Transpl Int (2002) 15: 226–232
DOI 10.1007/s00147-002-0399-8

ORIGINAL ARTICLE

Ulf P. Neumann
Jan M. Langrehr
Udo Kaisers
Martina Lang
Volker Schmitz
Peter Neuhaus

Simultaneous splenectomy increases risk for opportunistic pneumonia in patients after liver transplantation

Received: 12 October 2000
Revised: 15 October 2001
Accepted: 20 January 2002
Published online: 6 April 2002

U.P. Neumann ✉ · J.M. Langrehr
M. Lang · V. Schmitz · P. Neuhaus
Department of Surgery,
Virchow Clinic, Humboldt University,
Augustenburger Platz 1, 13353 Berlin,
Germany
E-mail: ulf.neumann@charite.de
Tel.: +49-30-450552001
Fax: +49-30-450552900

U. Kaisers
Department of Anesthesiology
and Intensive Care Medicine,
Virchow Clinic, Humboldt University,
Augustenburger Platz 1, 13353 Berlin,
Germany

Abstract Life threatening pneumonias after liver transplantation are often caused by opportunistic pathogens such as *Legionella pneumophila*, *Pneumocystis carinii*, *Aspergillus* species and cytomegalovirus (CMV). Due to the high incidence of morbidity and mortality caused by these pneumonias we reviewed 700 liver transplants for risk factors for the development of opportunistic pneumonia. Immunosuppression was commenced as either cyclosporin A- or tacrolimus-based protocols. In a subgroup of patients, splenectomy was performed simultaneously with liver transplantation ($n = 57$). Overall 60 opportunistic pneumonias occurred in 700 liver transplants. Using a stepwise logistic regression analysis, we found that OKT3 treatment and simultaneous splenectomy revealed a significantly increased risk for opportunistic pneumonia. Our study identified splenectomy as a major risk factor for the development of opportunistic pneumonia after liver transplantation. In these patients prophylactic protocols and early diagnosis may improve the long-term outcome.

Keywords Opportunistic pneumonia · Liver transplantation · Splenectomy · OKT3

Introduction

Pneumonia after orthotopic liver transplantation (OLT) occurs in up to 15% of the liver graft recipients and has led to a high incidence of morbidity and mortality [5, 17, 25]. The most common pathogens identified are aerobic gram-negative bacilli, which usually originate from the recipient's enteric flora [8, 13]. However, the spectrum of infectious pathogens in OLT recipients is different from that in the general population. Several reasons may account for the high incidence of pneumonias in patients after OLT. Abnormalities in pulmonary function have been reported in association with chronic liver diseases of various etiologies [16]. In contrast to immunocompetent patients, infections caused by opportunistic pathogens such as *Pneumocystis carinii*, *Aspergillus fumigatus*, cytomegalovirus (CMV), and *Legionella pneumophila* are more often seen in OLT recipients [9]. Long-term immunosuppression is the most probable factor for the development of these pneumonias [9], which commonly occur in the late period (> 1 month post-transplant) [35] after OLT. More recent protocols of immunosuppression, using quadruple regimens or tacrolimus-based primary immunosuppression for induction, are designed mainly to reduce rejection episodes. However, the risk of infectious complications associated with more aggressive immunosuppression remains, and may even develop as a more serious threat in patients undergoing OLT [28, 40].

In this retrospective analysis, the spectrum of opportunistic pneumonias (OPs) was analyzed in 700 liver transplants to identify risk factors for OP and investigate the outcome of OP after OLT.

Patients and methods

Study population

From September 1988 to December 1995 we performed 700 OLTs in 646 (373 male and 273 female) patients. Of these patients 633 (98%) were adults (median age: 47.5 years: range: 16–72 years) and 13 (2%) children (median age: 4.1 years: range: 0.7–15 years) below 16 years. Follow-up ranged from 1 to 98 months (median: 38 months). The underlying diseases leading to liver failure included post-necrotic cirrhosis, cholestatic diseases, malignancies, acute liver failure, metabolic diseases, re-transplantations, and further miscellaneous diagnoses as depicted in Table 1.

Retrospectively, data were collected on all pulmonary infections and classified by the pathogen identified. Analyzed risk factors for impaired outcome and higher incidence of pneumonia were age, gender and underlying disease. Surgical factors analyzed included the overall number of surgical procedures, the duration of the transplantation procedure, simultaneous splenectomy and the intra- and peri-operative blood transfusion requirement during the 24 h after OLT.

