

Perioperative synbiotic treatment to prevent infectious complications in patients after elective living donor liver transplantation. -A prospective randomized study-

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**KEYWORDS:** Synbiotic therapy; Living donor liver transplantation; Infectious complication

**Abstract**

**BACKGROUND:** Although the effect of synbiotic therapy using prebiotics and probiotics has been reported in hepatobiliary surgery, there are no reports of the effect on elective living donor liver transplantation (LDLT).

**METHODS:** Fifty adult patients undergoing LDLT between September 2005 and June 2009 were randomized into a group receiving 2 days preoperative and 2 weeks postoperative synbiotics therapy (*Bifidobacterium breve*, *Lactobacillus casei* and galactooligosaccharides: BLO group) and a group without symbiotic therapy (Control group). Postoperative infectious complications were recorded as well as fecal microflora before and after LDLT in each group.

**RESULTS:** Only one systemic infection occurred in the BLO group (4%), while the Control group showed 6 infectious complications with 3 sepsis and 3 urinary tract infections with *Enterococcus* species (24%,  $p=0.033$  vs. BLO group). No other type of complication showed any difference between the groups.

**CONCLUSIONS:** Infectious complications after elective LDLT significantly decreased by perioperative administration of synbiotic therapy.

The bowel has bacterial flora, in which a hundred billion of bacteria are present with a weight of one kg.<sup>1</sup> Bacterial translocation can occur if the intact environment is compromised, thus leading to the provocation of several cytokine networks and multiple organ failure in the end.<sup>2-5</sup> Especially, liver transplant recipients usually have a long history of liver disease and suffer portal hypertension, which leads to malnutrition.<sup>6</sup> Therefore, the mucosa of their bowel could be atrophic and more susceptible to bacterial translocation, which leads to endotoxemia and multi-organ failure.<sup>7-9</sup>

Synbiotic therapy is the medical term for comprehensive prebiotic therapy combined with probiotic therapy.<sup>10</sup> It has been used for amelioration of stool character, suppression of toxic substance and immunomodulation for various infectious diseases and is reported to provide good therapeutic efficacy.<sup>9-11</sup> Probiotics are bacteria which can provide beneficial effect through maintaining the balance of resident bacteria in the bowel such as bifidus bacterium and lactobacterium etc.<sup>12,13</sup> Generally, probiotics increase the intestinal motility, and stabilize the intestinal barrier for bacterial location.<sup>14,15</sup> Furthermore, probiotics, which are living bacteria, can protect

the innate immune system with cytokine modulation. On the other hand, prebiotics are an ingredient made from food and delivered to the large bowel, which can stimulate the proliferation of beneficial bacteria such as bifidus bacterium. Prebiotics can reach the colon without any transformation and serve as nutrition for probiotics.<sup>2</sup> Synbiotics therapy (administration of prebiotics plus probiotics) reduces the rate of infection after a pylorus preserving pancreaticoduodenectomy,<sup>16</sup> major hepatectomy for bile duct cancer<sup>17</sup>, whole liver deceased donor liver transplantation<sup>18,19</sup>, acute pancreatitis<sup>20</sup>. However, no reports have indicated whether infectious complication can be reduced by synbiotics therapy after living donor liver transplantation (LDLT). Since LDLT is always partial liver transplantation, postoperative portal hypertension is higher in LDLT as compared to whole liver transplantation<sup>21</sup>.

Therefore, this study was a prospective randomized control study in order to determine if synbiotics therapy during the perioperative period is effective to reduce infectious complications for a recipient undergoing LDLT.

## **Patients and Methods**

This prospective study was approved by the local Institutional Review Board in Nagasaki University Hospital, and written informed consent was obtained from all patients.

**Patients.** Fifty liver transplant recipients at Nagasaki University Hospital, Japan treated between June 2005 and June 2009 were enrolled in this study. The  $\alpha$ -error was set at 5% with a power of 80%. According to the previous report, infectious complications occur in 40% of liver transplant recipients and it could be reduced by symbiotic therapy to 10%.<sup>18,19</sup> Therefore, the calculated sample size was 25 patients for each group.

Patients were randomly assigned to the group that received synbiotics therapy (n=25) or did not (n=25). The characteristics of the patients are shown in Table 1. Primary endpoint of this study is to investigate if synbiotic therapy can reduce infectious complications after LDLT.

