation (kidney alone or in combination).

There was no thromboembolic event under anticoagulation and we did not observe any major bleeding event during the observation period. One patient died in another hospital two months after the observation period due to a cerebral hemorrhage. This patient was listed for a third liver transplantation because of transplant cirrhosis Child-Pugh B. He was treated with apixaban for PVT, but with a reduced dose of 2 × 2.5 mg due to a concomitant thrombocytopenia (47×10^9/l). The apixaban plasma levels were within the low expected range (trough level 20 ng/ml, peak level 32 ng/ml, measured 11 months before the event).

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Table 1. Characteristics of 47 patients after solid organ transplantation at the time of initiation of DOAC therapy.

Demographics	Age (median, range)	65 (34–81) years
	Male gender	29 (61.7%)
Type of organ transplant, *n* (%)	Kidney	19 (40.4%)
	Liver	23 (48.9)
	Combined	5 (10.7%)
	Kidney + liver	2 (4.3%)
	Kidney + pancreas	2 (4.3%)
	Liver + pancreas	1 (2.1%)
Immunosuppression, *n* (%)	CNI-Monotherapy	8 (17.0%)
	CNI + MMF	23 (49.0%)
	CNI + mTORi	10 (21.3%)
	mTORi Monotherapy	4 (8.5%)
	mTOR + MMF	2 (4.3%)
Indication for DOACs, *n* (%)	Atrial fibrillation	25 (53.2%)
	Venous thromboembolism	16 (34.0%)
	Deep vein thrombosis	11 (23.4%)
	Pulmonary embolism	5 (10.6%)
	Portal vein thrombosis	5 (10.6%)
	Thrombosis of cardiac bypass	1 (2.1%)
DOACs, *n* (%)	Rivaroxaban	26 (55.3%)
	Apixaban	20 (42.6%)
	Edoxaban	1 (2.1%)
Intervals	Time between transplantation and initiation of DOAC (median, range)	85 (2–224) months
	DOAC exposure (median, range)	17.3 (3.1–52.0) months
	Cumulative DOAC exposure in all patients	1015 months (84.6 patient-years)
Laboratory values at DOAC initiation	Platelet count (median, range)	211 (42–360) Gpt/l
	Thrombocytopenia (<150 Gpt/L)	9 (19.1%)
	ALT at initiation of DOAC (mean, SD)	0.63 ± 0.2 μkat/l
	GFR at initiation of DOAC (mean, SD)	49.7 ± 20.7

CNI, calcineurin inhibitor; MMF, Mycophenolate mofetil; mTORi, inhibitor of mammalian target of rapamycin; DOAC, direct oral anticoagulant; ALT, alanine amino-transferase; GFR, glomerular filtration rate; SD, standard deviation.

Changes in immunosuppressive therapy after initiation of DOAC therapy

Data regarding changes in the immunosuppressive therapy after DOAC initiation were available for 34 patients (rivaroxaban, $n = 17$; apixaban, $n = 16$; edoxaban, $n = 1$), the median interval between DOAC initiation and the first measurement of the immunosuppressive drug level was 51 (range 2–228) days.

In patients receiving combined therapy with CNI and mTORi ($n = 7$), changes in both substances were analyzed separately (tacrolimus $n = 20$, cyclosporine $n = 8$, everolimus $n = 9$, sirolimus $n = 4$). The mean change of the immunosuppressive drug level after DOAC initiation was + 18.8 (± 29.6)%, without significant differences between the immunosuppressive agents. In the matched control cohort the increase in the trough level was significantly lower (+3.0 ± 16.5%, $P = 0.004$), Fig. 2. The level of tacrolimus increased by 22% after initiation of edoxaban ($n = 1$). There was no difference in the change of the immunosuppressive drug levels when DOAC was started early (<18 months, $n = 9$) compared to late (>18 months, $n = 32$) after transplantation (23.7 ± 37.4% vs. 17.5 ± 27.5%, $P = 0.649$).

