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Pneumocystis jirovecii infection in liver transplant, is prophylaxis needed?

Infección por Pneumocystis jirovecii en el trasplante hepático, ¿es necesaria la profilaxis?

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LIST OF ABBREVIATIONS

BDG: β-D-Glucan
CMV: cytomegalovirus
HT: heart transplantation
IR: incidence rate
IS: immunosuppression
KT: kidney transplantation
LT: liver transplant
LuT: lung transplantation
MMF: mycophenolate mofetil
mTORi: inhibitors of the mammalian target of rapamycin
NA: not applicable
OKT3: monoclonal antibody targeted at the CD3 receptor
PJP*: Pneumocystis jirovecii* pneumonia
PTY: person transplant years
q.d.: daily
q.w.: once a week
SLF-PYT: sulfadoxine/pyrimethamine
SOT: solid organ transplant
TCMR: T cell-mediated rejection
t.i.w.: three times a week
TMP-SMX: Trimethoprim-sulfamethoxazole
TRANSNET: Transplant-Associated Infection Surveillance Network

ABSTRACT

Background: In liver transplant (LT) recipients*, Pneumocystis jirovecii* pneumonia (PJP) is most frequently reported before 1992 when immunosuppressive regimens were more intense. It is uncertain whether universal PJP prophylaxis is still applicable in the contemporary LT setting.

Aims: to examine the incidence of PJP in LT recipients followed at our institution where routine prophylaxis has never been practiced and to define the prophylaxis strategies currently employed among LT units in Spain.

Patients and methods: we retrospectively reviewed all LT performed at our center from 1990 to October 2019. The identification of PJP cases was performed through the individual review of medical records and through microbiological and hospital discharge records. All 25 adult LT units in Spain were queried via email to specify their current prophylaxis strategy against PJP.

Results: during the study period 683 LT procedures were carried out on 631 patients. Five cases of PJP were identified, with only one occurring within the first 6 months after the LT. The cumulative incidence and incidence rate were 8.2 cases per 1000 patients and 0.99 cases per 1000 persons transplant year. All LT units responded to our query, the majority of which provide prophylaxis (80%). PJP prophylaxis for the first 6 months was the most frequent duration reported (48%). In contrast, 20% of the centers do not apply prophylaxis.

Conclusions: the low incidence of PJP in our unprophylaxed cohort, with most cases occurring beyond the usual recommended period of prophylaxis, do not support a one-size-fits-all approach to PJP prophylaxis. A significant heterogeneity in prophylaxis strategies exists among Spanish LT centers.

Key words: Prophylaxis, Pneumocystis jirovecii, liver transplantation.

RESUMEN

Introducción: En los receptores de trasplante hepático (TH), la neumonía por *Pneumocystis jirovecii* (PJP) era informada con mayor frecuencia antes de 1992, cuando los regimenes inmnosupresores que se aplicaban eran más intensos. No se sabe con certeza si la profilaxis universal de la PJP sigue siendo recomendable en el contexto actual del TH.

Objetivos: Examinar la incidencia de PJP en los receptores de TH seguidos en nuestra institución donde nunca se ha llevado a cabo profilaxis frente PJP, y definir las estrategias de profilaxis empleadas actualmente en las distintas unidades de TH en España.

Métodos: Revisamos retrospectivamente todos los TH realizados en nuestro centro desde 1990 hasta octubre de 2019. La identificación de los casos de PJP se llevó a cabo mediante la revisión individual de la historia clínica de cada paciente y a través de los registros del Servicio de Admisión y del Servicio de Microbiología. Las 25 unidades de TH de España fueron consultadas por correo electrónico para especificar su estrategia de profilaxis actual contra la PJP.

Resultados: Durante el periodo de estudio se realizaron 683 TH en 631 pacientes. Se identificaron cinco casos de PJP, de los que solo uno ocurrió en los primeros 6 meses después del TH. La incidencia acumulada y la tasa de incidencia fueron de 8.2 casos por cada 1000 pacientes y 0.99 casos por cada 1000 pacientes trasplantados-año. Todas las unidades de TH respondieron a nuestra consulta, la mayoría de las cuales administran profilaxis (80%). La profilaxis en los primeros 6 meses fue la más frecuente (48%). En cambio, el 20% de los centros no la administra.

Conclusiones: La baja incidencia de PJP en nuestra cohorte de pacientes sin profilaxis, donde la mayoría de los casos ocurrieron tras el periodo usualmente recomendado de profilaxis, no respalda un enfoque único para la profilaxis de la PJP. Existe una significante heterogeneidad entre las estrategias de profilaxis de los diferentes centros españoles con unidad de TH.

Palabras clave: Profilaxis, Pneumocystis jirovecii, trasplante hepático.

