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Analysis of early-stage infection risk factors after living donor liver transplantation in 25 children

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Living donor liver transplantation (LDLT), which was first demonstrated in 1989, has now been widely used to cure many late-stage hepatic diseases and has achieved huge success, especially in the pediatric field. However, accumulated studies indicate that early-stage infection after LDLT is the main cause leading to its failure and the death of the patient. Here, we retrospectively analyzed 25 cases (from 2006 to 2009) of infections of children in our hospital, underwent LDLT and their correlated factors. To identify the factors most closely related to early stage infection, we categorized early stage infection levels as either mild, moderate or severe and we compared their related risk factors, including total length of stay in hospital (TLSH), length of stay in the intensive care unit (LSI), duration of catheterization (DC), length of pre-/post-operative antimicrobial agent application, length of immuno-suppressant agent application (LISA) and underlying diseases postoperatively. The results revealed the following. (i) Of 25 patients, 24 recipients were infected to various extents: 6 cases of mild infection, 14 cases of moderate infection and 4 cases of severe infection (one patient died). (ii) TLSH, LSI and durations of preoperative (DPAA) and postoperative (DPOAA) antimicrobial agent application were statistically different between the severe and moderate infection groups (P = 0.03 (TLSH), 0.004 (LSI), 0.003 (LAPP), 0.005 (DPOAA). (iii) Between the severe and mild infection groups, the TLSH (P = 0.016), LSI (P = 0.015), DAPP (P = 0.007) and DPOAA (P = 0.001) were also significantly different. (iv) In the case of LISA, only methylprednisolone (P = 0.01) and the calcineurin inhibitor ciclosporin A (P = 0.009) showed statistically significant differences between the moderate and mild infection groups. (v) Biliary atresia is the predominant underlying liver disease. (vi) In contrast to previous findings, there was no significant difference in DC among the three infection groups. (vii) Of all the 25 patients, 24 survive till now and the only one died was diagnosed as portal vein thrombosis (PVT) combined with high white blood count (>30 x 10^9/l) in venous blood. DPAA, DPOAA and biliary atresia are the risk factors most closely related to infection during liver transplantation in children, an observation worthy of future investigation. To avoid long term DPAA and long term DPOAA induced early severe infection, nosocomial infection control and drug resistant bacteria monitoring are worthy to be concerned after LDLT in children.

Key words: Living donor liver transplantation, risk factor, children.

INTRODUCTION

Living donor liver transplantation has been used for end-stage liver disease treatment since 1989 (Raia et al., 1989; Lee et al., 2001). LDLT has basically resolved the problem of donor organ shortages. However, early complications postoperatively and long-term life quality are issues that still need to be resolved. Infection and secondary complications postoperatively are major issues contributing to morbidity and mortality (Kawecki and Sawicka-Grzelak, 2008). It has been reported that the postoperative infection rate for liver transplant

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recipients is 13.6% (Zhu and Gao, 2009), of which 90% occurred in the early stage after the operation (Fishman and Rubin, 1998). In addition, bacterial infection is mainly caused by gram-negative strains, enterococcus or aureus.

Fungal infection is likely to attack high-risk patients who have undergone repeated transplant operations, continuous hemodialysis, preoperative chemotherapy or multi-antibiotic treatment. The latest studies show that early pulmonary bacterial and fungal infection rates after liver transplantation in adults are 21.3 and 3.7%, respectively (Zhu and Gao, 2009). Besides hepatitis B and hepatitis C, the main pathogenic viruses responsible for infection in children include cytomegalovirus, Epstein-Barr virus and Herpes Simplex Keratitis (Singh et al., 1997). The degree of postoperative infection is influenced by various factors, such as immunosuppressive agent application, prophylactic use of antibiotics (antimicrobial agents), regional bacterial epidemiology and invasive ducts. Furthermore, the hospital environment and management in different clinical divisions also affect the infection status. Therefore, we should assess the number of risk factors to reduce the incidence of infections and their complications.

In the present study, we retrospectively analyzed 25 cases of children who underwent LDLT in our hospital from 2006 - 2009. Patient information including age, gender, LSI, TLSH, DC, LISA, DPAA, DPOAA, underlying liver disease and etiological data (Broering et al., 2004; Kim et al., 2009) were evaluated. The results indicate that DAPP, DPOAA and biliary atresia are the main risk factors related to post-operative infection.

