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TRANSPLANT INFECTIOUS DISEASE

ORIGINAL ARTICLE

Perfusion fluid-related infections in liver transplant recipients: A 5-year, single-center, retrospective study

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Abstract

Background: Perfusion fluid (PRF) is employed in liver transplantation (LTx) to maintain graft viability. Still, it represents a new potential way of infection transmission in LTx recipients (LTRs). Currently, no systematic research has investigated this topic.

Methods: Five-year single-center retrospective study conducted on LTRs from January 2017 to December 2021. We analyzed the incidence of positive PRF culture (PRF+) and perfusion fluid-related infections (PRF-RI) and their associated factors. We also assessed 1-year mortality, both overall and infection-related.

Results: Overall, 234 LTx were included. PRF+ were found in 31/234 (13.2%) LTx for a total of 37 isolates, with >1 isolate identified in 5 (2.1%) cases. High-risk microorganisms (Enterobacterales 13/37, *Enterococcus* spp. 4/37, *S. aureus* 3/37, *P. aeruginosa* 2/37) were isolated in 25/37 (67.6%) LTRs, the remaining being coagulase-negative staphylococci (12/37, 32.4%). Antimicrobial prophylaxis was administered to all LTRs, always active against the isolate even if suboptimal in 19 cases (61.3%). PRF-RI devel-

List of Abbreviations: BSI, bloodstream infections; CoNS, coagulase-negative Staphylococci; ESLD, end-stage liver disease; HOPE, hypothermic oxygenated machine-perfusion; IAI, intraabdominal infections; IQR, interquartile range; LTR, liver transplant recipients; LTX, liver transplantation; MDR, multidrug resistance; OR, odds ratio; PF, preservation fluid; PF+, positive preservation fluid culture; PFRI, PF-related infections; PRF, perfusion fluid; PRF-RI, PRF-related infections.

oped in 4/234 LTx (1.7%), and prophylaxis was considered suboptimal in 2/4 of them. The isolation of >1 microorganism in PRF culture was associated with an increased risk of developing PRF-RI (OR 37.5 [95%CI 2.6–548.4], $p = .01$). PRF-RI were associated with longer ICU stays ($p = .005$) and higher 1-year mortality, both overall and related to infections ($p = .001$).

Conclusion: Despite PRF+ being infrequent, only a minority of patients develops PRF-RI. Nonetheless, once occurred, PRF-RI seems to increase morbidity and mortality rates.

KEYWORDS
infection, liver transplantation, perfusion fluid, prophylaxis

1 | INTRODUCTION

Liver transplantation (LTx) is a potentially curative treatment option for end-stage liver disease (ESLD) and acute liver failure. Unfortunately, liver transplant recipients (LTR) are at a high risk of post-operative infections, which have a relevant impact in terms of morbidity and mortality. Indeed, infections are the most frequent cause of death 30–180 days after LTx.[1] Early infections often originate from the surgical site and relate to microorganisms carried by the donor or the recipient at transplantation.[2,3] About donor-derived infections, a possible vehicle of microorganisms to the recipient is represented by preservation fluid (PF) or perfusion fluid (PRF).

PF is a group of solutions developed to statically store organs in "as-optimal" condition and to provide maximally safe times to transport organs to potential recipients.[4] While the isolation of microorganisms in preservation fluid culture (PF+) is commonly reported in the literature (28.9%—98%), PF-related infections (PF-RI) are far less frequently described (7.4-10%). However, a high mortality rate secondary to PF-RI has been reported (35%, 95%CI: 21%—53%).[5–12]

Instead, PRF consists of solutions actively circulated using machine perfusion technology into the procured organs before implantation. This approach can allow marginal grafts to regain adequate characteristics for their safe transplantation or can replenish energy stores during preservation, offering more excellent protection to the organ better primed for reperfusion.[13] Due to the continuous and growing organ demand, machine perfusion is expected to be employed with increasing frequencies.[14] The risk of microbial contamination during machine perfusion and subsequent infection development, based on the absence of a fully functional immune system in the ex-situ setting, is an acknowledged fact.[15] Currently, no systematic research has addressed the role of PRF in causing infections (PRF-RI) among LTRs, with only two case reports describing PRF-RI. The first, recently proposed by our group, is a case of PRF-RI due to *Escherichia coli* ESBL-producers in LTR who received a graft that underwent hypothermic oxygenated machine-perfusion (HOPE).[16] The second, proposed by Hann et al., is a case of PRF-RI due to *E. coli* in LTR who received a graft that underwent normothermic machine perfusion.[17] In both cases, the germ was primarily isolated in the PF, leading to a severe systemic infection with subsequent isolation of the bacteria in the bloodstream.