Furthermore, we analyzed the incidence of OP depending on the cold ischemia time, the immunosuppressive induction protocol, rejection episodes, and additional anti-rejection treatment.

Surgery

Grafts were preserved exclusively using University of Wisconsin solution. OLT was performed by the standard technique using a venous–venous bypass and completion of all vascular anastomoses prior to reperfusion, as previously described [27].

Immunosuppression

Standard immunosuppression was commenced as either cyclosporin-A (CsA)- or tacrolimus-based regimens (Table 2). CsA-based protocols consisted of triple therapy (CsA, azathioprine, steroids) or quadruple drug induction regimen including an antithymocyte globulin preparation (ATG, Fresenius, Bad Homburg, Germany), or a monoclonal anti-interleukin 2-receptor antibody (BT563, Biotest, Dreieich, Germany) [22, 26] (Table 2).

In the tacrolimus protocol, which was part of the European multicenter trial [11], tacrolimus was administered as a single i.v. dose of 0.075 mg/kg bodyweight within 8 h of OLT. Prednisolone was given once daily, tapered to a dose of 15 mg per os as a maintenance dosage [11].

The dual tacrolimus was changed to oral intake (2×0.05 mg/kg/day) also during the immediate postoperative course and started with 40 mg prednisolone per os postoperatively [29]. Tacrolimus-based triple therapy used an initial oral dosage of 2×0.03 mg/kg/day as well as oral azathioprine (2×1–2 mg/kg/day) and prednisolone (20 mg per os) [29]. In addition, a quadruple immunosuppressive induction regimen including tacrolimus (2×0.05 mg/kg/day orally), ATG (5 mg/kg/day), azathioprine and prednisolone was compared with a dual protocol containing tacrolimus (2×0.1 mg/kg/day) and prednisolone.

Table 1. Indications for 700 liver transplantations in 646 patients

Indication	$n=700$	%
Post-necrotic cirrhosis	331	47.2
Autoimmune cirrhosis	21	3
Cholestatic diseases	95	13.6
Acute liver failure	43	6.1
Metabolic diseases	29	4.1
Malignancies	71	10.1
Re-transplantation	57	8.1
Miscellaneous	53	7.5

Table 2. Incidence of OP depending on the immunosuppressive protocol. Patients receiving antithymocyte globulin preparation (*ATG*) or a monoclonal anti-interleukin 2-receptor antibody (BT563) had a risk of 7.8% to 8.9% of developing OP after OLT. Notable was the low incidence of OPs in patients on tacrolimus-based protocols compared with those on CsA-based protocols. The incidence of OP was not significantly altered by the different immunosuppressive induction regimens

Immunosuppression induction therapy	Incidence of OP
CsA, prednisolone, azathioprine	2/33 (6. 1%)
ATG, CsA, azathioprine, prednisolone	25/279 (8.9%)
BT563, CsA, azathioprine, prednisolone	12/153 (7.8%)
Tacrolimus, prednisolone	8/147 (5.4%)
Tacrolimus, prednisolone, azathioprine	2/28 (7.1%)
ATG, tacrolimus, prednisolone, azathioprine	2/60 (3.3%)
CsA-based	39/465 (8.4%)
Tacrolimus-based	12/235 (5.1%)

Management of rejection

Rejection episodes were suspected in the case of rising liver serum enzymes, reduced bile flow, altered bile color, fever, clinical deterioration and confirmation by graft biopsy. Initial treatment of acute cellular rejection episodes consisted of 500 mg methylprednisolone for 3 days. Steroid-resistant rejection episodes were again treated with a monoclonal CD3 (OKT 3: 5 mg/day, Orthoclone, Janssen-Cilag, Neuss, Germany) antibody for 5–7 days. Since tacrolimus became available to our transplant center in May 1990, it has been introduced as a rescue agent for OKT-3 non-responders, or as soon as changes indicating a chronic rejection become evident.