PATIENTS AND METHODS

Patients

Total number of 25 children with 12 boys and 13 girls who underwent LDLT from June 2006 to May 2009 at the Children's Hospital of Chongqing Medical University were analyzed. The age distribution of the patients was from 2 months to 13 years and 15 patients were less than 1 year old. All liver sources were from living donor legally. In details, 11 from mother, 11 from father, 1 from grandmother and 2 from cousin.

Definition of infection

The definition of infection is based on the criteria of the Centers for Disease Control and Prevention (CDC) (Garner et al., 1988; Chang et al., 1998). Bacterial or fungal infections, defined as specific changes combined with clinical symptoms such as fever, cough and hypoxemia etc., were confirmed by at least one positive blood culture or image results (X-ray, MRI, CT). Viral (CMV, EBV, HSV) infections were diagnosed when specific IgM showed positive results with the ELISA assay, as described previously (Singh et al., 1994). The clinical diagnosis should be consistent with the symptoms in all definitions. Infection grades are assigned based on the systemic inflammatory response syndrome (SIRS) defined by the Society of Critical Care Medicine (SCCM) in 2008. For each patient, we measured his/her blood parameters for many times. The white blood count (WBC) and C response protein (CRP) concentration in blood we used to define infection level in this paper is derived from the first time the patient exhibit infection phenotypes. Therefore it can well represent the early stage infection after LDLT.

Mild infection: Detection of bacteria without image changes, WBC < 12 X 10^9/l, CRP < 30mg/l.

Moderate infection: Detection of bacteria and image changes, WBC > 12 X 10^9 /l, 30mg/l < CRP < 200mg/l.

Severe infection: Detection of bacteria, image changes and complications with fungal infections. The patients also received antifungal drug treatment, WBC > 12 X 10^9 /l, CRP > 200mg/l. Note: "Image" refers to X-ray, MRI, or CT.

Antimicrobial application

The anti-bacterial agents include cefotaxime sodium (0.6 g/day IV), gentamicin (60,000 units/day IV), furbencillin sodium (0.8 g/day IV), piperacillin sodium (1.1256 g/day IV), cefuroxime axefil (750 mg/day IV), micronomicin sulfate (160 mg/day) and levofloxacin (40 mg/day IV). Each patient used several of the agents listed above with respect to their own situation. Amphotericin B, 5-fluorocytosine, fluconazole, traconazole and voriconazole (3 - 6 mg/day/kg) were utilized for fungal infections.

Immunosuppressive therapy

All patients received immunosuppressive therapy after their operations. Pharmacological agents that were used included methylprednisolone, ciclosporin, tacrolimus, dexamethasone, prednisone acetate, methylprednisolone, dexamethasone acetate, prednisone and meglumine adenosine cyclophosphate. For each patient, the doctor selected several types of immunosuppressants at minimum effective doses based on their personal situations.

Statistical analysis

ANOVA was used to analyze the continuous variables and Fisher's exact test was used to analyze the categorical variables. Statistical differences between two groups were determined with the least significant difference test (LSD). Statistical analysis was performed using the SPSS version 14.0 software package and $p < 0.05$ was considered as statistically significant. P^o indicates a comparison among three levels of infection by ANOVA. P' indicates a comparison between mild infection and moderate infection. P'' indicates a comparison between mild infection and severe infection. P''' indicates a comparison between moderate infection and severe infection

RESULTS

Population characteristics and clinical parameters

Before undergoing LDLT, only one child presented with a fever, prompting infection, and that child was diagnosed with cholangitis. None of the other 24 children presented with any signs of infection. As we described in the methods, we categorized the infection level according to blood parameter, bacteria detection and image changes.

Table 1. Population information by the infection level distribution and underlying diseases.