The levels of the immunosuppressive drugs were within the therapeutic range in all DOAC patients. The change in the trough level of immunosuppressive agents was considered clinically relevant if a dose adjustment was necessary. This occurred in 10 (24.4%) DOAC patients and in 5 (12.2%) controls ($P = 0.25$). Changes in all immunosuppressive drug levels as well as the proportion of patients with dose adjustments were not different between the immunosuppressive agents (Table 2), and between rivaroxaban (5/17) and apixaban (5/16).

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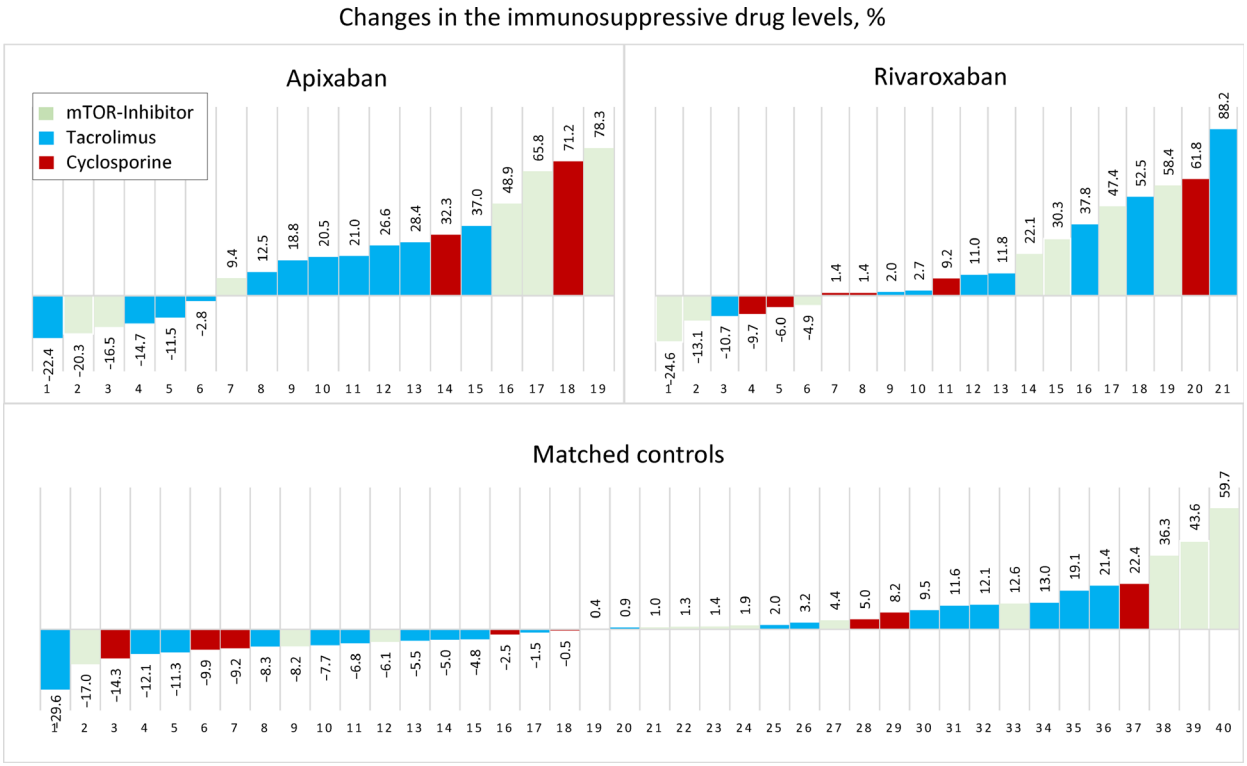

Figure 2 Changes in the immunosuppressive drug levels after the initiation of direct oral anticoagulants.

Table 2. Change of the trough level of immunosuppressive agents and dose adjustments after the initiation of DOAC.

