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PRESERVATION FLUID CULTURES. CLINICAL SIGNIFICANCE IN LIVER TRANSPLANTATION

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Abstract The aim of this study was to determine the incidence of preservation fluids (PF) bacterial positive cultures, identify the germs involved, determine their correlation with infections in recipients during the postoperative period and compare outcomes in terms of morbidity, hospital stay and both patient and graft survival. We describe incidence and etiology of germs developed in PF cultures in our series and evaluate its impact on recipients. A prospective study in deceased donor liver transplants (LT) recipients was carried out from January 2014 to December 2017. Back table PF cultures were analized considering positive the development of any germs and negative to no signs of growth after 5 days. PF were classified as contamination or pathogens. Targeted antibiotic therapy was administered in the last ones. Recipients were divided in: PF (-) and PF(+). Recipients infections related to positive PF were analyzed. These were identified as "direct correlation" when the same germ grew up in PF. Hospital stay and 30 days follow up were compared. Eighty-eight patients PFs were included, 38% (33) had positive cultures, 28 (85%) of these were considered contamination and only 5 as pathogens. We found no differences in postoperative infections (p 0.840), ICU and total hospital stay (p 0.374 and 0.427) between both groups. Postoperative infections and hospital stay seem not to be influenced by PF cultures positivity. Treatment of isolated pathogens could have prevented infections, therefore, those groups that perform PF cultures should consider treatment in these cases and conclude prophylaxis when PF is negative or contaminated.

Key words: liver transplantation, culture techniques, organ preservation solutions

Resumen ***Cultivos de líquido de preservación en trasplante hepático. Significancia clínica.*** Las infecciones bacterianas son frecuentes en pacientes sometidos a trasplante hepático. Describimos la incidencia y etiología de los cultivos de líquidos de preservación (LP) positivos en nuestra serie y analizamos su importancia clínica. Se trata de un trabajo prospectivo de pacientes trasplantados hepáticos, entre enero 2014 a diciembre 2017. Se analizaron muestras de LP tomadas al finalizar la mesa de banco, considerándose positivo el desarrollo de cualquier germen y negativo la ausencia del mismo luego de 5 días. Los LP positivos se clasificaron en: con contaminantes y con patógenos. Los pacientes con LP patógenos recibieron tratamiento antibiótico de acuerdo al antibiograma. Los pacientes fueron divididos en dos grupos: con LP + y LP-. Las infecciones relacionadas a los LP fueron analizadas. Se consideró "correlación directa" cuando el mismo germen desarrolló en el LP y en el recipiente. Se comparó estadía hospitalaria en ambos grupos. Se incluyeron 88 pacientes, 38% (33) presentaron LP+, de los que el 85% (28) fueron por contaminación y 5 por patógenos. No se hallaron diferencias significativas en infecciones postoperatorias (p 0.840) y estadía hospitalaria (p 0.427) entre ellos. No hubo casos de "correlación directa". Las infecciones postoperatorias y la estadía hospitalaria de los pacientes no parecen estar influidas por la positividad de los cultivos de LP. El tratamiento dirigido a los gérmenes aislados como patógenos pudo prevenir infecciones, por lo tanto, los grupos que realizan cultivos de rutina deberían considerar el tratamiento en estos casos y finalizar la profilaxis cuando el LP sea negativo o contaminado.

Palabras clave: trasplante hepático, técnicas de cultivo, soluciones de preservación de órganos

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KEY POINTS

- Postoperative infections are one of the main causes of morbimortality in patients undergoing liver transplantation. We described the incidence and etiology of germs developed in preservation fluid (PF) cultures in our series and evaluate its impact on recipients.
- Postoperative infections and hospital stay seems not to be influenced by PF cultures positivity. The treatment of isolated pathogens could have prevented infections, therefore, those groups that perform PF cultures should consider treatment in these cases and conclude prophylaxis when PF is negative or contaminated.

Postoperative infections are one of the main causes of morbimortality in patients undergoing liver transplantation (LT)[1]. In recent years, the development of better immunosuppressive agents has decreased the rates of acute and chronic rejection with a raise in infections by uncommon microorganisms as a counterpart[2]. Although many groups have focused their attention on studying all possible pathways of infections transmission in LT, there are few reports that discuss the positivity of preservation fluids (PF) cultures and their clinical impact in recipients[3, 4]. We are not aware of the existence of published studies on this issue in Latin America. The aim of this study was to determine the incidence of PF positive cultures, identify the germs involved, determine their correlation with infections in recipients during the postoperative period and compare outcomes in terms of morbidity, hospital stay and both patient and graft survival.