		Mild infection	Moderate infection	Severe infection	P^o
		n = 6	n =14	n = 4	
Age (Year)		7.96 ± 4.97	1.05 ± 1.49	6.36 ± 5.22	
Sex (Male)		50% (3/6)	35.7% (5/14)	75% (3/4)	0.333
Virus	CMV		42.9% (6/14)	25% (1/4)	1.000
Diagnosis					0.004
	Biliary atresia	16.6% (1/6)	85.7% (12/14)	25% (1/4)	
	Hepatolenticular degeneration	33.3% (2/6)		25% (1/4)	
	Cavernous transformation of the portal vein	33.3% (2/6)		25% (1/4)	
	Hepato-glycogenosis	16.7% (1/6)	7.14% (1/14)		
	Biliary cirrhosis			25% (1/4)	
	Subacute fatal hepatitis		7.14% (1/14)		
Combined disease					1.000
	Biliary cirrhosis	33.3% (2/6)	64.3% (9/14)		
	Hemophagocytic syndrome		7.14% (1/14)		
venous blood WBC		(10.3±1.4)x10^9/l	(20.7 ± 3.1) x10^9/l	(23.2±8.1) x10^9/l	
Venous blood CRP		CRP < 12 mg/l	(63.3 ± 29.4) mg/l	CRP > 200 mg/l	

Annotation: P^o indicates a comparison among three levels of infection by ANOVA; P^o <0.05 indicates statistical significance; Age was analyzed by ANOVA, while virus, diagnosis and combined disease were analyzed by Fisher's exact test.

After the LDLT operations, six patients had mild infection (including the preoperative infection cases), 14 patients had moderate infection and 4 patients had severe infection. The white blood cell concentrations in venous blood among mild, moderate and severe infection groups are (10.3 ± 1.4) x 10^9/l, (20.7 ± 3.1) x10^9/l and (23.2 ± 8.1) x10^9/l. Further more, the values of C response protein in blood are <12 mg/l (mild infection), (63.3 ± 29.4) mg/l (moderate infection), and >200 mg/L (severe infection). The mean ages of children with mild infection, moderate infection or severe infection were 7.96, 1.05 and 6.36 years, respectively. There was no statistical difference in the case of liver source. The underlying diseases of these patients are shown in Table 1. Impressively, biliary atresia (14/24) was the predominant disease in all subjects. There was a statistically significant difference between the various underlying diseases and the infection level (P^o = 0.004). However, gender and the presence of combined diseases, such as biliary cirrhosis and hemophagocytic syndrome, did not result in significant differences among the three groups. Of all the 25 patients, 24 children survive till now and the only one died case was diagnosed as portal vein thrombosis (PVT). Infection is a known cause to PVC and in this case high blood WBC (>30x10^9/l) was observed indicating that the infection may contribute to the PVT process.

Frequency of infection and pathogen distribution

Among the 25 children who underwent LDLT, 96% (24/25) developed infections. In total, 100 bacteria strains were isolated from sputum, ascites, catheters, stool, urine, drainage fluid, pus, throat swabs and urethral orifice secretions. Of those strains, 42, 32, 12, 7 and 7% were isolated in the 1st, 2nd, 3rd and 4th week and 1st month after LDLT, respectively (Table 2). In the severe infection group, 27 fungal strains were identified in 4 patients from sputum samples, throat swabs and stool samples.

Consistent with the trends of the bacterial infections, most fungal infections (16/27) occurred within the first two weeks after the operations. Out of the 16, Four (4/27) and six (6/27) fungal strains were isolated in the third and four weeks, respectively. The isolated bacterial strains were mainly obtained from the respiratory tract and abdomen. As shown in Figure 1, 43% were obtained from sputum and 25% were obtained from ascites. Meanwhile, *Staphylococcus epidermidis* (21%) is considered to be the leading pathogen among gram-posi-tive strains. Among gram-negative strains, *Pseudomonas aeruginosa* (11%) and *Klebsiella pneumoniae* (11%) are predominant. Among fungal strains, *Candida albicans* was the most common type. 22 strains of *C. albicans* were isolated from sputum, throat swabs and stool samples. The pathogen distribution is shown in Figure 2.

Impact factors for post-operative infection

Parameters including TLSH, LSI, DC, DPAA, DPOAA and LISA were compared in the three infection groups (Table 3). The respective TLSH and LSI values in the mild,

Table 2. Bacterial infection time and sample source after LDLT.