INTRODUCTION

Pneumocystis jirovecii, formerly Pneumocystis carinii, is a ubiquitous, opportunistic fungus that causes *Pneumocystis jirovecii* pneumonia (PJP) in immunocompromised individuals, including solid organ transplant (SOT) recipients. This infection leads to substantial morbidity and mortality and prior to the broad implementation of prophylaxis, the risk of developing PJP among SOT recipients was approximately 5-15% [1]. This figure exceeds the recommended incidence threshold of 3-5% for using prophylaxis [2], and accordingly, current guidelines recommend anti**-**PJP prophylaxis for at least 6-12 months for all SOT recipients due to the higher degree of immunosuppression during these first months [1,3-5]. For lung and small bowel transplant recipients requiring higher intensity of immunosuppression or in case of prior PJP infection or cytomegalovirus infection, guidelines recommend considering prolonged prophylaxis[1]. Trimethoprim-sulfamethoxazole (TMP-SMX) is the prophylactic drug of choice with two meta-analysis reporting a reduction in the risk of PJP occurrence of 85-91% in non-human immunodeficiency virus immunocompromised patients when compared to no prophylaxis [2,6].

The evidence supporting the use of anti-PJP prophylaxis in liver transplant (LT) recipients, however, is less clear. PJP incidence varies with the type of organ transplanted, the geographic region, the immunosuppressive regimen utilized, and the period studied [1]. The high incidences of PJP in the absence of prophylaxis reported in LT cohorts from the 1980´s [7,8] contrast with those from recent series in which PJP incidence is below 3% [9-13] and even similar to incidences from LT recipients using prophylaxis [14-24] **(Table 1)**. Moreover, only one study concerning LT patients was included in the two meta-analysis reporting the efficacy of TMP-SMX prophylaxis, and this randomized clinical trial did not include a control group without prophylaxis as it assessed the efficacy and safety of weekly sulfadoxine/pyrimethamine compared with daily TMP-SMX [17]. These data question the risk-benefit ratio of a systematic PJP prophylaxis in LT recipients and may lead to variability in prophylactic strategies among centers. Little data are available in this latter regard and, to our knowledge, are restricted to pediatric SOT setting [25,26].

In this report, we aim to examine the incidence and characteristics of PJP in LT recipients followed at our transplant center where routine prophylaxis is not practiced since the beginning of our LT program in 1990 and to define the prophylaxis strategies currently employed for PJP prevention among LT units in Spain.

AIMS

The object of the present study is to examine the incidence and characteristics of PJP in LT recipients followed at our institution where routine prophylaxis has never been practiced and to define the prophylaxis strategies currently employed among LT units in Spain.

MATERIAL AND METHODS

Patients

The Marques de Valdecilla University Hospital (Santander, Cantabria, Spain) is an urban, academic tertiary care center with great expertise in organ transplantation. We conducted a retrospective review regarding PJP infection of all LT performed at our institution since the beginning of our adult LT program in November 1990 to October 2019. In the initial years our center performed all LT, not only from Cantabria, but also from several others Spanish autonomous communities such as Galicia, Basque Country, Canary Islands, Asturias, La Rioja and Castilla y Leon. These regions progressively developed their own LT programs over the following 12 years, and since 2009 our program is responsible of all LT performed in Cantabria and La Rioja. The organ donation activity in these two Spanish autonomous communities is the highest of our country (above 80 donors per million of population), and as of January 1, 2019, their combined population was 895,212 inhabitants. All patients received an ABO-compatible primary orthotopic LT from deceased donors using the piggyback operation [27] and no prophylaxis against PJP was undertaken, except for some patients with combined liver-kidney transplant.

In order to evaluate the local prevalence of PJP infection in other solid organ transplant (SOT) recipients at our institution, a retrospective review regarding this infection was also conducted in recipients of kidney (KT), heart (HT), and lung transplantation (LuT). The KT program was the first type of SOT to be performed at our center in February 26, 1975. At its beginning, it covered all the transplant activity from several Spanish regions (Cantabria, Castilla y Leon, Asturias and Basque Country), but currently this activity is restricted to Cantabria. However, the program still undertakes all combined kidney-pancreas transplants from Asturias, Basque Country, and La Rioja and KT in hyperimmunized patients from several other regions. Universal anti-PJP prophylaxis with TMP-SMX for 6 months is performed in all KT recipients since 1996. The HT program began in December 17, 1984. It has one of the highest HT rates of the country and performs all HT from various Spanish regions (Cantabria, Basque Country, and La Rioja). No prophylaxis against PJP is applied. Finally, the LuT program began in March 29, 1997. It currently covers all the LuT activity from Cantabria, Asturias, Basque Country, Navarra, La Rioja, and several provinces of Castilla y Leon (Palencia, León, Valladolid, Burgos, Salamanca and Zamora). Lifelong prophylaxis against PJP with TMP-SMX is established in all LuT recipients since the beginning of the program.

Cases

PJP cases were defined by the following criteria: (1) new onset of respiratory symptoms; 2) radiological findings consistent with PJP infection **(Figure 1)**; 3) microbiological demonstration of PJP infection (i.e. Real-time quantitative PCR, and/or Grocott methenamine silver stain performed in samples from bronchial alveolar lavage (BAL), sputum (spontaneous or induced), and transbronchial or open lung biopsy) **(Figure 2)**. Cases without microbiological confirmation were also included if the clinical and radiological picture supported the diagnosis of PJP. Information on demographics, indication for LT, time period between LT and PJP, diagnostic method, clinical

presentation, treatment and outcome of PJP, co-existing infections, immunosuppressive regimens used at PJP diagnosis, and previous acute or chronic rejection were retrieved for all LT patients.

