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Therapeutic drug monitoring (TDM) of Leflunomide as an immunosuppressive treatment in thoracic (cardiac and/or lung) transplant recipients at The Georges POMPIDOU European Hospital-Paris-France

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Abstract

Leflunomide is an immunosuppressant indicated in the treatment of rheumatoid arthritis. This drug has a particular pharmacokinetics. Therapeutic Drug Monitoring (TDM) has been recommended for this drug because of its hepatic and hematological toxicities. We present here the prospective and retrospective analysis of a preliminary experience of the use of leflunomide as an alternative immunosuppressant in heart and/or lung transplantation of patients with or without cystic fibrosis. This study was conducted in 17 heart transplant patients (n=8) and/or lung transplant patients (n=9, 7 of whom had cystic fibrosis) who received treatment with leflunomide between April 2005 and June 2008. The indication for leflunomide was generally intolerance to the other immunosuppressants. The residual concentrations measured in patients with cystic fibrosis (C_0 = 12.8 ± 5.5 mg/L) were statistically lower than those measured in patients without cystic fibrosis (C_0 = 44.0 ± 24.2 mg/L) ($p < 0.05$). However, the dose related to weight in patients with cystic fibrosis (D = 0.32 ± 0.08 mg/Kg) tends to be slightly higher than that in patients without cystic fibrosis (D = 0.26 ± 0.10 mg/Kg). In terms of evolution, two patients died, one patient was lost to follow-up and leflunomide was stopped in 2 patients. With a mean follow-up of 12 months, the outcome was acceptable in the 12 patients in whom treatment was maintained. This experience must be evaluated over the longer term so that it can be extended to a larger cohort or proposed earlier after transplantation.

Keywords: Therapeutic Drug Monitoring; Leflunomide; Immunosuppressant; Cystic Fibrosis; Transplantation

1. Introduction

Therapeutic Drug Monitoring (TDM) consists of monitoring the blood concentrations of some drugs to be in the therapeutic range to avoid a possible underdose that would lead to therapeutic ineffectiveness or a possible overdose that would increase toxicity. The TDM mainly concerns drugs with a narrow therapeutic index with a large interindividual variability in terms of pharmacokinetics and/or pharmacodynamics. This process allows the individualization of treatment to find the appropriate dose for each patient that allows a target concentration to be reached within the therapeutic range while avoiding toxic thresholds (1)(2). At the Georges POMPIDOU European Hospital, the Cardiovascular Surgery department adopted a distinct immunosuppressive strategy depending on the type

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of transplant (cardiac or pulmonary). Indeed, heart transplant patients were usually put on corticosteroids and ciclosporin ± mycophenolate mofetil, and in case of intolerance, patients were switched to an inhibitor of proliferation signal (everolimus or sirolimus). While lung transplant patients were put on corticosteroids and tacrolimus ± mycophenolate mofetil and as previously, directed towards everolimus or sirolimus in case of intolerance or failure. But the department's strategy had changed following the observation of serious adverse effects, especially with the inhibitors of proliferation signal, which prompted doctors to prescribe leflunomide as an immunosuppressive alternative to these patients. Leflunomide (N-(4-trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide) is an immunosuppressive drug from the malononitrilamides class, initially developed for organ transplantation (kidneys), but this development had been stopped, in particular due to a risk of liver toxicity. However, the development of this drug continued in the indication of autoimmune diseases such as rheumatoid arthritis, leading to marketing in this indication (MA obtained on 09/02/1999) (3). But its use is complicated by specific pharmacokinetics because it is a prodrug that will release an active metabolite, teriflunomide "A77 1726" which has a very long half-life (2 weeks). It is necessary to wait several weeks (12 weeks) to reach the plasma balance state, which will complicate the TDM. In addition, this product has a strong binding to plasma proteins, particularly to albumin. Elimination is mainly by metabolization in the liver with the formation of a glucuron-conjugated derivative which will be excreted in the urine. There is a part of biliary excretion of teriflunomide in the unchanged form which will be found in the feces, accompanied by a significant enterohepatic cycle. The clearance of this product is mainly hepatic, also requiring 12 weeks after stopping administration for the body to eliminate this drug, which penalizes the flexibility of use (4). The therapeutic dose indicated in rheumatoid arthritis is 10 mg to 20 mg per day after a loading dose of 100 mg per day for 3 consecutive days, to reach a steady-state concentration of around 35 mg/L (3)(5). The monitoring methods include a TDM due to his hepatotoxicity and also his hematotoxicity and nephrotoxicity, which implies the monitoring of hepatic and renal functions as well as the blood count. This TDM is also necessary because of the teratogenic risk which, combined with the long half-life, requires information on the negative circulating concentrations in both men and women before starting procreation. In case of lung transplantation in patients with cystic fibrosis, particularly pediatric patients, the risk of prolonged underdosing due to increased clearance and potential decreased absorption must be assessed, which will justify special monitoring of blood concentrations in these patients to avoid possible graft rejection or poor control of a possible BK virus infection. The objective of our study is to retrospectively describe the cohort of cardiac and/or pulmonary transplant patients at The Georges POMPIDOU European Hospital who received leflunomide as an alternative immunosuppressant in order to determine the optimal dosage regimen and monitoring methods during treatment.