Infection prophylaxis

Infection prophylaxis consisted of peri-operative (48 h) administration of antibiotics (cefotaxime 4 g/day i.v.; metronidazole 1 g/day i.v.; and tobramycin 120 mg/day i.v.). Selective bowel decontamination with oral polymyxin B (100 mg/day), tobramycin (80 mg/day) and nystatin (0.5 MU/day) was started after the patient had been listed with Eurotransplant, and was continued for 3 weeks postoperatively. The same antibiotic combination was used as an oral paste four times/day [34, 41]. After February 1992 all patients received oral ciprofloxacin (2×250 mg/day) for prophylaxis of *Legionella pneumophila* and oral prophylaxis against *Pneumocystis carinii* pneumonia with 480 mg trimethoprim/sulfamethoxazole (SMZ/TMP) was applied three times a week. This prophylactic regimen was continued for 4 weeks after the patient was discharged from the hospital. For anti-viral prophylaxis, CMV hyperimmunoglobulin (1 ml/kg bodyweight, Syntex & Grünenthal, Aachen, Germany) was given on days 1 and 14 after transplantation, and low-dose acyclovir (3×200 mg/day) was administered orally 6 weeks after OLT.

Pneumonia

Clinical symptoms indicating pneumonia were: cough, dyspnea, fever, leukocytosis, impaired oxygen saturation, and appearance of new pulmonary infiltrates on the chest X-ray. Bacterial pneumonia was diagnosed by positive bacterial cultures from blood, sputum or bronchoalveolar lavage (BAL) fluid in association with the clinical symptoms described above.

Common opportunistic micro-organisms which cause pulmonary infections in immunocompromised patients are *Legionella pneumophila*, *Aspergillus fumigatus*, CMV, *Pneumocystis carinii*, and *Mycobacterium tuberculosis* [9, 35]. Legionellosis was diagnosed by urinary antigen detection and the direct immunofluorescence test [33, 42]. *Aspergillus fumigatus* pneumonia was determined in the presence of a positive culture of the fungus in the BAL fluid and positive antigen detection in peripheral blood probes (titer > 1:4). *Pneumocystis carinii* pneumonia (PCP) or CMV pneumonia were diagnosed in patients with dry cough, dyspnea, interstitial infiltrates on repeated chest X-rays associated with the detection of *Pneumocystis carinii* or CMV in BAL fluids. Suspected PCP was confirmed by Grocott-Gomori methenamine-silver nitrate stain [14]. Assessment for CMV was performed by testing the immediate early antigen, or detecting the virus in the BAL using polymerase chain reaction (PCR) [39].

Treatment

Treatment of *Legionella pneumophila* was commenced with erythromycin (1.5–2 g/day in three to four doses), and six patients received a combination therapy of erythromycin and rifampicin (1.2 g daily in two doses).

Antifungal treatment was with amphotericin B in combination with flucytosin, and dosing was performed according to the drug serum levels and kidney function.

PCP was treated with trimethoprim/sulfamethoxazole (TMP 20 mg/kg/day i.v.; SMZ 100 mg/kg/day i.v.). The dosage was adjusted according to the renal function of the patients.

CMV pneumonia was treated with ganciclovir (10 mg/kg/day in two daily doses) and CMV hyperimmunoglobulin (1 ml/kg/day). Non-responders to this treatment received foscarnet at 90 mg/kg/day in one daily dose.

Statistical methods

Data were depicted as mean ± SD. Risk factors for opportunistic infections were analyzed by univariate and stepwise logistic regression. Categorical data were analyzed by Fisher's exact-test for simple cross-tables and likelihood ratio chi-square test for higher dimensional (2×3) tables. Continuous variables were tested with the Mann-Whitney rank-sum test. Significance was accepted when $P < 0.05$. Potentially confounding variables associated with the development of an OP ($P < 0.1$) were identified as true confounders. Subsequently, the likelihood of factors being responsible for the development of postoperative OP was investigated by stepwise logistic regression.

Results

The actuarial 1-, 3-, and 5-year patient survival values of the entire study population ($n = 646$) were 91%, 89% and 85%, respectively. The overall mortality rate of all patients after OLT was 83/646 (12.8%). In 20 of these 83 (24.1%) patients an OP was responsible for their death. Altogether, 121 pneumonias occurred in 99/646 (15.3%) patients, with 60 opportunistic infections occurring in 51/646 (7.9%) patients. Distribution of micro-organisms responsible for pneumonia, and outcome, are shown in Table 3. Eighteen of 51 (35%) female and 33/51 (65%) male patients experienced one or more severe opportunistic lung infections after transplantation. The median age of patients experiencing an OP was 47.9 years (21–66 years) compared with 46 years (0.7–72 years) in patients without an OP. Underlying disease and preoperative child score did not contribute to an increased risk of OP after OLT (Table 4).

