Table 1. Correlation between each NLR cut-off and HCC recurrence using the Kaplan–Meier method.

Cut-off value of NLR	1-yr RFS*	3-yr RFS*	5-yr RFS*	Chi-square	*p* value
NLR ≥6 (n = 11)	72.7% *vs.* 92.4%	42.4% *vs.* 87.6%	42.4% *vs.* 85.8%	11.7	0.0006
NLR ≥5 (n = 18)	83.3% *vs.* 92.0%	59.1% *vs.* 87.9%	59.1% *vs.* 86.0%	6.86	0.009
NLR ≥4 (n = 26)	78.8% *vs.* 93.2%	60.5% *vs.* 89.0%	30.3% *vs.* 89.0%	15.2	<0.0001
NLR ≥3 (n = 52)	81.4% *vs.* 95.7%	72.6% *vs.* 90.4%	66.0% *vs.* 90.4%	9.98	0.002
NLR ≥2 (n = 83)	84.4% *vs.* 98.5%	76.1% *vs.* 94.4%	72.5% *vs.* 94.4%	9.77	0.002
NLR ≥1 (n = 131)	90.5% *vs.* 95.2%	83.5% *vs.* 90.2%	81.4% *vs.* 90.2%	0.55	0.456

*RFS in the high *vs.* the low NLR group.
HCC, hepatocellular carcinoma; NLR, neutrophil–lymphocyte ratio; RFS, recurrence free survival.

Table 2. Demographic and clinical characteristics of patients in the low NLR and high NLR groups.

	NLR <4 (n = 132)	NLR ≥4 (n = 26)	*p* value
Patient background			
Recipient's age (yr), mean (min.-max.)	58 (21-68)	54 (40-73)	0.06
Recipient's sex (male/female), n	77/55	15/11	0.95
Recipient's BMI (kg/m^2), mean ± SD	23.8 ± 0.3	24.0 ± 0.7	0.89
Etiology (HBV/HCV/NBNC), n	21/92/19	4/22/0	0.11
MELD score, mean ± SD	11.0 ± 0.4	12.1 ± 1.3	0.50
CRP (mg/dl), mean ± SD	0.50 ± 0.1	1.2 ± 0.2	<0.0001
Pretransplant therapy for HCC (yes/no), n	83/49	18/8	0.53
Operative factor			
Operative time (min), mean ± SD	860 ± 49	795 ± 110	0.84
Intraoperative bleeding (ml), mean ± SD	5060 ± 588	8315 ± 1352	0.21
Graft (LL/RL/PS/Dual), n	79/49/3/1	19/7/0/0	0.10
Immune suppression (FK506/CyA), n	53/79	16/10	0.04
Tumoral factor			
α-fetoprotein (ng/ml), mean (min.-max.)	446 (1.9-26,525)	3289 (1.6-43,107)	0.79
Des-gamma-carboxy prothrombin (mAU/ml), mean (min.-max.)	315 (3-5934)	879 (7-13,691)	0.63
Maximum tumor size (cm), mean ± SD	2.2 ± 0.1	2.4 ± 0.3	0.99
Number of tumors, n	3.8 ± 0.3	3.5 ± 0.7	0.69
Tumor differentiation (well/moderate/poor), n	14/83/35	4/13/9	0.47
Vascular invasion (yes/no), n	47/85	12/16	0.31

BMI, body mass index; CRP, C reactive protein; CyA, cyclosporine; FK506, tacrolimus; HBV, hepatitis B virus; HCV, hepatitis C virus; LL, left lobe; MELD, model for end-stage liver disease; NBNC, non-HBV, and non-HCV; PS, posterior segment; RL, right lobe.

immunosuppressive agents. Of the patients in the low-NLR group, 53 received FK506 and 79 received cyclosporine A; of the patients in the high-NLR group, 16 received FK506 and 10 received cyclosporine A ($p = 0.04$). We also observed a significant difference in the low and high NLR groups in pretransplant C-reactive protein (CRP) concentration (0.50 mg/dl *vs.* 1.2 mg/dl, $p < 0.0001$). NLR did not correlate with any tumor factor, including serum tumor markers, tumor number, size, or microvascular invasion.

When we compared survival outcomes in the two groups, we found that the 1-, 3-, and 5-year OS rates were significantly lower in the high (80.1%, 66.6%, and 57.1%, respectively) than in the low (95.9%, 88.4%, and 84.1%, respectively) NLR group ($p = 0.002$, Fig. 1A). We also found that NLR ⩾4 significantly correlated with HCC recurrence following LDLT, with 1-, 3-, and 5-year RFS rates being significantly lower in the high (78.8%, 60.5%, and 30.3%, respectively) than in the low (93.2%, 89.0%, and 89.0%, respectively) NLR group ($p < 0.0001$; Fig. 1B). Interestingly, all 12 patients in the high-NLR group who experienced recurrences did so within 3 years of LDLT, suggesting that NLR may be a marker of early HCC recurrence after LDLT.

To date, the MC has been the gold standard for selecting HCC patients as candidates for LT. To confirm whether NLR predicts the outcome of LDLT for HCC patients independent of MC, a multivariate analysis was performed. As shown in Table 3, NLR ⩾4 was an independent factor affecting HCC recurrence after LDLT, with a HR of 6.24 ($p = 0.0002$). In addition, we performed multivariate analysis including all tumor factors shown in Table 2, which still showed NLR ⩾4 was significant factors associated HCC recurrence after LDLT (Supplementary Table 1).

We also compared survival outcomes in patients with high and low NLR who did or did not meet the MC. Of the 94 recipients who met the MC, 15 had high NLR, with 1-, 3-, and 5-year RFS rates of 100%, 73.9%, and 73.9%, respectively; in contrast, none of the 79 recipients with low NLR showed HCC recurrence ($p = 0.0008$, Fig. 1C). Similarly, among the 64 recipients who did

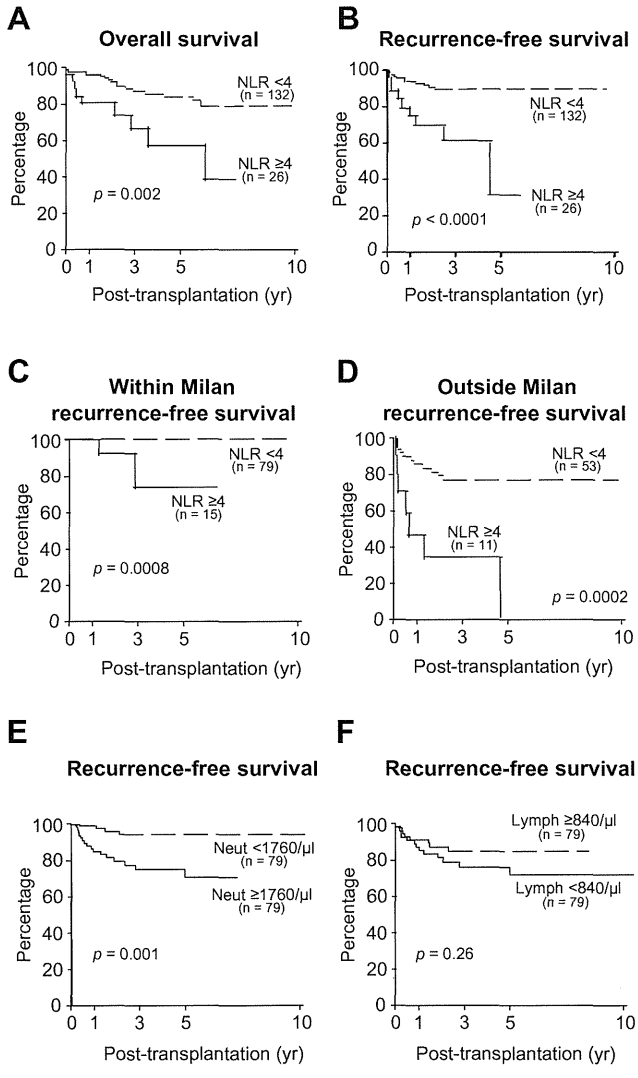

Fig. 1. Survival outcomes in patients with high (⩾4) and low (<4) NLR. (A) OS rates and (B) RFS rates in the high- and low-NLR groups. (C and D) RFS rates in patients with high and low NLR who did (C) and did not (D) meet the MC. (E and F) RFS rates in patients above and below the (E) median neutrophil count (1760/μl) and the (F) median lymphocyte count (840/μl).

Table 3. Multivariate analysis of factors affecting HCC recurrence after liver transplantation.

Variables	HR	95% CI	*p* value
Milan Criteria	15.9	4.58-100	<0.0001
NLR ≥4	6.24	2.52-15.0	0.0002

HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; NLR, neutrophil–lymphocyte ratio.

not meet the MC, 11 with high NLR showed poorer survival outcomes than 53 with low NLR (Fig. 1D). The 1-, 3-, and 5-year RFS rates were 84.6%, 76.1%, and 76.1%, respectively, in the low-NLR group, and 46.7%, 35%, and 0%, respectively, in the high-NLR group ($p = 0.0008$). These results suggest that LT recipients with high NLR should be monitored carefully for HCC recurrence, even if they meet the MC, and that recipients with low NLR outside the MC may be feasible candidates for LDLT.

Correlation between neutrophil rather than lymphocyte counts and HCC recurrence

We also compared survival outcomes relative to neutrophil and lymphocyte counts. The 158 LT recipients were divided into two groups according to the median neutrophil count, those with high (⩾1760/μl, n = 79) and low (<1760/μl, n = 79) neutrophil groups. The 1-, 3-, and 5-year RFS rates were 97.1%, 93.6%, and 93.6%, respectively, in the low-neutrophil group, and 84.3%, 74.9%, and 70.5%, respectively, in the high-neutrophil group ($p = 0.001$, Fig. 1E). In contrast, when we divided the 158 recipients according to the median lymphocyte count, we found that the 1-, 3-, and 5-year RFS rates were 92.9%, 87.6%, and 87.6%, respectively, in the high-lymphocyte group (⩾840/μl, n = 79), and 88.8%, 81.2%, and 77.7%, respectively, in the low-lymphocyte group (<840/μl, n = 79) ($p = 0.26$, Fig. 1F). We also observed that serum CRP concentration was higher in patients with NLR ⩾4 than with NLR <4 (Table 2). This finding suggested that the association between NLR and HCC recurrence was due to inflammatory cytokines rather than depletion of lymphocytes.