Materials and methods

A non-randomized, prospective, consecutive and monocenter study with deceased donor LTs was carried out during the period of January 2014 through December 2017. We only excluded live donor LTs.

We sent specimens of PF as well as a tissue sample for culture of fungi, aerobic and anaerobic bacteria once back-table was finished prior to implantation.

We considered positive PF the development of one or more germs in the samples and negative PF when there was no growth of germs after 5 days of cultures in the case of bacteria and 42 days for fungi. The isolation of methicillin-sensitive (MS) *Staphylococcus*, negative coagulase *Staphylococcus*, polymicrobial flora, corinebacterias and *Streptococcus viridans* were considered contamination. On the other hand, the following were classified as pathogenic: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus* (MRSA), *Streptococcus pyogenes*, enterobacterias, *Enterococcus*, Gram-negative anaerobic bacilli, Gram-negative aerobic bacteria (*Pseudomonas aeruginosa*) and any fungi.

Broad-spectrum prophylaxis with 3 g of intravenous ampicillin-sulbactam was routinely indicated in all recipients, 30 minutes before skin incision and four times a day up to 48 hours after surgery. In case of positivity in PF or in other donor cultures (routinely performed blood cultures or any other tissue or fluid considered potentially infected), antibiotic therapy was indicated in all cases according to the corresponding antibiogram (except in cases of positive cultures of PF considered contamination). No scheduled cultures were performed in recipients during their hospital stay in absence of suspected infection. When fever (axillar temperature ≥ 38° C) or hypothermia (axillar temperature ≤ 36°), combined with any other sign or symptom of infection were present, such as tachypnea (> 20 breaths per minute), pCO_2 > 32mmHg, tachycardia (> 90 bpm), leukocytosis (> 12 000 WBC/ml) or leukopenia (< 4000 cells/ml), or more than 10% immature neutrophils[5], blood cultures and urine cultures were performed. Sputum, bronchoalveolar lavage, abdominal fluid or collections cultures were only performed after clinical or imaging suspicion of these localized infections. Percentage of positive cultures of PF were determined, distinguishing contamination from pathogenic germs. Isolated germs in each case were grouped by frequency of appearance.

We identified as "direct correlation" when the same germ with the same sensitivity and resistance to antimicrobials according to antibiogram grew up in PF and in any culture from the recipient. No genotypic study was done to identify germs.

Demographic data of both groups were analyzed, considering age of the recipients, gender, body mass index (BMI), Model of End Stage Liver Disease (MELD) score, days on waiting list, blood transfusions during surgery and early orotracheal extubation. Ninety days post-LT infections in both groups (positive PF, PF+; and PF-) were compared regardless of the isolated germ in the recipient. We classified infections as bacteremia, urinary tract infections (UTI), lower respiratory infections (tracheobronchitis, pneumonia and pleural effusion), superficial and deep surgical site infections (SSI) and catheter-associated infections according to WHO criteria[6].

ICU and Hospital stay were analyzed in days. Donor risk factors for PF+ were analyzed, including age, sex, BMI, days of hospitalization and of mechanical ventilation and a history of positive blood cultures.

Statistical analysis was performed with SPSS 21 software. Continuous variables were expressed as mean and range and were analyzed between groups with Student and Mann-Whitney test. The categorical variables were presented by frequency and percentage and were compared with the chi square method and Fisher's test. Survival was analyzed with Kaplan Meyer curve using the Long Rank for survival comparison. In all cases, the level of statistical significance was considered with a $p < 0.05$.

Results

Eighty eight LT were included in the observed period. Donors, PF cultures and postoperative evolution were analyzed. The mean age of recipients was 49 years (range 1-70). Demographic data is presentad in Table 1. PF cultures were positive in 33 cases (38%): twenty eight cases of PF+ (85%) were identified as contamination and 5 as infection (15%). These 5 cases received directed antibiotic therapy once the germ was isolated. Germs are detailed by frequency in Table 2.

PF+ group did not present higher infection rate in general within the first 90 days (38% vs. 35.5%, p 0.84). No case of direct correlation was identified.

ICU stay between study groups did not show significant differences (11.1 vs 8.17, p: 0.374) as well as hospital stay in days (19.4 vs 15.8, p 0.427).