Immunosuppression

The immunosuppressive induction protocol had no significant impact on the incidence of OP (Table 2). A total of 465 patients received a CsA-based triple or quadruple induction regimen. The incidence of OP for the triple- and the quadruple treated group of patients was not significantly different (6.6% vs. 8.9%). The incidence of OP tended to be lower in tacrolimus-based immunosuppressive regimens, reaching 12/235 (5.1%), than in CsA-treated patients, with an incidence of 39/465 (8.4%) (not significantly different, Table 4). The incidence of OP in patients with steroid bolus anti-rejection treatment was 4% (7/168) vs. 6.5% (25/380) in patients without rejection episodes (not significantly different). Ninety-eight patients received either tacrolimus- or OKT3-rescue treatment.

Patients receiving steroid bolus- and OKT3-treatment (54 patients) showed a significantly higher risk for OP [14/54 (26%), Table 4] than patients without OKT3 treatment (40/592, 6.7%, $P < 0.01$). The incidence of OP in patients with steroid-bolus treatment and tacrolimus-rescue therapy [12/69 (17.4%)] was not significantly increased compared with patients without rejection treatment (Table 4).

Surgery

To assess the impact of surgical factors on the incidence of OP after OLT, we reviewed the length of the transplantation procedure, the number of re-transplantations, the number of repeat laparotomies and blood transfusion requirements. Using a stepwise logistic regression analysis we found that the incidence of OP was independent of the duration of the transplantation

Table 3. Distribution of pathogens, incidences, and clinical outcome of pneumonias after liver transplantation. Overall 60 OPs occurred in 51 patients

Pathogen	Incidence	Mortality
Pneumocystis carinii	8 (6.6%)	7 (87.5%)
Legionella pneumophila	15 (12.4%)	2 (13.3%)
Aspergillus fumigatus	8 (6.6%)	5 (62.5%)
CMV	24 (19.8%)	6 (25%)
Mycobacterium tuberculosis	5 (4.4%)	0
Bacterial	61 (50.4%)	1 (1.6%)
Total	121 (100%)	21 (17%)

Table 4. Risk factors for the development of an OP after OLT. Variables potentially associated with the occurrence of OP were evaluated by a univariate and multivariate stepwise logistic regression. Data are depicted as mean ± standard deviation: $P < 0.05$ was considered statistically significantly different. Only patients with OKT3 treatment and splenectomized patients yielded a significantly increased risk for OP in this analysis

Risk factors	Patients with OP ($n = 51$)	Patients without OP ($n = 595$)	Univariate P	Multivariate P
Rejection episodes (n)	26	266	0.185	–
OKT3 treatment (n)	14	40	0.001	0.005
Tacrolimus rescue (n)	12	57	0.02	0.1
Tacrolimus vs. CsA	12 vs. 39	223 vs. 426	0.1	0.37
Underlying disease	–	–	0.226	–
Repeat laparotomies (n)	11	79	0.05	0.34
Re-transplantation (n)	6	51	0.285	–
Splenectomy (n)	14	43	0.006	0.017
Gender (male/female)	33/18	353/242	0.38	–
Preoperative child classification (A/B/C)	9/18/24	121/273/201	0.42	–
Age (years)	47.3 ± 10.1	45 ± 12.3	0.3	–
Duration of the OLT (h)	6.7 ± 2.1	5.8 ± 1.9	0.02	0.1
Cold ischemia time (h)	10.5 ± 4.6	10.1 ± 3.6	0.9	–
Intraoperative blood requirement (U)	8.1 ± 7	8.2 ± 5.2	0.2	–

procedure. Patients undergoing a re-transplantation had a similar risk for OP (11%) as did a patient after primary OLT (9%, n.s.) (Table 4). Cold ischemia time (10.5 ± 4.6 h vs. 10.1 ± 3.6 h) and intraoperative blood transfusion requirements during the transplantation procedure (8.1 ± 7 U vs. 8.2 ± 5.2 U) were similar in patients both with OP and without OP (Table 4).