2. Materials and Methods

2.1. Inclusion criteria

Heart and/or lung transplant patients treated with leflunomide as part of immunosuppressive therapy and/or patients with BK virus infection between April 2005 and June 2008.

2.2. Studied population

This study was conducted on 17 heart transplant patients (n=8) and/or lung transplant patients (n=9, 7 of whom had cystic fibrosis) from The Georges POMPIDOU European Hospital who received treatment with leflunomide. This treatment with leflunomide was always associated with other immunosuppressants (Ciclosporin for heart transplant patients and Tacrolimus for lung transplant patients, ± mycophenolate mofetil ± corticosteroids ± everolimus ± sirolimus. The demographic characteristics of this population are detailed (Table 1).

Table 1 Demographic characteristics of the studied population are listed in chronological order to the initiation of leflunomide in our center

Patient	Sex	Age (years)	Weight (kg)	Cystic fibrosis	Type of transplantation	Transplantation date	Hemodialysis
1	M	54	92	no	Heart-Lung	01/31/2004	
2	M	61	78	no	Heart	10/07/2003	
3	M	30	54	yes	Lung-Liver	05/02/2005	
4	M	33	54	yes	Lung	07/05/2005	yes
5	M	68	93	no	Heart	01/04/1990	

6	M	19	60	yes	Lung	06/24/2004	
7	F	55	70	no	Heart-Lung	03/17/1999	
8	M	53	112	no	Heart	11/10/1994	
9	M	58	69	no	Heart	09/09/1995	
10	M	37	47	yes	Lung	08/22/1995	
11	M	55	67	no	Heart	01/01/2006	
12	F	48	48	no	Lung	10/18/2003	
13	M	31	61	yes	Lung	08/24/2001	
14	M	14	25	yes	Lung	05/03/2002	yes
15	F	60	52	no	Lung	12/30/2006	
16	M	60	75	no	Heart	10/30/1988	
17	M	24	53	yes	Lung-Liver	11/21/2006	

2.3. Data collection

The study was based on the collection of patient data from their medical records, this collection highlighted the following information: individual clinical indication for leflunomide, date of initiation and duration of treatment, initial dose as well as any subsequent dosage adjustments, residual plasma concentrations of teriflunomide "active metabolite of leflunomide", assessment of hepatotoxicity "dosing of AST, ALT, and total bilirubin", assessment of hematotoxicity "leukocytes, platelets, and hemoglobin level", assessment of nephrotoxicity "creatininemia, estimated glomerular filtration rate", collection of other unusual or serious adverse effects affecting patients during treatment, analysis of the outcome of patients under leflunomide, collection of doses and corresponding concentrations of other immunosuppressants before the administration of leflunomide and at 3 months "considered the time for plasma balance" after the introduction of leflunomide).

