



# Epidemiology, clinical characteristics and outcome of early bacterial and fungal infections in liver transplantation: a single-centre, nine-year experience

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EP0157

## ABSTRACT

**Purpose** The aim of our study was to define the epidemiology, clinical characteristics and outcome of early bacterial and fungal infections in a cohort of patients who underwent liver transplantation (LT) at the University Hospital of Ancona over a nine year period.

**Methodology** All consecutive patients who underwent ortotopic LT in our center were considered. We compared LT-recipients with early infections (study group) and patients without early infections (control group). An early infection was defined as occurring in the first month post-transplantation.

**Results** A total of 330 LT-recipients were considered. In 95 of these patients (28.8%) at least one infection was detected. In 56 cases only one site was involved, in 39 cases ≥2 sites. There were 46 (29.7%) pneumonia, 44 (28.4%) surgical site infections, 35 (22.6%) blood stream infections, and 30 (19.4%) urinary tract infections. Gram-negative bacteria accounted for 62% of the culture-positive cases, followed by Gram-positive bacteria (32%) and fungi (6%). A high proportion of multi-drug-resistant strains was found within either Gram-negative (50%) or Gram-positive (72%) bacteria. One-year survival was significantly lower in the study group (68%) than in the control group (95%). Factors independently associated with a higher risk of infection were: Child Pugh class C, Roux-en-y biliary anastomosis, intraoperative red blood cell transfusions ≥ 5 units, post-transplant ICU stay ≥ 5 days and renal replacement therapy.

**Conclusion** Overall, these data show that the occurrence of an early infection has an adverse impact on survival and indicate a need for preventive measures employed at several stages during the management of LT-recipients.

## BACKGROUND

Liver transplantation (LT) has come to be established as a lifesaving procedure for the treatment of many end-stage liver diseases. However, infections, one of the main complications, remain a leading cause of morbidity and mortality among LT-recipients. Bacterial infections are the most frequent type of infectious diseases post-transplant, followed by fungal, viral and protozoal infections.

The aim of our study was to define the epidemiology and clinical characteristics and determine the predisposing factors and outcome associated with bacterial and fungal infections in the early post-transplant period patients in a large cohort of patients undergoing LT in the current era.

## PATIENTS AND METHODS

### Study design

This was a retrospective, observational study conducted at the Università Politecnica delle Marche, Ancona, Italy from August 2005 to October 2014. The patient population included consecutive patients who underwent orthotopic LT (OLT) and who survived more than 48 hours after transplantation.

### Definitions and microbiology

An early infection was defined as that occurring in the first month post-transplantation. Infections were identified through active surveillance in the LT ward, and through reviews of outpatient medical records. The criteria used for defining and classifying infections were those proposed by the Centers for Disease Control and Prevention. In particular, the following were considered: pneumonia, surgical site infections (SSIs, including deep intra-abdominal infections), blood stream infections (BSIs, including vascular catheter-related infections) and urinary tract infections (UTIs). Bacterial susceptibility patterns were reviewed and classified according to the ESCMID guidelines.

### Data collection

Data were collected from both groups for the following characteristics: donor variables, preoperative recipient variables, intraoperative recipient variables and postoperative recipient variables (Tables 1, 3 and 4).