The identification of PJP cases was performed using three approaches: 1) Individual review of the medical records of each LT recipient; 2) List of all laboratory-confirmed PJP cases from the Department of Microbiology; 3) Hospital discharge records. The latter consisted of a list of all patients admitted to our hospital with diagnosis upon discharge of PJP registered as code 136.3 of the International Classification of Diseases, Ninth Revision, Clinical Modification, listed in any position in the Hospitalization Minimum Data Set, that is the National database of hospital discharge records in Spain. These microbiological and discharge records were cross-referenced by medical record number against a secure intramural database of all LT recipients transplanted at our center. The search of PJP cases in the other SOT recipients did not include the individual review of their medical records and was limited to data obtained from the microbiological and discharge records, and also from each SOT database.

Immunosuppressive drug regimens in liver transplantation

From 1990 to 1999, postoperative immunosuppression was based on triple therapy with cyclosporine A, azathioprine and steroids. In subsequent years tacrolimus replaced cyclosporine as first-line therapy due to its better long-term graft and patient survival [28]. Similarly, mycophenolate mofetil (MMF) replaced azathioprine as the antimetabolite agent of choice and it was generally used for treatment of T cell-mediated rejection (TCMR) and/or for patients who had renal dysfunction limiting the dose of tacrolimus. The remaining patients received dual therapy with tacrolimus and steroids. The latter were tapered slowly during the first year at the beginning of the program and hereinafter were discontinued 4–6 months post-LT, except for those patients at higher immunological risk (e.g. immune-mediated diseases such autoimmune hepatitis). Inhibitors of the mammalian target of rapamycin (mTORi) were generally used in case of intolerance to MMF and/or development of de novo malignancy after LT. In the last decade, induction therapy with the interleukin-2 receptor blockers (basiliximab) was given as a calcineurin-sparing agent to patients with prior or postoperative significant renal impairment (i.e. creatinine clearance <60 mL/min). Long-term immunosuppression was adjusted to the recipient characteristics, etiology of primary liver disease and magnitude of alloimmune activation, with the aim of minimizing immunosuppression as much as possible. In the event of moderate and severe TCMR, management consisted of pulses of steroids (typically 1 g of methylprednisolone daily for 3 days) and an increase in calcineurin inhibitor therapy with or without addition of other agents (antimetabolites or mTORi). Mild TMCR was generally treated by increasing calcineurin inhibitor therapy.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Ethics Committee for Clinical Research of Cantabria. A waiver of informed consent was provided since the study was considered a retrospective review.

Prophylaxis strategies against *Pneumocystis jirovecii* in Spanish liver transplants units

All the 25 adult LT units in Spain were queried via email to specify their current prophylaxis strategy against PJP: drug of choice, dosage, and duration.

Statistical analysis

Quantitative variables were expressed as median and interquartile range and qualitative variables as proportions. Cumulative incidence was determined by the number of new PJP cases during the study period divided by the size of the population at risk (i.e. patients transplanted) per 1000. Incidence rate of PJP was determined in units of the reciprocal of person transplant years (PTY) calculated through April 2019, death, or loss to follow-up. Statistical analysis was performed with IBM SPSS Statistics v22.0 for Mac (IBM Corp., Armonk, NY, United States).

RESULTS

Incidence of *Pneumocystis jirovecii* in liver transplant recipients

From November 1990 to October 2019, 683 LT procedures were carried out on 631 patients. The most frequent liver disease and indication of LT was alcoholic liver disease and decompensated cirrhosis, respectively. Fifty-two patients were re-transplanted and 29 received other transplants, the most frequent of which was combined kidney-liver transplantation **(Table 2)**. Prophylaxis against PJP was established in 21 of these 29 recipients of other additional transplants (20 KT and one bone marrow transplantation) following the corresponding protocols of each program. All of them were given TMP-SMX and none developed PJP. The other patient who received a bone marrow transplantation died early after the third day and no prophylaxis was undertaken, whereas the reason for not initiating prophylaxis in the remaining KT patients could not be clarified after reviewing the medical record.

In the whole LT cohort five cases of PJP were identified, giving an overall cumulative incidence of 7.9 cases per 1000 patients and an incidence rate of 0.95 cases per 1000 PTY. Excluding the 21 patients in whom prophylaxis was undertaken, the cumulative incidence and incidence rate were 8.2 cases per 1000 patients and 0.99 cases per 1000 PTY, respectively.

Clinical presentation and outcome of *Pneumocystis jirovecii* infection in liver transplant recipients

The risk factors for PJP, clinical features, treatment and outcome of the five LT patients that developed PJP are shown in **Table 3**. Of the five patients only one was diagnosed within the first 6 months post LT and in two the infection occurred several years after LT. Three cases were diagnosed in the 1990´s and had more intense immunosuppressive regimens following the common practice at that time. Pulse steroid therapy for moderate/severe TCMR preceded PJP in two cases and co-existing infections were present in all but one patient. The most frequent symptom and radiological finding were fever with productive cough and ground glass opacities, respectively. In two cases no microbiological confirmation could be achieved, and diagnosis was based on clinical and radiological findings after discarding other etiologies. In another patient a lung biopsy was needed in order to rule out everolimus-induced interstitial lung pneumonitis. PJP was severe in two patients, causing death in one of them. All but one patient with severe pancytopenia were treated with TMP-SMX.