Although CRP concentration was associated with NLR, CRP concentration itself did not statistically affect RFS (Supplementary Fig. 2A and B). When we divided patients into two groups relative to various cut-offs for neutrophil and lymphocyte counts, we found that a neutrophil count ⩾2000/μl was negatively correlated with HCC recurrence after LDLT (Supplementary Fig. 2C), whereas a neutrophil count ⩾3000/μl was not correlated with RFS (Supplementary Fig. 2D). Lymphocyte counts of <600/μl (Supplementary Fig. 2E) and <500/μl (Supplementary Fig. 2F) did not correlate with RFS.

VEGF and IL-8 expression did not correlate with NLR

To determine whether elevated neutrophil levels were a primary source of VEGF and IL-8, the major angiogenesis or tumor growth factors, we measured the intra and peritumoral levels of *VEGF* and *IL-8* mRNAs. We found that neither intra nor peritumoral expression of *VEGF* and *IL-8* mRNA was correlated with NLR (Supplementary Fig. 4A and B). Moreover, ELISA assays of serum VEGF and IL-8 showed that they did not correlate with NLR (Supplementary Fig. 4C) either. Taken together, these findings indicate that none of these angiogenesis and tumor growth factors were involved in the mechanism by which NLR correlated with HCC recurrence after LDLT.

IL-17 expression significantly correlated with NLR

To determine whether IL-17 is involved in the relationship between NLR and HCC recurrence, we measured the expression of this cytokine. Since IL-17 is produced by some helper T cells (Th17 cells), not by hepatocytes, little *IL-17* mRNA is present in RNA extracted from liver tissue (data not shown). We therefore used immunohistochemical staining to investigate the intra and peritumoral expression of IL-17. Most IL-17-producing cells were present in the peritumoral region (Fig. 2A). Although intratumoral IL-17 expression did not differ between the high- and low-NLR groups ($p = 0.32$), peritumoral IL17 expression was significantly higher in the high-NLR group ($p = 0.03$, Fig. 2B). Furthermore,

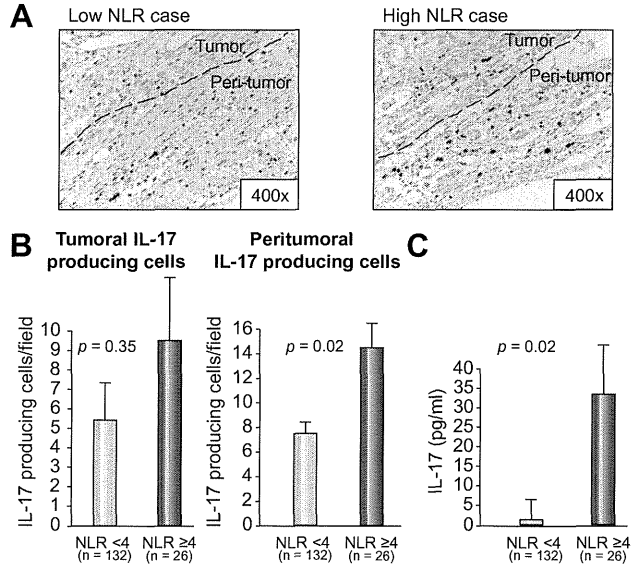

Fig. 2. Hepatic and systemic IL-17 production relative to NLR. (A) Immunohistochemical staining for IL-17-producing cells in paraffin-embedded blocks of liver tissue samples. The left panel shows a sample with low NLR and the right panel shows a sample with high NLR. In both groups, there were more IL-17-producing cells in peritumoral than intratumoral regions. (B) Count of IL-17-producing cells according to NLR in intratumoral and peritumoral regions. (C) IL-17 concentration in sera collected at the time of transplantation from patients with high and low NLR. (This figure appears in color on the web.)

serum IL-17 concentration was significantly higher in the high- than in the low-NLR group (1.3 *vs.* 33.6 pg/ml, $p = 0.02$, Fig. 2C). These findings indicated that the proinflammatory cytokine IL-17 was significantly associated with NLR.

Next, the expression of IL-17 was compared between patients who had received pre-transplant treatment for HCC and those who had not. The tumoral, peritumoral, or serum IL-17 expression was not different between the two groups (data not shown).

Tumor-associated activation of macrophages is upregulated in the high-NLR group

We also investigated the correlation of NLR with CD163-positive tumor associated macrophages (TAMs). The density of CD68, a marker ubiquitously expressed on macrophages, was not associated with NLR, either in or around the tumors (Fig. 3A). However, the number of CD163-positive TAMs around, but not within, the tumor was significantly higher in the high-NLR group (Fig. 3B). Moreover, the density of TAMs correlated significantly with that of IL-17-producing cells ($R^2 = 0.17$, $p = 0.04$, Fig. 3C). The expression of TAMs was not associated with whether patients had received pretransplant therapies or not (data not shown).

TAMs have recently been found to originate from splenic monocytes [17]. Although the RFS outcomes were similar in recipients who had ($n = 94$) and had not ($n = 64$) undergone splenectomy, the 1-, 3-, and 5-year RFS rates in the 19 patients with high NLR who had undergone splenectomy (88.5%, 68.1%, and 33.3%, respectively) were significantly higher than in the 7 patients with high NLR who had not undergone splenectomy (68.1%, 50.3%, and 16.7%, respectively; $p = 0.02$, Fig. 4). In the low-NLR group, there was no difference in HCC recurrence between the 75 patients who had undergone splenectomy and the 57 who had not ($p = 0.63$, Supplementary Fig. 4).

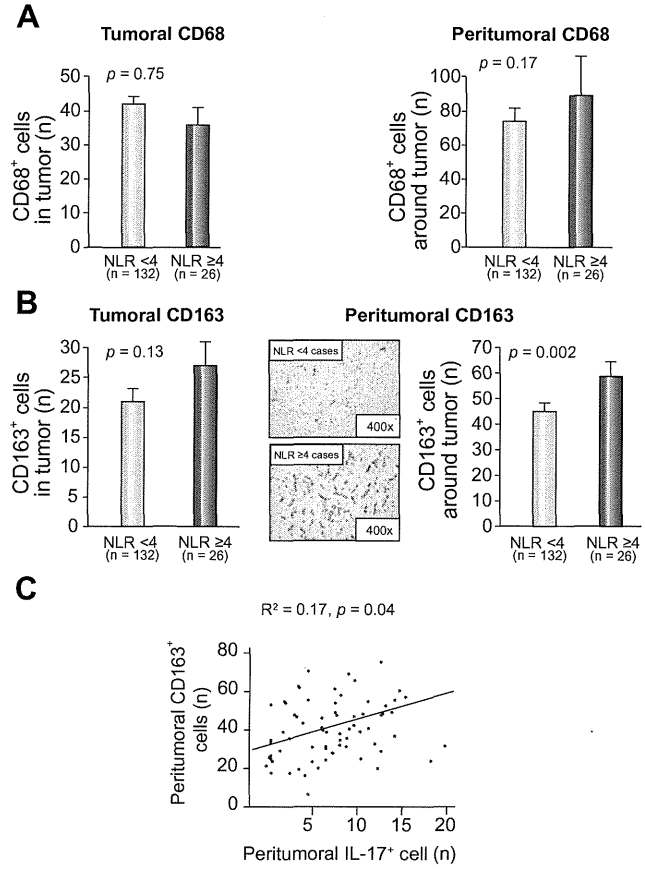

Fig. 3. Immunohistochemical assays for CD68- and CD163-positive macrophages. (A) Cells positive for CD68, a ubiquitously expressed macrophage marker, were counted in the tumor and peritumoral region. (B) Cells positive for CD163 (TAM marker) were counted in the tumor. CD163 immunohistochemical staining of the peritumoral region of samples with low and high NLR. Staining was greater in the high-NLR group ($p = 0.005$,). (C) Relationship between the density of CD163-positive cells and IL-17-producing cells in the peritumoral region ($R^2 = 0.17$, $p = 0.04$). (This figure appears in color on the web.)

Discussion

Many studies to date have shown that higher NLR is correlated with adverse survival outcomes in patients with various solid tumors [8–12,18]. Despite the total replacement of the liver, HCC recurrence following DDLT was correlated with pretransplant NLR [12,18]. To expand these findings, we assessed whether pretransplant NLR was correlated with HCC recurrence after LDLT. We found that NLR $\geqslant 4$ showed the greatest correlation with recurrence; in contrast, other studies have used NLR $\geqslant 5$ as the cut-off value [8–12,18]. Moreover, to our knowledge, this study is the first to describe the molecular mechanism involved in the relationship between NLR and cancer recurrence.

Previous studies [8–12] have shown that high NLR reflects relatively depleted lymphocytes, impairing the host immune response to malignancy. Elevated neutrophils were regarded as a reservoir of VEGF. In contrast, we found that the lymphocyte number was not associated with survival outcomes, whereas the neutrophil count was. Furthermore, the expression of tumoral, peritumoral, and circulating VEGF did not show any correlation with NLR. We also found that expression of IL-8,

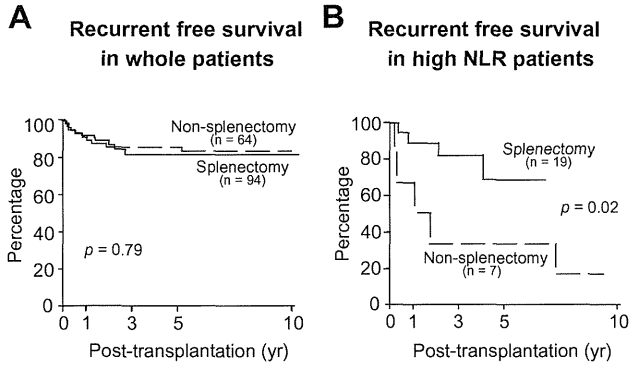

Fig. 4. Relationships between RFS and splenectomy. (A) RFS rates in patients who had and had not undergone splenectomy. (B) RFS rates in patients with high NLR who had and had not undergone splenectomy.

another angiogenesis and tumor growth factor that can promote neutrophil recruitment [19], was not associated with HCC recurrence after LDLT. In contrast, we found that serum CRP concentration was positively correlated with NLR. Taken together, these results indicate that elevated NLR promotes HCC recurrence via some sort of inflammatory microenvironment, not via angiogenesis alone.

IL-17 is a proinflammatory cytokine that promotes HCC growth [20,21]. In addition, IL-17 is an initiator of neutrophil recruitment by CXC chemokines, such as CCL2 released from IL-17-producing T cells [15,21,22]. We observed a correlation between elevated NLR and upregulation of IL-17 production in both peritumoral regions of the liver and peripheral blood. IL-17 may therefore be a key molecule involved in the relationship between NLR and HCC recurrence.