### Immunosuppression

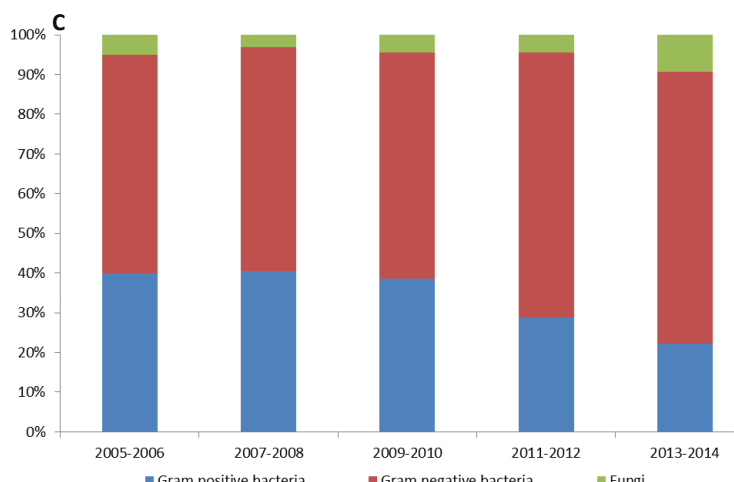
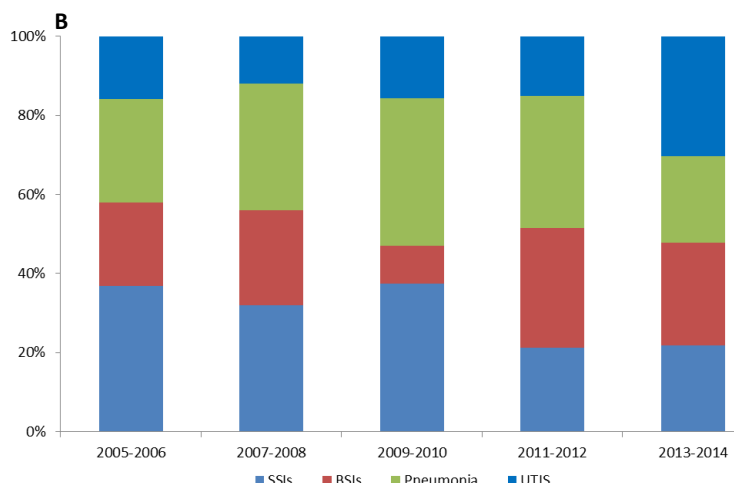
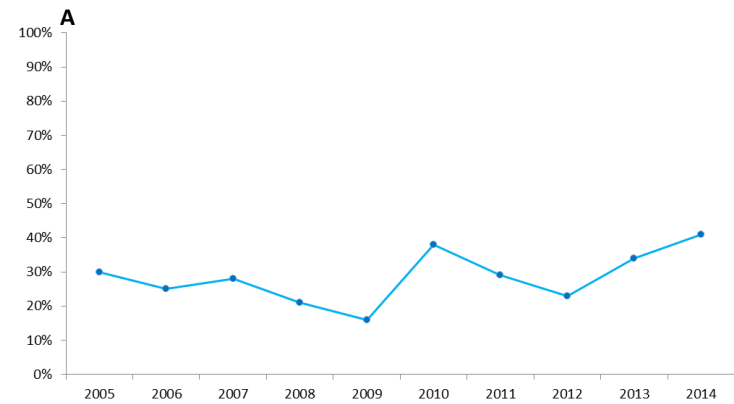
In the majority of the patients, standard immunosuppression consisted of tacrolimus in the first 15 days after LT followed by cyclosporine or everolimus. When combination immunosuppression therapy was necessary, mycophenolate mofetil was the most frequently used agent. A minority of patients received cyclosporine as primary immunosuppressive agent. Drug levels of immunosuppressive drugs were performed every three days.

### Statistical analysis

Quantitative data are depicted as median with interquartile (Q1- Q3) ranges and compared by U Mann-Whitney test. Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the X<sup>2</sup> test with Yates' correction or Fisher's exact test. The diagnostic accuracy of selected risk factors was evaluated using receiver operating curve (ROC). We analyzed the factors associated with the presence of early infections by using a stepwise binary logistic regression model in which variables found to be significant at the univariate level (P-value <0.05) were introduced. Patient survival was evaluated using the Kaplan-Meier curves and compared with the log-rank test. All significance tests were two-tailed. Statistical analysis was performed using SPSS, version 20 (Statistical Package for Social Sciences Inc., Chicago, IL).

