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# Interstitial pneumonitis caused by everolimus: a case–cohort study in renal transplant recipients

Marije C. Baas,[1]* Geertrude H. Struijk,[1]* Dirk-Jan A. R. Moes,[2] Inge A. H. van den Berk,[3] René E. Jonkers,[4] Johan W. de Fijter,[5] Jaap J. Homan van der Heide,[1,6] Marja van Dijk,[6] Ineke J. M. ten Berge[1] and Frederike J. Bemelman[1]

1 Renal Transplant Unit, Department of Nephrology, Division of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands
2 Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, the Netherlands
3 Department of Radiology, Academic Medical Center, Amsterdam, the Netherlands
4 Department of Pulmonology, Academic Medical Center, Amsterdam, the Netherlands
5 Renal Transplant Unit, Division of Internal Medicine, Department of Nephrology, Leiden University Medical Center, Leiden, the Netherlands
6 Renal Transplant Unit, Division of Internal Medicine, Department of Nephrology, Groningen University Medical Center, Groningen, the Netherlands

**Keywords**
area under the curve, case–cohort study, everolimus, pneumonitis, renal transplant recipients.

**Correspondence**
Marij C. Baas MD, PhD, Department of Nephrology, Huispost 464, Postbus 9101, 6500 HB Nijmegen, the Netherlands.
Tel.: +31(0)24 3614761;
fax: +31(0)24 3540022;
e-mails: m.c.baas@amc.uva.nl, m.baas@nier.umcn.nl

**Conflicts of Interest**
We have no conflict of interest to declare.

*Both authors contributed equally to this manuscript.

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## Summary

The use of inhibitors of the mammalian target of rapamycin (mTORi) in renal transplantation is associated with many side effects, the potentially most severe being interstitial pneumonitis. Several papers have reported on sirolimus-induced pneumonitis, but less is published on everolimus-induced pneumonitis (EIP). Data on risk factors for contracting EIP are even more scarce. In the present case–cohort study in renal transplant recipients (RTR), we aimed to assess the incidence and risk factors of EIP after renal transplantation. This study is a retrospective substudy of a multicenter randomized controlled trial. All patients included in the original trial and treated with prednisolone/everolimus were included in this substudy. RTR who developed EIP were identified as cases. RTR without pulmonary symptoms served as controls. Thirteen of 102 patients (12.7%) developed EIP. We did not find any predisposing factors, especially no correlation with everolimus concentration. On pulmonary CT scan, EIP presented with an organizing pneumonia-like pattern, a nonspecific interstitial pneumonitis-like pattern, or both. Median time (range) to the development of EIP after start of everolimus was 162 (38–407) days. In conclusion, EIP is common in RTR, presenting with an organizing pneumonia, a nonspecific interstitial pneumonitis-like pattern, or both. No predisposing factors could be identified (Trial registration number: NTR567 (www.trialregister.nl), ISRCTN69188731).

## Introduction

Inhibitors of the mammalian target of rapamycin (mTORi), sirolimus and everolimus, are potent immunosuppressive drugs widely used after organ transplantation. They have been introduced in renal transplantation because of their supposed lack of nephrotoxicity and potential anti-oncogenic and anti-atherosclerotic effects [1–5].

Unfortunately, the use of mTORi is associated with many side effects like edema, impaired wound healing, mouth ulcers, anemia, proteinuria, development of lymphoceles, hyperlipidemia, and hypertriglyceridemia [6]. Also interstitial pneumonitis may complicate treatment with an mTOR inhibitor. There are many reports of sirolimus-induced pneumonitis (SIP) [7]. Estimates of the incidence of SIP vary between 5 and 15% in solid organ transplant recipients. Clinical presentation ranges from asymptomatic to respiratory failure, but published reports suggest that SIP generally has a mild course and resolution of symptoms usually occurs after dose reduction or discon-



 

tinuation of sirolimus. Far less is known on everolimus-induced pneumonitis (EIP): case reports of EIP do exist in solid organ transplantation and oncology, but systematic case–control studies have not been performed in renal transplant recipients (RTR).

The mechanism responsible for pulmonary toxicity by mTORi is not completely understood. Some suggest a dose-dependent risk [8–10], but there are also reports of cases with low mTORi trough levels [11,12]. Apart from the dose of mTORi, other possible risk factors have been identified in patients with non-small cell lung cancer, like smoking and pre-existing pulmonary disease [13]. Other studies found plasma creatinine and glomerular filtration rate (GFR) to be risk factors for the development of EIP [14], indicating that the tolerance to mTORi may be altered in the presence of severe renal insufficiency.

