Artificial Liver Support System Combined with Liver Transplantation in the Treatment of Patients with Acute-on-Chronic Liver Failure

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Abstract

Background: The search for a strategy to provide temporary liver support and salvage the patients with acute-on-chronic liver failure (ACLF) remains an important issue. This study was designed to evaluate the experience in artificial liver support system (ALSS) combined with liver transplantation (LT) in the treatment of ACLF.

Methodology/Principal Findings: One hundred and seventy one patients with HBV related ACLF undergoing LT between January 2001 and December 2009 were included. Of the 171 patients, 115 received 247 sessions of plasma exchange-centered ALSS treatment prior to LT (ALSS-LT group) and the other 56 received emergency LT (LT group). The MELD score were 31 ± 6 and 30 ± 7 in ALSS-LT group and LT group. ALSS treatment resulted in improvement of liver function and better tolerance to LT. The average level of serum total bilirubin before LT was lower than that before the first time of ALSS treatment. The median waiting time for a donor liver was 12 days (2–226 days) from the first run of ALSS treatment to LT. Compared to LT group, the beneficial influences of ALSS on intraoperative blood loss and endotracheal intubation time were also observed in ALSS-LT group. The 1-year and 5-year survival rates in the ALSS-LT group and LT group were 79.2% and 83%, 69.7% and 78.6%.

Conclusions/Significance: Plasma exchange-centered ALSS is beneficial in salvaging patients with ACLF when a donor liver is not available. The consequential LT is the fundamental treatment modality to rescue these patients and lead to a similar survival rate as those patients receiving emergency transplantation.

Introduction

Liver failure remains a disease associated with high mortality. In China, because of the high prevalence of hepatitis B, hepatitis B virus-related acute-on-chronic liver failure (ACLF) is a common cause of liver failure, which is much different from the western countries where drugs, alcohol, and hepatitis C are the major causes. According to Asian Pacific Association for the Study of the Liver, ACLF has been recently defined as ‘acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease’ [1].

Liver transplantation (LT) is the best treatment for salvaging patients with ACLF. However, LT is not always possible because of donor shortage. Enormous attempts at providing temporary liver support have been made, with an aim to increase survival rate or improve the condition of the patient until a donor is available. In the past decades, a variety of artificial liver support system (ALSS), including plasma exchange (PE), hemoperfusion, PE plus continuous hemodiafiltration, MARS and fractionated plasma separation, adsorption and dialysis system, have been employed in the management of liver failure [2].

Our previous study has demonstrated that ALSS can efficiently decrease the mortality of patients with severe hepatitis of early and middle stages [3]. However, for patients with ACLF, since hepatocytes undergo massive denaturation, necrosis and dysfunction, it is difficult to completely reverse the clinical course of the disease with routine medical management and even combined with ALSS. Under this circumstance, LT is usually indispensable. In the present study, we described our experience of PE-centered ALSS combined with LT in the rescue of patients with ACLF.
Methods

2.1 Patients

This study was approved by the First Affiliated Hospital, Zhejiang University School of Medicine and the current regulation of the Chinese Government, and the Declaration of Helsinki were strictly followed for each organ donation and transplant performed in our center. Written informed consents from each donor and recipient were obtained. No donor livers were harvested from executed prisoners. All the data was analyzed anonymously.

From January 2001 to December 2009, 796 patients underwent LT at our hospital. United Network for Organ Sharing status was used as the organ allocation system before December 2002, and Model for End-Stage Liver Disease (MELD) score was applied after January 2003. Their primary diseases included hepatic malignancies (n = 510) and benign end-stage liver diseases (n = 486). Malignancies, retransplantation, and combined transplantation were excluded. Among the 486 cases of benign end-stage liver diseases, 171 cases of ACLF with a known history of chronic hepatitis B or cirrhosis were finally enrolled in this retrospective study. Of these 171 cases, 115 received 247 sessions of ALSS treatment before LT (ALSS-LT group), whereas the other 56 cases fortunately gained appropriate donor livers and received emergency LT within 72 h after their referral to our centre, without any prior ALSS treatment (LT group). The baseline characteristics including age, gender, serum total bilirubin, MELD scores, and major complications of the above two groups were collected at the time of admission and summarized in Table 1. MELD score was calculated as 9.57×loge (Cr mg/dl) +3.78×loge (TB mg/dl)+11.20×loge (international normalized ratio)+6.43 [4]. Lamivudine combined with low-dose intramuscular hepatitis B immunoglobulin therapy was applied in all patients [5]. Immunosuppressive regimen was triple therapy incorporating tacrolimus or cyclosporin A, mycophenolate and steroid [6].