Overall, data on the characteristics and outcomes of PRF+ and PRF-RI are missing, and there is no consensus about the management and treatment of these conditions. We conducted a retrospective study to assess the incidence and clinical relevance of PRF+ and PRF-RI and identify variables associated with these events.

2 | METHODS

A 5-year, single-center retrospective study on all consecutive LTx performed from 01/01/2017 to 31/12/2021 at IRCCS Ospedale Maggiore Policlinico of Milan, Italy, was carried out. The primary study endpoint was the incidence of PRF+ and PRF-RI and the variables associated with these outcomes. Secondary outcomes included identifying factors related to these events and 1-year overall and infection-related mortality.

For each patient, demographic and clinical data were extracted from electronic records. Regarding surgical procedures, we evaluated the need for red blood cells, platelets, fresh frozen plasma transfusions, and vascular or biliary complications. Any deviation from the ordinary postoperative course was defined according to the Clavien-Dindo classification.

PRF samples were collected from the reservoir of the machine perfusion system at the end of the machine perfusion of livers, put in sterile test tubes and sent immediately for microbiological procedures. One ml of the specimen was inoculated in brain-hearth enrichment broth, incubated at 36 ± 1°C for 3 days and subcultured on chocolate agar and blood agar plates (incubated at 36 ± 1°C in CO_2 for 48h). Furthermore, 10 μl of the sample were directly inoculated on chocolate agar and blood agar and incubated at 36±1°C in CO_2 for 48 h and Shaedler agar and incubated for 48 h in anaerobic conditions at 36 ± 1°C. Transport medium for anaerobes was not used. PRF+ was defined as the growth of any microorganism in PRF culture, with germs classified as high-risk microorganisms (i.e., gram-negative bacilli, *Staphylococcus aureus*, β-haemolytic streptococci, *Bacteroides* spp. and *Candida* spp.)

and low-risk microorganisms (i.e., Coagulase-negative Staphylococci [CoNS], *Streptococcus viridans*, and *Corynebacterium* spp.). Multi-drug resistance (MDR) was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories.

PRF-RI was defined as identifying the same microorganism in both PRF and other LTR samples (i.e., blood, urine, abdominal drainage fluid) in the presence of signs and symptoms of infection. According to the infection site, we distinguished bloodstream infections (BSI), catheter-related BSI, pneumonia, urinary tract infections, intra-abdominal-infections (IAI), sepsis, and peritonitis.

As antimicrobial prophylaxis, piperacillin/tazobactam was administered before surgical incision and discontinued 24 h after the end of surgery to all LTRs who were not colonized by MDR microorganisms, in accordance with the protocol currently employed in our center. Antifungal prophylaxis was added in high-risk LTR (re-LTx, acute hepatic failure, renal replacement, MELD > 30). In MDR-colonized LTRs, targeted prophylaxis was defined according to clinical evaluation. For cases showing PRF+, all the antimicrobial agents used as prophylaxis were retrospectively evaluated to distinguish active against isolate from suboptimal against isolate prophylaxis. We defined active against isolate prophylaxis as the use of the antimicrobial agent of choice as well as the use of an antimicrobial agent with documented activity against the PRF isolate. Suboptimal against isolate prophylaxis was defined as the use of antimicrobial agents with activity against PRF isolate that did not represent the best available therapy (i.e., usage of piperacillin/tazobactam for methicillin-susceptible CoNS). Pre-emptive therapy was defined as posttransplant administration of targeted antimicrobial treatment against the isolates of PRF+ without clinical signs of active infection.