The presence of lymphocytes and eosinophils in broncho-alveolar lavage fluid suggests an immune-mediated reaction [7,10,15]. It has been hypothesized that sirolimus binds to plasma proteins and that this complex is processed by antigen-presenting cells in the lungs with consecutive T-cell recognition and recruitment of inflammatory cells like macrophages [7]. Others suggested that sirolimus exposes cryptic alveolar antigens evoking an ongoing cellular immune response [10]. Both mTOR inhibitors, despite inhibiting the adaptive immune response, enhance innate immunity [16,17], thereby possibly contributing to the development of pulmonary inflammation. Histopathologic patterns include bronchiolitis obliterans organizing pneumonia, lymphocytic interstitial pneumonia, non-necrotizing granulomatous inflammation and vasculitis that support the immune-mediated hypothesis [7,8,10,18,19]. The mechanisms involved in EIP are speculative due to the lack of detailed studies. However, a recent study suggests a similar immunologic mechanism for EIP [12], although there are also reports of resolution of SIP after conversion to everolimus [20–22]. In conclusion, ongoing exposure to mTORi may lead to a persistent inflammatory response in the lungs presenting clinically as pneumonitis.

With the present case–cohort study, we aimed to describe the incidence, clinical presentation, radiologic findings and predisposing factors of EIP in RTR.

## Patients and methods

### Patients

This study was conducted as part of a larger prospective, multicenter randomized trial studying the effects of withdrawal of cyclosporin A (CsA) from an immunosuppressive regimen containing an IL-2 antagonist (basiliximab), CsA, prednisolone (P), and mycophenolate sodium (MPS) early after transplantation. Three university hospitals in the Netherlands participated in this trial from January 2005 until December 2009: the Academic Medical Center in Amsterdam (AMC), the Leiden University Medical Center (LUMC), and the University Medical Center in Groningen (UMCG). Institutional review board approval has been obtained. The study was conducted in accordance with the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. Informed consent was obtained from every patient. The details and results of an interim analysis of this trial have previously been published (trial registration number: NTR567 (Dutch trial registry), ISRCTN69188731, www.trialregister.nl) [23]. In short, RTR, receiving their first or second renal transplant, were treated with quadruple immunosuppressive therapy consisting of P, CsA, MPS, and basiliximab. After 6 months, RTR were (in the absence of rejection, proven by renal biopsy) randomized to one of three immunosuppressive regimens: P/CsA, P/MPS, and P/everolimus. Drug exposure of CsA and everolimus was monitored by AUCs at fixed moments. The target value of the AUC for CsA was 5400 μg h/l in the first 6 weeks and 3250 μg h/l thereafter. The target AUC for everolimus was 150 μg h/l for fluorescence polarization immunoassay (FPIA) and 120 μg h/l for liquid chromatography tandem mass spectrometry (LC-MS/MS), corresponding to the average 23% overestimation of FPIA [24]. The primary outcome was interstitial graft fibrosis and hyalinosis. Secondary outcome was, among others, graft rejection. Patients who received a third or fourth transplant were excluded, as were patients with >50% panel reactive antibodies.

### Case definition

For this retrospective substudy, all RTR who were randomized to treatment with P/everolimus and/or effectively switched to treatment with P/everolimus during the study were included. Pulmonary problems in patients using everolimus were detected by the trial reports of (serious) adverse events and review of the charts of all included patients. Charts were analyzed for clinical signs (e.g., dyspnea, cough, or fever) and radiologic signs of pulmonary involvement (abnormal chest X-ray and pulmonary CT scans). RTR, who developed symptoms of an EIP, were identified as cases. We used the following criteria for EIP [10]: (i) exposure to everolimus before the onset of pulmonary symptoms, (ii) exclusion of other pulmonary disease, especially infection, (iii) radiographic findings on CT of the chest not compatible with other diagnoses, and (iv) resolution of pulmonary symptoms after discontinuation of everolimus. When available, histopathologic diagnosis consistent with drug-induced lung toxicity was considered gold standard.

Renal transplant recipients who were treated with P/everolimus, but did not develop pulmonary symptoms, served as control patients. Patients in whom everolimus

was discontinued because of pulmonary symptoms, but in whom no CT imaging was performed, were excluded from the analysis. These patients were classified as possible EIP.

The following data were retrospectively collected from medical records: sex, age, race, original renal disease, organ origin (living related or deceased), data on rejection episodes and CMV infection, analysis of BAL fluid, dialysis mode, history of pulmonary disease, smoking, everolimus AUCs, and trough levels. Chest X-rays and (HR)CT of the chest from possible cases were re-analyzed by two independent reviewers [radiologist (IB) and pulmonologist (RJ)], who were blinded to the clinical information of patients. New abnormalities (compared to a pretransplantation chest X-ray) were scored. Pulmonary function tests (when performed) were also recorded. The course of the EIP was analyzed, and time to clinical recovery was noted.