2.2 ALSS treatment

Blood access was established through a double-lumen catheter via the patient’s jugular or femoral vein. The methods of ALSS included PE, plasma perfusion, continuous hemodiafiltration and MARS. PE was performed with plasma separator Plasmacure PS-06 (Kuraray Co., Tokyo, Japan). The total volume of exchanged plasma was about 3300 ml, and the exchange rate of plasma was 22–25 ml/min. Continuous hemodiafiltration was performed with Diafilter D-30NR (Minntech Co., Minneapolis, MN). Plasma perfusion utilizing Adsorba 300C contained 300 g cellulose coated charcoal (Gambrro Dialysatoren GmbH Co., KG, Hechingen, Germany). The MARS system (MARS monitor, Teraklin AG, Rostock, Germany) was used, and its albumin circuit, containing 600 mL 20% human albumin, was driven at 150 mL/min. Dexamethasone (5 mg) and prophylactic antibiotics were routinely given. Totally 20–60 mg heparin and 10–30 mg protamine sulphate were given in one run of ALSS treatment.

The detailed methods of PE-centered ALSS were performed based on individuals’ conditions. For example, patients with coagulopathy were indicated for PE; when the patient had hepatic encephalopathy, we used PE plus plasma perfusion or continuous hemodiafiltration. For patients complicated with hepatorenal syndrome or imbalance of water or electrolytes, we applied PE plus continuous hemodiafiltration or MARS. In ALSS-LT group, 247 sessions of ALSS were applied to 115 patients, with PE 162 times, PE plus plasma perfusion 52 times, PE plus continuous hemodiafiltration 10 times and MARS 15 times.

2.3 Data Collection

Patient demographic, surgical, and postoperative data were collected by chart review and from surgical records. Serum parameters of serum total bilirubin, alanine aminotransferase, aspartate aminotransferase, total bile acid, creatinine, prothrombin time and electrolytes were closely monitored before and after every session of ALSS treatment or during the perioperative period.

2.4 Statistical analysis

The values were expressed as mean±SD. The data were statistically analyzed by SPSS 10.0 software package (SPSS Inc, Chicago, IL). The laboratory data were compared by Wilcoxon’s rank-sum test or Mann-Whitney U test. Chi-square test was used to compare categorical variables. Survival analysis was estimated using Kaplan-Meier method. A P value less than 0.05 was considered statistically significant.

Results

3.1 Efficacy of ALSS

Before ALSS treatment, 115 patients in ALSS-LT group were in poor general condition, complicated by cachexia, fatigue, loss of appetite, abdominal distention, jaundice, hepatorenal syndrome or hepatic encephalopathy. After ALSS treatment, general conditions and clinical symptoms including spirit, sleeping, appetite, and hepatic encephalopathy were improved. The changes of main laboratory parameters in 4 subgroups of ALSS are listed in Table 2. The levels of serum total bilirubin declined markedly by 50% on average in all subgroups. PE plus continuous hemodiafiltration and MARS obviously decreased serum creatinine level with the removal rates of 45±9% and 28±4%.

Table 1. The baseline patient characteristics.

|                      | ALSS-LT group (n = 115) | LT group (n = 56) | P value |
|----------------------|-------------------------|------------------|---------|
| Age (years)          | 46±10                   | 45±10            | NS      |
| Gender (male/female) | 100/15                  | 45/11            | NS      |
| TB (μmol/L)          | 557±195                 | 537±201          | NS      |
| MELD score           | 31±6                    | 30±7             | NS      |
| Infections           | 33                      | 15               | NS      |
| Encephalopathy       | 54                      | 24               | NS      |
| Hepatorenal syndrome | 29                      | 12               | NS      |

Abbreviations: ALSS, artificial liver support system; LT, liver transplantation; TB, total bilirubin; MELD, model for End-Stage Liver Disease; HBV, hepatitis B virus.