Continuous variables were described as median and interquartile range (IQR), differences between groups were searched using the Mann-Whitney U test. Categorical variables were expressed as numbers and percentages; Pearson's chi-square test was employed to compare groups. The incidence of PRF+ and PRF-RI was calculated. Risk factors associated with PRF+ and PRF-RI were investigated with logistic regression models. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated. Univariate survival analysis was performed employing the Kaplan–Meier method with log-rank test to compare groups. All statistical tests were two-tailed, and the significance threshold was $p < .05$. Analyses were performed with STATA 17.0 (STATA Corp., College Station, TX).

The study was approved by the local ethical committee and conducted following the Helsinki Declaration.

3 | RESULTS

During the study period, a total of 234 LTx have been performed in 218 LTRs. Baseline demographic and clinical characteristics of enrolled patients are summarized in Table 1. Most LTRs were male (171/218, 78.4%), with a median age of 57.2 years (IQR 12) at transplantation. Hepatocellular carcinoma was the leading cause of transplantation (49%, 107/218), followed by ESLD (35.6%, 78/218). Among the 234 LTx, surgery complications occurred in 218 (92.7%). Biliary complications were the most frequently reported (88/234, 37.6%), followed by vascular complications (18/234, 7.7%). Details regarding rectal colonization status at transplantation and antimicrobial prophylaxis administered are reported in Supplementary Table 1-2. One-year mortality for infectious causes was 3.7% (8/218), with early onset (39 days, IQR 152) from LTx, as shown in Table 2.

TABLE 1 Demographic and clinical characteristics of patients included in the study.

Patients	*N* = 218	
Male gender, *N* (%)	171 (78.4)	
Age at transplantation, years	57.2 (12)	
Transplantation indication, *N* (%)	HCC, *N* (%)	107 (49)
	ESLD, *N* (%)	78 (35.6)
	AHF, *N* (%)	12 (5.5)
	PSC, *N* (%)	8 (3.65)
	BA, *N* (%)	1 (0.5)
	IEM, *N* (%)	1 (0.5)
	Others, *N* (%)	11 (5)
Retransplantation, *N* (%)	15 (6.9)	
Second retransplantation, *N* (%)	1 (0.4)	

Note: Data are expressed as median and IQR.
Abbreviations: AHF, acute hepatic failure; BA, biliary atresia; ESLD, end-stage liver disease; HCC, hepatocellular carcinoma; IEM, inborn errors of metabolism (Alpha-I antitrypsin, Crigler-Najjar disease, Type I, Byler's disease, Glycogen storage disease Type I, Wilson's disease, Hemochromatosis, Tyrosinemia, Wolman's disease, Familial amyloidotic polyneuropathy, Primary hyperoxaluria Type 1); PSC, primary sclerosing cholangitis.

The incidence of PRF+ was 13.2% (31 out of 234). PRF+ never isolated an MDR germ, and in five cases multiple isolates were found, in one patient being three, so the total number of isolates was 37. Most isolates were considered high-risk microorganisms (67.6%, 25/37), while 32.4% (12/37) were deemed low-risk pathogens. *Klebsiella* spp. was the most frequently isolated microorganism in the high-risk group (5/37), followed by *E. coli* (4/37) and *S. aureus* (3/37). Antimicrobial prophylaxis was considered active against isolate in all PRF+ (31/31, 100%), suboptimal in 19/31 cases (61.3%). Pre-emptive therapy against the PRF isolates was given in a single patient (3.2%), according to the clinician's decision. PRF culture results, antimicrobial prophylaxis administered at LTx and the number of PRF-RI are detailed in Table 3. A more detailed description of the microrganism(s) isolated and the relative antimicrobial prophylaxis administered is reported in Supplementary Table 3.

Overall, four (1.7%) males receiving their first single LTx developed PRF-RI, three IAI and one BSI; all received a first LTx. Administered prophylaxis was suboptimal in half of them, and clinical infections appeared from three to 9 days after LTx. Specific antimicrobial therapy to treat PRF-RI was introduced in three of four of individuals, the remainder being the patient who received a pre-emptive treatment. Early death occurred in three of these LTR, and in two of them it was

TABLE 2 Outcome summary.