2.4. Plasma dosing of teriflunomide

The dosage of teriflunomide, the active metabolite of leflunomide, was carried out by High-Performance Liquid Chromatography (HPLC) on the plasma of patients after deproteinization of the sample by acetonitrile (6). Detection is done in UV at the wavelength $\lambda = 295$ nm.

2.5. Statistical analysis of data

Comparison of mean doses between patients with and without cystic fibrosis was performed by a student's t-test on an unpaired series ($p<0.05$). A comparison of mean concentrations between patients with and without cystic fibrosis was performed by a student's t-test on an unpaired series with Welch's correction ($p<0.05$).

3. Results

3.1. Clinical indication of leflunomide treatment

The indication for leflunomide treatment collected in each patient's file is indicated (Table 2).

Table 2 Clinical indication of leflunomide treatment

Patient	The time between transplantation and initiation of Leflunomide (years)	Indication of Leflunomide
1	1.2	Leukopenia under Mycophenolate mofetil
2	1.7	Renal failure + mouth ulcers under Sirolimus + acute cellular rejections + repeated infections

3	0.3	Mycophenolate mofetil toxicity (leukopenia, thrombocytopenia)
4	0.3	Neutropenia + thrombotic microangiopathy
5	16.2	Renal failure + impossibility of Everolimus (Italian patient and impossibility of dosage in Italy)
6	1.7	Leukopenia under Mycophenolate mofetil
7	7.6	Severe neutropenia under Mycophenolate mofetil
8	12.6	Mouth ulcers under Everolimus
9	12.7	Renal failure + drug-induced pneumonitis with Everolimus
10	12.8	Renal failure +++ (contraindication to treatment With Tacrolimus and corticosteroids)
11	12.8	Renal failure +++ and as soon as the doses of Ciclosporin are reduced -> rejection
12	12.9	Significant diarrhea with the combination of Everolimus + Mycophenolate mofetil
13	13	Episodes of severe neutropenia (bone marrow suppression) under Mycophenolate mofetil
14	13.1	BK-Virus Nephropathy
15	13.2	Renal failure (discontinuation of Everolimus) + reactivation of CMV
16	13.3	Renal failure + mouth ulcers under Everolimus
17	13.3	Leukopenia under Everolimus

3.2. Analysis of exposure to leflunomide

Dates and durations of leflunomide treatment are listed in (**Table 3)**. Treatment durations ranged from 1 to 33 months. Two patients died, one patient was lost to follow-up after a major clinical event, and a fourth patient had treatment stopped following a bi-nephrectomy for control of both severe high blood pressure and BK virus infection.

Table 3 Duration and outcome of leflunomide treatment

Patient	**Date of initiation under leflunomide**	**Initial dose of leflunomide (mg/24h)**	**Duration of Treatment with leflunomide (months)**	**Becoming patients**
1	04/12/2005	10	12	Dead
2	06/19/2005	10	1	Stop after one month
3	09/03/2005	10	33	current medication
4	10/27/2005	20	32	current medication
5	12/14/2005	20	30	current medication
6	03/02/2006	20	27	current medication
7	09/21/2006	20	17	lost sight of
8	04/18/2007	20	24	Stop after 24 months
9	06/20/2007	20	12	Stop after 12 months
10	09/17/2007	10	9	Stop after 9 months

11	01/24/2008	20	6	Stop after 6 months
12	02/19/2008	20	6	Stop after 6 months
13	03/19/2008	20	6	Stop after 6 months
14	04/30/2008	10	3	Planned stop
15	05/15/2008	20	1	Dead
16	05/28/2008	20	3	Stop after 3 months
17	05/29/2008	20	3	Stop after 3 months