Pneumocystis jirovecii in other solid organ transplant recipients

Table 4 shows the number of transplants, cumulative incidence and outcome of PJP infection in each type of SOT. KT had the highest cumulative incidence with 8.8 cases per 1000 patients. Eight of the 14 KT recipients had been transplanted before the implementation of universal prophylaxis with TMP-SMX for the first 6 months in 1996. In these patients, PJP infection was diagnosed within 6 months in five of them (62.5%). From this period onwards, only one of the 6 cases of PJP (16.7%) was diagnosed within

this time frame. Mortality was high regardless of the duration of time since KT. Only one PJP case was identified in LuT and HT, with a cumulative incidence of 1.6 and 1.4 cases per 1000 patients, respectively. The LuT patient received prophylaxis with pentamidine due to sulfonamide allergy. Both cases occurred within the first 6 months and could be successfully treated.

Prophylaxis strategies against *Pneumocystis jirovecii* in Spanish liver transplants units

All 25 adult LT units in Spain responded to our query **(Figure 3)**, the majority of which provide PJP prophylaxis (80%). All of these centers reported TMP-SMF as their drug of choice and all use the same dosage, 160 mg of TMP and 800 mg of SMX (i.e. double strength) orally three times weekly. Duration of PJP prophylaxis, however, varied, with the administration of TMP-SMF for the first 6 months being the most frequent duration reported (n=12, 48%). In contrast, five centers (20%) do not indicate prophylaxis against PJP. All of them argued a perceived low incidence of PJP infection at their institution as the primary reason for not employing PJP prophylaxis.

DISCUSSION

In liver transplant recipients, PJP is most frequently reported before 1992 when immunosuppressive regimens were more intense [7,8]. As these regimens have evolved over time, it is uncertain whether universal PJP prophylaxis is still applicable in the contemporary LT setting. The results of the present study show, in the largest unprophylaxed LT cohort published to date, a very low incidence of PJP over a 30-year period, with most cases occurring beyond 6 months and during the first decade of the program when higher immunosuppression was prescribed. The survey to LT units in Spain indicates that while anti-PJP prophylaxis with TMP/SMX is generally implemented in most centers, there is a wide degree of variability within that practice, and there is also an increasing number of centers that do not apply prophylaxis.

The low incidence of PJP in our cohort is in line with recent series in which this infection occurred in less than 3% of LT recipients in the absence of prophylaxis [9-13]. These figures are below the recommended threshold for establishing anti-PJP prophylaxis in SOT patients [1,2], suggesting that previously reported attack rates, on which the current practice of PJP prophylaxis is based, may have lost validity due to less aggressive immunosuppression regimens and to improvements in the quality of the pre- and post-transplant patient care. Two of our cases occurred far beyond the first year which is in agreement with increasing reports of late-onset PJP [23,24]. Both of them had risk factors for its development, which include low total and CD4+ lymphocyte counts, cytomegalovirus infection, hypogammaglobulinemia, graft rejection, and patient age [1,8,23]. These risk factors, however, do not provide an accurate individual risk assessment and in order to decrease the morbidity of this infection but also to avoid unnecessary chemoprophylaxis because of its associated toxicity well-standardized criteria to establish PJP prophylaxis are most needed. Local PJP prevalence should also be taken into account when assessing this risk, as outbreaks of PJP may occur in nosocomial settings, possibly due to person-to-person spread [1,13]. Our data support a negligible nosocomial transmission at our institution given the absence of outbreaks and the low PJP incidence in the other SOT.

This change in the epidemiology of PJP in LT recipients may lead to different prophylactic strategies among transplant centers. Based on the responses of our survey, there is a lack of consistent or unified approach across LT units in Spain. In line with current guidelines, most of the centers (80%) employ universal anti-PJP prophylaxis, but there is large variability regarding its duration, with a trend towards a shorter period of treatment. This is not surprising as duration of prophylaxis has relied on expert consensus and not on high quality evidence [1]. All these centers used the same drug and dosage, TMP-SMF (160 mg / 800 mg) three times weekly. The most striking finding was that 20% of the units did not prescribe prophylaxis due to a perceived low incidence of PJP infection at their institutions.

The main limitations of our study are related to its retrospective design and to the fact that we do not provide risk factors to better identify patients at high risk for PJP. Our low incidence, however, makes this latter analysis unreliable. Given the thorough examination and the non-restrictive case definition for PJP (we included patients without microbiological confirmation) we believe in the accuracy of the reported incidence among LT recipients. Nevertheless, we acknowledge this incidence might be underestimated in the other SOT patients as the identification of PJP cases was based

solely on administrative and microbiological records. It must be highlighted, however, that these sources proved to be acceptably reliable since they identified 80% of PJP cases in LT recipients. Finally, we did not investigate the impact of our strategy on the occurrence of infections caused by other opportunistic agents sensitive to TMP-SMF (e.g. Toxoplasma gondii or Nocardia) [1].