Transplants	*N* = 234	
Hospitalization length, days	22 (21)	
ICU stay, days	2 (3)	
Intra-operative red blood packed cell transfusion, *N* (%)	182 (77.7)	
Intra-operative fresh frozen plasma transfusion, *N* (%)	203 (86.7)	
Intra-operative platelet unit transfusion, *N* (%)	63 (27)	
Acute graft rejection episodes, *N* (%)	20 (8.5)	
Graft loss, *N* (%)	9 (3.8)	
Vascular complications, *N* (%)	18 (7.7)	
	PVT, *N* (%)	6 (2.6)
	HAT, *N* (%)	6 (2.6)
	HAS, *N* (%)	3 (1.3)
	HAP, *N* (%)	2 (0.8)
	HAR, *N* (%)	1 (0.4)
Biliary complications, *N* (%)	88 (37.6)	
	Leaks, *N* (%)	71 (30.3)
	Strictures, *N* (%)	12 (5.12)
	Haemobilia, *N* (%)	3 (1.28)
	Biloma, *N* (%)	1 (0.4)
	Stones and clots, *N* (%)	1 (0.4)
Surgical complications, *N* (%)	218 (92.7)	
Clavien-Dindo classification	II	74 (31.6)
	IIIa	50 (21.4)
	IIIb	32 (13.7)
	I	31 (13.2)
	IVa	13 (5.5)
	IVb	11 (4.7)
	V	6 (2.6)
Reinterventions, *N* (%)	97 (41.4)	
One-year mortality, *N* (%)	15 (6.9)	
One-year mortality, days	36 (262)	
One-year infection-related mortality, *N* (%)	8 (3.7)	
One-year infection-related mortality, days	39 (152)	

Note: Data are expressed as median and IQR. ICU, intensive care unit; RBC, red blood cells; PLT, platelet; PVT, portal vein thrombosis; HAT, hepatic artery thrombosis; HAS, hepatic artery stenosis; HAP, hepatic artery pseudoaneurysm; HAR, hepatic artery rupture.

TABLE 3 Perfusion fluid culture results, antimicrobial prophylaxis administered at transplantation and number of perfusion fluid-related infections.

Transplants	*N* = 234	
Perfusion fluid culture positive, *N* (%)	31 (13.2)	
Perfusion fluid culture with two isolates, *N* (%)	4 (1.7)	
Perfusion fluid culture with three isolates, *N* (%)	1 (0.4)	
Prophylaxis active against isolate(s), *N* (%)	31 (100%)	
Prophylaxis suboptimal against isolate(s), *N* (%)	19 (61.3)	
Pre-emptive therapy against isolate(s), *N* (%)	1 (3.2)	
Perfusion fluid-related infection, *N* (%)	4 (1.7)	
Total isolates	***N*= 37**	
Low risk, *N* (%)	12 (32.4)	
	CoNS	12 (33.3)
High risk, *N* (%)	25 (67.6)	
	Klebsiella spp.	5 (13.5)
	E. coli	4 (10.8)
	S. aureus	3 (8.1)
	Enterobacter spp.	2 (5.4)
	P. aeruginosa	2 (5.4)
	E. faecalis	2 (5.4)
	E. faecium	1 (2.7)
	S. mitis	1 (2.7)
	S. oralis	1 (2.7)
	E. gallinarum	1 (2.7)
	S. pneumoniae	1 (2.7)
	P. mirabilis	1 (2.7)
	Citrobacter spp.	1 (2.7)
MDR isolates on perfusion fluid, *N* (%)	0	

Abbreviations: CoNS, coagulase-negative staphylococci; MDR, multi-drug resistant.

related to PRF-RI, the latter dying because of aortic arch dissection unrelated to infection. Table 4 summarizes clinical features of PRF-RI, management, and outcome.

In Table 5 outcome was sorted according to PRF results. No significant differences were detected in hospitalization length, ICU stays, 1-year overall mortality and 1-year infection-related mortality between PRF+ and PRF- groups. Instead, compared with PRF-RI-LTR, those who developed PRF-RI experienced longer hospitalization (39.5 vs. 22 days, $p = .05$) and ICU stay (7.5 vs. 2 days, $p = .005$) than PRF-RI negative group. Similarly, both 1-year overall mortality and 1-

TABLE 4 Perfusion fluid-related infections, management, and outcome.