Finally, no significant differences were found in donor risk factors in both groups (described in Table 3). Same

TABLE 1.– *Demographic data of both groups, preservation fluid (PF) positive and negative*

	PF+(n:33)	PF- (n:55)	p
Age (mean)	52	48	0.2
Gender, male	25 (75.8%)	31 (56.4%)	0.07
BMI (mean)	28.7	29	0.75
MELD (mean)	23.8	22.5	0.46
Blood transfusions (mean)	2.9	3.3	0.31
Days in waiting list (mean)	361	226	0.23
Early extubation (mean)	17(51.5%)	21(39.2%)	0.26

MELD: Model for end-stage liver disease; BMI: Body max index

TABLE 2.– *Frequency of isolated germs in preservation fluids*

Germ (preservation fluid)	n	%
Polymicrobial flora	11	33.3
Staphylococcus epidermidis	10	30.3
*Klebsiella pneumoniae**	2	5.7
*Staphylococcus aureus**	2	5.7
Corynebacterium spp	3	8.5
*Enterobacter cloacale**	1	2.9
Klebsiella oxytoca	1	2.9
Micrococcus luteus	1	2.9
Staphylococcus warneri	1	2.9
Staphylococus caprae	1	2.9
Total	33	100

** Considered pathogens*

TABLE 3.– *Donor risks factors for PF positivity*

	33 PF+	55 PF-	p
Age (mean) (%)	44.9	42.8	0.58
Male gender (%)	75.8	56.4	0.67
Body mass index (mean)	28.7	29.1	0.76
Days in mechanical ventilation (mean)	3.4	3.3	0.82
Positive blood cultures (%)	55.0	32.4	0.06

germ in PF and donor blood culture was identified in only 1 case without direct correlation in recipient.

Discussion

Modern immunosuppressive regimens among other advances have increased LT graft survival by reducing the incidence of acute and chronic rejection, with an counterpart increased risk of opportunistic infections in recipients[2]. At the same time, the organ scarcity and thus unproportioned growth of the organ transplant list compared to available organs has increased the use of marginal donors[7], including those with an increased risk of infection transmission. However, very few reports have focused on the post-transplant impact of PF cultures[3,4], being completely unknown in

our region. Having post-LT infections a significative impact on both patient and graft survival[8-10], and the lack of prospective studies on this subject, we consider ours relevant. For this purpose, the authors carried out this prospective study that showed a positivity rate of 33% in PF cultures, very similar to previous reports[3,4] requiring specific antibiotic treatment only in 15% of cases (pathogenic germs). We collected culture samples after back-table was finished. Although some groups described collecting cultures at different times[3], we considered our approach practical for our aim to analyze impact in recipients outcomes regardless the moment of contamination, to contemplate all moments of possible contamination within manipulation of the graft prior to implantation.

Antibiotic profilaxis prior to transplantation and up to 48 hours later would prevent transmition of germs considered as contamination in both, PF and surgical site[11]. When pathogenic germs were isolated in PF, directed antibiotic therapy was supplied, without registering any "direct correlation" in recipients who presented any positive culture, in contrast to the results shown by Cerutti et al, that registered a correlation in 4% of cases[8]. The treatment may have prevented the appearance of any transmition and therefore may have incurred a bias per se. However, in his work Cerutti does not specify if direct correlation was secondary to isolated germs in the preservation fluid or in any other donor cultures. Whenever a positive correlation is found, a genotypic study of isolated germs should be done if we want to confirm the same origin[12, 13]. However, the authors consider that in terms of costs and clinical relevance this study would not be necessary.

Regardless of correlation, positivity in PF (whether it was contamination or pathogenic, in which case targeted antibiotic treatment was implemented) did not incur in a higher rate of infections in recipient as well as in differences in ICU and total hospital stay, so we can conclude that should not be considered as a risk factor. Even so, a prospective work with higher number of patients is needed to confirm these.

We found no significate correlation between donor risks factors and PF positivity. In our study, mechanical ventilation days and donor age did not increase the incidence, as described by Ruiz et all[3]. However, authors identified almost significant correlation in PF positivity when donors had a history of positive blood cultures (p = 0.06). Perhaps, with a higher N of patients, such differences could become significant. A blood culture in one case developed the same germ as the preservation fluid (*Klebsiella pneumoniae*), antibiotic therapy was done in recipient and no direct correlation was observed, that's mean, that the patients didn't present that germen in any culture.

In conclusion, in our experience, when there was a positive PF for contamination germs, no treatment was required for the recipient, without a negative consequence in terms of infection. And when the PF fluid culture was positive for a pathogenic germ, if directed treatment was conducted, there were no infections in the recipient as well. We recommend routine culture of PF and management of positive PF cultures as outlined before.

Conflicts of interest: None to declare

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