**Liver transplant methods** All partial liver grafts were preserved in

University of Wisconsin solution and implanted using a piggy-back technique as previously described<sup>22</sup>. Surgeons experienced in microscopic surgery anastomosed all of the hepatic arteries with the aid of a surgical microscope. Graft selection was based on the results of a volumetric study using computed tomography (CT) to obtain a ratio of graft volume to standard liver volume of more than 35% in the recipients. All patients received intravenous prophylaxis with amoxicillin and cefotiam for 4 days as a standard protocol. Empiric therapy was initiated in the event of infection and subsequently antibiotics were narrowed based on the resistance index.

A dual or triple immunosuppressive regimen was used, which included Tacrolimus or Cyclosporine A, prednisolone, and/or mycophenolate mofetil. Patients with a compromised renal function were administered induction therapy with IL-2 antibodies. Only biopsy-proven rejections were treated if clinical and laboratory signs mandated steroid bolus treatment. Rituximab (anti-CD20 antibody) was used preoperatively for immunosuppression in ABO-incompatible cases.

The variables of age, gender, primary liver disease, ABO incompatibility, median graft volume versus standard liver volume, Model

for End-Stage Liver Disease (MELD) score at time of LDLT as well as concomitant hepatocellular carcinoma were compared between the group receiving synbiotics and the controls.

Subsequently, at 24 hours after LDLT, all patients underwent enteral nutrition with ELENTAL® (Ajinomoto pharmaceutical. Ltd., Tokyo, Japan), which is elemental diet, through a tube jejunostomy made during liver transplantation. The initial infusion rate with 1 kcal/ml was 20 ml/h and if tolerated it was increased 60 ml/h until sufficient oral intake was possible. The composition of ELENTAL was described previously elsewhere.<sup>23</sup>

### **Synbiotic therapy**

All patients had started oral administration of Yakult BL antifatulent (Yakult Honsha, Tokyo, Japan) containing 20 mg of living *Lactobacillus casei* strain Shirota; 15 mg of living *Bifidobacterium breve* strain Yakult; and galactooligosaccharides (Oligomate 55, Yakult Honsha; 15 g/day) 3 times per day from 2 days prior to elective LDLT, and had continued for 2 weeks after LDLT via either a tube jejunostomy or per os. Usually, both prebiotics and probiotics were taken with 10 ml of tap water. We selected this formula of

synbiotic based on previous report on major hepatectomy.<sup>16</sup>

The rate of infectious complications and patient survival were recorded, while stool cultures were also performed.

## **Statistics**

All data are expressed as the median values with ranges. The statistical analysis was performed using the Mann-Whitney u-test for continuous values and the chi-square test for categorical values. Statistical difference was defined as a p-value of less than 0.05. The StatView 5.0 statistical software package (Abacus Concepts, Berkeley, CA, USA) was used for all statistical analyses.

## **Results**

All patients tolerated synbiotic therapy throughout the study period. There was no difference in the patient characteristics between the groups (Table 1). Figure 1 shows the result of cultured bacteria in the feces. Generally, *Escherichia* species, *Enterobacter* species and *Klebsiella* species were regarded as normal bacterial flora in the stool. There was no significant



pattern of the change of bacterial species between the groups. However after LDLT under immunosuppression, Enterococcus species became evident in both groups in around 25% of the patients.

Table 2 that infectious complication occurred after LDLT in 6 of 25 (24%) of the patients in the control group, while in one of 25 (4%) in the BLO group ( $p < 0.05$ ). In particular, the rate of urinary infection was higher without synbiotic therapy. The rate of intraabdominal infection was not statistically different. Enterococcus species and MRSA were main bacteria related to the infection. The postoperative date of infection varied. Some infectious complication occurred after termination of synbiotics therapy.

Table 3 shows there was no significant difference between the groups in other complications after LDLT. In addition, there was no difference in the ICU period, hospitalized period and mortality rate between the groups.