The initiation doses and their dosage adjustments are collected **(Table 4).**

Table 4 Adaptation of doses (mg/24h) of leflunomide based on the TDM

Patient	**Month 1**	**Month 1,5**	**Month 2**	**Month 3**	**Month 6**	**Year 1**	**Year 2**
1	10	10	20	20	20	10	dead
2	10	Stop after one month					
3	10	10	10	20	20	20	20
4	20 then 10	10	10	10	20	20	20
5	20	20	20	20	20	20	40 then 20
6	20	20	20	20	20	20	20
7	20	20	20	20	20	20	lost sight of
8	20	20	20	20	20	20	20
9	20	20	20	20	20	20	Stop after 12 months
10	10 then 20	20	20	20	40	Stop after 9 months	
11	20	20	20	20	20	Stop after 6 months	
12	20	20	20	20	20	Stop after 6 months	
13	20	20	20	20	20	Stop after 6 months	
14	10 then 20	30	40	50	Planned stop		
15	20	Dead					
16	20	20	20	20	Stop after 3 months		
17	20	20	20	20	Stop after 3 months		

12 of the 17 patients included were put on leflunomide at the initial dose of 20 mg/24 h. The other 5 patients, whose initial dose was 10 mg/24 h, underwent an adaptation and an increase in the dose up to the dose of 20 mg/24 h in the majority of cases. The mean doses of 16 ± 5 mg/24 h and 18 ± 4 mg/24 h respectively in patients with and without cystic fibrosis are comparable. The dose relative to weight in patients with cystic fibrosis (D = 0.32 ± 0.08 mg/kg/24h) is not statistically different but tends to be slightly higher than that calculated in patients without cystic fibrosis (D = 0.26 ± 0.10 mg/kg/24h).

The study of the evolution of leflunomide concentrations over time (Table 5, Figure 1) confirms that the treatment is balanced after 3 months of exposure.

Table 5 Evolution of leflunomide concentrations (mg/L) over time (muco + = Cystic fibrosis)

Patient	Month 1	Month 1,5	Month 2	Month 3	Month 6	Year 1	Year 2
1							
2							
3							
4 (muco +)							8.2
5							197
6 (muco +)							25.5
7							
8						68	
9					20.5	22.5	
10 (muco +)	11.4						
11		38.8		67.3	79.5		
12							
13 (muco +)	6.7						
14	5	7.8	11.3	14	19.6		
15							
16	19		37				
17 (muco +)	16		13.8	15			

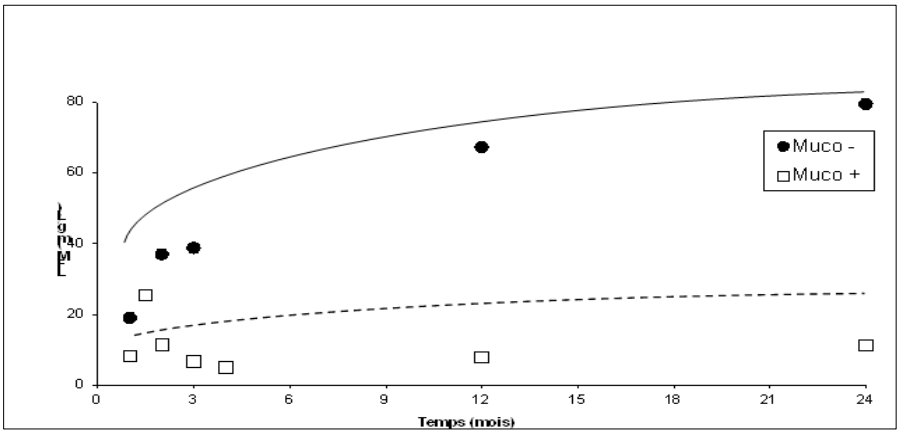

Figure 1 Evolution of leflunomide concentrations (mg/L) over time in patients with (Muco+) or not (Muco-) cystic fibrosis

On the other hand, the residual concentrations measured in patients with cystic fibrosis (C_0 = 12.8 ± 5.5 mg/L) are statistically lower than those measured in patients without cystic fibrosis (C_0 = 44.0 ± 24.2 mg/L) ($p<0.05$). The statistical analysis was performed by excluding patient 5 due to a significant overdose at 197 mg/L. Patient 14, although not suffering from cystic fibrosis, had a low exposure at 19.6 mg/L for which we have no explanation

3.3. Analysis of tolerance to leflunomide

Tolerance to leflunomide was assessed by analyzing patients' biological constants after they were started on leflunomide (Table 6).