### Radiologic classification

Imaging findings on chest CT scan were classified into three distinct patterns (a simplified version of the approach by Endo *et al.* [25]): (i) multifocal areas of airspace consolidation with a predominantly peribronchial and/or subpleural distribution and bronchial wall thickening, compatible with OP, (ii) extensive bilateral ground-glass attenuation or airspace consolidation with traction bronchiectasis, compatible with a NSIP, or (iii) a combination of OP and NSIP.

### Measurements

Plasma creatinine was measured with an enzymatic PAP+ (phenol/4-aminoantipyrine) assay on a Roche Modular analyzer (Roche, Almere, the Netherlands). Estimated GFR was calculated using the abbreviated MDRD formula: $GFR = 175 \times (Pcr \div 88.4)^{-1.154} \times age^{-0.203}$ (female: multiply result by 0.742, black: multiply result by 1.210).

Cytology, Ziehl-Neelsen staining, bacterial, viral, and fungal cultures were routinely performed on all BAL fluid specimens.

$AUCs_{0–12\ h}$ for everolimus were calculated from blood samples drawn at $T = 0$, 1, 2, 3, 4, 5, and 6 h after administration. The everolimus $AUCs_{0–12\ h}$ consisted of full AUCs (seven or six time points) and sparsely sampled AUCs (four time points), calculated using linear trapezoidal rule. Everolimus levels were determined by immunoassay (Innofluor® Certican® Assay System) according to manufacturers' instructions (Seradyn Inc., Indianapolis, IN, USA) or by a validated LC-MS/MS method [24]. As there is an average overestimation of 23% by FPIA [24], the average $AUC_{0–12\ h}$ measured with LC-MS/MS was corrected by this 23% to eliminate the differences between both methods.

Pulmonary function (VC and DCLO) was measured using standard testing procedures.

### Statistical analysis

All statistical analyses were performed using SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA). Univariate analysis was performed to identify risk factors associated with EIP. Associations of discrete variables with EIP are expressed in terms of exact odds ratios with their 95% confidence interval and analyzed with a chi-square test. Associations of continuous variables were analyzed with a Mann–Whitney *U*-test. A *P*-value $<0.05$ was considered statistically significant. Area under the curves ($AUCs_{0–12\ h}$) were calculated using the linear trapezoidal rule with everolimus trough concentrations used as 12-h values. AUCs were grouped into three different time periods (range): 1 month (0.2–3.5), 6 months (4.0–8.1), and 12 (9.4–14.5) months after start of everolimus. If one patient had multiple AUC measurements within one time period, the average AUC was calculated and used in the analysis.

## Results

### Presentation of EIP

One hundred and two RTR were treated with prednisolone (P) and everolimus during the study period. At 6 months, 96 patients were randomized to P/everolimus [23]. Six additional patients who switched to P/everolimus for various reasons outside the study protocol were also included in this case–cohort study. We identified 13 cases, corresponding with an incidence of 12.7% (i.e., 13/102). Seven cases were classified as 'possible cases' and were excluded from the definite analysis. A detailed description of these patients can be found as supplementary data (Table S1).

Eighty-two RTR who did not develop pulmonary symptoms served as control patients. Table 1 shows the demographic data of cases and control patients. The characteristics of the 13 patients who developed an EIP are listed in Table 2. The median (range) time on everolimus of all patients was 752 (32–1502) days. In the cases, the median time (range) on P/everolimus until confirmation of EIP by computed tomography (CT) was 162 (38–407) days. Beyond 407 days, no more EIP occurred (Fig. 1). The most common presenting symptoms were dyspnea and cough (10/13 cases). Fever was present in 8/13 cases. One patient was asymptomatic; however, 2-deoxy-2-($^{18}$F)fluoro-D-glucose (FDG)-positive pulmonary infiltrates were discovered on a PET scan performed because of multiple unexplained bone fractures. A consecutive HRCT scan showed an image compatible with drug-induced pneumonitis.