Case	Gender	Age at transplantation, years	Re-LTx	Microorganisms isolated on perfusate	Microorganisms responsible for the infection	Type of infection	Days from transplant to infection	Prophylaxis active against isolate-s (drug-s)	Prophylaxis suboptimal against isolate-s	Pre-emptive therapy against isolate (drug-s)	Specific therapy for infection (drug-s)	One-year mortality (days since transplantation)	Death due to infection
1	Male	63	No	*S. mitis* + *S. oralis*	*S. mitis* + *S. oralis*	IAI	3	Yes (AMC)	No	No	No	Yes (36)	No
2	Male	52	No	*Klebsiella* spp.	*Klebsiella* spp.	IAI	6	Yes (TZP + CAS)	No	Yes (MEM)	Yes (MEM)	Yes (11)	Yes
3	Male	70	No	*Enterobacter* spp. + *Citrobacter* spp.	*Citrobacter* spp.	BSI	9	Yes (TZP + CAS)	Yes	No	Yes (FEP)	Yes (42)	Yes
4	Male	70	No	*E. coli* + *E. faecium*	*E. faecium*	IAI	6	Yes (TZP)	Yes	No	Yes (VAN + TGC)	No	-

Abbreviations: Re-LTx, re-transplantation; IAI, intrabdominal infection; BSI, bloodstream infection; AMC, amoxicillin/clavulanate; TZP, piperacillin/tazobactam; CAS, caspofungin; MEM, meropenem; FEP, cefepime; VAN, vancomycin; TIG, tigecycline.

TABLE 5 Outcomes according to positive perfusion fluid culture results (A) and perfusion fluid-related infection (B) among 234 liver transplants.

(A)			
Variable	**PRF+ (31, 13.2%)**	**PRF- (203, 86.8%)**	***p*-Value**
Hospitalization length, days (median, IQR)	30 (27)	21 (19)	.14
Length of ICU stay, days (median, IQR)	2 (3)	2 (3)	.57
Vascular complications, *N* (%)	2 (6.4)	16 (7.9)	.78
Biliary complications, *N* (%)	10 (32.3)	78 (38.4)	.51
Reinterventions, *N* (%)	12 (38.7)	85 (42.1)	.72
Retransplantations, *N* (%)	3 (9.7)	12 (5.9)	.42
Acute graft rejection episodes, *N* (%)	3 (9.7)	17 (8.4)	.81
One-year mortality, *N* (%)	4 (12.9)	11 (5.42)	.11
One-year mortality, days (median, IQR)	39 (176)	93 (233)	.92
One-year infection-related mortality, *N* (%)	2 (6.4)	6 (2.9)	.87
One-year infection-related mortality, days	39 (91)	93 (152)	.81
(B)			
Variable	**PRF-RI+ (4, 1.7%)**	**PRF-RI- (230, 98.3%)**	***p*-Value**
Hospitalization length, days (median, IQR)	39.5 (9)	22 (19)	**.05**
Length of ICU stay, days (median, IQR)	7.5 (22.5)	2 (2)	**.005**
Vascular complications, *N* (%)	1 (25)	17 (7.4)	.19
Biliary complications, *N* (%)	0	88 (38.3)	.12
Reinterventions, *N* (%)	0	97 (42.4)	.08
Retransplantations, *N* (%)	0	15 (6.5)	.60
Acute graft rejection episodes, *N* (%)	1 (25)	19 (8.3)	.24
One-year mortality, *N* (%)	3 (75)	12 (5.2)	**.001**
One-year mortality, days	36 (31)	151 (260)	.42
One-year infection-related mortality, *N* (%)	2 (50%)	6 (2.61)	**.001**
One-year infection-related mortality, days	42 (176)	36 (133)	.94

year infection-related mortality were significantly higher in LTR who developed PRF-RI (3/4 [75%] vs. 12/230 [5.2%], $p = .001$; 2/4 [50%] vs. 6/230 [2.61%], $p = .001$).

Detection of >1 isolate in PRF was the only risk factor positively associated with PRF-RI development (OR 37.5, 95% CI 2.56–548.36), as reported in Table 6.