**Table 1:** Demographic and clinical characteristics of the study cohort

Characteristics	Patients			p value
	All (n=330)	No early infection (n=235)	Early Infection (n=95)	
Age (years)	54 (48-60)	55 (48-60)	52 (47-60)	0.180
Gender Male	254 (77)	178 (76)	76 (80)	0.406
BMI	24 (22-27)	24 (22-26)	25 (22-28)	0.441
MELD score	15 (11-21)	14 (10-19)	18 (14-28)	<0.0001
MELD score ≥25	49 (15)	22 (9)	27 (28)	<0.0001
MELD-Na score	18 (13-24)	17 (12-22)	21 (16-29)	<0.0001
MELD-Na score ≥25	75 (23)	42 (18)	33 (35)	0.001
Child-Pugh stage C	106 (34)	63 (28)	43 (49)	<0.0001
Ascites	190 (58)	126 (54)	64 (67)	0.030
Pre-LT hospitalization	45 (14)	24 (10)	21 (22)	0.008
Previous Abdominal Surgery	64 (19)	50 (21)	14 (15)	0.228
Indication for LT – Viral	197 (60)	139 (59)	58 (68)	0.845
HCV positivity	163 (49)	110 (47)	53 (56)	0.175
HBs-Ag positivity	61 (19)	47 (20)	14 (15)	0.338
HIV positivity	22 (7)	13 (6)	9 (10)	0.291
Presence of HCC	107 (32)	78 (33)	29 (31)	0.735
Retransplantation	15 (5)	7 (3)	8 (8)	0.041



**Figure 1:** Early infections in LT recipients over the nine-years considered (the year 2005 included only five months [August to December], the year 2014 included only 10 months [January to October]). Incidence rate (A). Types of infections diagnosed (SSIs [Surgical Site Infections], BSIs [Blood Stream Infections], UTIs [Urinary Tract Infections]) (B). Types of isolated microorganisms (C).

## RESULTS (I)

A total of 330 LT were included in the study. Demographic and clinical characteristics of these transplant recipients are shown in Table 1. Most common indication for transplantation (60% of the cases) was ESLD due to viral hepatitis.

In all 28.8% (95/330) of the patients had at least one infection documented within 30 days after LT for a total of 155 infections. The incidence rate ranged from 16% (in 2009) to 41% (in 2014, [Fig. 1a]). The incidence of specific type of infection is shown in Fig. 1b. There were 46 (29.7%) pneumonia, 44 (28.4%) SSIs, 35 (22.6%) BSIs and 30 (19.4%) UTIs. In 56 cases only one site was involved, in 23 cases two sites, while in the remaining 16 cases three or more sites of infection were documented. The majority of the infections (122/155, 78.7%) were monomicrobial, 26 infections (16.8%) were caused by two microorganisms, while the remaining seven (4.5%) were due to three or more microorganisms.

There were 144 (93%) bacterial infections, 6 (4%) fungal infections and 5 (3%) mixed infections. Gram-negative bacteria accounted for 62% of the culture-positive cases, followed by Gram-positive bacteria (32%) and fungi (6%, [Fig. 1c and Table 2]). Among Gram-negative bacteria, the most frequently isolated pathogen was *K. pneumoniae* (30%) followed by *E. coli* (23%) and *P. aeruginosa* (20%), while among Gram-positive bacteria the most frequent organism was *E. faecium* (55%) followed by *S. aureus* (22%) and *S. epidermidis* (10%).

Of 121 Gram-negative bacteria, 61 isolates (50%) were antibiotic resistant organisms and included 59 MDR and two XDR. Within the former group of isolates there were 23 *K. pneumoniae*-carbapenemase-producing (KPC) and 13 extended-spectrum beta-lactamase-producing (ESBL) bacteria. Among 63 Gram-positive bacteria, there were 50 MDR isolates (79%), including six methicillin-resistant *S. epidermidis* (MRSE) and five methicillin-resistant *S. aureus* (MRSA). Additionally, there were two vancomycin-resistant Enterococcus isolates one of which was also MDR.