Table 6 Biological parameters corresponding to each patient

		Creatininemia (µmol/L)	**GFR (ml/min)**	**Total bilirubin (µmol/L)**	**AST (UI/L)**	**ALT (UI/L)**	**Leukocytes (G/L)**	**Platelets (G/L)**	**Hemoglobin (g/L)**
Normal values		**M = 60 à 115 F = 45 à 105**	**>60**	**5 à 17**	**4 à 40**	**4 à 40**	**4 à 10**	**150 à 450**	**M= 130 à 170 F= 120 à 160**
Patient	**Month**								
1	M0	139		7	15	24	7.1	132	123
	M3	123		8	12	15	2.7	142	94
	M6	125		10	14	17	10.1	153	136
	M12	141	48	6	11	14	7.2	189	107
2	M0	254		12	21	21	2.6	148	106
	M3	170		8	10	6	3.3	206	106
	M6	141		9	24	26	5.2	162	102
	M12	270	22	8	17	16	5.8	136	128
3	M0	114		9	30	98	10.4	20	129
	M3	134		8	21	50	6.8	17	113
	M6	113	70	11	14	50	3.1	19	118
	M12	143	53	12	21	27	1.3	21	107
4	M0	340		10	108	311	4	153	113
	M3	382	17	8	15	25	6	196	97
	M6	650	9	10	30	48	6	153	129
	M12	424	15	9	42	58	4.8	194	107
5	M0	118		23	22	21	7.4	186	131
	M6	118	57	25	33	35	5.8	232	122
	M12	117	57	19	22	11	7.1	227	121
6	M0	115	76	19	11	13	3.9	203	119

	M3	153	54	18	14	16	3.8	181	119
	M6	152	55				5.4	185	106
	M12	189	42	22	19	20	5.9	151	119
7	M0	188	26				3.9	341	104
	M3	132	39	11	25	25	3.9	307	106
	M6	126	41	13	19	24	4.2	313	102
8	M0	168	40	13	24	18	7.6	217	138
	M12	171	39	9	28	27	4.2	181	123
9	M0	189	34	16	14	15	9.7	210	153
	M12	257	24	12	29	35	4	140	127
10	M0	194	36	8	33	36	5.3	161	122
11	M0	181	36		63	136	6.8	198	114
	M3	183	36		58	115	4	177	107
	M6	130	53				4.3	160	94
12	M0	268	18	8	30	14	7.9	260	89
	M3	190	26						
	M6	151	34	5	34	28	2.5	369	89
13	M0	116	68	9	32	76	3.8	274	145
	M6	90	91	10	31	60	7	192	154
14	M0	508	15	9	29	40	9.8	287	109
15	M0	123	41		28	24	5.7	231	88
16	M0	204	31		25	25	4.1	131	109
17	M0	89	97		13	26	2	282	91
	M3	88	98	4	18	31	3.8	156	104

This table shows that most patients had impaired renal function at the start of treatment with 2 cases of severe renal failure. The liver function appears to be preserved except for 4 cases with a transient increase in liver enzymes. At the hematological level, we observed 7 cases of frank leukopenia with 2 severe cases that normalized following treatment with leflunomide, and a few cases of thrombocytopenia including one severe case that persisted with leflunomide.

3.4. Other immunosuppressive treatments

Daily doses of immunosuppressive treatments before leflunomide administration and 3 months after its introduction are indicated **(Table 7)**.