TAMs have been reported to be a major component of the tumor inflammatory microenvironment and to promote proliferation and tumor angiogenesis [16]. Monocytes are recruited from the circulation into local tissue or malignant sites, where they are recognized by CD68-expressing residential macrophages. In response to inflammatory cytokines released by tumors, some of these residential macrophages differentiate into CD163-expressing TAMs. In contrast to CD68-positive macrophages, CD163-positive TAMs are suppressors of the antitumor immune response. Furthermore, IL-17-producing cells have been found to interact with TAMs in HCC patients [20,23]. We observed a correlation between IL-17-producing cells and the density of CD163, confirming their collaboration. Interestingly, both IL-17 producing cells and CD163 positive TAMs produce the same family of CXC chemokines that promote the recruitment of monocytes and neutrophils [21,24,25]. Moreover, both cell types promote tumor migration mediated by matrix metalloproteinase [26,27] and downregulate the antitumor immune response resulting from the expansion of FoxP3-positive regulatory T cells [25,26] or programmed death-1-positive T cells [28,29].

In summary, IL-17-producing T cells are thought to release CXC chemokines that recruit neutrophils, leading to elevated NLR, and promote the differentiation of tissue macrophages in peritumoral regions into TAMs. Both IL-17-producing cells and TAMs accelerate tumor progression and antitumor T cell exhaustion. Our findings and other studies [12,18] demonstrate the association between elevated NLR and HCC recurrence in LT recipients, from whom tissue macrophages and IL-17-producing T cells in the liver have been completely removed. However, elevated preoperative serum IL-17 has also been found to promote tumor recurrence [30]. Circulating IL-17 may recruit TAMs into sites of tumor recurrence even after LT. Recurrent HCC following LT may be an indication for resection, but it remains unclear whether TAMs are involved at recurrent sites, suggesting the need for additional investigation using animal models. Monocytes that differentiate into TAMs have been recently reported to originate from the spleen [17]. We found that RFS rates were significantly lower in LT recipients with high NLR who had not undergone splenectomy than those who had, suggesting the continuous feeding of splenic TAMs with high IL-17 concentrations following LT. Although investigations involving larger numbers of patients are required, our findings suggest that splenectomy may be a useful strategy for preventing tumor recurrence after LT in HCC patients with high NLR.

In conclusion, we found that elevated NLR was significantly correlated with HCC recurrence after LDLT via an inflammatory tumor microenvironment provided by TAMs and IL-17-producing cells.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2012.08.017.

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ORIGINAL ARTICLE

Etiologies, Risk Factors, and Outcomes of Bacterial Pneumonia After Living Donor Liver Transplantation

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The prevalence and clinical characteristics of bacterial pneumonia after living donor liver transplantation (LDLT) have not yet been elucidated. We performed a retrospective analysis of 346 LDLT recipients. Fifty patients (14.5%) experienced bacterial pneumonia after LDLT, and they had a higher short-term mortality rate (42.0%) than patients with other types of bacterial infections after LDLT. Gram-negative bacteria accounted for 84.0% of the causative pathogens. A multivariate analysis showed that preoperative diabetes ($P < 0.01$), United Network for Organ Sharing status 1 or 2A ($P < 0.01$), and an operative blood loss > 10 L ($P = 0.03$) were significant risk factors for bacterial pneumonia after LDLT. Post-LDLT pneumonia was associated with the following post-LDLT events: the prolonged use of mechanical ventilation (≥3 days), a prolonged stay in the intensive care unit (≥7 days), the creation of a tracheostomy, primary graft dysfunction, the use of mycophenolate mofetil, and the need for renal replacement therapy. Among patients with bacterial pneumonia, the mortality rate was higher for patients with delayed-onset pneumonia, which occurred at least 10 days after transplantation (n = 15), and it was significantly associated with graft dysfunction. A combination of broad-spectrum antibiotics and aminoglycosides provided cover for most gram-negative bacteria except *Stenotrophomonas maltophilia*, which was associated with a longer period of mechanical ventilation and was resistant to commonly used broad-spectrum antibiotics. Delayed-onset bacterial pneumonia is a serious type of bacterial infection after LDLT and is frequently associated with graft dysfunction. The multidrug resistance of *S. maltophilia* is an issue that needs to be addressed. ***Liver Transpl 18:1060-1068, 2012.*** © **2012 AASLD.**

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Bacterial pneumonia is a major cause of severe hospital-acquired infections, and the severity of bacterial pneumonia is largely determined by the patient's underlying condition.[1] Liver transplant recipients are at particularly high risk because of immunosuppression, massive blood loss and transfusions during surgery, systemic edema with fluid accumulation, and the prolonged period of mechanical ventilation.[2-9] As a result, bacterial pneumonia is a major cause of morbidity and mortality for liver transplant recipients.[3-8]

The types of causative bacteria and their propensity for hospital-acquired pneumonia have been studied in general intensive care unit patients and in patients

Abbreviations: ABPC, ampicillin; CAZ, cefazolin; CFPM, cefepime; DDLT, deceased donor liver transplantation; GM, gentamicin; GRWR, graft-to-recipient weight ratio; GV, graft volume; LDLT, living donor liver transplantation; LVFX, levofloxacin; MELD, Model for End-Stage Liver Disease; MEPM, meropenem; PIPC, piperacillin; PVF, portal vein flow; PVP, portal vein pressure; SBT, sulbactam; SLV, standard liver volume; TAZ, tazobactam; UNOS, United Network for Organ Sharing.

Toru Ikegami contributed to the conception, design, and drafting of the article. Ken Shirabe contributed to revisions of the article for important intellectual content. Rumi Matono contributed to the study design, data collection, and analysis. Tomoharu Yoshizumi contributed to the study design and data analysis. Yuji Soejima contributed to the data collection and analysis and figure development. Hideaki Uchiyama, Hiroto Kayashima, and Kazutoyo Morita contributed to the data collection. Yoshihiko Maehara gave final approval for the article.

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undergoing deceased donor liver transplantation (DDLT).[3-8] To date, however, very few studies have examined the etiologies, risk factors, and outcomes of bacterial pneumonia in patients undergoing living donor liver transplantation (LDLT).[5] The main difference between LDLT and DDLT is that LDLT involves a smaller graft, which is also the main disadvantage of LDLT because it results in increased portal vein pressure (PVP), intestinal edema and bacterial translocation, prolonged ascites, and hyperbilirubinemia.[10] All these events might increase the frequency and severity of bacterial pneumonia after LDLT. Because bacterial pneumonia is a serious condition after organ transplantation (including LDLT), the details should be elucidated.

Therefore, the aim of this study was to examine the etiologies, risk factors, and treatment outcomes of bacterial pneumonia after LDLT.

PATIENTS AND METHODS

Patients

Patients who underwent LDLT (n = 346) at Kyushu University Hospital between May 1997 and July 2011 were included in the current study. The graft types included left lobe grafts (n = 218), right lobe grafts (n = 123), and posterior segment grafts (n = 5). All LDLT procedures were performed after full informed consent was obtained from the patients; this study was approved by the liver transplantation committee and the institutional review board of Kyushu University in compliance with the Declaration of Helsinki. Medical information recorded in the LDLT database of our institute and microorganism records were reviewed for possible bacterial pneumonia, and medical charts were reviewed so that the details could be identified. Early graft loss was defined as graft mortality within the first 6 months after LDLT. The mean observation period was 4.4 ± 3.5 years.

Surgical Procedures and Preoperative and Postoperative Care

Recipients were usually admitted to the hospital a few days before LDLT was scheduled. However, patients whose general condition was deteriorating and who needed intensive medical treatment remained hospitalized and underwent LDLT. Deteriorating conditions were categorized as United Network for Organ Sharing (UNOS) status 1 or 2A. Status 1 indicated fulminant hepatic failure, primary graft nonfunction, or hepatic artery thrombosis within the first 7 days after transplantation. Status 2A indicated hospitalization for chronic liver failure with a Child-Pugh score > 10 and one of the following conditions: active variceal hemorrhaging, hepatorenal syndrome, refractory ascites, refractory pleural effusion, or hepatic encephalopathy unresponsive to medical therapy.

The LDLT surgical procedures are described in detail elsewhere.[11] The major differences between our procedures and those of other centers include a wide indication for splenectomy and the division of major shunt vessels (>10 mm). Splenectomy was indicated for hypersplenism, a PVP after reperfusion > 20 mm Hg, and a hepatitis C–positive status.[12] A pneumococcal vaccine (Pneumovax NP, Merck Sharp and Dohme Co., Inc., Whitehouse Station, NJ) was routinely administered at least 2 weeks before LDLT in order to prevent overwhelming postsplenectomy sepsis.

After LDLT, patients were transferred to the intensive care unit and were set under mechanical ventilation. If the ratio of the partial pressure of arterial O_2 to the fraction of inspired O_2 was >300 to 350, extubation was indicated within the first 24 hours after LDLT under stable cardiovascular, graft, and renal conditions.

Renal replacement therapy in the form of continuous venovenous hemodiafiltration was indicated for oliguria or anuria patients with medically refractory pulmonary edema, metabolic acidosis, hyperkalemia, and uremic encephalopathy (Hemofeel CH polymethyl methacrylate membrane hemofilter, Toray Medical Co., Ltd., Tokyo, Japan). The operating conditions were set as follows: a blood flow rate of 80 to 100 mL/minute, a dialysate flow rate of 100 to 600 mL/hour, and a filtration rate of 100 to 600 mL/hour.

Primary graft dysfunction was defined as graft dysfunction without apparent technical, anatomical, immunological, or hepatitis-related issues after LDLT and was characterized by persistent hyperbilirubinemia (total bilirubin > 20 mg/dL) for >7 consecutive days after postoperative day 7.[13] Graft dysfunction that was possibly due to a combination of a smaller graft for the recipient, an older donor, a deterioration of the recipient's condition, and other minor factors was called primary graft dysfunction.

Immunosuppression

The basic immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. The target tacrolimus level was 10 to 14 ng/mL in the first month after LDLT and was decreased to 7 to 10 ng/mL over the next few months. The target cyclosporine level was 150 to 250 ng/mL in the first month after LDLT and was decreased to 100 to 150 ng/mL over the next few months. Mycophenolate mofetil was started at the daily dose of 2 g, was tapered to 1 g daily over 1 to 3 months, and was tapered off at 6 months. One gram of methylprednisolone was given after reperfusion, and the dose was decreased from 200 mg to 20 mg daily over 1 week; patients were then switched to oral prednisolone, which was tapered off at 3 months.