## Discussion

This prospective randomized study demonstrated that synbiotics therapy successfully reduced the rate of infectious complications after LDLT, which has a greater chance to induce temporary portal hypertension leading to bacterial translocation. The portal venous pressure after LDLT should have been elevated in the current series of patients because the graft volume versus standard liver volume ratio was around 40%.<sup>21</sup> Therefore, synbiotic therapy may be potentially more effective in patients after LDLT than deceased donor liver transplantation. In addition, LDLT is partial liver transplantation in which liver regeneration should occur to support the patient's life. Infection itself was reported to reduce the magnitude of liver regeneration, thus symbiotic therapy should be used for the patients undergoing LDLT.<sup>14</sup>

The patients in the present study received enteral nutrition, which could reduce the rate of infection from 29% to 14%.<sup>25,26</sup> This is probably why the rate of infection in this study was lower than previous reports with symbiotic therapy. In addition, the rate of acute cellular rejection (ACR) was not changed by symbiotic therapy. The rate of ACR is reduced from 44% to

7% by enteral nutrition after whole liver transplantation.<sup>27</sup> There was no difference in the rejection rate even though there were more ABO blood type incompatible LDLT patients in the symbiotic group than in the control group.

Methicilline resistant staphylococcus aureus (MRSA) and Enterococcus species were the principle bacteria causing sepsis although gram-negative gut derived bacteria is thought to be found in septic patients. Although there was no explanation for the gram-positive bacteria in this series, Enterococcus was frequently observed as the dominant bacteria after LDLT in the feces.<sup>28</sup> Immunosuppression and duration of our antibiotics use might cause Enterococcus sepsis in partial liver transplant recipients. In addition, reduction of urinary tract infections was reported in a previous study, which is consistent with the current data, indicating that symbiotic therapy is likely to be responsible for the reduction of urinary tract infection.<sup>29</sup> Previous authors speculated that in addition to their impact on bacterial translocation, probiotics act via several other mechanisms. For instance, they can reduce and eliminate potentially pathogenic microorganisms, reduce and eliminate various toxins and mutagens from the

urine and feces, modulate innate and adaptive immune defense mechanisms, promote apoptosis and release numerous nutrients, antioxidants and growth factors from consumed fibers. These functions might all be important for reduction of infections in surgical patients. However, definite mechanism regarding the reduction of urinary tract infection should await further investigation <sup>3, 25, 29, 30</sup>).

In conclusion, infectious complications after LDLT were significantly decreased with synbiotics therapy. It is possible to achieve the ecological liver transplantation using symbiotic therapy while maintaining an intact environment in the body.

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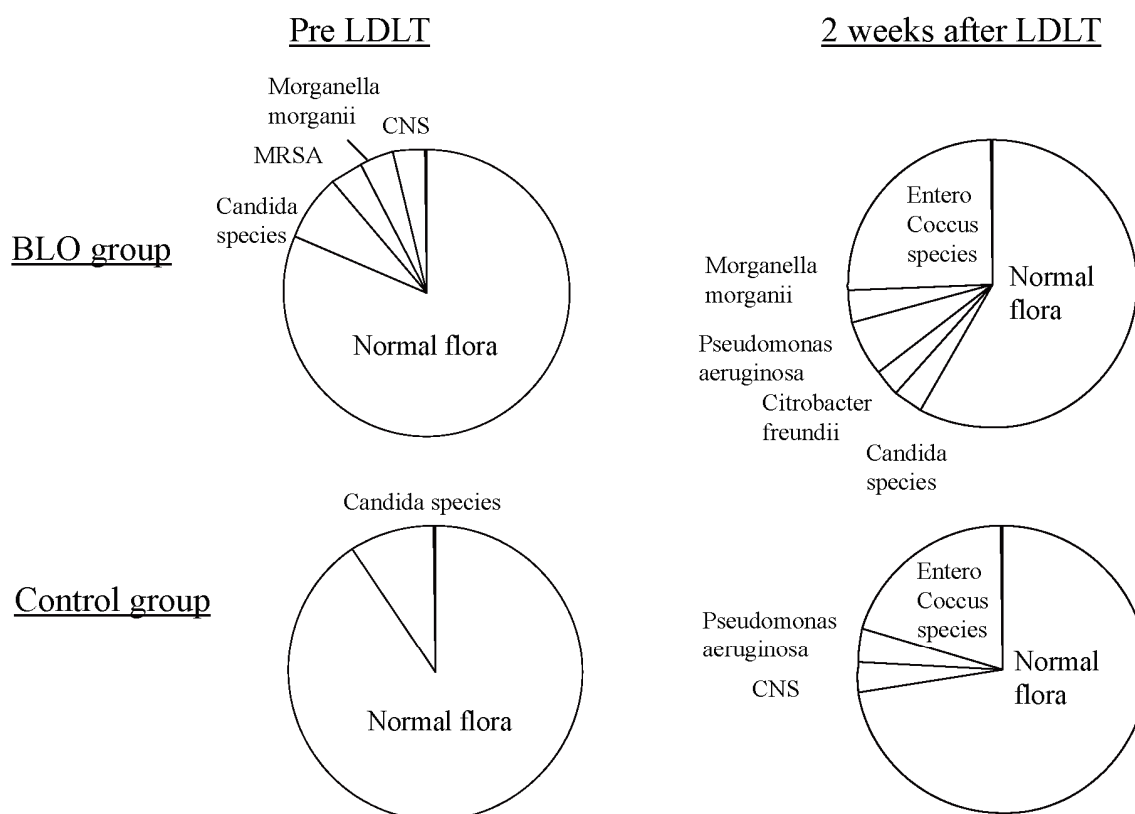