		Change in immunosuppression level (%)			Dose adjustment	
Immunosuppressive agent	Number of samples	Mean	SD	*P*	*n*, %	*P*
Cyclosporine A	*n* = 8	20.2	31.4	*P* = 0.78	1 (12.5%)	*P* = 0.38
Tacrolimus	*n* = 20	16.5	25.8		6 (30.0%)	
Everolimus	*n* = 9	16.5	33.5		2 (22.2%)	
Sirolimus	*n* = 4	33.2	43.3		1 (25.0%)	

IS, immunosuppression; SD, Standard deviation.

Prospective part

Bleeding questionnaire

The bleeding questionnaire was completed by 30 patients treated with DOACs (rivaroxaban *n* = 15, apixaban *n* = 15). Minor bleedings were reported by 17 patients (56.6%) and were more frequent under rivaroxaban (12/17, 70.6%) compared to apixaban (5/17, 29.4%, *P* = 0.04). CRNMB were reported by 3/30 (10%) patients. All patients had epistaxis and one patient concomitant hematuria due to cystic renal degeneration.

Eight patients underwent surgical interventions. Anticoagulation was bridged postoperatively with LMWH in five patients (inguinal hernioplasty, incisional hernioplasty, hip prosthesis, vulva resection, knee prosthesis), discontinued without bridging in two patients (tooth extraction, lymph node extirpation) and continued during a minor wrist surgery in one patient. No extended bleeding or wound healing complications were reported.

The questionnaire was also completed by 19 patients using the VKA phenprocoumon. Minor bleedings were reported by 17 (89.5%) patients. This was comparable to the rivaroxaban group (*P* = 0.634). There were no clinically relevant bleeding episodes in the VKA group. One surgery

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was reported among patients on phenprocoumon (hemorrhoidal resection), without complications under bridging with LMWH. The INR values were available in all patients treated with phenprocoumon ($n = 19$) and were within the therapeutic range in 17 (89.5%).

There were no arterial or venous thromboembolic events. The results of the questionnaires are summarized in Table 3.

The role of renal and liver function and type of transplantation on bleeding events. The mean glomerular filtration rate (GFR) was higher in patients after liver compared to kidney transplantation (57.0 ± 22.0 ml/min vs. 41.0 ± 17.8 ml/min, $P = 0.014$), but there was no difference in the proportion of patients with chronic kidney disease (CKD) stage 3 and 4 (liver 15/24, kidney 19/23, $P = 0.193$). There was a trend toward a lower proportion of minor bleeding events in liver transplant recipients (liver 5/14, kidney 12/16, $P = 0.063$). The mean GFR in patients reporting minor bleeding events was not different between kidney and liver transplant recipients (42.1 ± 16.3 ml/min vs. 42.6 ± 11.2 ml/min, $P = 0.991$) and between patients reporting minor bleedings and patients without minor bleedings (42.1 ± 14.6 ml/min vs. 50.3 ± 25.2 ml/min, $P = 0.306$). There was no difference in the mean GFR between rivaroxaban and apixaban treated patients (47.8 ± 17.9 ml/min vs. 50.5 ± 25.6 ml/min, $P = 0.695$), and in the proportion of patients on rivaroxaban after kidney compared to liver transplantation (9/16 vs. 6/14, $P = 0.72$). Transaminases were elevated in three patients to a maximum of twice the upper limit of normal but only in one patient with an available bleeding questionnaire. Transaminases were not different between patients with or without minor bleedings.

In multivariate analysis including GFR, type of DOAC (apixaban vs. rivaroxaban), type of transplantation (liver vs. kidney) and immunosuppressive agents (CNI vs. mTORi vs. CNI + mTORi) only anticoagulation with rivaroxaban (OR 9.2 [95% CI: 1.4–60.0], $P = 0.020$) and kidney transplantation (OR 6.4 [95% CI: 1.0–40.0], $P = 0.050$) were independent predictors of minor bleeding events. After exclusion of patients on extended prophylaxis with a reduced DOAC dose, rivaroxaban (OR 12.4 [95% CI: 1.2–128.6], $P = 0.035$) and kidney transplantation (12.3 [95% CI: 1.2–125], $P = 0.035$) persisted as predictors for minor bleeding events.