CONCLUSIONS

In conclusion, our findings demonstrate both a low incidence of PJP in our unprophylaxed transplant cohort, with infection occurring in most cases beyond the usual recommended period of prophylaxis, and a significant heterogeneity among prophylaxis strategies across Spanish LT centers. These data do not support a one-size-fits-all approach to PJP prophylaxis and call for new studies that allow for a better characterization of high risk PJP groups in whom prophylaxis should be implemented.

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FIGURES

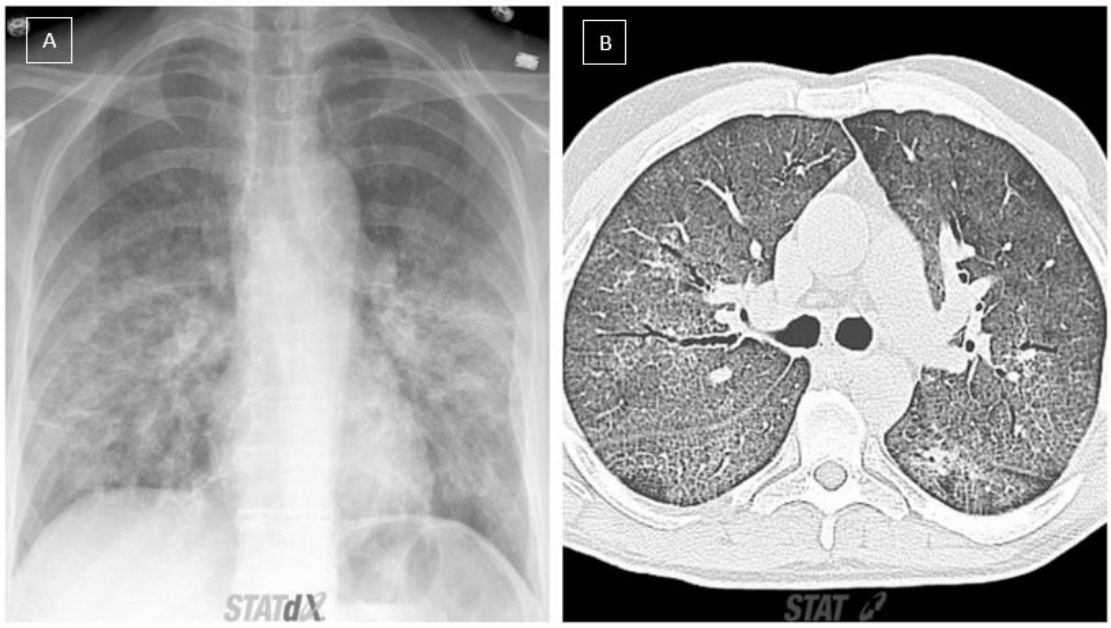

Figure 1. A. PA chest radiograph shows Pneumocystis pneumonia manifesting with diffuse bilateral heterogeneous opacities. **B.** Axial HRCT of a patient with Pneumocystis pneumonia shows diffuse bilateral ground-glass opacities on a background of reticular opacities (the so-called crazy-paving pattern).
*Images obtained via STATdx

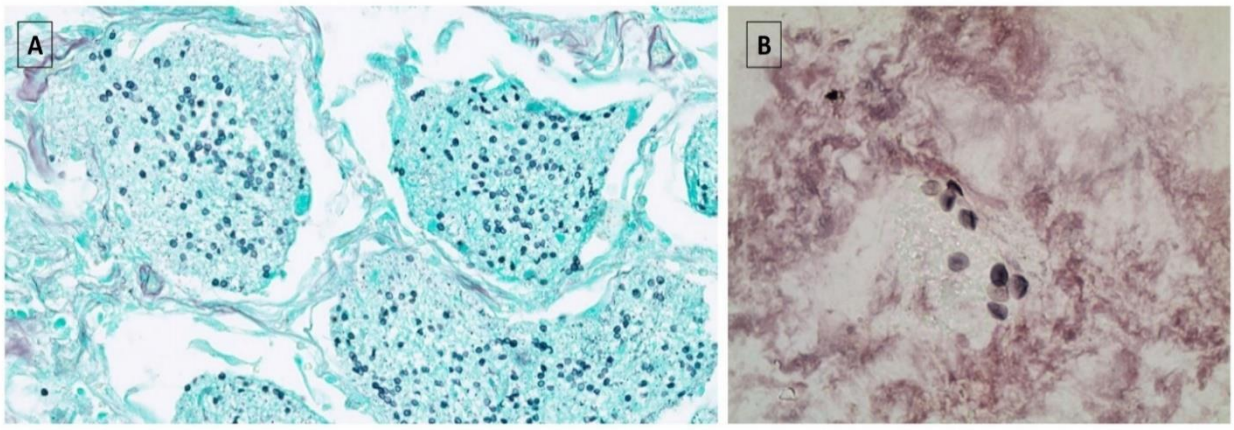

Figure 2. Diagnostic specimens for microbiological demonstration of *Pneumocystis jirovecii* infection. A. Lung biopsy showing intraalveolar proteinaceous exudates with the presence of numerous *Pneumocystis jiroveci* cysts. Grocott methenamine silver stain (at x400 magnification). **B.** Induced sputum showing the presence of numerous *Pneumocystis jiroveci* cysts. Grocott methenamine silver stain (at x100 magnification).