The incidence of OP was increased in patients undergoing liver transplantation with simultaneous splenectomy, compared with patients without simultaneous splenectomy. In this subgroup, 14/57 (24.5%) splenectomized patients developed OP after liver transplantation, in contrast to 43/589 (7.3%) patients without simultaneous splenectomy ($P < 0.05$, Table 4).

In 90 of 700 transplants one or more repeat laparotomies were performed up to the 28th postoperative day. Of these patients, 11/90 (12%) developed an OP, compared with 33/610 (5.4%) without re-operation after the liver transplantation.

Discussion

Diagnosis of OP remains difficult, because the parameters used to define pneumonia might be misleading. In the post-transplant period, patients developing fever, leukocytosis, and non-specific radiological signs in the chest X-rays might not necessarily have pneumonia. Rejection or other infections such as sinusitis or phlebitis from intravenous catheters can cause fever and leukocytosis. Radiological patterns are not specific for pneumonia, and even in apparent pneumonia, an etiological diagnosis may not be defined by the presence of radiological findings. Another helpful tool in such cases might be high-resolution computer tomography of the thorax, which can give better and quicker information about pathological changes of the lungs than can the plain X-ray [18].

To obtain an etiological diagnosis for pneumonia, several methods, including collection of sputum and BAL, are in use. Two reported meta-analyses concluded that bronchoscopic methods should not necessarily be accepted as standard for diagnosis of pneumonia in the ICU for non-immunocompromised patients [3, 6]. Due to the high mortality rates of OP in patients after OLT, an early recognition of OP is a necessity to start a specific therapy early and improve the outcome of OP after OLT. Therefore, we believe that invasive diagnostics are justified in this patient population. However, because of possible complications due to invasive diagnostic measures, it would be helpful to identify patients at special risk of development of OP. Previous studies have described various factors associated with the development of opportunistic lung infections after OLT, including surgical time, repeat laparotomies, number of rejection episodes and OKT3 treatment [9, 20, 31, 32, 36]. In our study the incidence of OP was independent from the immunosuppressive induction regimen, however, there was a low incidence of OP in patients with tacrolimus-based induction therapy compared with CsA-based protocols. This difference did not reach significance, but clearly indicates that tacrolimus-based immunosuppression is not associated with a higher incidence of OP after OLT.

We observed a significantly higher incidence of OP in patients undergoing OKT-3 therapy for steroid-resistant rejection episodes, as described previously [9], which can be explained by the increased immunosuppression in those patients. The duration of the transplantation procedure, number of repeat laparotomies and transfusion requirement had no influence on the occurrence of OP in this study, which stands in contrast to that by Paya et al. who reported these factors to be responsible for the occurrence of severe bacterial and CMV infections [32]. Reasons for these differences may be the use of leukocyte-depleted transfusions from CMV-negative

donors in our study. For the first time, our study pointed to simultaneous liver transplantation and splenectomy as an independent risk factor for the occurrence of OP in this study population. Changes in response to specific antigens and/or altered T-cell function might be responsible for the increased incidence of OP in splenectomized recipients of liver grafts, however, the etiology remains unclear [38]. There are contradictory reports regarding the effect of splenectomy after OLT. During the early days of liver transplantation, splenectomy was used to reduce the immunological response of the recipient. Additionally, the nearly universal presence of pancytopenia in liver-transplant candidates and consequent problems with azathioprine dosing was improved [37]. In our retrospective analysis splenectomy was performed in patients with excessive hypersplenism, hypersplenomegalia or steal syndrome. The preoperative status and child score of patients with a splenectomy were not impaired when compared with those of patients without a splenectomy. Also, splenectomy was not associated with more blood transfusions, which increases the evidence that long-term changes in the immunological response of the patient due to the splenectomy are responsible for the high risk of opportunistic infections after OLT. These data are supported by a study by Samimi et al. who could clearly show that splenectomy concomitant with OLT produces a significantly higher mortality rate [37]. In this study the high mortality rates were caused mainly by infectious septic complications. Similar results in the past have been reported after bone-marrow transplantation [12].