For patients with severe hepatic encephalopathy or renal insufficiency, no calcineurin inhibitor was given for the first 3 days, and mycophenolate mofetil was started at the daily dose of 3 g with regular steroid tapering. A calcineurin inhibitor was started on the fourth day after LDLT, and the dose was increased to the lower target level: 5 to 8 ng/mL for tacrolimus and 80 to 150 ng/mL for cyclosporine. Once patients

had recovered from their neural or renal problems, the daily dose of mycophenolate mofetil was decreased to 2 g, and the calcineurin inhibitor level was pushed up to the regular dose.

For a patient with apparent posttransplant infectious complications (including pneumonia), immunosuppression was adjusted: the tacrolimus level was 4 to 7 ng/mL, the cyclosporine level was 80 to 150 ng/mL, mycophenolate mofetil was discontinued, and prednisolone was tapered to 5 mg.

Bacterial Pneumonia and Sepsis

Pneumonia was defined as the presence of a new or progressive and persistent infiltrate on a chest X-ray, a positive sputum culture, and at least 2 of the following: a body temperature > 38°C or < 36°C; a leukocyte count > $10 \times 10^3/\mu L$ or < $3.5 \times 10^3/\mu L$; new-onset purulent sputum, changes in the characteristics of sputum, or increased respiratory secretions; new-onset or worsening coughing, dyspnea, tachycardia, rales, or bronchial breath sounds; and worsening gas exchange (ie, oxygen desaturation, increased oxygen requirements, or increased mechanical ventilator support), as reported by Beck and Gastmeier.[14] Only cases with a positive culture and the aforementioned clinical picture were diagnosed with pneumonia. A microbiological diagnosis was obtained with a positive culture of bronchial sampling from a plugged telescopic catheter in all patients receiving mechanical ventilation. A tracheal aspiration culture was performed only for spontaneously breathing patients. The first episode of pneumonia in each patient was counted in this study.

Bacterial sepsis was defined as the isolation of bacteria other than common skin contaminants from a single blood culture within 3 months after transplantation along with clinical symptoms, including a high fever, shivering, dyspnea, an altered mental status, tachycardia, and hypotension.[15]

Perioperative Antibacterial Management

Perioperative prophylaxis consisted of intravenous cefotaxime (4 g/day) and ampicillin (ABPC)/sulbactam (SBT; 6 g/day) 4 times per day for 3 days after LDLT, and it was started at the time of the laparotomy. Selective digestive decontamination was not performed. Patients with clinically suspected bacterial pneumonia were empirically treated with broad-spectrum antibiotics in combination with aminoglycosides. Vancomycin was administered to patients who were suspected to have methicillin-resistant *Staphylococcus aureus* on the basis of preoperative screening. The most frequently used empirical antibiotic therapy regimen consisted of meropenem (MEPM) with or without gentamicin (GM) and with or without vancomycin. Within 48 hours of the initiation of microbiological investigations, the antibiotic treatment was adapted according to the results of susceptibility tests.

For the prevention of significant renal toxicity induced by antibiotics, target blood levels were monitored and controlled. One gram of vancomycin was administered on a daily basis, and the trough level was checked at the third dose in order to keep the level between 8 and 12 μg/mL for patients with a serum creatinine level < 1.5 mg/dL. For other patients with renal insufficiency or on dialysis, 1 g was administered, and this was followed by daily random level monitoring. When the level came down to <8 μg/mL, another gram of vancomycin was given. As for GM, 2.5 g/kg was administered on a daily basis, and the trough level was checked at the third dose in order to keep the level at < μg/mL for patients with a serum creatinine level < 1.5 mg/dL. For other patients with renal insufficiency or on dialysis, 2.5 g/kg was administered, and this was followed by daily random level monitoring. When the level came down to <1 μg/mL, another dose was administered. As for MEPM, 0.5 g was administered every 12 hours.

Statistical Analysis

All values are expressed as means and standard deviations. Categorical variables were compared with the χ^2 test, and continuous variables were compared with the Mann-Whitney test. Cumulative survival analyses were performed with the Kaplan-Meier method and the log-rank test. Multivariate analyses were performed with a logistic regression model, and odds ratios and 95% confidence intervals were calculated. P values < 0.05 were considered statistically significant. All statistical analyses were performed with StatView software (Abacus Concepts, Berkeley, CA).

RESULTS

Characteristics of the Recipients, Donors, and Grafts

The mean age of the recipients was 51.5 ± 11.8 years. The mean Model for End-Stage Liver Disease (MELD) score was 17.4 ± 7.1. Approximately half of the patients [n = 169 (48.8%)] were UNOS status 1 or 2A before LDLT. The indications for LDLT included acute liver failure [n = 51 (14.7%)], cholestatic cirrhosis [n = 73 (21.1%)], postnecrotic viral or nonviral cirrhosis [n = 210 (21.1%)], and others [n = 12 (3.5%)]. The majority of the patients were Child class C [n = 181 (61.4%)]. Seventy patients (20.2%) had diabetes.

The mean age of the donors was 35.9 ± 11.2 years. Seventeen grafts (4.9%) were blood type–incompatible. The graft types included left lobe grafts [n = 218 (63.2%)], right lobe grafts [n = 123 (35.6%)], and posterior segment grafts [n = 5 (1.2%)]. The mean graft volume (GV)/standard liver volume (SLV) ratio was 41.8% ± 8.7%, and the mean graft-to-recipient weight ratio (GRWR) was 0.81% ± 0.19%.

Splenectomy was performed in 261 patients (47.1%), and duct-to-duct biliary reconstruction was performed in 181 patients (75.7%). The mean cold and warm ischemia times were 87 ± 55 and 39 ± 10

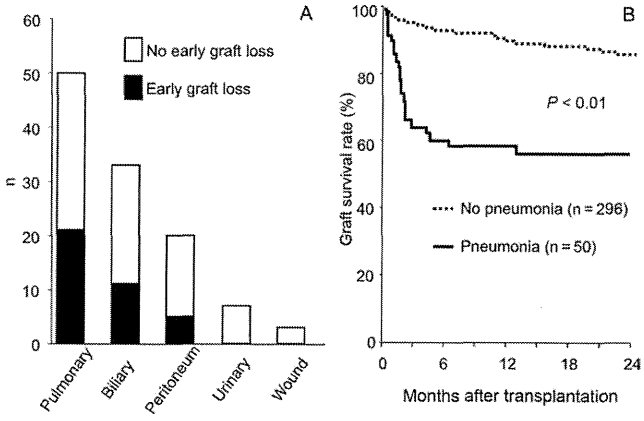

Figure 1. (A) Incidence of pulmonary, biliary, peritoneal, urinary tract, and surgical site bacterial infections and associated early graft loss. (B) Cumulative graft survival rates for patients with bacterial pneumonia and patients without bacterial pneumonia.

minutes, respectively. The mean PVP at the end of the surgery and the mean portal vein flow (PVF)/graft weight (GW) ratio were 17 ± 4 mm Hg and 341 ± 137 mL/100 g, respectively. The mean operative time was 802 ± 178 minutes, and the mean blood loss was 6.1 ± 7.5 L. After LDLT, 54 patients (15.6%) experienced acute cellular rejection, and 92 patients (26.6%) had a cytomegalovirus infection. The 1- and 5-year cumulative graft survival rates were 87.7% and 79.1%, respectively.

Posttransplant Bacterial Pneumonia

Fifty patients (14.5%) experienced bacterial pneumonia within the first 3 months after LDLT, and pneumonia was the leading cause of bacterial infections after LDLT (Fig. 1A). The incidence of early graft loss was 42% (21/50) with pulmonary bacterial infections, 33% (11/33) with biliary bacterial infections, 25% (5/20) with peritoneal bacterial infections, 0% (0/7) with urinary tract bacterial infections, and 0% (0/4) with surgical site bacterial infections. Eighteen patients with bacterial pneumonia (36.0%) experienced sepsis.

All the patients with pneumonia were intubated for a microbiological determination of the diagnosis and treatment. The mean length of mechanical ventilation (5.8 ± 6.0 versus 1.6 ± 2.3 days, $P < 0.01$) and the mean stay in the intensive care unit (4.9 ± 2.9 days versus 8.9 ± 7.3 days, $P < 0.01$) were significantly prolonged in the patients with pneumonia versus the patients without pneumonia. The 6-month and 2-year cumulative graft survival rates were 59.8% and 55.5% for the patients with bacterial pneumonia (n = 50) and 93.4% and 85.9% for the patients without bacterial pneumonia (n = 296, $P < 0.01$; Fig. 1B).

Pathogens of Bacterial Pneumonia

The causative organisms of bacterial pneumonia in the patients included *Pseudomonas aeruginosa* (n = 13), *Stenotrophomonas maltophilia* (n = 10), methicillin-resistant *S. aureus* (n = 7), *Klebsiella pneumonia* (n = 4), *Klebsiella oxytoca* (n = 1), *Acinetobacter baumannii* (n = 9), *Enterobacter cloacae* (n = 4), *Serratia marcescens* (n = 1), and *Enterococcus faecalis* (n = 1). Gram-positive bacteria accounted for 16% (8/50) of the organisms causing sepsis, and gram-negative bacteria accounted for 84% (42/50). *Pseudomonas aeruginosa* and *S. maltophilia* were the most common bacteria.

The length of mechanical ventilation was significantly longer for patients with pneumonia caused by *S. maltophilia* (32.7 ± 19.9 days) versus patients with pneumonia caused by other types of bacteria (6.8 ± 10.6, $P < 0.01$).

Risk Factors for Bacterial Pneumonia After LDLT

A univariate analysis showed that the rates of UNOS status 1 or 2A (68.0% versus 45.8%, $P < 0.01$), diabetes (36.0% versus 17.6%, $P < 0.01$), and an operative blood loss > 10 L ($P < 0.01$) were significantly higher for patients with bacterial pneumonia versus patients without bacterial pneumonia (Table 1). Post-LDLT pneumonia was associated with the following post-LDLT events: the prolonged use of mechanical ventilation (≥3 days; $P < 0.01$), a prolonged stay in the intensive care unit (≥7 days; $P < 0.01$), the creation of a tracheostomy ($P < 0.01$), primary graft dysfunction ($P < 0.01$), the use of mycophenolate mofetil ($P = 0.03$), and the need for renal replacement therapy ($P < 0.01$).

Donor, recipient, graft, and recipient surgical factors were entered into a multivariate risk analysis. The analysis revealed that the presence of diabetes (yes: odds ratio = 2.8, 95% confidence interval = 1.4-5.6, $P < 0.01$), UNOS status 1 or 2A (yes: odds ratio = 2.3, 95% confidence interval = 1.2-5.1, $P < 0.01$), and an operative blood loss > 10 L (yes: odds ratio = 2.3, 95% confidence interval = 1.1-4.8, $P = 0.03$) were significant risk factors for posttransplant bacterial pneumonia (Table 2).