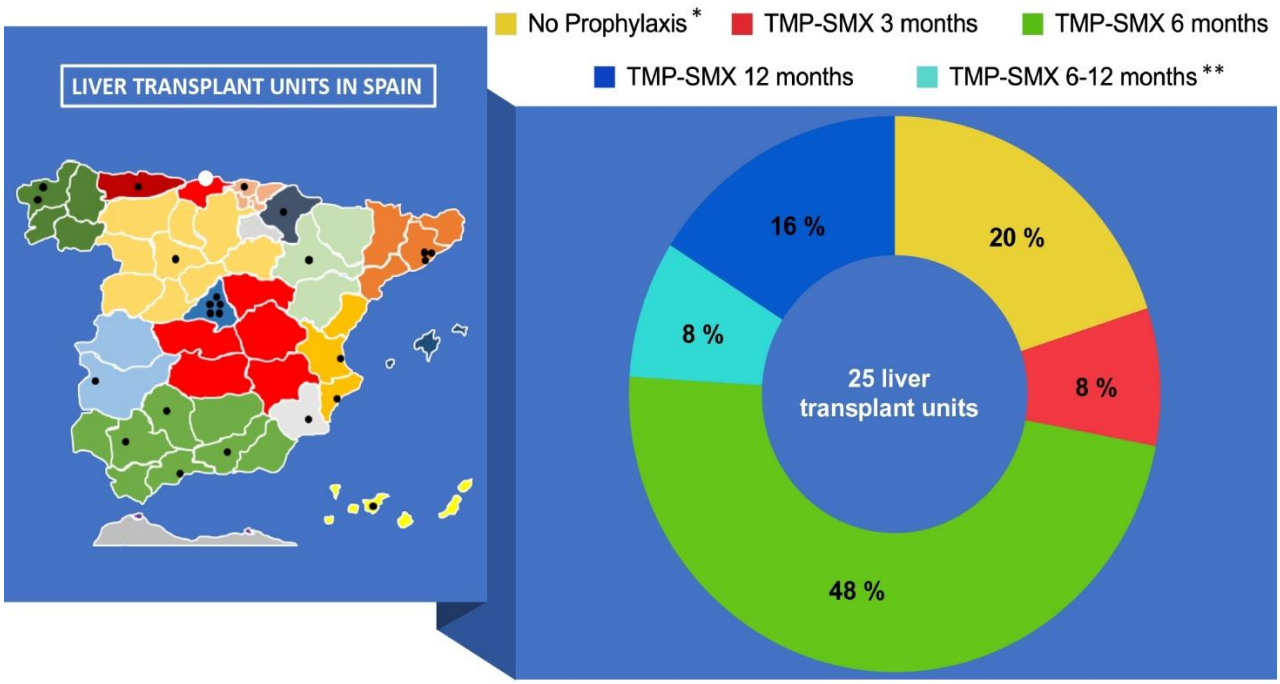

Figure 3. Prophylaxis strategies against *Pneumocystis jirovecii* in all liver transplant units in Spain.

All centers indicating prophylaxis used trimethoprim-sulfamethoxazole (160 mg / 800 mg) three times weekly irrespective of the prophylaxis duration. Black dots indicate the location of the other liver transplant units in Spain, whereas the bigger white dot indicates our center in Santander (Cantabria).

* One center indicates trimethoprim-sulfamethoxazole prophylaxis only in patients with human immunodeficiency virus infection and another center indicates it in the rare cases in which anti-thymocyte polyclonal antibodies are used (1 case out of the last 200 liver transplants at this center).

** These two centers maintain prophylaxis for 12 months if steroids are not stopped at 3 months in one center and at 6 months in the other. Otherwise, prophylaxis is stopped at 6 months.

TABLES

Table 1. Large studies evaluating the incidence of *Pneumocystis jirovecii* in liver transplant recipients in the presence or absence of prophylaxis *