Legionnaires' disease has been recognized as a potentially fatal cause of bacterial pneumonia in immunocompromised, non-transplanted patients, producing a progressive pneumonia with a mortality rate reaching 30% [24] and has been reported in patients after renal, cardiac or bone-marrow transplantation [7, 19, 23], which facts are also in line with our observations where *Legionella pneumophila* was responsible for 15/60 (25%) OP, with a mortality rate of 2/15 (13%). *Pneumocystis carinii*, originally classified as a protozoan, was recently reclassified as a fungus [10]. PCP has been described as occurring in up to 10% of pediatric patients [4] and in 5.2% [14] of adults after OLT. In contrast, we observed a much lower incidence of PCP in our study population [1% (8/646)]. The clinical symptoms of patients with PCP included fever, dry cough, hypoxia and interstitial infiltrates in the chest X-ray and were non-specific and often indistinguishable from CMV pneumonia [15]. It cannot be diagnosed by examination of the sputum alone. Therefore, in our patients, diagnosis of PCP was established by BAL. The high mortality rate from PCP was caused by co-existing liver graft dysfunction and simultaneous CMV infection. None of these patients with PCP was on prophylactic SMZ/TMP treatment at the time of the onset of PCP. This result confirms previous reports on PCP in liver- and bone-marrow-transplant recipients, which proved SMZ/TMP to be effective in the prevention of PCP in immunocompromised patients [1].

The biological significance of isolated *Aspergillus* species in any clinical specimen is still unclear [21]. A major cause for the high mortality rate [5/8 (62.5%)] in our patient population might be the difficulty of diagnosing an *Aspergillus* infection early, as previously mentioned by others [2]. Isolation of *Aspergillus* species in clinical specimens ranges between 2% and 8% in a general population without an infection [43]. Isolation of *Aspergillus* species from respiratory specimens does not imply disease, as this fungus may be a colonizer or a laboratory contaminant. Because of severe side effects of antifungal treatment with amphotericin B and flucytosin (nephrotoxicity, hepatotoxicity), early distinction between colonization and invasive aspergillosis appears to be mandatory in improving the outcome of patients with positive *Aspergillus* detection after OLT. Previous studies demonstrated that two or more positive cultures of *Aspergillus* species may be helpful in distinguishing between infection and colonization. Yu et al. observed that positive cultures rarely indicate disease in non-compromised patients but are virtually indicative of invasive aspergillosis in leukemic patients with neutropenia [45]. Therefore, in recent years, to prevent serious complication we started antifungal treatment after the first positive *Aspergillus* culture in the BAL associated with an increase in *Aspergillus* antigen in peripheral blood.

Infection with human CMV is a major cause of morbidity in immunocompromised patients and is known to occur frequently in patients after solid organ transplantation. Introduction of newer diagnostic methods and therapeutic agents have improved the outcome of CMV infection after OLT during the past decades [39]. Several prophylactic protocols are used today to prevent CMV infection [44]. Patients receiving a graft from a CMV IgG positive donor are especially at high risk of developing a CMV infection [30]. Our study demonstrated a significantly increased risk of CMV pneumonia in patients treated with OKT3 for steroid-resistant rejection episodes. CMV was the most frequent pathogen leading to an opportunistic infection in this study population. However, 18/24 patients undergoing CMV pneumonia were treated effectively with i.v. ganciclovir or Foscavir, and in 3/6 patients who died after developing CMV pneumonia, simultaneous PCP had occurred.

In our study we identified patients at high risk for OP after liver transplantation. The time-related spectrum of pathogens after transplantation has immediate clinical implications for screening, prophylaxis and therapy following OLT. Using logistic regression analysis we showed that patients undergoing OKT3 treatment or simultaneous splenectomy and liver transplantation are

at high risk of developing severe OP after OLT. In patients at high risk for OP, prophylactic protocols and early invasive diagnostics may be mandatory to improve the outcome after OLT. Splenectomy should be indicated only very cautiously in patients undergoing liver transplantation. In patients with excessive hypersplenomegalia, hypersplenism or a steal syndrome we now recommend banding of the splenic artery to reduce the arterial blood flow. However, the immunosuppressive induction protocol did not influence the incidence of OP significantly. SMZ/TMP proved to be effective in preventing PCP after OLT and is now given to all our patients after OLT. Especially in those patients at high risk of OP developing clinical features of pneumonia, an early establishment of the diagnosis is essential so that a specific therapy may be started that hopefully prevents fatal pulmonary complications.

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