INCIDENCE AND CLINICAL IMPACT OF POSITIVE CULTURE PRESERVATION FLUID MICROBIOLOGICAL FINDINGS IN LIVER TRANSPLANTATION

Incidência e impacto clínico dos achados microbiológicos de cultura positiva do líquido de preservação no tranplante hepático

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ABSTRACT

Purpose: Postoperative infection is considered one of the most important causes of morbidity and mortality after liver transplantation. Few studies have examined the incidence of culture-positive preservation fluid and the outcome of related recipients. We studied the incidence and clinical impact of infections in preservation fluids for liver transplantation. **Methods**: We cultured Celsior® preservation fluid from 225 liver transplantations for 4 consecutive years under a post-transplant infection prophylactic protocol consisting of a third generation cephalosporin plus netilmicin in high risk patients for 48 hours. **Results**: Seventy preservation fluids were found to be positive with one to three pathogens. Thirty-one percent of these were skin saprophytic flora; nevertheless in 29 cases (41.1%) we isolated high virulent pathogens. Only eight patients developed postoperative fever due to the pathogen isolated in the preservation fluid. **Conclusion**: Positive cultures of preservation fluids were observed in 31.1% patients, although one third was skin saprophytic flora. Our results do not support routine culturing of the preservation solution provided that there is an adequate antibiotic prophylactic regimen.

Keywords: Liver Transplantation; Organ Preservation; Infection; Bacteria; Fungi.

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INTRODUCTION

Postoperative infection is considered one of the most important causes of morbidity and mortality after LT.[1,2,3] The risk of infection is very high due to the surgical procedure, multiple blood transfusions, central venous catheters, extended intensive care unit stay and immunosuppressant therapy.[4] A potential source of infection could be an infected donor, contamination at the time of the infusion or packaging, back-table procedure, and during the transplantation technique. Nevertheless, in most cases, the primary source of infection is not identified.[5,6,7] Therefore, it is necessary to control all possible foci of infection. A sterile preservation medium in which the graft is preserved after harvesting, such as Celsior®, is prone to microorganism colonization

owing to its chemical and physical proprieties (table 1). It has been illustrated that 7-24% [8,9,10] of preservation solutions may contain multiple strains of pathogens such as bacteria and fungi. However, this does not always imply septic complications. Still, very few studies in the literature report the incidence of culture-positive PF and the outcome of related transplants, as there are no widely accepted guidelines to assess the infusion solution.

The aims of the present study were to determine the incidence of positive PF cultures in our transplant center, to describe the microorganisms responsible by positive PF cultures and to assess the outcome associated with contaminated PF.

Table 1 *- Qualitative and quantitative composition of the organ preservation solution Celsior®.*

	Pentafraction (nmol/L)	*50g/L (g/L)*
Glutathione	3	0,921
Mannitol	60	10,93
Lactobionic acid	80	28,664
Glutamic acid	20	2,942
Sodium hydroxide	100	4,0
Potassium chloride	15	1,118
Magnesium chloride	13	2,642
Histidine	13	4,65

PATIENTS AND METHODS

From January 2013 to December 2016, 225 adult LT with cadaveric grafts were performed in the Pediatric and Adult Liver Transplantation Unit from Coimbra University Hospital, Portugal. Retransplantation was excluded, since these patients were already under immunosuppressive therapy, thus prone to infectious agents. Microbiologic cultures obtained from the PFs were retrospectively reviewed and all positive results were identified.

Fluid used for preserving grafts was Celsior®. The storage process of the solution is very controlled and safe, with conservation at 4ºC. In the operating room, there is an appropriate place and dedicated team to prepare the solution; at the proper time, PF samples were systematically collected when the liver from the donor is removed from the fluid to the back-table procedures and are sent to our microbiology laboratory. After the microscopically examination, each specimen is cultured in a thioglycolate broth and on two blood agar plates that are aerobically and anaerobically incubated, respectively, at 37ºC for 5 days. When a microorganism is isolated from such cultures, an antibiogram is performed. The results of PF cultures are reported to the clinicians as soon as available.

During the studied period, the antimicrobial prophylaxis in LT recipients consisted of a third cephalosporin, and in patients considered to have higher risk for infection (those with hepatic cirrhosis, recent hospital admission and previous upper gastrointestinal bleeding episodes) netilmicin 150mg 12/12 hours was added. This protocol was intraoperatively administered, and discontinued at the end of the surgical procedure. In addition, immunosuppressive therapy consisted of corticosteroid therapy (methylprednisolone 40mg 24/24 hours), a single dose of basiliximab 20mg, and also a single dose of rituximab 20mg, followed by tacrolimus for plasma levels between 10 and 15 micrograms/L.

The follow up period considered for infection related to the PF microorganism was 14 days. Recipient infection related to positive PF culture was considered present when patient presented clinical signs and symptoms of infection, the same pathogen was isolated from the host and they shared the same antibiogram profile.