Early Pneumonia and Delayed Pneumonia After LDLT

The time to the onset of bacterial pneumonia is shown in Fig. 2. The incidence of bacterial pneumonia was highest on postoperative day 6, and there was a decline on postoperative days 8 and 9. Therefore, pneumonia occurring within the first 10 days after LDLT was defined as early-onset pneumonia (n = 35), whereas pneumonia occurring at least 10 days after LDLT was defined as delayed-onset pneumonia (n = 15). The incidence of early graft loss after LDLT was significantly higher among patients with delayed-onset pneumonia versus patients with early-onset pneumonia (73.3% versus 25.7%, $P < 0.01$; Fig. 3).

We next compared the clinical characteristics of patients with early-onset pneumonia and patients with delayed-onset pneumonia (Table 3). Overall, we

TABLE 1. Univariate Analysis of the Risk Factors for Bacterial Pneumonia After LDLT

	Posttransplant Pneumonia		
	Yes (n = 50)	No (n = 296)	*P* Value
Recipient factors [n (%)]			
Male sex	23 (46.0)	143 (48.3)	0.75
Age > 60 years	14 (28.0)	73 (24.7)	0.61
Child class C	31 (62.0)	150 (50.7)	0.12
MELD score > 25	6 (12.0)	52 (17.6)	0.33
UNOS status 1 or 2A	34 (68.0)	135 (45.6)	<0.01
Acute liver failure	8 (16.0)	45 (15.2)	0.88
Diabetes	18 (36.0)	52 (17.6)	<0.01
Smoking	11 (22.0)	37 (12.5)	0.88
Ventilator use	10 (20.0)	35 (11.8)	0.11
Era: 2006 or later	24 (48.0)	141 (47.6)	0.98
Donor factors [n (%)]			
Male sex	33 (66.0)	193 (65.2)	0.95
Donor age > 45 years	17 (34.0)	78 (26.4)	0.26
Graft factors [n (%)]			
Left lobe graft	28 (56.0)	190 (64.2)	0.25
GV/SLV < 40%	22 (44.0)	130 (43.9)	0.99
GRWR < 0.8%	28 (56.0)	148 (50.0)	0.43
Recipient surgery [n (%)]			
Splenectomy	25 (50.0)	136 (45.9)	0.66
PVF/GV ratio < 260 mL/100 g	37 (74.0)	206 (69.6)	0.53
PVP > 20 mm Hg at closure	9 (18.0)	59 (19.9)	0.09
Duct to duct	40 (80.0)	221 (74.7)	0.42
Operative time > 15 hours	14 (28.0)	60 (20.3)	0.26
Blood loss > 10 L	14 (28.0)	38 (12.8)	<0.01
Posttransplant course [n (%)]			
Renal replacement therapy	22 (44.0)	34 (11.5)	<0.01
Cytomegalovirus infection	14 (28.0)	78 (26.4)	0.82
Acute rejection	4 (8.0)	50 (16.9)	0.11
Tracheostomy	13 (26.0)	2 (0.6)	<0.01
Mechanical ventilation ≥ 3 days	34 (68.0)	55 (18.6)	<0.01
Intensive care unit ≥ 7 days	31 (62.0)	55 (18.6)	<0.01
Tacrolimus	23 (46.0)	112 (37.8)	0.28
Mycophenolate mofetil	41 (82.0)	198 (66.9)	0.03
Primary graft dysfunction	18 (36.0)	27 (9.1)	<0.01

TABLE 2. Multivariate Analysis of the Preoperative and Intraoperative Risk Factors for Bacterial Pneumonia After LDLT

Variable	Odds Ratio	95% Confidence Interval	*P* Value
Diabetes	2.8	1.4-5.6	<0.01
UNOS status 1 or 2A	2.3	1.2-5.1	<0.01
Operative blood loss > 10 L	2.3	1.1-4.8	0.03

found no differences in the preoperative characteristics of recipients or the characteristics of donors between the 2 groups. However, the warm ischemia time (45 ± 11 versus 36 ± 9.4 minutes, $P = 0.01$), intraoperative blood loss (13.5 ± 12.8 versus 6.6 ± 7.5 L, $P = 0.03$), red blood cell transfusions (40 ± 30 versus 14 ± 9 U, $P < 0.01$), frozen plasma transfusions (40 ± 28 versus 20 ± 12 U, $P < 0.01$), PVP at closure (20.4 ± 6.9 versus 16.1 ± 3.5 mm Hg, $P < 0.01$), and hepatic artery flow (125 ± 42 versus 88 ± 57 mL/minute, $P = 0.03$) were significantly greater in the delayed-onset group versus the early-onset group. The PVF/GV ratio was significantly lower in patients with delayed-onset pneumonia versus patients with early-onset pneumonia (229 ± 80.5 versus 378 ± 140 mL/100 g, $P < 0.01$). Early graft function on postoperative day 14 was significantly worse in patients with delayed-onset pneumonia (n = 15) according to the total bilirubin level (17.9 ± 11.9 versus 10.1 ± 8.3 mg/dL, $P = 0.010$) and the prothrombin time/international normalized ratio (1.6 ± 0.2 versus 1.2 ± 0.2, $P < 0.01$). Gram-negative bacteria were present in 80% (28/35) of the patients with early-onset pneumonia and in 93% (14/15) of the patients with delayed-onset pneumonia.

The clinically causative events for delayed-onset pneumonia included primary graft dysfunction (n = 10), hepatic artery stenosis and radiological intervention (n = 1), decreased portal inflow and relaparotomy for shunt closure (n = 1), severe venous congestion of

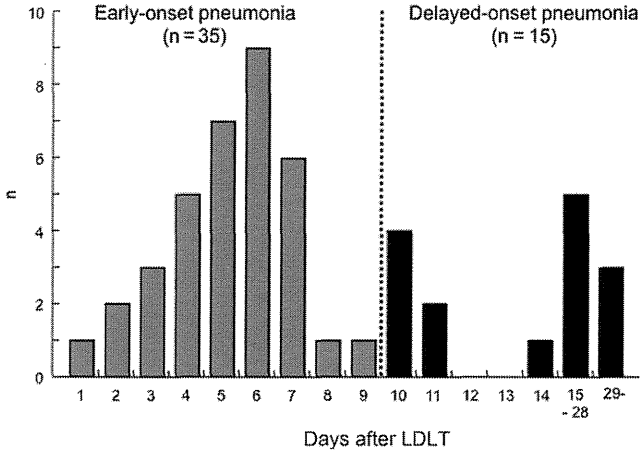

Figure 2. Time to the first episode of bacterial pneumonia. The pneumonia patients were divided into 2 groups: early-onset pneumonia (n = 35) and delayed-onset pneumonia (n = 15).

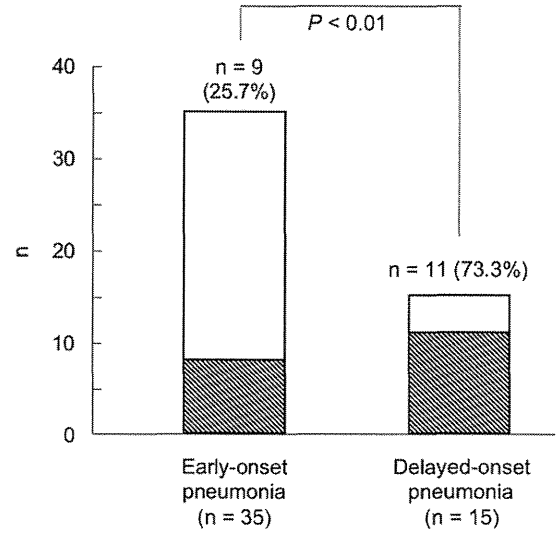

Figure 3. Early graft loss in patients with early-onset or delayed-onset bacterial pneumonia. The shaded portion of each bar shows the number of patients who experienced early graft loss.

the anterior segment of a right lobe graft (n = 1), portal vein thrombosis (n = 1), and prolonged mechanical ventilation due to pulmonary hypertension (n = 1).

Susceptibilities of Gram-Negative Bacteria Causing Pneumonia to Antibiotic Regimens

The susceptibility of gram-negative bacteria in patients with pneumonia (n = 42) is summarized in Fig. 4. The gram-negative bacteria had low susceptibility to cefazolin (CAZ; 5.3%) and ABPC/SBT (17.9%) and moderate susceptibility to cefepime (CFPM; 59.4%), piperacillin (PIPC)/tazobactam (TAZ; 61.5%), MEPM (66.7%), and GM (66.7%). The gram-negative bacteria were more susceptible to levofloxacin (LVFX; 74.4%) and combinations of broad-spectrum antibiotics with GM (CFPM and GM, 72.7%; PIPC/TAZ and GM, 74.4%; MEPM and GM, 74.4%; and LVFX and GM, 76.9%). *Stenotrophomonas maltophilia* (n = 10) accounted for 23.8% of the gram-negative bacteria responsible for pneumonia, and it showed high resistance to broad-spectrum antibiotics (except for minocycline hydrochloride). LVFX and combinations of broad-spectrum antibiotics with GM provided cover for almost all gram-negative bacteria except *S. maltophilia.*

DISCUSSION

In a 1996 study of patients who underwent DDLT, Singh et al.[6] reported that 14.8% of the patients experienced pneumonia with a mortality rate of 53% after transplantation. In contrast, Weiss et al.[3] reported that 15.5% of patients developed pneumonia within 6 days after transplantation, but the mortality rate was much lower: 21.7%. The mortality rate of patients with early-onset pneumonia was 25.7% in our study of patients after LDLT and was thus similar to the rate reported by Weiss et al. The mortality rate of patients with delayed-onset pneumonia, however, was quite high in our study: 73.3%. The precise impact of graft dysfunction on delayed-onset pneumonia is difficult to evaluate because both events could affect or cause each other.[10]

Only 1 study has investigated the prevalence of postoperative bacterial pneumonia in LDLT. Saner et al.[5] analyzed 55 LDLT recipients with a mean MELD score of 14.2 and found that 18.2% of the patients experienced pneumonia with a low 1-year survival rate of 42%. They concluded that the high mortality rate might be due to the longer warm ischemia time and the smaller graft size. Although GRWR was not described in their report, our series used small LDLT grafts (mean GRWR = 0.81%) and had the same warm ischemia time (39 minutes), but the mean MELD score was 17.4. The main difference between the Essen series[5] and our series is that the Essen series included more patients with alcoholic cirrhosis (21.8% versus 4%) and had fewer patients (55 versus 346); this suggests some learning curve effect.