In all identified cases, the pulmonary CT scan revealed consolidations matching an organizing pneumonia (OP), a nonspecific interstitial pneumonitis (NSIP)-like pattern, or a combination of the two (Fig. 2). In one patient, no CT scan could be retrieved, but EIP was confirmed with

**Table 1.** Univariate analysis of risk factors for everolimus-induced pneumonitis among renal transplant recipients.

| | Cases ($n$ = 13) | Control patients ($n$ = 82) | Odds ratio (CI) | $P$-value |
|---|---|---|---|---|
| Male gender *n* (%) | 9 (69.2) | 50 (61.0) | 0.69 (0.2–2.5) | 0.57 |
| Recipient age, median (range) | 50.0 (32–71) | 53.5 (22–70) | – | 0.37 |
| Caucasian *n* (%) | 11 (84.6) | 70 (85.4) | 1.06 (0.2–5.4) | 0.94 |
| Underlying renal disease | | | | |
| Vascular | 3 (23.1%) | 15 (18.3%) | 1.00 | 0.25 |
| Immunologic | 4 (30.8%) | 22 (26.8%) | 0.91 (0.2–4.7) | |
| Urological | – | 10 (12.2%) | 0.00 | |
| Other | 3 (23.1%) | 28 (34.1%) | 0.54 (0.1–3.0) | |
| Unknown | 3 (23.1%) | 7 (8.5%) | 2.14 (0.3–13.4) | |
| Renal transplant type (living) *n* (%) | 6 (46.2) | 43 (52.4) | 1.29 (0.4–4.2) | 0.67 |
| Smoking | | | | |
| Yes | 1 (7.7) | 17 (22.1) | 0.2 (0.0–2.6) | |
| Stopped prior to Tx | 4 (30.8) | 19 (24.7) | 1.1 (1.3–4.0) | 0.52 |
| No | 8 (61.5) | 41 (53.2) | 1.0 | |
| Pulmonary disease *n* (%) | 4 (30.8) | 14 (17.1) | 0.46 (0.1–1.7) | 0.25 |
| Rejection episode *n* (%) | 1 (7.7) | 16 (19.5) | 2.91 (0.4–24.0) | 0.32 |
| Time on RRT (months) | 48.1 (0–277) | 28.8 (0–344) | – | 0.23 |
| Dialysis mode *n* (%) | | | | |
| Preemptive | 1 (7.7) | 13 (15.9) | 1.0 | 0.34 |
| HD | 7 (53.8) | 23 (28.0) | 4.0 (0.4–35.8) | |
| PD | 3 (23.1) | 31 (37.8) | 1.3 (0.1–13.2) | |
| HD & PD | 2 (15.4) | 15 (18.3) | 1.7 (0.1–21.4) | |
| GFR (ml/min) | | | | |
| 6 months after Tx | 59.1 (30.8–87.8) | 52.4 (17.4–110.2) | – | 0.10 |
| 9 months after Tx | 54.5 (35.8–79.5) | 52.8 (20.6–102.8) | – | 0.53 |
| 12 months after Tx | 50.4 (35.5–75.4) | 51.2 (11.7–96.8) | – | 0.65 |
| 18 months after Tx | 54.2 (37.0–93.3) | 50.1 (14.3–101.6) | – | 0.84 |
| 24 months after Tx | 58.8 (22.6–97.8) | 47.0 (10.1–104.6) | – | 0.45 |
| Time on EVL (days) | 157.5 (32–485) | 864.5 (69–1502) | – | <0.001 |
| AUC EVL 1 month after start (μg h/l) | 173 (65–447) | 169.5 (77–439) | – | 0.97 |
| AUC EVL 6 months after start (μg h/l) | 172 (164–238) | 171 (98–356) | – | 0.40 |
| AUC EVL 12 months after start (μg h/l) | 237 | 169 (89–261) | – | NA |
| Trough level EVL 1 month after start (μg/l) | 9.2 (3.8–25.4) | 9.1 (4.0–28.1) | – | 0.98 |
| Trough level EVL 6 months after start (μg/l) | 10.8 (8.0–14.0) | 9.4 (2.9–22.0) | – | 0.44 |
| Trough level EVL 12 months after start (μg/l) | 14.5 | 8.9 (4.5–14.7) | – | NA |
| CMV infection *n* (%) | | | | |
| Primary infection | 1 (7.7) | 7 (8.5) | 1.12 (0.1–9.9) | 0.92 |
| Reactivation | 3 (23.1) | 28 (34.1) | 1.73 (0.4–6.8) | 0.43 |

AUC, area under the curve; CI, confidence interval; CMV, cytomegalovirus; EVL, everolimus; HD, hemodialysis; NA, not available; PD, peritoneal dialysis; RRT, renal replacement therapy; Tx, transplantation. GFR estimated by the abbreviated MDRD. Associations of discrete variables with everolimus-associated pneumonitis are expressed in terms of exact odds ratios with their 95% confidence interval and analyzed with a chi-square test. Associations of continuous variables are analyzed with Mann–Whitney *U*-test.

pulmonary biopsy. Eight cases underwent a broncho-alveolar lavage (BAL). No pathogenic microorganisms could be detected. In all cases, everolimus was discontinued. In 6/13 cases, everolimus was only discontinued when antibiotic therapy did not result in improvement. The absence of any microorganisms in the BAL fluid and the failure of empirical antibiotic treatment ruled out infection in these patients. Corticosteroids were administered in three cases. Pulmonary function tests were performed just after the onset of symptoms in 6/13 cases, showing normal to mildly lowered VC 90.2% (range 68–112), normal forced expiratory volume in 1 s (FEV1) 84.8% (70–100) with a decreased single-breath diffusion capacity for carbon monoxide (DCLO) in all, 56% (range 38–75).