Finally, 1-year survival was slightly reduced in the PRF+ group compared to PRF- LTR (Figure 1), even if it did not reach significance (p = .12). Considering PRF-RI, 1 year after LTx, 25% (1/4) of patients who

TABLE 6 Association between selected variables and the development of perfusion fluid-related infection among 31 patients with positive perfusion fluid culture.

Variable	PRF-RI+ (4, 12.9%)	PRF-RI- (27, 87.1%)	*p*-Value	OR	95%CI
Age (*N*, %)					
<60 years	1 (25)	16 (59.3)	.20	1.00	Reference
>60 years	3 (75)	11 (40)	-	4.46	0.40–47.6
Suboptimal prophylaxis, *N* (%)					
No	2 (50)	10 (37)	.62	1.00	Reference
Yes	2 (50)	17 (63)	-	0.59	0.07–4.85
High-risk germ isolation, *N* (%)					
No	0	13 (48.1)	.07		
Yes	4 (100)	14 (51.8)	-		
>1 isolate, N (%)					
No	1 (25)	25 (92.6)	**.01**	1.00	Reference
Yes	3 (75)	2 (7.4)	-	**37.5**	**2.56–548.36**
Vascular complications, *N* (%)					
No	3 (75)	26 (96.3)	.10	1.00	Reference
Yes	1 (25)	1 (3.7)	-	8.66	0.42–177.32
Biliary complications, *N* (%)					
No	4 (100)	17 (63)	.14		
Yes	0	10 (37)	-		
Reintervention, *N* (%)					
No	4 (100)	15 (55.6)	.08		
Yes	0	12 (44.4)	-		
Acute graft rejection episodes, *N* (%)					
No	3 (75)	25 (92.6)	.27	1.00	Reference
Yes	1 (25)	2 (7.4)	-	4.16	0.28–60.93
Red blood cell transfusion, *N* (%)					
No	0	6 (23.1)	.28		
Yes	4 (100)	20 (76.9)	-		
Plasma transfusion, *N* (%)					
No	1 (25)	4 (15.4)	.63	1.00	Reference
Yes	3 (75)	22 (84.6)	-	0.54	0.04–6.65
Platelet transfusion, *N* (%)					
No	1 (25)	17 (65.4)	.12	1.00	Reference
Yes	3 (75)	9 (34.6)	-	5.6	0.51–62.6

Abbreviations: PRF, perfusion fluid; PRF-RI, perfusion fluid-related infection; MDR, multi-drug resistant; ICU, intensive care unit.

developed an infection due to a germ carried by PRF were still alive compared to 93.2% (218/234) of those who did not ($p = .001$).

4 | DISCUSSION

Our retrospective study is the first one assessing the role of PRF in infections occurring in LTx. Overall, a microorganism was isolated in the PRF in a relevant number of LTx (almost 15%). Still, one-third of these isolates were considered low-pathogenicity, and only a minority of LTx (1.7%) developed a PRF-RI. Isolation of >1 microorganism was associated with an increased risk of developing a PRF-RI, which led to longer ICU stays. It was also linked with higher 1-year mortality, both overall and related to infections, even though the number of recorded events was limited. Of note, all patients with PRF+ received antimicrobial prophylaxis at the time of surgery, which was considered adequate, even if, in some cases, suboptimal against the microorganisms(s) subsequently identified.

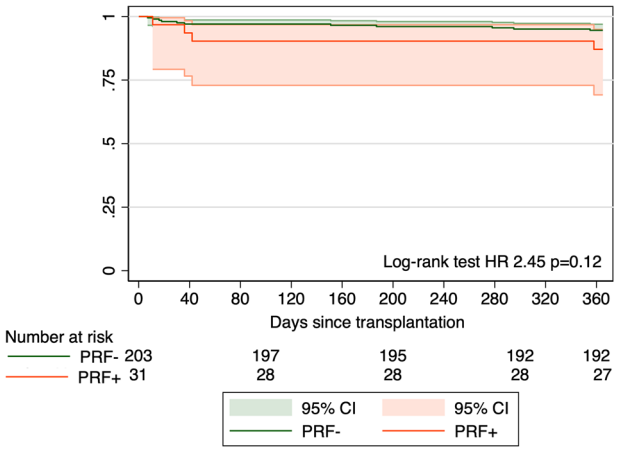

FIGURE 1 Kaplan–Meier curve of 1-year survival according to perfusion fluid culture results. PRF+, positive perfusion fluid culture; PRF-, negative perfusion fluid culture; CI, confidence interval.