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**FIGURE LEGENDS**

**Figure 1** Bacterial profile in fecal culture



**FIGURE 1** Bacterial profile in fecal culture.

Cultured bacteria in the feces of the patients undergoing living donor liver transplantation in each group.

MRSA: methicilline resistant Staphylococcus aureus

CNS: Coagulase -negative staphylococci

**Table 1** Patient characteristics

	BLO group (n=25)	Control group (n=25)	
Median Age (range)	56 (33-66)	57 (25-68)	N.S.
M : F	13 : 12	16 : 9	N.S.
Primary disease	LC-C (9), LC-B (5) LC-AI(3), LC-AIH (2) PSC(3),PBC(2), LC-unknown(1)	LC-C (13), LC-B (7) Caroli (1), FHF (1) LC-AI (1), PV thrombus (1) PSC (1)	N.S.
ABO incompatibility	9 (36%)	4 (16%)	N.S.
Median GV/SLV (range)	39% (24.8-61)	41.5 % (23.6-57)	N.S.
Median MELD (range)	15 (2-34)	16 (4-41)	N.S.
Concomitant HCC	12	12	N.S.

LC-C:liver cirrhosis due to hepatitis C virus, LC-B: LC-C:liver cirrhosis due to hepatitis B virus,  
 LC-AI:liver cirrhosis due to alcohol intoxication, LC-AIH:liver cirrhosis due to autoimmune hepatitis,  
 PSC:primari sclerosing cholangitis, PBC:primary biliary cirrhosis, FHF:fulminant hepatic failure  
 LC-unknown:liver cirrhosis due to unknown origin, PV:portal vein, N.S.:not significant, HCC:hepatocellular carcinoma  
 GV/SLV:graft volume versus standard liver volume ratio, MELD:model for end-stage liver disease

**Table 2** Infectious complications after LDLT

	BLO group (n=25)	Control group (n=25)	
Type of infection	1 catheter infection (POD 19)	3 sepsis (POD 11,10,9) 3 urinary tract infection (POD 7, 8, 5)	p<0.05
Bacteria cultured in blood	1 Enterobacter asburiae (POD 19)	2 MRSA(POD 10, 9), 1 MRSA+candida glabrata (POD 11) 3 Enterococcus faecium (POD7, 8, 5)	
Intraabdominal Infection	1 (4%) Klebsiella oxytoca,+Enterococcus faecium (POD19)	2 (8%) 1 Enterobacter asburiae (POD 19) 1 Enterococcus faecium (POD 14)	N.S.

MRSA: methicilline resistant Staphylococcus aureus

N.S.: not significant

POD:postoperative day

**Table 3** Other complications

	BLO (n=25)	Control (n=25)	
Others	2 ACR	3 ACR	N.S.
	3 CMV	3 CMV	
	1 HAT	1 HAT	
	1 HPS	1 HPS	
	1 TMA	1 NOMI	
	1 adrenal insufficiency		
ICU period (days)	7 (4-35)	7 (2-48)	N.S.
Hospitalized period (days)	40 (16-132)	33 (16-97)	N.S.
Mortality	3	3	N.S.

ACR:acute cellular rejection, CMV:cytomegalovirus, HAT:hepatic arterial thrombus,  
HPS:hemophagocytic syndrome, TMA:thrombolytic microangiopathy,  
NOMI:non-occlusive mesenteric ischemia, N.S.:not significant  
ICU: intensive care unit