### Follow-up after EIP

All patients had a full clinical recovery within 1 year. In nine cases, this was a subjective recovery because of the absence of follow-up with CT scan or pulmonary function tests. Only in one case, pulmonary function tests were performed after discontinuation of everolimus, showing an

**Table 2.** Characteristics of renal transplant recipients with an everolimus-induced pneumonitis.

| Patient | Age | Gender | Time on EVL until symptoms (days) | Symptoms | Radiologic findings on pulmonary CT | Broncho-alveolar lavage | Treatment | Time to recovery |
|---|---|---|---|---|---|---|---|---|
| 1 | 66 | Male | 109 | Dyspnea, coughing | OP/NSIP | NA | Discontinue EVL | <3 months |
| 2 | 49 | Male | 206 | Dyspnea, coughing, fever | NSIP | NA | Discontinue EVL | <12 months |
| 3 | 61 | Male | 162 | Dyspnea | NSIP/OP | Negative | Discontinue EVL | <6 months |
| 4 | 48 | Female | 279 | Coughing, fever | OP | NA | *AB + discontinue EVL | <3 months |
| 5 | 32 | Female | 385 | None | OP | NA | Discontinue EVL | |
| 6 | 49 | Male | 130 | Dyspnea, coughing, fever | OP with GG | Negative | Discontinue EVL | <1 month |
| 7 | 71 | Female | 14 | Dyspnea | OP/NSIP | NA | Discontinue EVL | <12 months |
| 8 | 50 | Male | 58 | Coughing, fever | OP | Negative | Discontinue EVL | Unknown |
| 9 | 70 | Male | 41 | Dyspnea, coughing, fever | VATS: OP | Negative | †AB + discontinue EVL + corticosteroids | <3 months |
| 10 | 38 | Female | 106 | Dyspnea, coughing, fever | OP | Negative | ‡Discontinue EVL + AB + corticosteroids | <1 month |
| 11 | 60 | Male | 41 | Dyspnea, coughing, fever | OP | Negative | §AB + discontinue EVL + corticosteroids | <3 months |
| 12 | 64 | Male | 109 | Coughing, fever | OP/NSIP | Negative | ¶AB + discontinue EVL | <3 months |
| 13 | 48 | Male | 7 | Dyspnea, coughing | OP | Negative | **Discontinue EVL | Unknown |

AB, antibiotics; OP, organizing pneumonia; CT, computed tomography; EVL, everolimus; NA, not available; NSIP, nonspecific interstitial pneumonia; VATS, video-assisted thoracoscopy. Negative BAL means that no microorganisms were detected. BAL fluid was not analyzed for type of leukocytes.
*First AB (ceftriaxone) was given, which did not improve the pulmonary symptoms. Hereafter, ceftriaxone was stopped and everolimus was discontinued.
†First AB (amoxicilline/clavulanic acid) was given which did not improve the pulmonary symptoms, and AB was discontinued. After histopathologic prove of organizing pneumonia, everolimus was discontinued and 60 mg prednisolone was started.
‡Everolimus was discontinued, and AB (ciprofloxacine and co-trimoxazole) together with 40 mg prednisolone was given. Sputum cultures revealed no bacteria, some Candida species. After 1 day, oseltamivir was added and 3 days later voriconazol.
§First AB (doxycycline) was given which did not improve the pulmonary symptoms, and AB was discontinued. Then everolimus was discontinued, 30 mg of prednisolone was administered, and pulmonary symptoms resolved.
¶AB (cefuroxime) was given due to 10–100 colonies of *Escherichia coli* in sputum, because of lack of improvement, everolimus was discontinued, and pulmonary symptoms resolved.
**One month before pulmonary CT, patient was admitted with suspected pneumonia. AB was given. BAL cultures remained negative, and everolimus was discontinued. Because of continuing pulmonary symptoms, patient was readmitted 1 month later (while on prednisolone and tacrolimus). CT revealed OP and pulmonary embolism, and anticoagulation was started.