Samples	1st week	2nd week	3rd week	4th week	>4 week	Total	
	(n)	(n)	(n)	(n)	(n)	No.(n)	Percentage
Sputum	24	11	5	2	0	42	42
Ascites	9	7	3	4	2	25	25
Catheter	6	7	1	0	0	14	14
Stool	3	3	0	1	0	7	7
Urine	0	1	3	0	0	4	4
Drainage fluid	0	0	0	0	4	4	4
Pus	0	2	0	0	0	2	2
Throat swab	0	0	0	0	1	1	1
Urethral orifice secretions	0	1	0	0	0	1	1
Total	42	32	12	7	7	100	100

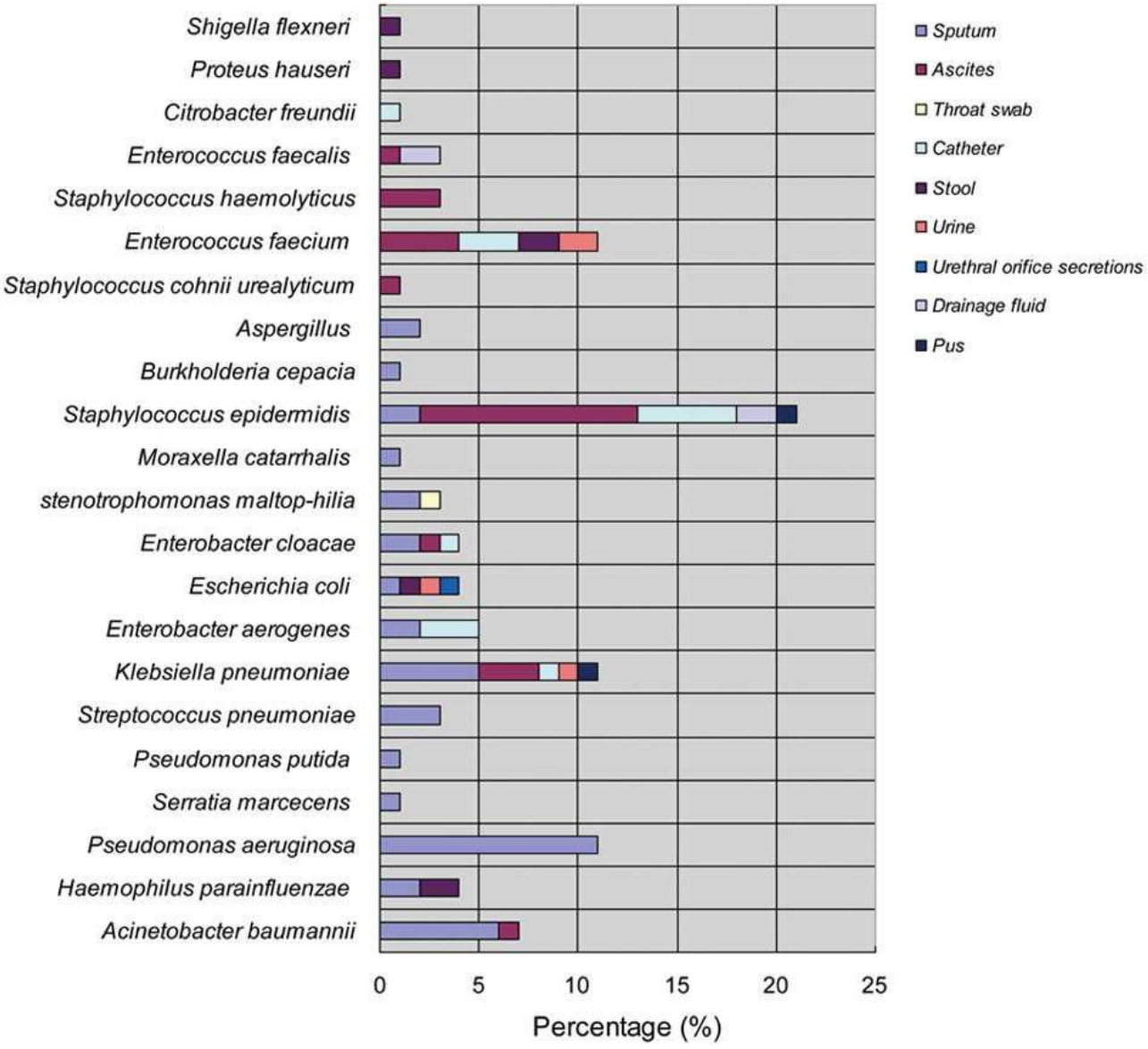

Figure 1. Source of bacterial pathogens isolated from children.