Systematic research about the incidence of PRF+ and PRF-RI in LTx are absent, with only two case reports describing systemic infection due to microorganisms isolated in the PRF. In the report from our group, we described a bloodstream infection due to *E. coli* ESBL-producer isolated in the PRF after HOPE usage. Of note, the isolate was resistant to the standard surgical prophylaxis used in our center (piperacillin/tazobactam). We hypothesized that PRF contamination occurred during the in-situ cooling, necessary for ex-situ organ preservation and transport, that the oxygen supplementation during HOPE acted as an additional factor supporting aerobic microorganisms' growth and that the suboptimal prophylaxis administered facilitated the systemic spread of the bacteria.[16] Similarly, Hann et al. described a severe systemic infection, associated with early allograft dysfunction, due to *E. coli* identified in the PRF after normothermic machine perfusion of the liver. Also in this case, the bacterium was resistant to the surgical prophylaxis administered to the recipient (piperacillin/tazobactam).[17] Although it is difficult to draw general conclusions from such a small number of observations, it is noteworthy that the case of BSI due to a microorganism isolated from PRF reported in our study (patient 3) was due to an Enterobacterales (*Citrobacter*) and that the surgical prophylaxis administered was considered suboptimal (consequently to the possible expression of AmpC gene by bacteria belonging to the *Citrobacter* genus). Overall, this suggests how effective surgical prophylaxis is a critical element in preventing the dissemination of potential pathogens from PRF, and how the isolation of Gram-negative bacteria of the Enterobacterales family must be considered in the management decision process.

Instead, several studies have been performed evaluating the role of PF. A recent systematic review and meta-analysis[7] assessing the incidence of PF+ and its impact on solid organ transplant (SOT) recipients found an overall incidence of PF+ of 37% and an incidence of PF-RI among SOT recipients with pathogenic microorganisms of 10%. In their multicenter prospective study, Oriol et al.[8] found a high prevalence (62.5%) of culture-positive PF (PF+), but only one-quarter of these isolated were highly pathogenic microorganisms. The incidence of PF-RI was similar to the rate of PRF-RI in our series (1.3%), and the authors identified the administration of a pre-emptive therapy as a protective factor against PF-RI. In our cohort, only one case received pre-emptive therapy. Still, the totality of patients with PRF+ received antibiotic prophylaxis at surgery active against the isolate(s), suggesting how this prophylaxis can prevent the majority of PRF-RI. Of note, two of our patients with PRF-RI received antibiotic prophylaxis which was considered sub-optimal against the isolate(s). Overall, PRF+ seem less frequent than PF+ and are associated with fewer related infections, at least in our setting, where antibiotic prophylaxis was effective against all the isolates recorded.

Regarding the microorganisms isolated, our results differ from those of another study, performed again on PF, with CoNS as the most frequent bacteria isolated and relevant pathogens found only in 28% of cases.[11] In our cohort, CoNS was the single most frequently isolated species (12/37, 33.3%), but germs considered relevant were more frequent overall, representing the remaining 67%. Considering how in the study we are referring to PF+ were found in 92% of cases, we can assume that PRF is less frequently contaminated, but when contamination occurs is often due to a germ of relevant pathogenicity. This probably reflects the contamination of PF during the manipulation of the graft, with microorganisms widespread on the skin surface, like CoNS. In contrast, PRF is contaminated by bacteria of gut/biliary origin during the perfusion process. Supporting that, we can observe how enteric microorganisms like *Klebsiella* spp., *E. coli* and Enterococci represented the most high-risk germs isolated in our study.