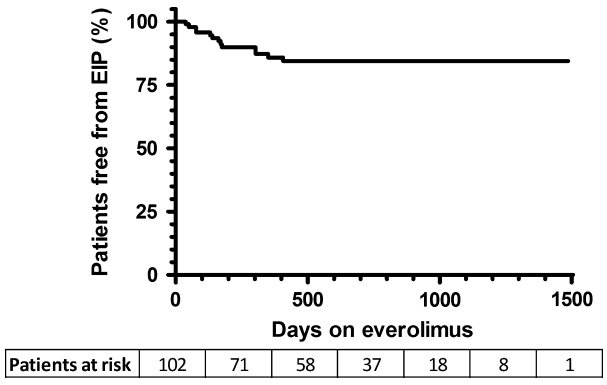


**Figure 1** Kaplan–Meyer curve demonstrating the time to development of everolimus-induced interstitial pneumonitis (EIP) in 13/102 (12.7%) renal transplant recipients treated with everolimus.

improvement of pulmonary function (data not shown). In three cases, follow-up CT scans were made after the diagnosis of EIP, which showed complete resolution of pulmonary abnormalities compatible with pneumonitis seen on earlier CT scans. None of the patients were rechallenged with everolimus.

Follow-up data on renal outcome were available for 12/13 patients. Of those 12 patients, 7/12 switched to P/CsA, 2/12 switched to P/tacrolimus, 2/12 switched to P/CsA/MPS (of those one continued later on P/MPS), and 1/12 switched to P/MPS. None of these patients developed a rejection after conversion. The median time from the switch from everolimus to another immunosuppressive regimen and last follow-up was 658 (0–1217) days. In that period, eGFR declined with a median (range) of 4.5 (−14.1 to 24.2) ml/min, corresponding with a median decline of 2.8 (range −5.1 to 18.3) ml/year. Kidney function in the patients on everolimus who did not develop an EIP remained stable after switch from P/CsA/MMF at 6 months until 2 years after transplantation (median (range) GFR change + 1.3 (−24.2 to 13.4) ml/min/year).

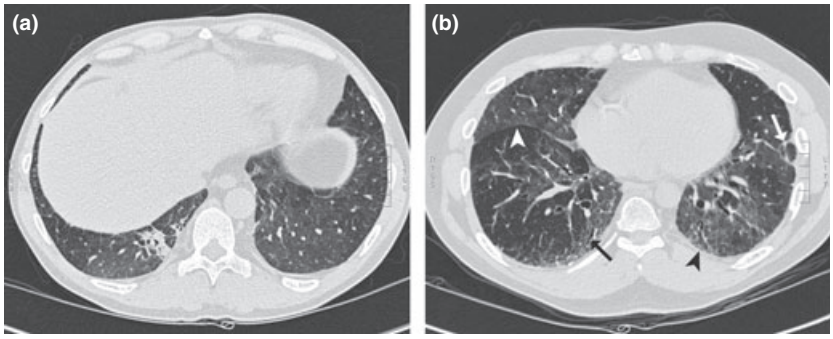


**Figure 2** (a) Organizing pneumonia: sharply demarcated consolidation, with a peribronchial and subpleural localization in the right-sided dorsal pleural sinus. Both lungs reveal a mosaic pattern. (b) Nonspecific interstitial pneumonitis: subpleural and peribronchovascular ground-glass opacities (white arrow head). Bronchodilation (black arrow) and thickened interlobular septa (black arrow head) within these ground-glass opacities. Furthermore, perilobular septal thickening (white arrow) compatible with a component of organizing pneumonia.

### Risk analysis for EIP development

We could not identify any predisposing factors to EIP, for example a known prior pulmonary history or smoking, nor was there a difference in renal function between cases and controls. Exposition to everolimus, expressed as area under the curve (AUC) or trough levels, was similar in cases and control patients (Table 1). According to the study protocol, everolimus exposure was monitored by AUCs 1 month, 6 months, 12 months, and 18 months after the initiation of everolimus. Additional everolimus AUC or trough level measurements were only performed when asked for by the treating nephrologist.

In cases, median time between confirmation of EIP by CT scan and most recent AUC was 69 (6–318) days. In case of patient compliance, the AUC is expected to be stable. The (median) AUC of everolimus was 207 (108–266) μg h/l, corresponding with trough levels of 10.7 (6.6–15.2) μg/l. During follow-up, 68.4% and 50% of the AUCs measured in the cases were >150 and >200 μg h/l, respectively, versus 69.0% and 32.2% in the control patients (NS). 73.7% and 38.9% of the trough levels measured in cases versus 69.4% and 23.1% in control patients, respectively, were >8 and >12 μg/l.