All early fungal infections were due to invasive candidiasis; *Candida albicans* accounted for 36.4% of the isolated yeasts followed by *C. glabrata* (27.3%) (Table 2)

**Table 2:** Pathogens isolated in early infections of the study cohort

Microorganisms <sup>a</sup>	n (%)	Types of infection <sup>b</sup>				Resistant strains (%) <sup>c</sup>
		Pneumonia	SSIs	BSIs	UTIs	
<i>K. pneumoniae</i>	36 (30)	12	10	8	6	23 KPC (64) 2 ESBL (6) 3 MDR (8) 1 XDR (3)
<i>E. coli</i>	28 (23)	9	3	7	9	10 ESBL (36) 10 MDR (36)
<i>P. aeruginosa</i>	24 (20)	13	6	2	3	4 MDR (17)
<i>S. maltophilia</i>	9 (7)	5	3	1	-	-
<i>A. baumannii</i>	4 (3)	3	-	1	-	1 MDR (25) 1 XDR (25)
Other Gram-negative	20 (17)	4	3	6	7	5 MDR (25) 1 ESBL (25)
<b>Total 121 (62%)</b>						
<i>E. faecium</i>	35 (55)	2	24	7	2	33 MDR (94) 1 VRE (3)
<i>S. aureus</i>	14 (22)	5	6	3	-	5 MRSA (36)
<i>S. epidermidis</i>	6 (10)	-	1	4	1	6 MRSE (100)
Other Gram-positive	8 (13)	3	3	-	2	6 MDR (75)
<b>Total 63 (32%)</b>						
<i>C. albicans</i>	4 (36)	-	1	-	3	ND
<i>C. glabrata</i>	3 (27)	-	2	1	-	ND
<i>C. tropicalis</i>	2 (18)	-	-	-	2	ND
Other fungi	2 (18)	1	1	-	-	ND
<b>Total 11 (6%)</b>						

<sup>a</sup> Others included:  
<sup>a</sup> Gram negative bacteria: *E. cloacae* (n° 5), *E. aerogenes* (n° 1), *S. marcescens* (n° 4), *K. oxytoca* (n° 2), *A. baumannii* (n° 2), *A. junii* (n° 1), *H. influenzae* (n° 1), *C. braakii* (n° 1), *Prevotella* spp. (n° 1), *B. uniformis* (n° 1), *M. morgani* (n° 1);  
<sup>b</sup> Gram positive bacteria: *E. faecalis* (n° 2), *S. pneumoniae* (n° 2), *S. haemolyticus* (n° 2), *S. pseudointermedius* (n° 1), *Staphylococcus* spp. (n° 1);  
<sup>c</sup> Fungi: *C. dubliniensis* (n° 1), *A. flavus* (n° 1).  
<sup>d</sup> SSIs, surgical site infections; BSIs, blood stream infections; UTIs, urinary tract infections.  
<sup>e</sup> KPC, Klebsiella pneumoniae-carbapenemase-producing; ESBL, extended-spectrum beta-lactamase; XDR, extensive drug resistant; MDR, multi drug resistant; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis; VRE, vancomycin-resistant Enterococcus;  
<sup>f</sup> -, not present; ND not done; \*, there was one VRE strain within the 33 MDR.

## CONCLUSIONS

- We documented that early bacterial and fungal infections are common in patients undergoing OLT and that pneumonia and SSIs were the most common types of infections in our center over a nine-year period.
- While Gram-negative bacteria were the most common causative agents, a high proportion of MDR strains was found within either Gram-negative or Gram-positive bacteria.
- We identified five specific modifiable risk factors and found that the occurrence of an early infection has a significant and adverse impact on survival.
- Overall, these data indicate a need for a wide range of preventive measures employed at several stages during the management of LT-recipients.

## RESULTS (II)

A significantly higher proportion of patients with early infections were Child Pugh class C, had MELD score ≥ 25 and ascites (Table 1). Additionally, they were more often hospitalized in the month before LT and underwent a retransplantation more frequently than the controls (Table 1).

None of donor variables were significantly different for the patients with and without early infections (Table 3).

Surgical and clinical postoperative variables in the study and control groups are depicted in Table 4. A significantly higher proportion of patients with early infections underwent a Roux-en-y anastomosis, and required transfusion with ≥ 5 RBC units or ≥ 10 plasma units. Additionally, ICU stay ≥ 5 days, renal replacement therapy (RRT), early graft dysfunction and CMV infection were significantly associated with early infections. Serum levels of immunosuppressive drugs (tacrolimus and cyclosporine) are reported in Figure 2. Both drug levels were not significantly different between study and control groups (Table 4).