DOAC levels and thrombin generation parameters

All DOAC levels were within the expected range for the applied dose according to the product information [9,10] DOAC plasma levels and TG parameters from patients after SOT receiving apixaban (2 × 5 mg, $n = 5$; 2 × 2.5 mg, $n = 9$) or rivaroxaban (1 × 20 mg, $n = 6$) were compared with data from nontransplant controls under stable anticoagulation with apixaban (2 × 5 mg, $n = 18$; 2 × 2.5 mg $n = 14$) and rivaroxaban (1 × 20 mg, $n = 14$). There was a significantly lower GFR in transplant patients compared to controls. Beside a higher apixaban trough level after intake of 2 × 5 mg in transplant patients there were no other differences in apixaban or rivaroxaban trough and peak levels in both cohorts. There was a shorter TTP in transplant patients receiving 2 × 5 mg apixaban at peak level ($P = 0.024$) and a trend toward a shorter TTP at trough levels ($P = 0.073$) compared to controls. ETP was higher in transplant patients receiving 2 × 2.5 mg apixaban compared to controls.

At peak level, there were higher plasma drug levels, peak thrombin and velocity index and a lower TTP in transplanted patients and in the nontransplant controls receiving rivaroxaban compared to those on apixaban. There was no difference in plasma drug levels and thrombin generation parameters at trough level between rivaroxaban and apixaban treated patients after SOT and controls (Table S2).

Data summarizing DOAC plasma levels and thrombin generation parameters are displayed in Table 4.

Intrinsic hemostatic activity

An ex vivo neutralization of the DOAC activity with charcoal was performed in 30 patients after SOT and in 23 nontransplant controls receiving DOACs. There was a significantly shorter lag time and TTP and a significantly higher thrombin peak in transplant patients compared to the control group, but no difference in ETP (Table 5).

D-Dimer in patients after SOT and controls

D-Dimers were measured in 49 patients after SOT (28 on DOAC, 21 on phenprocoumon) and in 47 nontransplant controls receiving DOACs. There was no difference in D-Dimer levels between transplant patients and controls (0.64 [95% CI: 0.46–0.81] vs. 0.52 [95% CI: 0.30–0.72], $P = 0.378$) in the whole cohort and in the subgroup of 28 patients after SOT and 40 control patients receiving DOACs (0.78 [95% CI: 0.53–1.03] vs. 0.55 [95% CI: 0.30–0.81], $P = 0.210$). There was a trend toward a higher D-Dimer level in patients after SOT receiving DOACs compared to those on phenprocoumon

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Table 3. Summary of bleeding complications according to the patient questionnaire.

Patient questionnaire	Rivaroxaban, *n* = 15		Apixaban, *n* = 15*		Phenprocoumon, *n* = 19	
Minor bleedings	*n* (%) 12 (80.0%)	Frequency per month (median, range)	*n* (%) 5 (33.0%)	Frequency per month (median, range)	*n* (%) 17 (89.5%)	Frequency per month (median, range)
Spontaneous bleedings including spontaneous hematomas	5 (33.3%)	2.0 (0.5–4.0)	1 (6.7%)	1.0	2 (10.5%)	1.0 (1.0–1.0)
Hematoma after minor injury	9 (60.0%)	2.0 (0.5–4.0)	5 (33.3%)	2.0 (0.5–8.0)	12 (63.2%)	2.0 (0.5–2.0)
Epistaxis	5 (33.3%)	0.5 (0.2–4.0)	4 (26.7%)	0.8 (0.2–2.0)	6 (31.6%)	1.0 (0.2–4.0)
Gingival bleeding	2 (13.3%)	0.6 (0.2–1.0)	0	-	1 (5.3%)	2.0
Prolonged bleeding after cutting injury	7 (46.7%)		2 (13.3%)		6 (31.6%)	
Melena	0		0		0	
Clinically relevant bleeding	2 (13.3%)		1 (6.7%)		0	
Localization of bleeding?	Hematuria, Epistaxis		Epistaxis		-	
Blood transfusion	No		No		-	
Surgery	5		3		1	
Bridging with LMWH	2		3		1	
Discontinuation of oral anticoagulation	2		0		0	
Extended bleeding after surgery	0		0		0	
Menorrhagia	0/2†		1/2†		0/1†	
Other bleedings	1 (Hematuria)		0		2 (Hemorrhoidal bleeding, Colitis)	
Wound healing complications	3 (20%)		3 (20.0%)		2 (10.5%)	