Table 7 Daily doses (mg/24h) of immunosuppressants were used before and 3 months after the introduction of leflunomide

Patient	Tacrolimus		Ciclosporin		Mycophenolate mofetil		Everolimus		Sirolimus		Prednisone		Azathioprine	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	3	2									16	14		
2	4	3			2000	2000					14	14		
3	12	18									22	10		
4	7	4			1500	1500					30	10		
5			240	210	3000	3000					10	5		
6	16	2									10	10		
7			180	180							5	5	100	Stop
8	3	2			2000	2000								
9	5	3			1500	1500					5	5		
10	2	Arrêt			2000	2000			3	3	2	30		
11			100	Arrêt	1000	1000	1	2			11	11		
12	2	2					1.5	1			5	5		
13	3.5	3.5			2000	2000	3	3			6	6		
14	4	4							4	4	7	7		
15	4	4					4	4			10	10		
16			75	75	1500	1500								
17	4	4									20	20		

Also, some residual concentrations of these immunosuppressants before and after the introduction of leflunomide are indicated **(Table 8).**

Table 8 Residual concentrations (ng/mL) of immunosuppressants used before and 3 months after the introduction of leflunomide

Patient	Tacrolimus		Ciclosporin		Mycophenolate mofetil		Everolimus		Sirolimus	
	Before	After	Before	After	Before	After	Before	After	Before	After
1	13.3	6.5								
2	11.3	19.3								
3	10.4	13								
4	9.3	5								
5			190	115						
6	11.3	6.9								
7			155	110						
8	10.1	5.6								
9	11.0	6								
10	6.1								5.7	
11			100		2.9		8.2	5.5		
12	7.0						14.2	5		
13	6.5	6.5					5.2	5.2		
14	14.5	14.5							3.4	3.4
15	5	5					4.9	4.9		
16			50	50						
17	8.2	8.2								

These tables show a predominance of the association between Tacrolimus and corticosteroids + Mycophenolate mofetil + Everolimus or Sirolimus. After 3 months of treatment with leflunomide, we were able to completely stop an immunosuppressant in 3 cases, and in 12 cases we were able to reduce the dosage of another immunosuppressant.

4. Discussion

The two main objectives of TDM are, on the one hand, to reduce the rate of therapeutic failure linked to poor compliance and/or an insufficient dose, and on the other hand, to prevent adverse and/or toxic effects linked to an excessive dose (7). For a drug to be eligible for TDM, it must meet all of the following conditions: a narrow therapeutic index, a concentration/pharmacological effect relationship better than its dose/pharmacological effect relationship, a large variability of the dose/concentration relationship from one patient to another, a low or predictable variability of the dose/concentration relationship over time in the same patient, and a pharmacological response that is difficult to access by measuring the effect (8). TDM is particularly useful in groups of patients for whom the pharmacokinetic parameters are particular or unpredictable (slow metabolizers, ultra-rapid metabolizers, patients with reduced muscle mass, obese patients, etc.), as well as certain at-risk populations such as newborns, the elderly, patients with renal insufficiency, patients with hepatic insufficiency and patients with cystic fibrosis. Especially in the case of drug combinations with a risk of pharmacokinetic interactions (enzyme inducers or inhibitors) (9)(10). Leflunomide is an immunosuppressive drug indicated primarily for the background treatment of rheumatoid arthritis in adults. In addition to its immunosuppressive activity, leflunomide has anti-BK Virus antiviral activity (11)(12). This ambivalence of activity has favored its use in the case where the kidney transplant patient presents a BKV infection, which is a virus with essentially renal tropism (13). Opportunistic in nature, and following the immunosuppression generated in particular by tacrolimus and mycophenolate mofetil, this virus causes invasion of the kidneys and ureters in kidney transplant

patients, particularly pediatric patients, but this virus can also affect the kidneys in other types of transplantation because of immunosuppression, it should be noted that natural seroconversion occurs around the age of 10-12 years (14)(15)(16). Leflunomide can also be considered as a potentially interesting immunosuppressant as an alternative in the event of refractory rejection or especially as a fallback treatment in the event of the impossibility of using classic immunosuppressants conventionally.