Variables collected from the medical records were gender, age, underlying liver disease, duration of surgical procedure, pathogen from positive PF culture, occurrence of infection, microorganism from infected recipient, antimicrobial therapy and outcome after discharge.

High virulent pathogens considered were Staphylococcus aureus, Streptococcus agalactiae, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii, while skin saprophytic flora (SSF) accounted for were Staphylococcus coagulase-negative, Streptoccocus viridans and Staphylococcus epidermidis.

The absolute (n) and relative (%) frequencies were presented for qualitative variables. The non-parametric Chi-square (X2) test was used to check if the distribution of variables was similar. Statistical analysis was done by using the software SPSS® version 21.

RESULTS

Positive cultures were found in 70 out of 252 (31.1%) liquid specimens. Twenty-two of these (31.4%) were skin saprophytic flora, while 29 PFs (41.4%) had isolates of high virulent pathogens. The remaining were sterile.

Three distinct organisms were isolated from 6 (8.6%) PF and two germs from 10 (14.3%) samples, whereas the remaining 54 (77.1%) PFs were monomicrobial. Consequently, 33 different organisms were isolated from the 70 culture-positive PFs, in a total of 98 pathogens.

The germs most frequently observed were Staphylococcus epidermidis and Escherichia coli. It was recovered anaerobic bacteria in 8 cases and fungi in 7 samples. Thirty-one percent of these were skin saprophytic flora.

Nevertheless, in 29 cases (41.1%) we isolated high virulent pathogens (table 2). A significant number of transplanted patients were male (53; 75.7%), with mean age of 55 years [29-72].

Underlying liver diseases of the 70 patients who received LT with culture-positive PF are shown in table 3. The mean time of surgical procedure was 8h09min [5h10min-13h05min]. During the intensive care unit stay, infection caused by the pathogens found in the PF occurred in 8 (11.4%) of the 70 recipients (table 4). The median time of infection onset was 3.6 days [2-9].

In every case, the infectious disease was caused by a single agent, regardless the number of pathogens recovered from the PF. Infection in recipients occurred in 4 patients with PF testing positive for Enterobacteriaceae, 2 for streptococci, 1 for Pseudomonas aeruginosa and 1 for yeasts. No PF-related infection was caused by staphylococci nor anaerobic bacteria. Infectious complications caused by PF-related microorganisms included 4 intra-abdominal infections, 2 pneumonia, 1 primary bacteremia and 1 wound infection.

All infected patients were treated with antibiotics, according to the suspected focus of infection and were discharge home with no more complications.

The global mortality rate was 4.3% (n=3), and none of these patients had systemic infection during the hospitalization.

***Table 2** -Description of the 33 organisms isolated from the 70 preservation fluids.*

	Microorganism	n (%)
Aerobic bacteria 28 (84.8%)	Staphylococcus epidermidis	14 (42.4)
	ESBL negative Escherichia coli	9 (27.3)
	Streptococcus viridans	6 (18.2)
	Enterococcus faecalis	5 (15.2)
	Staphylococcus warneri	4 (12.1)
	ESBL negative Klebsiella pneumoniae	3 (9.1)
	Methicillin-sensitive Staphylococcus aureus	3 (9.1)
	Staphylococcus lugdunensis	3 (9.1)
	Acinetobacter baumannii	2 (6.1)
	Enterococcus durans	2 (6.1)
	Enterococcus gallinarum	2 (6.1)
	ESBL positive Klebsiella pneumoniae	2 (6.1)
	Pseudomonas aeruginosa	2 (6.1)
	Serratia marcescens	2 (6.1)
	Staphylococcus capitis	2 (6.1)
	Staphylococcus hominis	2 (6.1)
	Coagulase-negative Staphylococcus	2 (6.1)
	Streptococcus agalactiae	2 (6.1)
	Citrobacter freudii	1 (3.0)
	Enterococcus avium	1 (3.0)
	Enterococcus faecium	1 (3.0)
	Haemophylus parainfluenza	1 (3.0)
	Proteus mirabilis	1 (3.0)
	Methicillin-resistant Staphylococcus aureus	1 (3.0)
	Staphylococcus auricularis	1 (3.0)
	Streptococcus anginosus	1 (3.0)
	Streptococcus gallolyticus	1 (3.0)
	Streptococcus parasanguinis	1 (3.0)
Anaerobic bacteria 4 (12.1%)	Enterobacter cloacae	5 (15.2)
	Aeromonas hydrophila	1 (3.0)
	Bacillus species	1 (3.0)
	Fusobacterium necrophorum	1 (3.0)
Funghi 1 (3.0%)	Candida albicans	7 (21.2)

Table 3 - *Underlying liver diseases of the 70 patients who received preservation fluids with culture-positive PF.*