*4 patients on apixaban received extended prophylaxis, one of these patients reported minor bleedings.

†Number of premenopausal female patients.

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Table 4. Plasma levels of direct oral anticoagulants, glomerular filtration rate and thrombin generation parameters of patients after solid organ transplantation and in nontransplanted controls.

		Apixaban 2 × 5 mg					Apixaban 2 × 2.5 mg					Rivaroxaban 1 × 20 mg				
		Trough			Peak		Trough			Peak		Trough			Peak	
		SOT	Control	*P*	SOT	Control	*P*	SOT	Control	*P*	SOT	Control	*P*	SOT	Control	*P*
	n	5	13		6	18		9	14		9	14		4	10		5	14	
GFR	mean	54	82	0.013	54 (33–75)	75	*0.022*	39	81	*0.003*	39	76	*0.003*	57	81	*0.042*	56	834	*0.013*
	(95% CI)	(33–75)	(71–92)			(61–89)		(20–59)	(70–92)		(20–59)	(62–89)		(33–80)	(67–95)		(40–72)	(69–98)	
DOAC	mean	76	41	0.004	190 (145–234)	163	0.280	44†	34	0.268	92	67	0.172	67	59	0.945	318	338	0.754
level	(95% CI)	(65–88)	(30–53)			(121–205)		(28–60)	(22–46)		(62–122)	(43–92)		(–26–161)	(23–95)		(180–457)	(276–400)	
TTP	mean	5.6	8.2	0.073	6.7* (6.1–7.3)	9.8	*0.024*	5.7	6.5	0.194	6.6	7.4	0.132	6.4	8.4	0.260	11.7	18.8	0.260
	(95% CI)	(5.4–5.8)	(5.3–11.1)			(7.6–12.0)		(5.0–6.4)	(5.5–7.4)		(5.9–7.3)	(6.5–8.3)		(2.7–10.1)	(6.5–10.2)		(6.9–16.4)	(14.3–23.3)	
Peak	mean	287	289	0.775	198* (158–237)	190	0.638	312	364	0.189	252	263	0.718	258	257	1.00	95	103	0.754
	(95% CI)	(225–348)	(222–356)			(153–227)		(264–360)	(293–434)		(213–292)	(213–313)		(104–413)	(222–292)		(57–134)	(76–130)	
ETP	mean	1752	2230	0.117	1605*	1983	0.150	1964	2516	*0.009*	1848	2198	*0.037*	1995	2280	0.330	1459	1620	0.343
	(95% CI)	(1303–2203)	(1886–2575)		(1178–2033)	(1725–2240)		(1646–2282)	(2223–2808)		(1603–2093)	(1948–2449)		(1520–2470)	(2011–2549)		(1076–1842)	(1295–1946)	
VI	mean	110	98	0.336	68* (54–81)	60	0.403	118	137	0.423	90	82	0.571	87	69	0.604	21	13 (8–17)	0.257
	(95% CI)	(82–138)	(65–132)			(45–75)		(94–143)	(93–180)		(71–109)	(61–104)		(11–163)	(49–89)		(2–39)		

DOAC, direct oral anticoagulant; SOT, solid organ transplantation; GFR, glomerular filtration rate; LT, lag time; TTP, time to peak; Peak, peak thrombin; ETP, endogenous thrombin potential; VI, velocity index.