The indication for the introduction of leflunomide in our population of 17 thoracic transplant patients was dominated by intolerance to other immunosuppressive treatments, with only one case of BK virus infection. Following prolonged use of immunosuppressants, particularly the inhibitors of calcineurin (Tacrolimus, Ciclosporin), for several years, all these patients had renal failure at the introduction of leflunomide, including 2 on hemodialysis, one is a child with BK virus nephropathy and the other had Thrombotic Microangiopathy (TMA). In demographic terms, this is a predominantly male population, with only 3 women, including patients with cystic fibrosis, which is unusual (in general, the sex ratio is balanced in this cohort). Furthermore, the group of patients with cystic fibrosis is younger (14 to 37 years) and of lower weight compared to the group of patients without cystic fibrosis (48 to 68 years). For most patients, the introduction of leflunomide was carried out at a distance from the transplantation, and even very far away for some. Indeed, leflunomide was used as a last resort and alternative, after having exhausted all alternative resources such as the inhibitors of calcineurin (Tacrolimus and Ciclosporin), especially the inhibitors of proliferation signal (Everolimus and Sirolimus). The terrain of these patients is particularly heavy and complicated (cystic fibrosis, small bowel lymphoma cured but cicatricial perforation under inhibitors of proliferation signal, thrombotic microangiopathy, etc.). In terms of exposure, leflunomide was administered at an initial dose of 10 to 20 mg/day, progressively in the spirit of a relay to existing immunosuppressants, the dose was then increased to 20 mg/day in most cases. The plasma concentration target was chosen based on the experiences available in the literature in renal transplant patients with BKV and based on pharmacokinetic references and targets for rheumatoid arthritis around 35 mg/L, generally obtained for doses of 40 to 60 mg/day (17)(18)(19). In our patient population, 2 subgroups appear, a subgroup of patients with cystic fibrosis for which the mean concentration was 13.0 ± 5.5 mg/L, significantly lower than the mean concentration of patients without cystic fibrosis which was 42.0 ± 21.5 mg/L ($p < 0.05$). This specificity is quite classic, it is partly due to the younger age and the specific characteristics of the pathology on the absorption, distribution, and clearance of drugs. The level of concentration reached, particularly in this group of patients with cystic fibrosis, is quite low but can be considered sufficient in patients at a distance from transplantation, so the risk of acute rejection is lower, in addition to the concomitant administration of other immunosuppressants. The initial doses were comparable 16 to 18 mg/day but yet higher in the group of patients with cystic fibrosis in weight expression with 0.32 mg/kg/day in this group, while patients without cystic fibrosis had a weight dose of 0.26 mg/kg/day. Not surprisingly, we note that in the cohort of patients with cystic fibrosis, it is more difficult to increase plasma exposure toward the therapeutic target for this drug with hepatobiliary tropism in patients with impaired hepatobiliary function. The plasma balance of this drug requires several weeks, 2 to 3 months to be reached, a period which is perfectly compatible with the particularly high half-life of leflunomide. Only one significant overdose, although well tolerated, was recorded (197 mg/L) in an elderly patient of high weight, treated with 40 mg/day without Therapeutic Drug Monitoring (TDM) beforehand. The dosage has since been reduced to 20 mg/day after the initiation of this TDM, which constitutes an undeniable argument for the importance of the TDM for this drug. The study of all immunosuppressive treatments before the introduction of leflunomide revealed the majority of the association of Tacrolimus with corticosteroids + Mycophenolate mofetil + Everolimus or Sirolimus. After 3 months of treatment with leflunomide, we recorded the complete stop of an immunosuppressant in 3 cases and the reduction of the dosage of another immunosuppressant in 12 cases. Essentially, the use of leflunomide was made following hematological intolerances especially to the inhibitors of proliferation signal (Everolimus and Sirolimus) and to Mycophenolate mofetil and specific cutaneous-mucosal side effects due to the inhibitors of proliferation signal (mouth ulcers), combined with the impossibility of increasing the doses of the inhibitors of calcineurin due to already significant renal insufficiency. The observation period of exposure to leflunomide was on average 12 months, ranging from 1 month to 3 years. In terms of evolution, 2 deaths occurred under leflunomide, at 1 year for an infectious cause (bacterial pulmonary infection) and at 1 month for a cause probably of cardiovascular origin (suspected ruptured aneurysm) after the relay with leflunomide. One patient was lost to follow-up after a stroke recorded around the 17th month of treatment. Leflunomide was stopped in a heart transplant patient after 1 month of treatment in 2005 due to the occurrence of significant leukopenia which had justified the stopping of all hematotoxic treatments including Mycophenolate mofetil, and even after resolution of the leukopenia and because of better control of plasma concentrations of Tacrolimus, leflunomide was not reintroduced. Finally, the patient with BKV underwent a bi-nephrectomy after three months of treatment with leflunomide in this treatment was stopped. In terms of tolerance, renal, hepatic, and hematological functions were assessed, in fact on the renal level, we observed relative stability of the initial renal function at the introduction of leflunomide, with possibly some discreet improvements, arguing at least for the preservation of the residual renal function. However, in a 14-year-old child, on hemodialysis, suffering from cystic fibrosis and a lung transplant in addition to BKV nephropathy, the development of severe uncontrollable high blood pressure justified a bi-nephrectomy performed after 3 months of treatment. This