Author and year	N	Study period and type	Prophylaxis	Cumulative incidence (%)	Mortality (%)	Comments
Kusne *et al*, 1988 [7]	101	1984-1985 Prospective	No	10.9	27.3	All cases occurred within the first 6 months and the three deaths had simultaneous CMV infection. IR 10 per 1000 PTY
Hayes *et al*, 1994 [8]	154	1986-1992 Retrospective	No	5.2	12.5	All cases occurred within the first 6 months. Profile of high-risk patients: ≥1 episode of rejection, OKT3 treatment, or allograft dysfunction.
Wade *et al*, 1995 [9]	284	1990-1993 Prospective	No	0.7	0	Both cases occurred within the first 3 months.
Hadley et al, 1995 [14]	124	1990-1992 Retrospective	Since July 1991, TMP-SMX q.d.	0	NA	No prophylaxis before July 1991
Singh *et al*, 1997 [15]	130	1989-1995 Prospective	TMP-SMX q.d. indefinitely	0	NA	All patients received tacrolimus as the primary immunosuppressive agent
Gordon *et al*, 1999 [16]	265	1987-1996 Retrospective	1987-1991: No 1992-1996: TMP-SMX t.i.w. 1 year	3.8	NS	Cohort of 1,299 SOT patients. All but one case occurred in the first year and without TMP-SMX. IR 3.7 per 1000 PTY. Side effects of TMP-SMX not reported for LT
Torre-Cisneros *et al*, 1999 [17]	120	NS RCT	TMP-SMX q.d. (N=60) SLF-PYT q.w. (N=60)	1.6	0	The two cases occurred in the TMP-SMX group. No significant differences between groups. Side effects in 17-18% in each group without treatment discontinuation
Neuman *et al*, 2002 [18]	646	1988-1995 Retrospective	TMP-SMX t.i.w. until 4 weeks after discharge	1.2	87.5	Splenectomy as a risk factor. High mortality due to co-existing allograft dysfunction and CMV infection. No case was on prophylaxis. Side effects of TMP-SMX were not reported
Akamatsu *et al*, 2007 [19]	180	2000-2003 Prospective	TMP-SMX in 22% guided by BDG levels (> 40 pg/mL)	1.1	0	All living donor liver transplants. Low positive predictive value of BDG. All cases occurred within the first 6 months. Side effects of TMP-SMX in 28%
Trotter *et al*, 2008 [20]	853	1997-2007 Retrospective	TMP-SMX t.i.w. (first 3 months)	0	NA	Side effects of TMP-SMX were not reported
Pappas *et al*, 2010 [21]	378	2001-2006 Prospective	NS	0	NA	TRANSNET. Data shown correspond to the Surveillance Cohort. PJP 12-month cumulative incidence of 3% in the Incidence cohort with 16,808 SOT (4,468 LT)
Orlando *et al*, 2010 [10]	203	2001-2008 Retrospective	No	0	NA	The authors suggested that monotherapy IS may nullify the risk for PCP

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Author and year	N	Study period and type	Prophylaxis	Cumulative incidence (%)	Mortality (%)	Comments
Ohkubo *et al*, 2012 [22]	156	NS Retrospective	TMP-SMX guided by BDG levels (> 40 pg/mL)	2.6	50	All living donor liver transplants during a 6-year period
Wang *et al*, 2012 [11]	436	2001-2011 Retrospective	No	1.2	20	All five cases occurred within the first 7 months.
Sarwar *et al*, 2013 [12]	611	2000-2012 Retrospective	No	1,1	71.4	Four of the 7 cases (57%) occurred within the first 7 months
Iriart *et al*, 2015 [23]	345	2004-2010 Retrospective	TMP-SMX t.i.w. the first 6 months	1.4	NS	Case-control study. No case while on prophylaxis. IR 2.6 per 1000 PTY. Age, lymphocyte count, and CMV infection were identified as risk factors for PJP
Desoubeaux *et al*, 2016 [13]	285	2011-2014 Retrospective	No	2.1	50	Four of the six cases occurred during an outbreak of PJP pneumoniae due to nosocomial acquisition. Survival is only reported in these 4 patients (50%)
Neofytos *et al*, 2018 [24]	567	2008-2016 Retrospective	354 (62.4%) received prophylaxis	0.7	NS	Swiss Transplant cohort (2842 SOT). Three of the 4 cases in LT had received prophylaxis. Mean time post-LT 440 days (range 71-1163)

* The minimun number of patients to consider large a study is 100.

Abbreviations: CMV: cytomegalovirus; IR: incidence rate; PTY: person transplant-year; OKT3: monoclonal antibody targeted at the CD3 receptor; TMP-SMX: trimethoprim-sulfamethoxazole; q.d.: daily; NA: not applicable; t.i.w.: three times a week; SOT: solid organ transplantation; LT: liver transplantation; SLF-PYT: sulfadoxine/pyrimethamine; q.w.: once a week; IS: immunosuppression; BDG: β-D-Glucan; TRANSNET: Transplant-Associated Infection Surveillance Network; PJP: Pneumocystis jirovecii

Table 2. Characteristics of LT recipients.

Variable *	Population (N= 631)
Age (years)	55.1 (47.2-60.9)
Gender (male)	467 (74.0)
Race (Caucasian)	625 (99.0)
Primary liver disease	
Alcohol	294 (46.6)
Hepatitis C	129 (20.4)
Alcohol + hepatitis C	52 (8.2)
Hepatitis B	36 (5.7)
Primary biliary cholangitis	21 (3.3)
Autoimmune hepatitis	13 (2.1)
Toxic	10 (1.6)
Other	76 (12.0)
Indication of liver transplantation	
Decompensated cirrhosis	347 (55.0)
Hepatocarcinoma	206 (32.7)
Acute liver failure	35 (5.5)
Acute on chronic liver failure	3 (0.5)
Other	40 (6.3)
Retransplant	52 (8.2)
Hepatic artery thrombosis	14 (26.9)
Recurrence of primary liver disease	10 (19.2)
Biliary complications	9 (17.3)

Variable *	Population (N= 631)
Hepatocarcinoma	1 (1.9)
Other	18 (34.6)
Other transplants	29 (4.6)
Renal (simultaneous/Consecutive)	14 (2.2) / 12 (1.9)
Bone marrow	2 (0.3)
Heart	1 (0.2)
Death	306 (48.5)
Lost of follow up **	34 (5.4)
Median time of follow-up (years)	6.5 (1.8-13.0)

* Quantitative variables were expressed as median and interquartile range and qualitative variables as absolute value (proportion).