In multivariate analysis, factors independently associated with a higher risk of developing an early infection after LT were, Child Pugh class C (HR 2.128 [CI95% 1.206-3.755], p = 0.009), Roux-en-y biliary anastomosis (HR 4.935 [CI95% 1.547-15.739], p = 0.007), RBC transfusion ≥ 5 units (HR 2.430 [CI95% 1.230-4.800], p = 0.011), post-transplant ICU stay ≥ 5 days (HR 2.918 [CI95% 1.605-5.304], p <0.0001) and RRT (HR 3.171 [CI95% 1.208-8.323], p = 0.019, Table 5).

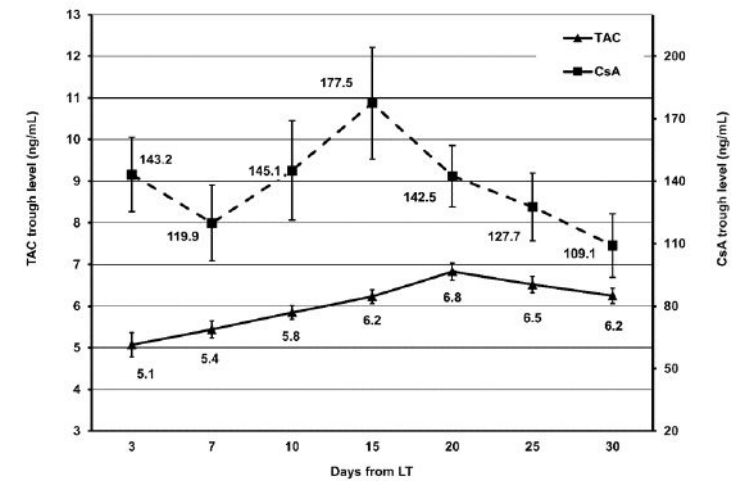
Kaplan-Meier 1-year survival curves for patients with and without early infections are presented in Figure 3. One-year survival was significantly lower in study group (68%) than in control group (95%; p <0.0001).

**Table 3:** Donor characteristics of the study cohort

Characteristics	Patients			p value
	All (n=330)	No early infection (n=235)	Early Infection (n=95)	
Age (years)	59 (43-72)	59 (43-72)	59 (44-73)	0.687
Gender male	188 (57)	131 (56)	57 (60)	0.559
BMI	25 (23-28)	25 (23-28)	26 (24-23)	0.464
Donor risk index	1.8 (1.5-2.0)	1.8 (1.4-2.0)	1.8 (1.5-2.0)	0.494
Cause of death				
CVA	201 (61)	144 (61)	57 (60)	0.988
Trauma	87 (26)	62 (26)	25 (26)	
Anoxia	36 (11)	25 (11)	11 (12)	
Others	6 (2)	4 (2)	2 (2)	
Macrovesicular steatosis ≥ 30%	16 (5)	11 (5)	5 (5)	0.783
ABO non identical match	24 (7)	13 (6)	11 (12)	0.093
Hemodynamic instability	80 (24)	58 (25)	22 (23)	0.880
ICU stay (days)	3 (2-5)	3 (2-5)	3 (2-5)	0.545