*$n = 5$.

†$n = 8$.

(0.78 [95% CI: 0.53–1.03] vs. 0.45 [95% CI: 0.28–0.68], $P = 0.053$).

Discussion

This observational study shows that DOACs can be safely administered together with CNIs and mTORi in patients after SOT. All DOAC levels were within the expected range in all patients after SOT. We have found a mild increase in the trough level of the immunosuppressive agents with no difference in patients treated with rivaroxaban or apixaban or between the immunosuppressive agents used. Except for some recent expert opinions [11], there are still no guidelines for using DOACs in this patient population.

Apixaban, rivaroxaban and edoxaban are substrates of the drug efflux transporter P-gp and breast cancer resistant protein (BCRP) and are partially metabolized via CYP3A4, which might cause drug–drug interactions (DDI) with CNI and mTORi. Patients with concomitant use of potential inhibitors or inductors of P-gp and CYP3A4 substrates (such as CNI and mTORi) were excluded from the DOAC approval trials, and there are no trials directly investigating the pharmacokinetics of DOACs in SOT recipients. In addition, many other factors influence the trough levels of the immunosuppressive medication in transplant patients, such as changes in gastric pH, hepatic and bile flow and gastrointestinal motility [12,13]. Some of these confounders have the highest effect in the first phase after transplantation [14]. Since the mean time span between transplantation and DOAC prescription in our cohort was seven years, the effect of these confounders should be limited.

We have observed a mean increase in the immunosuppressive drug level of 18.8% after DOAC initiation in our cohort. This was independent of the DOAC as well as of the immunosuppressive agent used. This finding is in line with reports for rivaroxaban, describing an increase of 9.2% and 12.7% in tacrolimus and cyclosporine levels, respectively [15] and was significantly higher than in the matched control cohort (3.0%, $P = 0.004$). This confirms the trend of increasing trough levels of the immunosuppressive agents after DOAC initiation. The rate of immunosuppressive dose adjustments following DOAC initiation in our cohort was 24.4%, compared to 12.2% in the controls ($P = 0.25$), suggesting lower clinical relevance of DDI in stable patients taking DOACs in the later phase after transplantation. Only two patients were started on DOAC less than one year and only 9 patients less than 18 months after transplantation in our cohort, making

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Table 5. Thrombin generation parameters of patients after solid organ transplantation and controls after DOAC neutralization with charcoal

	Patients after SOT, $n = 30$ mean (95% CI)	Controls, $n = 23$ mean (95% CI)	*P*
Lag time	1.74 (1.57–1.90)	4.76 (4.07–5.44)	<0.001
Time to peak	3.83 (3.63–4.02)	8.74 (7.69–9.79)	<0.001
Peak thrombin	414 (385–443)	246 (209–282)	<0.001
ETP	1983 (1814–2151)	2002 (1794–2209)	0.800
Velocity index	201 (185–217)	70.6 (55.6–85.7)	<0.001

SOT, solid organ transplantation; ETP; endogenous thrombin potential.

a final conclusion on the safety of DOACs in the early phase not feasible. However, based on the results obtained from the later phase after transplantation, we recommend monitoring trough levels of the immunosuppressant after DOAC initiation, especially in the early phase after transplantation, when higher immunosuppressant levels are targeted and therefore a further increase might be toxic.