Risk factors for developing pneumonia after LDLT included diabetes, pretransplant UNOS status 1 or 2A, and massive operative blood loss. A negative impact of diabetes on various infections has been reported within the context of abnormal neutrophil function (particularly impaired chemotaxis, phagocytosis, and bacterial killing).[16] Kornum et al.[17] performed a case-control study and showed that well controlled diabetes (hemoglobin A1c < 7%) and poorly controlled diabetes (hemoglobin A1c > 9%) were associated with increased risks of pneumonia (22% and 60%, respectively).

The deterioration of the patient's general status (ie, UNOS status 1 or 2A) is largely associated with poor short-term graft outcomes, as previously reported for DDLT.[18] Even for LDLT, it has been reported that both the disease severity and the general condition have a great impact on short-term graft survival,

TABLE 3. Characteristics of Patients With Early-Onset Pneumonia and Patients With Delayed-Onset Pneumonia

	Posttransplant Pneumonia		
	Early Onset (n = 35)	Delayed Onset (n = 15)	*P* Value
Recipient factors			
Male sex [n (%)]	16 (45.7)	7 (46.7)	0.95
Age (years)*	54.9 ± 10.6	49.1 ± 8.7	0.07
Child class C [n (%)]	7 (20.0)	9 (60.0)	<0.01
MELD score*	18.2 ± 7.6	18.3 ± 8.5	0.96
UNOS status 1 or 2A [n (%)]	12 (34.3)	14 (93.3)	<0.01
Acute liver failure [n (%)]	4 (11.4)	4 (26.7)	0.18
Major shunt vessels [n (%)]	19 (54.3)	7 (46.7)	0.62
Diabetes [n (%)]	14 (40.0)	4 (26.7)	0.37
Donor factors			
Male sex [n (%)]	26 (74.3)	8 (53.3)	0.15
Donor age (years)*	36.6 ± 11.8	42.3 ± 13.5	0.14
Left lobe graft [n (%)]	22 (62.9)	7 (46.7)	0.29
GV/SLV (%)*	42.1 ± 9.7	44.3 ± 7.0	0.42
GRWR (%)*	0.81 ±0.22	0.82 ± 0.17	0.93
Recipient surgery			
Cold ischemia time (minutes)*	78 ± 44	103 ± 47	0.07
Warm ischemia time (minutes)*	36 ± 9.4	45 ± 11	0.01
Hepatic artery flow (mL/minute)*	88 ± 57	125 ± 42	0.03
PVF/GV ratio (mL/100 g)*	378 ± 140	229 ± 80.5	<0.01
PVP at closure (mm Hg)*	16.1 ± 3.5	20.4 ± 6.9	<0.01
Splenectomy [n (%)]	18 (51.4)	7 (46.7)	0.76
Duct to duct [n (%)]	29 (82.9)	10 (66.7)	0.20
Operative time (minutes)*	803 ± 144	873 ± 210	0.18
Operative blood loss (L)*	6.6 ± 7.5	13.5 ± 12.8	0.02
Transfused red blood cells (U)*	14 ± 9	40 ± 30	<0.01
Transfused frozen plasma (U)*	20 ± 12	40 ± 28	<0.01
Postoperative factors			
Hepatic artery thrombosis [n (%)]	2 (5.7)	1 (6.7)	0.89
Portal vein thrombosis [n (%)]	0 (0.0)	1 (6.7)	0.12
Acute cellular rejection [n (%)]	4 (11.4)	1 (6.7)	0.61
Total bilirubin on day 14 (mg/dL)*	10.1 ± 8.3	17.9 ± 11.9	0.01
Ascites output on day 14 (L/day)*	0.67 ± 0.78	0.99 ± 1.2	0.27
Prothrombin time/international normalized ratio on day 14*	1.2 ± 0.2	1.6 ± 0.2	< 0.01

*The data are presented as means and standard deviations.

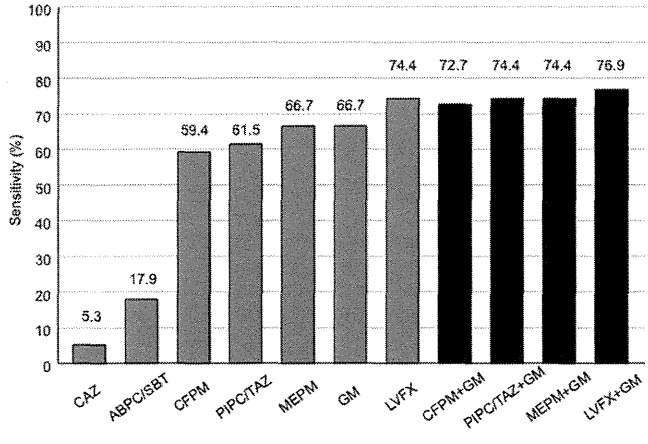

Figure 4. Susceptibility of gram-negative bacteria (n = 42). *Stenotrophomonas maltophilia* [n = 10 (23.8%)] was highly resistant to the commonly used broad-spectrum antibiotics.

although the impact of the MELD system on post-LDLT outcomes has been denied or is still under discussion.[19-21] In the current analysis, delayed-onset pneumonia was largely associated with poor graft dysfunction (whether the cause was primary or secondary). The prevalence of ventilator-associated nosocomial organisms, including *S. maltophilia* in the current series, could be attributed to the fact that secondary delayed-onset pneumonia was preceded by poorly functioning grafts. The duration of mechanical ventilation was significantly prolonged in patients with pneumonia caused by *S. maltophilia* versus patients with pneumonia caused by other types of bacteria.

Dysfunctional immunity caused by massive blood loss and resulting massive transfusions has also been reported.[22,23] Shorr et al.[22] reported a transfusion volume–dependent increase in ventilator-associated pneumonia in trauma patients. Meanwhile, Bernard

et al.[23] reported that packed red blood cell transfusions of 1, 2, or 10 U increased the risk of septic shock with odds ratios of 1.29, 1.53, and 2.29, respectively, in general surgery patients.

In our series of patients, gram-negative bacteria were the most common causative pathogens (84%), as compared with the earlier reports in DDLT. In patients with delayed-onset pneumonia, almost all of the bacteria responsible for pneumonia were gram-negative bacteria (93%). The most common bacteria isolated in this study were *P. aeruginosa* and *S. maltophilia*; the latter is an emerging and clinically significant causative pathogen for posttransplant infections. It is an aerobe gram-negative bacterium and is a relatively rare human pathogen.[24-26] However, this species is highly resistant to antibiotics because of its overproduction of β-lactamase and its high mutation rate.[24-26] It has been suggested that high-dose sulfamethoxazole/trimethoprim may be the only effective treatment for this species.[24-26] In the current series, the susceptibility of *S. maltophilia* to common antibiotics was as follows: 0% for CAZ, SBT/ABPC, CFPM, PIPC/TAZ, MEPM and sulfamethoxazole/trimethoprim; 10% for GM; 20% for LVFX; and 90% for minocycline hydrochloride.

Although it has been suggested that sulfamethoxazole/trimethoprim is the most effective treatment for *S. maltophilia*, our results do not support this. Instead, we found particularly high susceptibility to minocycline hydrochloride. Nevertheless, it is also true that a high sensitivity of *S. maltophilia* to minocycline hydrochloride has been demonstrated only in vitro and not in vivo.[24-26] Antimicrobial regimens such as LVFX, CFPM and GM, TAZ/PIPC and GM, and MEPM and GM are appropriate for posttransplant pneumonia because they cover most gram-negative bacteria, including *P. aeruginosa* (but not *S. maltophilia*). Further studies are necessary to better optimize the treatment of bacterial pneumonia after LDLT.

In conclusion, bacterial pneumonia (particularly delayed-onset pneumonia) is the most serious type of infection after LDLT. Risk factors for bacterial pneumonia include diabetes, the deterioration of the patient's general condition at the time of transplantation, and massive blood loss during surgery. Delayed-onset pneumonia and the emergence of *S. maltophilia* are major issues that need to be addressed.

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En Bloc Stapling Division of the Gastroesophageal Vessels Controlling Portal Hemodynamic Status in Living Donor Liver Transplantation

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Gastroesophageal shunts are commonly seen in patients with terminal liver disease requiring liver transplantation.[1] These shunts cause increased portal pressure in the gastroesophageal varices, increasing the risk of rupture and also allowing hepatofugal portal flow, which causes graft hypoperfusion and dysfunction after living donor liver transplantation (LDLT).[2,3] However, isolation and division of the vessels is difficult to achieve because of their anatomic properties. Moreover, obstruction of the shunt vessels may cause excessively high portal pressure, resulting in small-for-size graft dysfunction.[4] We describe a safe and rational technique for dividing the gastroesophageal hepatofugal shunts and left gastric arteries en bloc using end-stapling devices. Using this method, we can eradiate the shunts without increasing portal pressure.

METHODS

Indications for en bloc division of gastroesophageal vessels with major hepatofugal shunts include the larger caliber (>1 cm) vessels. In cases in which the left hepatic artery is replaced with a vessel originating from the left gastric artery, the technique is postponed until the graft arterialization is completed from other arterial sources in the recipient. Portal venous pressure is continuously monitored during LDLT surgery using a cannula (Medicut LCV-UK catheter 14G, Nippon Sherwood Inc) inserted into the superior mesenteric vein via a terminal jejunal vein.

We perform en bloc stapling division of the gastroesophageal vessels including huge hepatofugal shunts and the left gastric arterial systems as follows. After reperfusion of the graft, splenectomy is performed using a vessel-sealing system (LigaSure Atlas, Valleylab Inc) and endo-stapling devices (Echelon Flex Endopath Staplers 60–2.5, Ethicon Endo-Surgery Inc) to decrease the portal pressure, as previously described.[5] The gastrocolic and gastrosplenic ligaments are completely divided during splenectomy using the vessel-sealing system. Division of the left gastric ligament is started only when arterial reconstruction is complete because the gastric arterial system might be used for hepatic artery reconstruction.