## Discussion

Our study is the largest case series of everolimus-induced pulmonary disease in solid organ transplantation. Pneumonitis appears a common adverse event complicating the use of everolimus after renal transplantation, with an incidence of 12.7%. No clear predisposing factors are identified in our case–cohort study. Pulmonary CT scans reveal an OP or NSIP-like pattern. The course seems benign with disappearance of symptoms within 1 year after discontinuation of the drug.

The incidence of EIP (12.7%) reported in our study is higher than previously reported in RTR on mTORi, varying between 4 and 6.8% [26–28]. The true incidence of EIP in our cohort might even be higher because possible cases in whom pulmonary imaging with CT scan was lacking were excluded from analysis (Table S1, supplementary data). Furthermore, the reported incidence in our study is an underestimation of the true incidence of EIP, as EIP can be present on pulmonary CT scan without causing symptoms as demonstrated by White *et al.* [13] who routinely performed pulmonary CT scans in patients with advanced non-small cell lung cancer treated with everolimus. We identified one asymptomatic case in our cohort.

In patients treated with everolimus for renal cell carcinoma, the incidence of EIP has been reported to be around 25% [13,29,30]. This high incidence of EIP has been attributed to higher dosage of everolimus in these patients in combination with a higher detection level of EIP due to routinely performed pulmonary CT scans. In our study, drug exposure was relatively high with an AUC around 170 μg h/l and trough levels around 10 μg/ml because everolimus was prescribed as part of a double immunosuppressive regimen. However, everolimus exposure was not higher in the cases compared to controls. Remarkably, all patients developed EIP within 407 days; hereafter, no EIP occurred. When reviewing the literature, we found only two cases of EIP occurring beyond 407 days.

Much debate exists on the etiology of mTOR-induced pneumonitis. White *et al.* [13] showed that patients with interstitial lung disease on baseline CT scans, whether focal or diffuse, had a higher incidence of all types of pneumonitis. This may reflect the tendency of patients with underlying lung disease to develop more serious toxicity. Therefore, we hypothesized that previous pulmonary disorders (reported in the medical charts) could be a predisposing factor to the development of EIP in our patient cohort.

The incidence of an underlying pulmonary disease was 30.8 and 17.1% in cases and controls, respectively. This difference was not significant ($P = 0.25$), nor was the difference in smoking. Furthermore, we found no difference in GFR, which has also been suggested as a potential risk factor [14].

Therapeutic drug monitoring (TDM) of everolimus is essential due to the narrow therapeutic window in combination with highly variable pharmacokinetics. Moreover, direct toxicity of everolimus in the etiology of EIP is suggested [8]. As systematic everolimus AUCs and trough levels were determined in our study, we were able to