Apart from the potential influence on the immunosuppressive drug levels, we found that all DOAC plasma levels in our patient population were within the expected range. In a recent study in healthy volunteers, the AUC of apixaban increased by 20% after co-administration of CsA while the AUC decreased by 22% after tacrolimus [16]. An increase in the AUC of rivaroxaban by approximately 1.4 was demonstrated for the combination with fluconazole or erythromycin (both P-gp and moderate CYP3A4 inhibitors with comparable characteristics to CsA) [17]. This effect seems to be more pronounced in patients with moderate renal impairment [18]. The mean GFR in our cohort was 49.7 ml/min/1.73 m^2 and 22 (46.8%) patients were on a reduced DOAC dose due to moderate renal impairment, but we found no significant alterations in the DOAC plasma levels.

Although the use of mTORi is increasing, particularly in patients transplanted for hepatocellular carcinoma [19] and in those suffering from CNI adverse events [20], there are very few published data regarding interactions between mTORi and DOAC. Sirolimus shows the same pattern of inhibition of CYP3A4 and P-gp as tacrolimus in healthy volunteers [21]. In a recent retrospective analysis of 42 patients receiving DOACs after kidney transplantation, only four patients on both sirolimus and apixaban were included. No changes in tacrolimus levels were observed three days after DOAC initiation, but changes in the sirolimus trough levels were not reported [22]. In our cohort, trough levels of mTORi increased by a mean of 21 $\pm$ 35.8% and the rate of mTORi dose adjustment was not different to that of CNI.

Patients after kidney transplantation had a lower GFR and a trend toward more minor bleeding events compared to liver transplant recipients. The GFR was not significantly different between patients with and without minor bleeding events, which might be caused by the rather low number of patients included into our analysis. However, the results of the multivariate analysis suggest, that kidney transplant recipients may have an increased risk of bleedings independent of renal function and the type of immunosuppression.

In addition, the incidence of minor bleedings was lower in patients on apixaban (33.0%) than in those on rivaroxaban (80.0%) while it was 89.5% in patients on phenprocoumon. The reason for this relatively high number of bleeding episodes was because even hematomas were counted as minor bleeding events. Most interestingly, the GFR in patients treated with rivaroxaban and apixaban was comparable and there was no difference in the proportion of patients treated with rivaroxaban after kidney compared to liver transplantation. In addition, rivaroxaban was associated with an increased risk of minor bleedings in multivariate analysis. The lower incidence of minor bleedings with apixaban compared to that with rivaroxaban persisted even after the exclusion of patients on extended prophylaxis with apixaban (36.4% vs. 80.0%). The fact that apixaban is associated with a lower incidence of bleeding events compared with rivaroxaban is known from recent meta-analysis [23,24], but there is limited evidence about bleeding events in patients after SOT treated with DOACs. Ambrosi and colleagues reported a small case series of 11 rivaroxaban treated patients after heart transplantation, with two patients with severe renal

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impairment having an overdose leading to blood transfusion in one patient. In another retrospective study analyzing 37 patients mainly on rivaroxaban, seven minor bleeding events, one major bleeding and two breakthrough VTEs were reported during a median exposure time of 118 days. DDI were not found to be causative for the bleeding events (26.0% in patients with DDI vs. 7.1% without DDI, $P = 0.154$) which might be caused by the limited number of patients included [25].

There were only three CRNMBs in our cohort (two with apixaban and one with rivaroxaban). The only major bleeding happened in a patient with re-cirrhosis of the liver Child-Pugh grade B, thrombocytopenia and stable renal insufficiency (GFR 40 ml/min) on an extended prophylaxis with apixaban for PVT. Apixaban plasma levels measured eleven months before the hemorrhage were in the low expected range. These patients represent a major clinical challenge, as patients with Child-Pugh B cirrhosis and thrombocytopenia were not included into the clinical trials and DOACs are not approved for the treatment of PVT. However, the use of VKA is associated with an even higher risk for fatal and intracranial hemorrhage [26] and LMWH are inconvenient for long-term treatment and associated with a trend toward a higher intracranial bleeding risk in cancer patients [27]. Intagliata and colleagues reported a similar bleeding risk for traditional anticoagulants (LMWH and VKA) and DOACs in patients with liver cirrhosis [28]. Current expert opinions suggest the use of DOACs in patients with liver cirrhosis but with a restriction to patients with compensated cirrhosis (Child A or early Child B) and with platelet counts $> 50 \times 10^9$/l [29,30]. Our data indicate, that apixaban, particularly at a reduced dose is associated with a low bleeding risk. Nevertheless, the indication should be discussed on an individual basis.