patient was not the subject of an overdose (19.6 mg/L) of leflunomide but the causal link of this complication with this treatment cannot be ruled out. Finally, in this patient, leflunomide was stopped after 4 months of this intervention for a relay with Tacrolimus/Everolimus given that the nephrotoxicity of Tacrolimus is no longer an obstacle for its use in the absence of a renal target (bi-nephrectomy). On the hepatic level, the elements of the biological assessment appear generally normal at inclusion and during treatment, except for 4 isolated transient disturbances of one of the hepatic parameters finally normalized at 6 months. Hematologically, 7 frank leukopenias were observed, including 2 severe (< 2 G/L) which normalized for the most part during treatment with leflunomide, in particular after stopping everolimus or Mycophenolate mofetil. 3 episodes of leukopenia were also observed under leflunomide, including one isolated at 3 months and two others more persistent at 3 months and 6 months. Some moderate thrombocytopenia was observed with only one severe thrombocytopenia occurring in a lung-liver transplant patient, in connection with the residual splenomegaly of portal hypertension which was little modified during treatment. Overall, except for the pediatric patient with cystic fibrosis who underwent a bi-nephrectomy, tolerance proved to be rather acceptable with a follow-up of 12 months on average in complex, severe patients who had exhausted the resources of the immunosuppressive therapeutic arsenal.

5. Conclusion and perspectives

The precise assessment of the benefit/risk balance of leflunomide treatment in this context is the responsibility of specialized clinical and pharmacovigilance expertise due to the seriousness and complexity of these cases, particularly regarding cardiovascular events. However, our retrospective study demonstrates the possibility of using leflunomide as an alternative in cases of major intolerance to conventional immunosuppressive treatments in lung and heart transplantation. The 20 mg dose allows on average to obtain plasma concentrations compatible with a therapeutic range of 40 mg/L and the order of 15 mg/L in lung transplant patients with cystic fibrosis, with acceptable hepatic and hematological tolerance, even at a long distance from the transplantation. In most cases, leflunomide has preserved the existing level of renal function, except for one pediatric patient with BKV nephropathy. This preliminary experience, limited to 17 patients, with an average follow-up of 12 months, must be evaluated in the longer term on a larger number of patients so that it can be extended to a larger cohort or proposed earlier after transplantation to less impaired patients.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of informed consent

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