** All these lost were due to change of residence to another region and follow-up was undertaken by the corresponding liver transplant unit.

Table 3. Risk factors, clinical features, treatment and outcome of the five LT patients with PJP

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
Age at diagnosis (years)	65.7	51.5	47.4	68.6	69.3
Sex	Male	Male	Male	Male	Male
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Etiology of liver disease	Hepatitis C	Alcohol	Alcohol	Alcohol	Alcohol
Indication of liver transplantation	Hepatocarcinoma	Decomp. cirrhosis	Decomp. cirrhosis	Decomp. cirrhosis	Decomp. cirrhosis
MELD at transplant (points)	11	23		14	19
Child-Pugh at transplant (points)	5	9	7	10	10
Re-transplant	No	No	No	No	No
Other transplants	No	No	No	No	No
Year of liver transplantation	1995	1997	1998	2005	2015
Time from transplantation (months)	7.6	11.1	3.0	169.4	50.4
Body mass index (Kg/m^2)			21.5	29.4	34.4

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
Tabaquism	No	Former smoker	Former smoker	Former smoker	Former smoker
Diabetes	Yes	Yes	Yes	No	No
Chronic kidney injury	No	Grade 1	No	No	No
HIV	No	No	No	No	No
Liver allograft cirrhosis	No	No	No	No	Yes
Autoimmune disease	No	No	Psoriasis	Graves´ disease	No
Lung disease	No	No	No	COPD	No
Splenectomy	No	No	No	No	No
Immunosuppression					
Tacrolimus	No	No	No	No	Yes
Cyclosporine	Yes	Yes	Yes	Yes	No
Steroids	Yes	Yes	Yes	No	No
Mycophenolate mofetil	No	No	No	No	Yes

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
Azathioprine	Yes	No	Yes	No	No
mTORi	No	No	No	Everolimus	Everolimus
Acute rejection Pre-pneumocystis	No	No	Yes	No	Yes
Treatment of acute rejection			Pulses of steroids		Pulses of steroids
Chronic rejection	No	No	No	Yes	Yes
Co-existing infections	Ophthalmic zoster	CMV	Clostridium difficile	No	SBP
Symptoms					
Fever	Yes	Yes	Yes	Yes	Yes
Cough	Dry	Productive	Productive	No	Productive
Dyspnea	Yes	Yes	No	No	Yes
Thoracic pain	No	No	No	No	No
Leucocytes (x 10^3/µ)	5.5	6.2	3.8	6.2	3.0
Linfocytes (x 10^3/µ)	0.5	1.5	0.9	2	0.1

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
Polymorphonuclear (x 10^3/µ)	4.7	4.1	2.4	3.5	2.5
Chest CT	No	No	Yes	Yes	Yes
Radiological findings					
Nodules	No	No	No	No	No
Consolidations	No	No	Yes	Yes	Yes
Ground glass opacities	Yes	Yes	Yes	Yes	Yes
Pleural effusions	No	No	No	No	No
Bronchoscopy	No	No	Yes	Yes	Yes
Stain	Positive	Negative	Negative	Negative	Negative
PCR	No	No	No	Positive	Positive
Lung biopsy	No	No	No	Yes	No
Treatment of Pneumocystis					

Variable	Case 1	Case 2	Case 3	Case 4	Case 5
Antibiotic	TMP-SMX	TMP-SMX	TMP-SMX	TMP-SMX	Pentamidine
Corticoids	Yes	Yes	No	Yes	No
ICU admission	Yes	No	No	No	No
Death from Pneumocystis	No	No	No	No	Yes

Abbreviations: Decomp: decompensated; COPD; chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; mTORi: Inhibitors of the mammalian target of rapamycin; CMV: cytomegalovirus; SBP: spontaneous bacterial peritonitis; CT: computed tomography; TMP-SMX: trimethoprim-sulfamethoxazole; PCR; polymerase chain reaction; ICU: intensive care unit.

Table 4. Number of transplants, cumulative incidence and outcome of PJP infection in each type of SOT

Variables *	Kidney transplant	Lung transplant	Heart transplant
Number of patients	1600 **	642	705
Number of transplants	2085	653	720
PJP cases	14	1	1
Cumulative incidence (cases per 1000 patients)	8.8	1.6	1.4
Time from transplant to PJP diagnosis (months)	17.8 (2.0-103.6)	1.5	6.0
PJP diagnosis within 6 months	6 (42.9)	1 (100)	1 (100)
Death due to PJP	3 (21.4)	0 (0)	0 (0)

* Quantitative variables were expressed as median and interquartile range and qualitative variables as absolute value (proportion).

** Among these, 60 consisted of combined kidney-pancreas transplantation and 26 combined kidney-liver transplantation.

Abbreviations: PJP: Pneumocystis jirovecii.