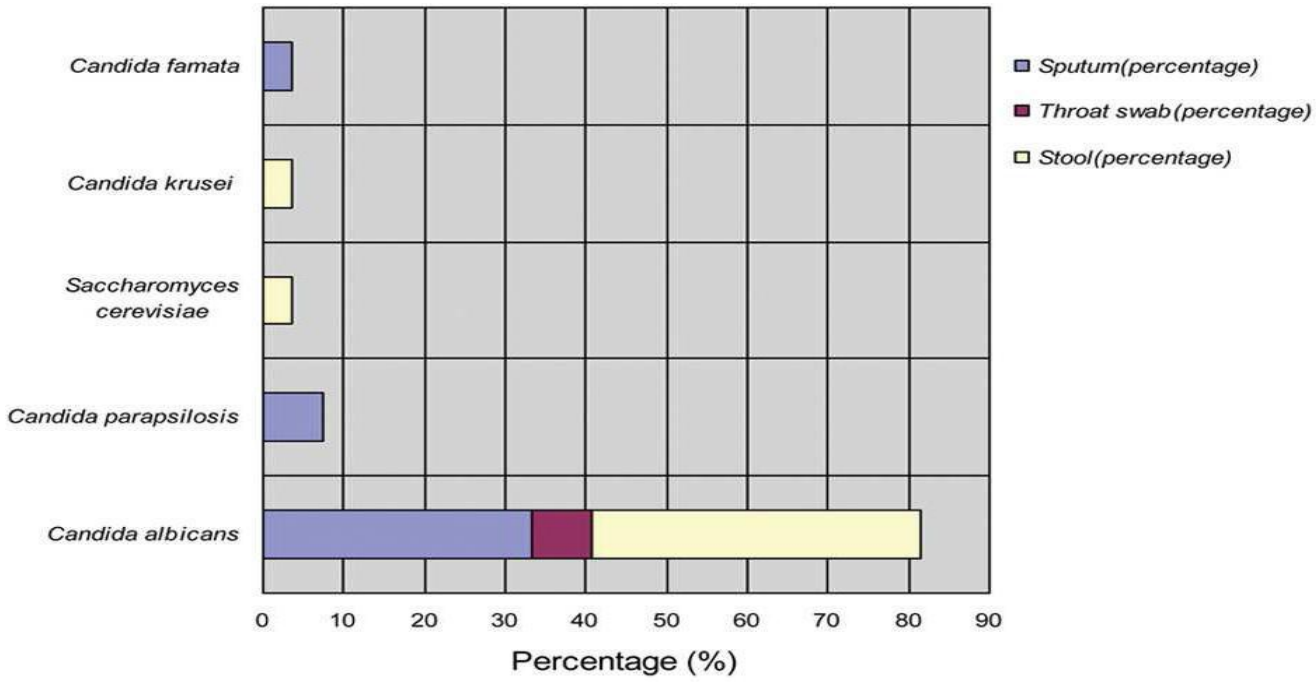

Figure 2. Source of fungal pathogens isolated from infected patients. In total, 27 fungal strains were isolated from patients. In sputum samples, 9 strains of *Candida albicans*, 2 strains of *C. parapsilosis*, and 1 strain of *C. famata* were isolated. In throat swab samples, 2 strains of *C. albicans* were isolated. In stool samples, 11 strains of *C. albicans*, 1 strain of *C. krusei* and 1 strain of *Saccharomyces cerevisiae* were isolated. Among the fungal strains, *C. albicans* (81.5%) was the predominant pathogen.