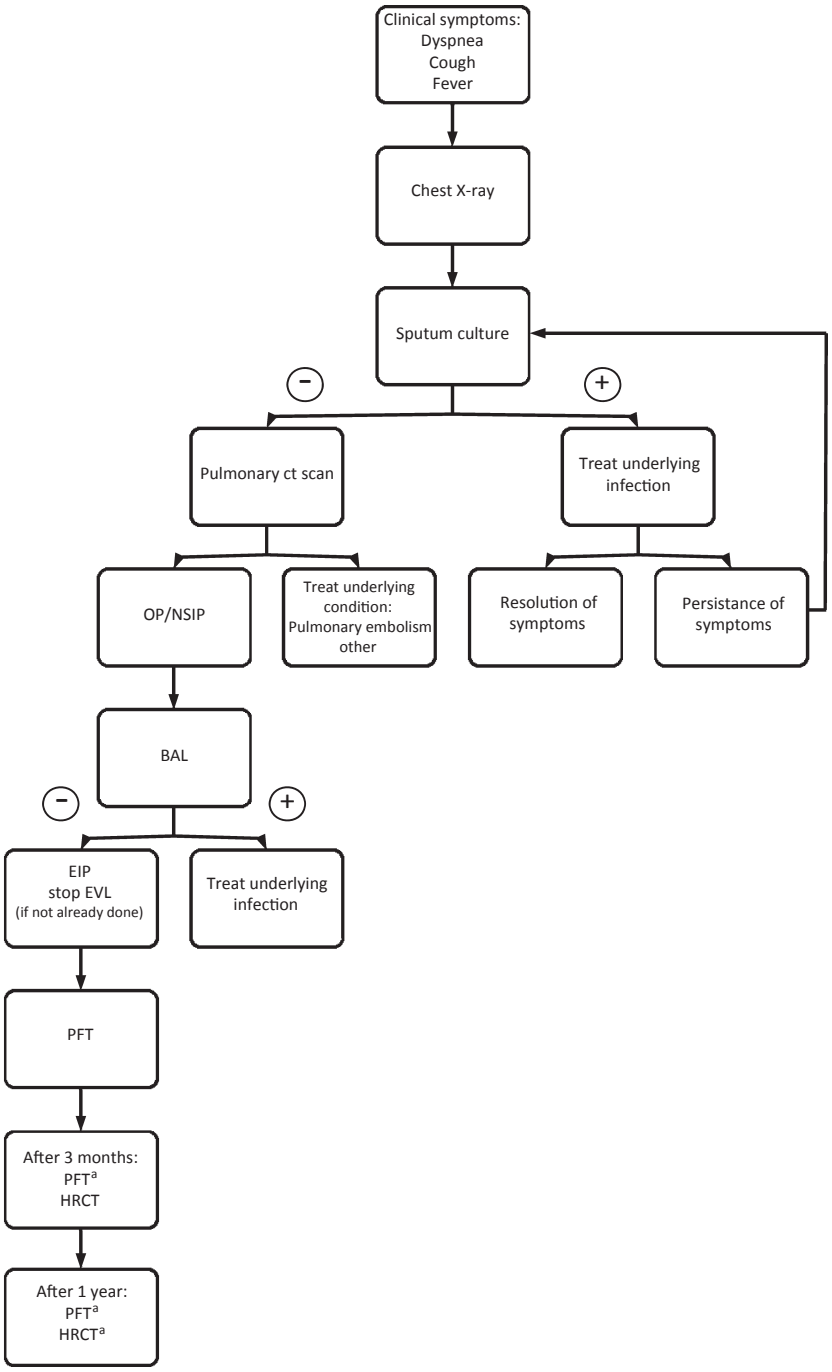


**Figure 3** Algorithm for the diagnosis everolimus-induced pneumonitis in patients using everolimus. OP, organizing pneumonia; NSIP, nonspecific interstitial pneumonitis; BAL, broncho-alveolar lavage; PFT, pulmonary function test; EIP, everolimus-induced pneumonitis; EVL, everolimus; HRCT, high resolution CT. [a]If abnormal in previous test.

accurately assess the exposure to everolimus in the cases and controls. Comparable exposure to everolimus in cases and controls makes toxicity simply based on higher exposure unlikely. We were not able to confirm the immune-mediated hypothesis, due to lack of flowcytometric analysis of BAL fluid.

Our study confirms the previous findings of EIP presenting radiographically with an OP-like pattern, NSIP-like pattern, or a combination of both, making CT imaging a valuable tool to discriminate infection from a direct everolimus effect. Limitations of this study are its retrospective design and the lack of a standardized follow-up of the patients. Although this is a large cohort of patients and we found an incidence of EIP of 12.7%, the absolute number of cases is still limited, which might have masked significant risk factors. Another limitation is that in some patients, a BAL to rule out pathogenic microorganisms was not performed and that previous use of antibiotics could have masked underlying infection in those patients who underwent a BAL. However, antibiotic treatment did not result in clinical improvement and recovery only occurred when treatment with everolimus was stopped. Three patients received additional corticosteroids. The effect of corticosteroids, administered at the same time as withdrawal of everolimus on the disappearance of symptoms is unclear. Some found that inhibition of mTOR blocks the anti-inflammatory effects of glucocorticoids in myeloid immune cells [31], suggesting that corticosteroids might not be beneficial in mTOR-induced pneumonitis. All patients subjectively recovered within 1 year. The long-term outcome after EIP is unclear because NSIP is known to potentially result in pulmonary fibrosis.

In conclusion, EIP is a common side effect of everolimus in RTR presenting radiographically with consolidations matching an organizing pneumonia, a nonspecific interstitial pneumonitis-like pattern, or a combination of both. No clear predisposing factors could be identified. As the presentation of EIP can be insidious or even asymptomatic, we recommend to perform radiographic imaging of the lungs when patients present with dyspnea, cough, or fever while on treatment with this drug according to the algorithm shown in Fig. 3. Moreover, as we did not find a correlation with exposure to everolimus between cases and controls, we advise to halt everolimus instead of reducing the dosage following EIP.

## Authorship

MCB and GHS: project design, collection, and interpretation of data and writing of the manuscript. DJARM: collection and interpretation of data and revision of the manuscript; IAHB and REJ: data interpretation and revision of the manuscript. JWF and JJHH: project design and revision of the manuscript. MD: data collection and revision of the manuscript. IJMB and FJB: project design, data interpretation, and revision of the manuscript.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1**. Characteristics of 7 renal transplant recipients with pulmonary symptoms not surely attributable to everolimus.

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