	Underlying liver disease	n (%)
Cirrhosis	Alcoholic	23 (32.9)
	Hepatocellular carcinoma	22 (31.4)
	Primary biliary cirrhosis	3 (4.3)
	Alcoholic and virus	2 (2.9)
	Virus (hepatitis B virus or hepatitis C virus)	2 (2.9)
	Non-alcoholic steatohepatitis	1 (1.4)
	Familial amyloid polyneuropathy	8 (11.4)
Non-cirrhotic hepatopathy	Primary sclerosing cholangitis	2 (2.9)
	Congenit hepatic fibrosis	1 (1.4)
	Hepatic polychistosis	1 (1.4)
	Rendu-Osler-Weber syndrome	1 (1.4)
Fulminant hepatitis	Autoimmune	2 (2.9)
	Virus (hepatitis E virus)	1 (1.4)
	Alcoholic	1 (1.4)

DISCUSSION

Recipients of a liver are at a high risk for infections owing to multiple risk factors. Issues that increase the risk are pre-operative colonization, prolonged hospitalization, long surgical procedure and presence of invasive devices. Consequently, infection in solid organ transplantation represents one of the most important cause of morbidity and mortality in these cases. Usually, infections are bacterial in origin and hospital acquired.[11] Contamination of the perfusion fluid used to preserve the liver graft may represent a source of infection in recipients in the early postoperative period. PF represents a potential medium in which microorganisms can easily grow.[6,12] However, little is currently known about the rate and significance of positive PF cultures routinely obtained after cadaveric liver transplantation.

In our study, for 4 consecutive years, 70 PFs had a positive culture. We observed a high rate of contamination of the PF with a 41.4% rate of positive cultures to virulent pathogens. Only 8 cases of systemic infection related to the pathogen collected in the specimen occurred with no mortality rate related to it. In the remaining 62 cases, contamination of the solution did not affect

Table 4 - *Characteristics of patients infected by the agent present in the preservation fluid.*

Patient	Gender	Age	Hepatic disease	Surgical time $	Microorganism	Sample	Days #
1	F	44	Hepatic polychistosis	8:35	P.aeruginosa	Blood	2
2	M	71	Hepatocellular carcinoma	6:50	K.pneumoniae *	Peritoneal fluid	3
3	M	43	Alcoholic cirrhosis	10:35	S.marcescens	Surgical wound	4
4	M	69	Hepatocellular carcinoma	8:10	E.coli **	Sputum	2
5	M	68	Familial amyloid polyneuro-pathy	7:40	C.albicans	Sputum	9
6	M	56	Alcoholic cirrhosis	6:20	S.epidermidis	Peritoneal fluid	5
7	M	65	Hepatocellular carcinoma	7:20	S.epidermidis	Peritoneal fluid	2
8	F	57	Primary biliary cirrhosis	8:00	K.pneumoniae **	Peritoneal fluid	2

patient outcome. Rarity of infection of the recipient in the early postoperative period is probably related to the low bacterial inoculum and administration of antibiotic prophylaxis.[5,13]

The absence of a control group is due to the fact that this is a retrospective study. On the other hand, we assumed, the infection was caused by the same PF microorganism strain as identified by the antibiogram. No bacterial strain typing was possible at the time of the preparation of this article.

CONCLUSION

Based on our findings, we can dismiss routine culture of the preservation solution, provided that there is an adequate post-transplant antibiotic prophylaxis. Genotyping of microorganisms will confirm a more accurate source of infection. Asepsis during procurement should be the prime goal. A focused alertness may decrease infection-related complications among transplanted patients.

RESUMO

Objetivo: A infeção no pós-operatório é considerada uma das mais importantes causas de morbi-mortalidade após transplante hepático. Poucos estudos analisam a incidência de culturas positivas do líquido de preservação do enxerto e o outcome dos respetivos recetores. Estudámos a incidência e o impacto clínico de infeção do líquido de preservação de orgão para transplante hepático. **Métodos**: Cultivámos o líquido de preservação (Celsior®) em 225 transplantes de fígado durante quatro anos consecutivos; os recetores de alto risco foram submetidos a um protocolo de profilaxia antibiótica de infeção pós-transplante, durante 48 horas, que consistia numa cefalosporina de 3ª geração e netilmicina. **Resultados**: Setenta líquidos de preservação foram considerados positivos com um a três patógenos identificados. Destes, 31% eram flora saprofítica da pele, porém, em 29 casos (41,1%), isolámos patógenos de alta virulência. Apenas oito doentes desenvolveram febre no pós-operatório devido ao microorganismo isolado no líquido de preservação. **Conclusão**: Foram identificadas culturas positivas em 31,1% dos casos, sendo que um terço corresponde a flora saprofítica da pele. Os nossos resultados não suportam a realização por rotina de cultura do líquido de preservação, desde que haja um regime profilático de antibióticos adequado.

Descritores: Transplante de Fígado; Preservação de Órgãos; Infeção; Bactérias; Fungos.

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