moderate and severe infection groups were 33.17 ± 20.66 and 12.50 ± 12.19 d, 29.57 ± 10.55 and 10.50 ± 4.88 d and 59.50 ± 22.13 and 37.00 ± 32.75 d. DPAA in the mild infection groups (2.50 ± 2.26 d) and moderate infection groups (2.64 ± 3.18 d) was very close, but it was longer in the severe infection group (10.00 ± 7.17 d). The more severe the infection, the longer the patient received antimicrobial agent therapy post-operatively. This observation is consistent with the DPOAA data. The DPOAA values in the mild, moderate and severe infection groups were 9.17 ± 6.62 d, 15.79 ± 8.29 d and 33.00 ± 17.26 d, respectively. In general, TLSH (P" = 0.016), LSI (P" = 0.015), DPAA (P" = 0.007) and DPOAA (P" = 0.001) were statistically different when comparing the severe and mild infection groups. The four parameters are also significantly different between the moderate and severe infection groups (TLSH P"' = 0.003, LSI P"' = 0.004, DPAA P"' = 0.003, DPOAA P"' = 0.005). In contrast to the expected results (Kim et al., 2009), there was no sta-tistically significant difference in the index of DC among the three levels of infection groups (P^{O} = 0.372). Glucocorticoids, calcineurin inhibitors (CNI) and alkylating agents are three commonly used types of immune-suppressive agents post-liver transplant in our hospital. We compared the length of different immune-suppressive agent applications among the three groups. The glucocorticoid subfamily, including prednisone acetate (P^{O} = 0.207), dexamethasone (P^{O} = 0.995), methyl-prednisolone (P^{O} = 0.327) and dexamethasone acetate (P^{O} = 0.718) and the alkylating agent sub-family member meglumine adenosine cyclophosphate did not result in any statistically significant differences among the three groups. Nevertheless, between the mild and moderate infection groups, methylprednisolone (P' = 0.01) and the CNI subfamily ciclosporin (P' = 0.009) resulted in significant differences.

DISCUSSION

To analyze various infection factors after liver transplant-tation in children, we categorized the degree of infection into three levels. Gram- negative and *Staphylococcus* bacteria were found to be the major bacteria responsible for infection, which is in line with most other reports (Kawecki and Sawicka-Grzelak, 2008; Fishman and Rubin, 1998; Snydman, 1999). Because children have immature immune systems and less antibiotic resistance to bacterial infection, the spectrum of isolated bacteria was slightly different compared to those isolated from adults following liver transplantation.

Biliary atresia, the predominant underlying disease in this study, had an incidence ranging from 5/100,000 to 32/100,000 in children. Liver transplantation is the only treatment method for end-stage liver disease (Chardot, 2006). For the bile duct fibrosis lesion that occurs in biliary atresia, a series of complications such as cholangitis, portal hypertension and hepatic ascites are

Table 3. Factors influencing early phase infections after IDLT.

			Mild infection	Moderate infection	Severe infection	P^{o}	P'	P''	P'''
Infection No.			**n = 6**	**n = 14**	**n =4**				
TLSH			33.17±20.66	29.57 ±10.55	59.50±22.13	0.009	0.64	0.016	0.003
LSI			12.50±12.19	10.50 ±4.88	37.00±32.75	0.012	0.777	0.015	0.004
DC			9.8 ± 6.80	11.21 ±7.26	20.00±26.6	0.372	0.773	0.191	0.212
DPOAA (day)			9.17± 6.62	15.79 ± 8.29	33.00±17.2	0.004	0.18	0.001	0.005
DPAA (day)			2.50 ± 2.26	2.64 ± 3.18	10.00±7.17	0.008	0.94	0.007	0.003
LISA (day)	Glucocorticoids	Methyl prednisolone	7.67 ± 4.08	14.14 ± 5.26	11.75±2.63	0.034	0.01	0.193	0.379
		Prednisone	5.17 ±11.25		21.50 ±29.03	0.019	0.398	0.052	0.006
		Prednisone acetate	0.50 ±1.23	3.57 4.52	5.25 ± 6.08	0.207	0.156	0.1	0.496
		Dexamethasone	5.00 ±11.30	3.79 ± 7.82	4.50 ± 4.80	0.955	0.771	0.928	0.883
		Methylprednisolone		5.00 ±10.00		0.327	0.207	1.000	0.275
		Dexamethasone acetate		1.14±4.28		0.718	0.494	1.000	0.555
	Calcineurin inhibitor (CNI)	Ciclosporin	3.50 ±5.43	15.64±8.63	6.25 ± 12.50	0.019	0.009	0.629	0.070
		Tacrolimus	2.00 ±4.00	0.79 ± 2.01	9.00 ± 15.38	0.095	0.698	0.102	0.033
	Alkylating agent	HLXGPA		0.86 ± 2.93		0.673	0.455	1.000	0.519

Annotation: P^{o} indicates a comparison among three levels of infection by ANOVA analysis; P' indicates a comparison between mild infection and moderate infection; P'' indicates a comparison between mild infection and severe infection; P''' indicates a comparison between moderate infection and severe infection; P<0.05 indicates statistical significance. Hospital days, days of stay in the ICU, days of stay with an abdominal-irrigating catheter, duration of antibiotic use after the operation, duration of antibiotic use before the operation, and duration of immunosuppressant use were analyzed by ANOVA. LDLT: Living donor liver transplantation; TLSH: Total length of stay in hospital; LSI: Length of stay in the ICU; DC: Duration of catheterization; DPAA: Duration of preoperative antimicrobial agent application; DPOAA: Duration of postoperative antimicrobial agent application; LISA: Duration of immunosuppressive agent application.