All patients in our study had DOAC levels within the expected range. At standard dose, apixaban but not rivaroxaban trough levels were higher in the SOT cohort compared to controls, which might be explained by the lower GFR in transplant patients and the twice-daily application of apixaban. However, this seems to be of low relevance as the bleeding tendency in patients receiving apixaban was lower compared with rivaroxaban in our cohort. In addition, we were able to show that the thrombin generation parameters LT and TTP were prolonged and peak thrombin and VI decreased in patients on rivaroxaban compared to apixaban treated patients at peak plasma levels. This effect was observed in our limited number of patients after SOT and in our controls which is similar to findings from healthy volunteers [31] and shows that our data might be representative despite the limited number of patients included. In addition, these data may explain the higher incidence of minor bleeding events in rivaroxaban treated patients because lower peak thrombin and longer lag time are associated with a higher probability of bleedings. This suggests, that patients with a high bleeding risk might rather be treated with apixaban than with rivaroxaban and in eligible patients the dose should be reduced to an extended prophylaxis. Unfortunately, a direct comparison of thrombin generation parameters in patients receiving DOACs and VKA is not feasible because of the different pharmacokinetics and the different behavior after TF stimulation in the CAT system [32]. Only the slightly lower D-Dimer level in VKA-treated patients gives a hint, that especially high-risk patients receiving VKA might have a better protection against thrombotic events, similar to that known from other high-risk entities like the antiphospholipid syndrome [33].

Interestingly, after *ex vivo* neutralization of DOAC activity, we have observed a shorter lag time and TTP and a higher thrombin peak and velocity index in transplant patients than in controls, which may be a sign of a more thrombophilic state. It remains unclear whether this is caused by the transplantation itself or a sequel of the underlying disease or an effect of the immunosuppressive medication. Nevertheless, available data indicate that patients after SOT are at high risk for thromboembolic complications [1,2].

Our study has several limitations. Data regarding DDI were collected retrospectively and patients were not treated by only one physician. Differences in the dose adjustment might have occurred between physicians, although a standard procedure for monitoring transplant patients is established in our hospital. However, the fact that the increase in the dose of the immunosuppressive agents was lower in the control cohort suggests that a close monitoring might be necessary especially in the early phase after transplantation. In addition, our data regarding DOAC plasma levels and thrombin generation parameters are limited by the small numbers of patients included, but we observed no major deviations from the control group although the transplanted patients had an impaired renal function.

Conclusion

Treatment with rivaroxaban and apixaban was safe and effective in our patients after liver and kidney transplantation. No major adjustments of immunosuppression

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were required and we did not observe relevant toxicities or adverse events. Although these data are encouraging, more data with more patients including other DOACs are needed.

Authorship

CP and AH: designed the study, collected and analyzed data and wrote the manuscript. AW: collected data, read and revised the manuscript. RS: performed laboratory analysis, read and revised the manuscript. CE, NA and DS: read and revised the manuscript. TB: designed the study, read and revised the manuscript. SP: designed the study, read and revised the manuscript.

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Conflicts of interest

Christian Pfrepper received speaker honoraria from BMS and Pfizer and worked as a medical advisor for Pfizer, Adam Herber received speaker honoraria from Pfizer, Cornelius Engelmann worked as a medical advisor for Novartis. The other authors have no conflicts of interest to declare.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Questions of the bleeding questionnaire.

Table S2 Plasma levels of direct oral anticoagulants, glomerular filtration rate and thrombin generation parameters of patients after solid organ transplantation and in non-transplanted controls.

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