The greater curvature of the stomach is manually lifted, the endo-stapling devices are applied to the base of the left gastric ligament including the left gastric artery, engorged coronary vein, and collateral vessels (Fig. 1A). The left gastric ligament is then divided en bloc using endo-stapling devices (Fig. 1B). Before the staples are fired, the esophagogastric junction, in which a nasogastric tube has been inserted, is manually palpated to prevent possibly injuring the esophagus. The tip of the endo-stapling device should be pointed vertically to the crus muscle for this purpose. Two sessions of this maneuver might be necessary to divide the ligaments, including the tortuous shunt vessels, therefore exposing the diaphragmatic crus. After division of the ligament, the stapled stump is mass-sutured using continuous 3–0 Prolene sutures with an SH needle (Ethicon Inc) to prevent postsurgical bleeding and occlude the retroperitoneal collateral veins. The stapled stump is also mass-sutured on the stomach side using the same sutures.

RESULTS

Between January 2011 and January 2012, 40 LDLTs were performed at Kyushu University Hospital. Among these cases, stapling division of the gastroesophageal vessels to control portal hemodynamic status was performed in 13 patients (32.5%) with simultaneous splenectomy. The mean Model for End-Stage Liver Disease (MELD) score of these patients was 15.5. ± 4.4. The endo-stapling devices were applied safely in all of the patients (Fig. 2A) without significant blood loss (Fig. 2B). The gastroesophageal shunt vessels (Fig. 2C) were successfully obstructed using

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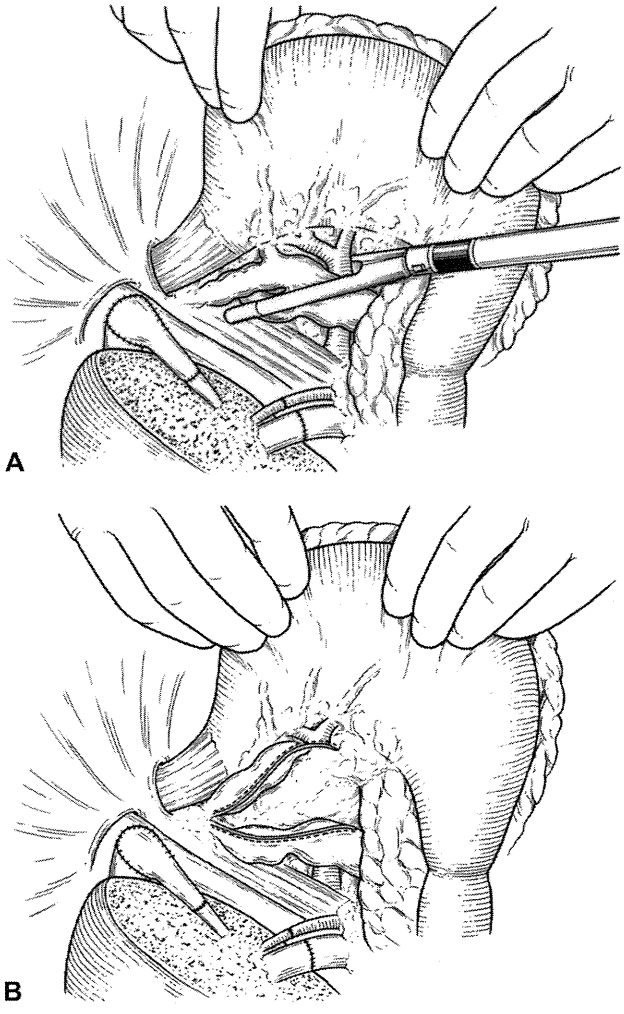

Figure 1. (A) The en bloc endo-stapling device was applied at the base of the left gastric ligament including the left gastric arterial system and the gastroesophageal shunts. (B) The left gastric ligament was divided en bloc.

this approach (Fig. 2D) in all of the patients. Although the left hepatic artery was replaced with a branch from the left gastric artery in 4 patients, our technique could be applied after graft arterial reconstruction using the right hepatic arteries.

The portal pressure decreased from 18.8 ± 5.6 mmHg to 17.4 ± 4.2 mmHg ($p = 0.02$, paired t-test) after stapling division of the gastroesophageal shunt vessels. There was no significant change in portal flow (1.65 ± 0.51 L/min vs 1.73 ± 0.60 L/min, $p = 0.79$, paired t-test) after division of the gastroesophageal shunt vessels. We did not perform pyloroplasty in this series because of the risk of postoperative complications including bleeding or leakage. However, no apparent gastroparesis or gastric stasis was observed.

DISCUSSION

Gastroesophageal shunt vessels are commonly seen in patients with terminal liver disease who undergo liver transplantation. However, surgical isolation and ligation of the shunts in LDLT is a technically difficult procedure often associated with massive bleeding. This en bloc procedure may also increase portal pressure, resulting in graft dysfunction.

Although gastroesophageal shunt vessels in terminal liver disease are derived from coronary or left gastric veins, their appearances differ greatly.[1] Gastroesophageal shunts are usually multiple in number, are coiled or tortuous in shape, engorged with a thin wall, and are buried in the retroperitoneum on the diaphragmatic crus. Therefore, manual isolation and ligation of such vessels is technically very difficult and may cause massive bleeding. In contrast, en bloc division of such vessels using endo-stapling devices is much safer, and does not require dissection or tying. In 1998, Hashizume and colleagues[6] first reported en bloc division of upper gastric vessels and splenectomy using endo-stapling devices under laparoscopy for patients with portal hypertension. Once the left gastric ligament is divided in two with the stapling devices, the retroperitoneal or gastric varices are easily mass-sutured for occlusion under a broad surgical field. Such mass-sutures are also useful for reinforcing the stapled stumps to prevent later bleeding or oozing. Moreover, stapling division followed by suturing could eliminate the multiple shunts; the isolation technique cannot.

Increases in portal pressure are also associated with isolated ligation of gastroesophageal shunt vessels.[4] It is well known that increased portal pressure is a significant cause of graft dysfunction in LDLT, and is characterized by prolonged cholestasis and intractable ascites. Therefore, to safely obstruct the portosystemic shunt vessels, the splanchnic or portal inflow should be controlled.[7] By simultaneous en bloc division of the left gastric arteries and the gastroesophageal shunts using endo-stapling devices, the portal pressure can be controlled. We previously reported splenic artery ligation in 2004[8] and splenectomy to control portal pressure in 2008.[9] The technique described here represents a third approach to controlling portal inflow in LDLT.

Although gastroesophageal varices or other portosystemic shunts might be improved during the years after LDLT, they might be causes of insufficient portal inflows into the transplanted grafts.[3] Therefore, we indicate the stapling division of the gastroesophageal vessels as major hepatofugal shunts including the larger caliber (>1 cm) vessels. Currently, we indicate the technique regardless of the portal pressure after reperfusion in LDLT. For other

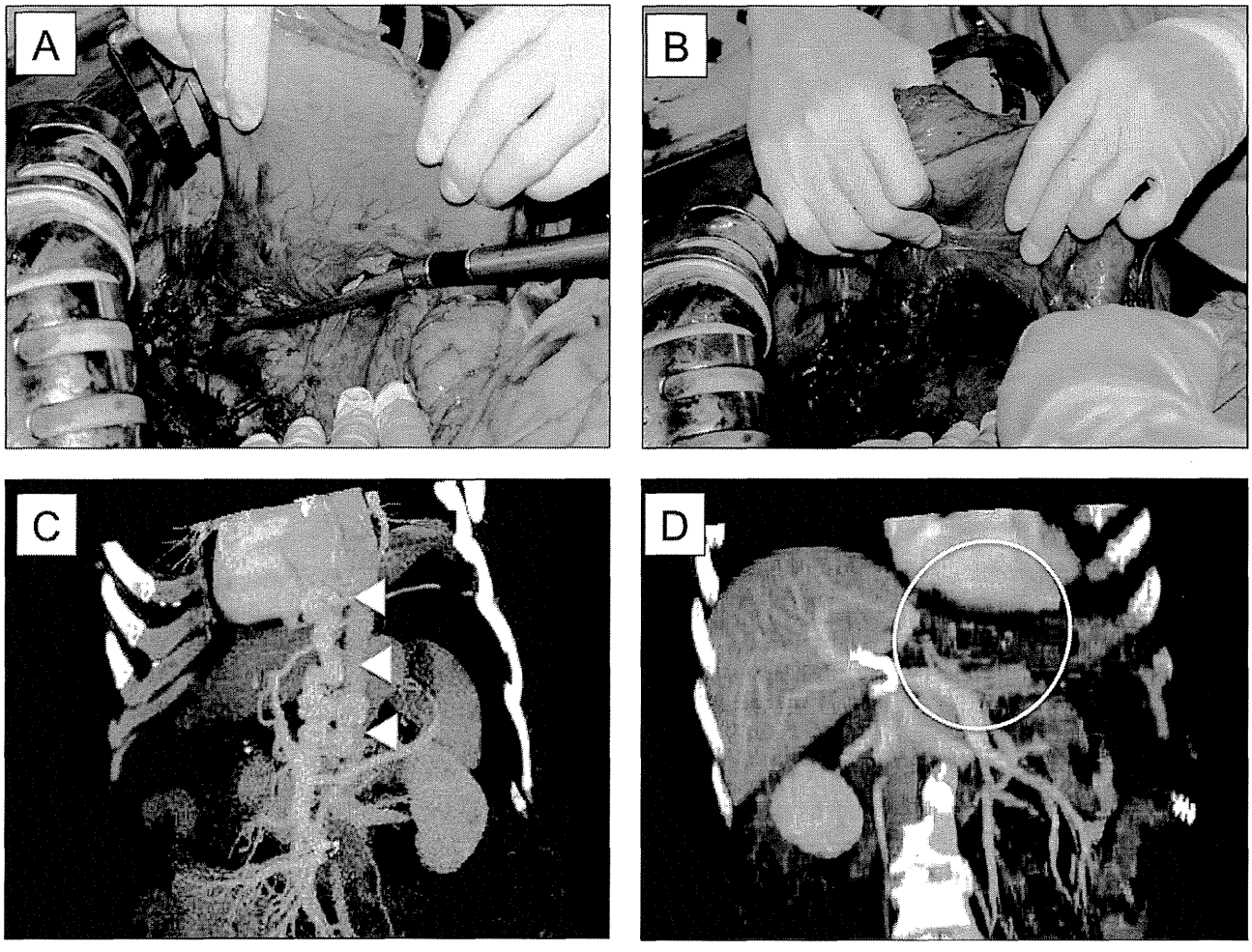

Figure 2. (A) Application and (B) firing of the endo-stapling devices. (C, white arrowheads) The major gastroesophageal shunt vessels (D, white circle) were eradicated, as confirmed by CT with 3-dimensional reconstruction.

types of major portosystemic shunts, including sprenorenal shunts and mesocaval shunts, we obstruct all such vessels during LDLT, simplifying the portal circulation system, because of the early experiences with portal steal phenomena, as Lee and associates[3] reported.