Table 4. Risk factors with p < 0.05.

		p
Underlying disease	biliary atresia	0.022
DPOAA (day)		0.026
DPAA (day)		0.009

Annotation: Risk factors were analyzed by multivariate statistical analysis; DPAA: Duration of preoperative antimicrobial agent application; DPOAA: Duration of postoperative antimicrobial agent application.

common in clinical practice. Therefore, the probability of infection increased as the incidence of biliary atresia developed. In this study, the percentage of biliary atresia cases in the mild infection group was 16.6%, which is significantly less than the 85.7% observed in the moderate infection group. Moreover, cavernous transformation of the portal vein and hepatolenticular degeneration induced fewer incidences of bacterial infection.

Viruses that are involved in liver transplantation mainly include CMV, EBV, HSV, HBV and HCV (Snydman, 2001). In children, the prevalent viruses during early and long- term postoperative stages are CMV, EBV and HSV, which are intimately correlated with excessive immunesuppression (Reyes et al., 2002). CMV and EBV infections in children presented negative results before liver transplantation in most cases. The application of high amounts of immunesuppressive agents, especially anti- lymphocyte antibody treatment, can induce and aggravate primary CMV infections. 70% of the children who received liver transplants developed primary CMV infections and the mortality rate was as high as 7%. It has been reported that ganciclovir combined with a reduced amount of an immune inhibitor can significantly improve a patient's prognosis (Campbell and Herold, 2004). In our study, CMV was the only virus detected in the patients. Although previous studies indicated that CMV can directly or indirectly enhance the incidence of opportunistic bacterial infections, thereby affecting

morbidity and mortality (Razonable and Paya, 2003; Razonable and Emery, 2004; Razonable, 2008), our research did not verify a statistically significant relationship between CMV attack and the level of bacterial infection.

Interestingly, both the mild and the moderate infection group exhibited shorter TLSH, LSI, DPAA, and DPOAA compared with the severe infection group. These results suggest that noso-comial infection is a vital factor that influences the infection level and therefore it should be paid more attention in the future. The duration of anti-microbial prophylaxis (in this paper, refers to DAPP) has long been a subject of controversy. Longer anti-microbial prophylaxis in this study not only had little effect on prevention but also increased the probability of fungal infection and it may even lead to flora imbalance and extend the hospitalization time. A long DPOAA length may also lead to the emergence of drug- resistant bacteria, which can cause severe infection. This result is supported by the work of Kim and colleagues (Kim et al., 2009). LISA is a dilemma that challenges doctors during transplant operations. The immune system attacks grafts when they are applied in lower amounts, whereas excessive application of LISA leads to a high risk of infection. We found that LISA methylprednisolone and the CNI sub-family ciclosporin influenced the level of infection. Between the mild and moderate infection groups, the longer the time that it is applied, the more severe the infection will be.

In our data, the only one died case was diagnosed as portal vein thrombosis (PVT). But the underlying lethal reason is complicated. High amount of WBC (>30 x 10^9/l) was observed in the venous blood. One possible reason is infection induce or accelerate the process of PVT.

Conclusion

Paul M. Arnow once reported that the risk factors during liver transplantation include (i) Age >20 years, (ii) Biliary atresia (iii) Lower pre- operative albumin concentration (iv) Gastrointestinal or vascular complications, (v) Hemodialysis and (vi) Longer LSI (Arnow, 1991). In the present study, only biliary atresia (P = 0.022), DPAA (P = 0.009) and DPOAA (P = 0.026) were found to be potential risk factors (Table 4). The results suggest that there may be some differences in the postoperative risk factors between adults and children. To avoid long term DPAA and long term DPOAA induced early stage severe infection, nosocomial infection control and drug - resistant bacteria monitoring are worthy to be concerned after LDLT in children.

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