**Table 4:** Intra- and post-operative characteristics of the study cohort

Characteristics	Patients			p value
	All (n=330)	No early infection (n=235)	Early Infection (n=95)	
Split graft	23 (7)	17 (7)	6 (6)	0.954
Vascular graft	16 (5)	8 (3)	8 (8)	0.085
Type of anastomosis				
Roux-en-y	19 (6)	7 (3)	12 (13)	0.001
Termino-terminal	311 (94)	228 (97)	83 (87)	
Duration of operation (min)	91 (360-475)	405 (360-461)	420 (366-500)	0.194
Total ischemia time (min)	131 (385-555)	470 (380-550)	455 (395-574)	0.513
RBC units	7 (4-13)	6 (3-11)	8 (5-19)	<0.0001
RBC units ≥ 5	215 (65)	138 (59)	77 (81)	<0.0001
Plasma units	8 (4-13)	7 (3-11)	10 (5-15)	0.004
Plasma units ≥ 10	100 (30)	59 (25)	41 (43)	<0.0001
ICU stay (days)	3 (2-6)	3 (2-4)	6 (3-18)	<0.0001
ICU stay (days) ≥ 5	111 (34)	53 (23)	58 (61)	<0.0001
RRT	31 (9.4%)	9 (3.8%)	22 (23.2%)	<0.0001
Rejection	92 (28)	63 (27)	29 (31)	0.585
Use of bolus corticosteroid	95 (29)	62 (26)	33 (35)	0.167
1 <sup>st</sup> month main immunosuppressant trough levels				
TAC (Time-weighted AUC) (ng/mL)	5.9 (4.7-7.4)	5.8 (4.8-7.4)	5.9 (4.6-7.1)	0.544
CsA (Time-weighted AUC) (ng/mL)	130.3 (90.5-165.1)	134.3 (85.8-165.1)	118.5 (104.7-149.5)	0.927
Bile leak	59 (18)	40 (17)	19 (20)	0.631
Primary non function	7 (2)	3 (1)	4 (4)	0.109
Early allograft dysfunction	125 (38)	77 (33)	48 (51)	0.006
Hepatic artery thrombosis	9 (3)	4 (2)	5 (5)	0.127
Diabetes	47 (15)	33 (14)	14 (15)	0.951
CMV infection	75 (23)	42 (18)	33 (35)	0.002

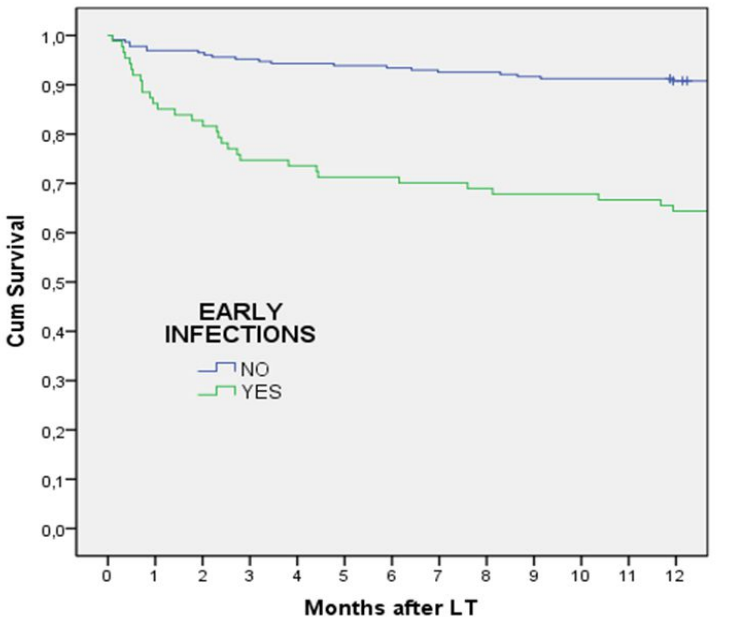


**Figure 2:** Mean (±SEM) Tacrolimus (TAC, 283 patients) and Cyclosporine (CsA, 30 patients) trough levels during the first month after liver transplantation (LT).

**Table 5:** Multivariate analysis of risk factors for early infections in the study cohort

Risk factors	Hazard ratio	CI 95%		p value
		Lower limit	Upper limit	
Child Pugh stage C	2.128	1.206	3.755	0.009
Roux-en-y biliary anastomosis	4.935	1.547	15.739	0.007
RBC ≥ 5 units	2.430	1.230	4.800	0.011
ICU stay ≥ 5 days	2.918	1.605	5.304	<0.0001
RRT	3.171	1.208	8.323	0.019

## Patient survival



**Figure 3:** One-year survival of OLT recipients with or without early infections

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