The low rate of PRF-RI we reported may be explained by the common use of piperacillin/tazobactam as surgical prophylaxis in our patients. A pre-emptive therapy was administered only in one case, in a patient that eventually died because of PRF-RI. In the studies which analysed the role of PF in LTx, both antimicrobial prophylaxis and pre-emptive therapy differed from ours. Reimondez et al.[6], referring to the use of ampicillin/sulbactam from surgery to 48 h after transplantation with tailored pre-emptive therapy in the case of PF+, reported no cases of PF-RI. Mularoni et al.[18] described that those recipients of organs from donors colonized/infected by carbapenem-resistant bacteria who received a pre-emptive and effective antimicrobial treatment after transplantation had a significantly lower risk of infection.

Our study has some limitations, mainly related to its retrospective nature. Notably, the number of PRF-RI is very scarce and limits the generalization of our conclusions. Furthermore, we did not perform a molecular typing to match the bacterial strain identified in the PRF with those identified in other biological materials of the patients. Therefore, the infections we have defined ad PRF-RI should probably be cautiously labeled as potentially-related PRF-RI. Moreover, minimal data were available about possible infectious events in the donors and their management. Finally, data about the number of organs procured, possibly including lungs and therefore exposing the surgical field to the high respiratory tract, from the same donor were lacking. Nonetheless, our study provides important information about the factors associated with the development of PRF-RI in patients who received a graft with PRF+, which is the ultimate event that is essential to prevent for clinicians.

Based on the results of our study, PRF-RI appears to be a marginal event despite PRF culture being positive in more than one in a tenth of grafts. This might be secondary to antimicrobial prophylaxis active against the microorganism subsequently identified in PRF, like all the patients in our cohort. It is possible to speculate that the graft contains a low number of microorganisms, which can be controlled and eliminated by the antimicrobials administered at the time of surgery, even when the drug is not the molecule of choice for these germs. This fragile equilibrium could be disrupted by the growing prevalence of MDR, which can require targeted antimicrobial regimens to be controlled and could hamper the efficacy of standard antimicrobial prophylaxis.[19]

Finally, the restricted sample size of our cohort has limited the certainty of our results and especially the identification of the variables associated with developing PRF-RI. Nonetheless, age >60 years, vascular complications, and acute graft rejection episodes seem more frequently associated with PRF-RI, despite not reaching statistical significance. Instead, the isolation of >1 microorganism in PRF is strongly associated with PRF-RI development, suggesting how when a polymicrobial culture is obtained, the evolution toward an evident infection is probably facilitated by a more compromised graft or a higher bacterial burden carried by the PRF.

Overall, PRF-RI is infrequent when the antimicrobial prophylaxis administered at the time of transplantation effectively against the germ subsequently isolated on PRF. Therefore, among patients with PRF+ which have received active prophylaxis, we do not recommend the standard administration of pre-emptive therapy. Instead, we suggest a case-by-case discussion, considering the administration of pre-emptive therapy when there are signs of an infection vehiculated by the graft, when PRF+ is polymicrobial, and when the prophylaxis administered is particularly suboptimal against the isolate. On this point, having a complete antimicrobial susceptibility test of the PRF isolate can be helpful. Contrariwise, when the isolate(s) is not susceptible to the surgical prophylaxis administered, we believe that a microbiological tests bundle should be performed even in the absence of symptoms, and pre-emptive antimicrobial treatment tailored to the isolate(s) should be instituted.

Larger, multicentric, prospective studies are needed to confirm our results and better identify the variables associated with the occurrence of PRF+ and development of PRF-RI to define the most effective antibiotic prophylaxis and definitively assess the utility of pre-emptive therapy.[20] It will also be interesting to assess the difference between hypothermic and normothermic machine perfusion systems regarding the risk of graft-derived infections.[21] Moreover, the growing menace of MDR microorganisms will probably affect this setting, and future studies should be aware of the possible transmission of these germs through graft and PRF.

AUTHOR CONTRIBUTIONS

AL, GR, AG, LC, BA, and AB conceived the study. GR, DD, EP, LDP, GV, AZ, CA, AM, CP, LA, EF, AM, GG, and FD collected the clinical data. CM performed microbiologic analysis. AL performed the statistical analyses. AL and GR wrote the first draft of the manuscript. All the authors reviewed the final version of the manuscript.

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CONFLICT OF INTERESTS STATEMENT

None related to the content of this publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request to the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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