CONCLUSIONS

En bloc stapling division of the gastroesophageal vessels is a safe and rational technique in obstructing gastroesophageal hepatofugal shunts without increasing the portal pressure in LDLT.

Author Contributions

Study conception and design: Ikegami, Yoshizumi, Soejima
Acquisition of data: Yoshizumi, Yoshiya, Toshima, Motomura, Uchiyama
Analysis and interpretation of data: Ikegami, Yoshizumi, Soejima
Drafting of manuscript: Ikegami
Critical revision: Shirabe, Maehara

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Primary Graft Dysfunction After Living Donor Liver Transplantation Is Characterized by Delayed Functional Hyperbilirubinemia

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The purpose of this study is to propose a new concept of primary graft dysfunction (PGD) after living donor liver transplantation (LDLT), characterized by delayed functional hyperbilirubinemia (DFH) and a high early graft mortality rate. A total of 210 adult-to-adult LDLT grafts without anatomical, immunological or hepatitis-related issues were included. All of the grafts with early mortality (n = 13) caused by PGD in LDLT had maximum total bilirubin levels >20 mg/dL after postoperative day 7 ($p < 0.001$). No other factors, including prothrombin time, ammonia level or ascites output after surgery were associated with early mortality. Thus, DFH of >20 mg/dL for >seven consecutive days occurring after postoperative day 7 (DFH-20) was used to characterize PGD. DFH-20 showed high sensitivity (100%) and specificity (95.4%) for PGD with early mortality. Among the grafts with DFH-20 (n = 22), those with early mortality (n = 13) showed coagulopathy (PT-INR > 2), compared with those without mortality ($p = 0.002$). Pathological findings in the grafts with DFH-20 included hepatocyte ballooning and cholestasis, which were particularly prominent in the centrilobular zone. PGD after LDLT is associated with DFH-20 caused by graft, recipient and surgical factors, and increases the risk of early graft mortality.

Key words: Donor age, graft dysfunction, hyperbilirubinemia, living donor liver transplantation, small-for-size

Abbreviations: DDLT, deceased donor liver transplantation; DFH, delayed functional hyperbilirubinemia; GRWR, graft recipient weight ratio; GV, graft volume; GW, graft weight; LDLT, living donor liver transplantation; MELD, model for end-stage liver disease; PGD, primary graft dysfunction; PNF, primary graft nonfunction; POD, postoperative day; PT-INR, prothrombin time international normalized ratio; PVF, portal venous flow; PVP, portal venous pressure; ROC, receiver operating characteristic curve; SLV, standard liver volume; T.Bil, total bilirubin.

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Introduction

In deceased donor liver transplantation (DDLT), primary graft nonfunction (PNF) is one of the most serious and life-threatening conditions in the immediate postoperative period (1–3). PNF has been attributed to graft steatosis, prolonged cold ischemic time and advanced donor age (1,2). Unfortunately, PNF is usually irreversible, which means that early retransplantation is still the only treatment option (3).

In living donor liver transplantation (LDLT), the clinical characteristics of functional graft failure, hereafter referred to as primary graft dysfunction (PGD), are expected to be very different from those of PNF after DDLT because of differences in graft quality, size and preservation time (4,5). In LDLT, a qualified liver graft is usually obtained after exhaustive donor selection processes, and is transplanted after a very short cold ischemic time (4–7). However, LDLT grafts are always small-sized liver grafts, which may not be sufficient for the recipient's requirements (8,9). Therefore, in the mid-2000s, many institutes started to recommend the use of larger grafts to prevent "small-for-size syndrome" (SFSS), which might be a typical presentation of PGD after LDLT, and is characterized by prolonged cholestasis and increased ascites volume, with reduced graft survival (6–10).

Nevertheless, transplantation centers are also considering smaller grafts, with improved outcomes under refined and established surgical techniques (11–14). Indeed, recent studies have documented that small grafts do not necessarily cause or correspond to PGD, which is attributed to multiple factors including disease severity, portal pressure, graft regeneration and donor age (15). Therefore, the term SFSS is now unsuitable to refer to delayed PGD occurring after LDLT because its characteristics differ from those of PNF after DDLT.

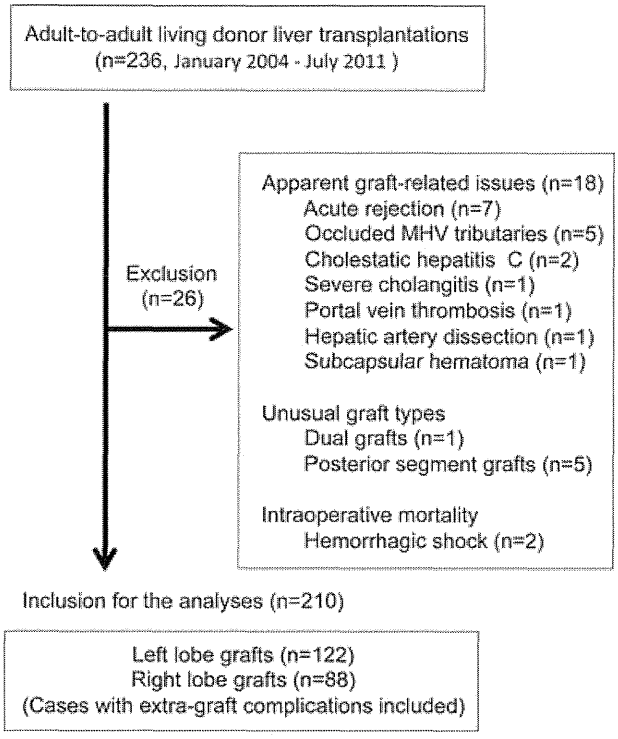

Figure 1: Twenty-six cases were excluded and 210 cases were included for the analyses. MHV = middle hepatic vein.

Therefore, the aim of this study was to characterize delayed PGD occurring after LDLT. We also sought to identify the factors associated with these disease processes and examine the pathological findings.

Materials and Methods

Patients

Between January 2004 and July 2011, a total of 236 adult-to-adult LDLTs were performed at Kyushu University Hospital, Fukuoka, Japan. Cases that underwent complex LDLT procedures using dual grafts (n = 1) or posterior segment grafts (n = 5), cases that died during surgery (n = 2) and cases with graft dysfunction caused by technical, immunological or recurrent hepatitis-related issues within 1 month of surgery (total, n = 18; acute rejection, n = 7; occlusion of the reconstructed middle hepatic vein tributaries, n = 5; cholestatic hepatitis C, n = 2; cholangitis, n = 1; portal vein thrombosis, n = 1; hepatic artery dissection, n = 1; and graft subcapsular hematoma because of percutaneous transhepatic cholangiogram, n = 1) were excluded from this study (Table S1). Cases with infectious complications (e.g. primary sepsis, pneumonia or spontaneous peritonitis) were not excluded in this analysis, because these complications overlapped with graft-oriented hepatic dysfunction in the same time in many cases, and it is difficult to delineate the cause and effect, as graft insufficiency could contribute to these infectious issues (15,16). Thus, 210 cases were included in this study (Figure 1). All of the LDLTs were performed after obtaining full informed consents from all patients and approval by the Liver Transplantation Committee of Kyushu University. The mean follow-up time was 3.4 ± 2.3 years.

Graft selection process

Grafts were selected as previously described (17). Left lobe grafts were considered to be the primary graft type if the desired graft volume (GV)/standard liver volume (SLV) is >35%. Right lobe grafts were considered if the simulated GV/SLV of the left lobe graft was <35% and the donor's remnant liver volume was >35%. Major middle hepatic vein tributaries >5 mm were maximally reconstructed to maintain uncongested GV/SLV >40% in right lobe grafts.

Surgical procedures

The donor parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (CUSA™, Valleylab Inc., Boulder, CO, USA) and a saline-linked radio-frequency dissecting sealer (Tissuelink™, Tissuelink Medical Inc., Dover, DE, USA) with the hanging maneuver (18). After donor hepatectomy, the graft was perfused, weighted and stored in University of Wisconsin solution (Viaspan ™, DuPont Inc., Wilmington, DE, USA). For right lobe grafts, the middle hepatic vein tributaries were reconstructed on the back table using the explanted portal vein or other vessels procured from the recipient (19). After recipient hepatectomy, the grafts were transplanted in a piggyback fashion. The orifice of the recipient hepatic vein was enlarged with an incision on the vena cava for the venous anastomosis to provide sufficient outflow. The venous anastomoses were performed using continuous 5–0 PDS-II™ sutures (Ethicon Inc., Somerville, NJ, USA). Reconstruction of the portal vein with continuous 6–0 PDS-II™ sutures was followed by reperfusion. Arterial reconstruction was performed using interrupted 8–0 Prolene™ sutures (Ethicon Inc.), under microscope. Bile duct reconstruction was performed by duct-to-duct biliary anastomosis or by hepaticojejunostomy using interrupted 6–0 PDS-II™ sutures.

Measurement of arterial and portal hemodynamics properties

Portal venous pressure (PVP) was continuously monitored during LT surgery using a cannula (Medicut LCV-UK catheter 14G™, Nippon Sherwood Inc., Tokyo, Japan) placed in the superior mesenteric vein via a terminal jejunal vein by direct cut down. Intraoperative blood flow was measured in the recipients after reperfusion using an ultrasonic transit time flow meter (Transonic System™, Ithaca, NY, USA) in the recipients after reperfusion. Hepatic arterial flow is expressed as mL/min and portal venous flow is expressed as L/min.

Immunosuppression

The basic immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. The target tacrolimus level was 10–15 ng/mL in the first month after LDLT month and was decreased to 5–10 ng/mL over the next few months. The target cyclosporine level was 200–250 ng/mL in the first month after LDLT and was decreased to 100 to 200 ng/mL over the next few months. One gram of methylprednisolone was given after reperfusion, and decreased from 200 mg to 20 mg daily over 1 week, then switched to oral prednisolone, tapered off at 3 months.

Clinical laboratory and ascites data

The serum total bilirubin (T.Bil) level, prothrombin time-international normalized ratio (PT-INR) and ammonia level were determined daily after LDLT and were analyzed in this study. The amount of ascites drained via the indwelling abdominal drains was also recorded. Prolonged coagulation profile (PT-INR > 1.8) was corrected by giving fresh frozen plasma. Fluid loss because of drainage of the ascites was corrected using intravenous 5% albumin solution to maintain circulatory stability and urinary output.

PGD and delayed functional hyperbilirubinemia (DFH)

PGD was defined as graft insufficiency with possible early graft loss, without technical, anatomical, immunological or hepatitis-related issues. DFH was