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respectively. Prothrombin time decreased significantly in PE involved ALSS subgroups (P<0.05).

### 3.2 Impact of ALSS on patients’ transplantability

One hundred and twenty five patients of ALSS-LT group were successfully bridged to LT after attaining proper donor organs. The average level of TB before LT was significantly lower than that before the first session of ALSS treatment (476±6 vs. 557±65 μmol/L, P<0.05). A similar trend was observed in the average ICU staying time but the reduction was not statistically significant (9.8±4.5 days vs. 10.5±4.7 days, P>0.05). (Table 4).

### 3.4 Impact of ALSS combined with LT on patients’ survival

In ALSS-LT group, the survival rates of 1-year and 5-year were 79.2% and 69.7%, respectively. Compared with that in ALSS-LT group, the survival of LT group did not show significant difference, with the 1-year and 3-year survival rate of 83% and 78.6%, respectively. No significant differences in survival were found between two groups (Figure 1). However, in another cohort of ACLF patients (n = 158) receiving only conventional medical therapy (no ALSS and no LT), the 1- and 3-month mortality was 79.7% and 90.3%, respectively.

### Discussion

The past two decades witnessed the progress of LT [7,8]. As the only efficient procedure to treat ACLF, LT has been applied with a perioperative mortality rate of less 5% and 1-year survival rate of exceeding 80% for recipients in some major transplant centers in China [9]. At our center, ACLF related to HBV infection has been one of the main indications of LT. The shortage of donor livers, however, will undoubtedly make the patients with critical condition lose the opportunity for LT. For those who undergo LT in a deteriorating status with cachexia and disturbance of internal environment, the outcome would be unsatisfactory. The increasing discrepancy between the number of potential candidates for LT and the number of donor livers available suggests that some therapeutic alternatives for temporary liver support to patients with ACLF should be necessary [10,11].

Recent studies have shown that extracorporeal liver support systems could temporarily support patients’ liver function, improve their preoperative condition, and enhance their tolerance to surgery, thus extending the waiting time for a donor liver as a bridge to LT [12–16].

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### Table 2. Changes of key laboratory parameters pre- and post-artificial liver support system (ALSS) treatment in different subgroups.

| Parameters                  | PE (n = 162) | PE+continuous hemodiafiltration (n = 52) | PE+Plasma perfusion (n = 18) | MARS (n = 15) |
|-----------------------------|-------------|----------------------------------------|-------------------------------|--------------|
| TB                          | 575±174     | 530±165                                | 558±183                       | 511±137      |
| Pre-treatment (μmol/L)      | 260±96      | 253±82                                 | 261±95                        | 331±115      |
| Post-treatment (μmol/L)     | 55±6        | 52±5                                   | 54±5                          | 34±4         |
| Removal rate (%)            | <0.05       | <0.05                                  | <0.05                         | <0.05        |
| P value                     | Cr          | 80±19                                  | 301±102                       | 78±15        |
| Pre-treatment (μmol/L)      | 76±15       | 169±52                                 | 74±13                         | 145±29       |
| Post-treatment (μmol/L)     | 4±5         | 45±9                                   | 4±4                           | 28±4         |
| Removal rate (%)            | >0.05       | <0.05                                  | >0.05                         | <0.05        |
| PT                          | 32.4±10.3   | 31.6±8.0                               | 31.2±7.7                      | 28.4±8.4     |
| Pre-treatment (s)           | 20.7±3.6    | 22.5±6.3                               | 23.6±5.6                      | 30.1±8.8     |
| Post-treatment (s)          | <0.05       | <0.05                                  | <0.05                         | >0.05        |

Removal rate was calculated as: (pre-treatment concentration—post-treatment concentration)/pre-treatment concentration.

Abbreviations: PE, plasma exchange; TB, total bilirubin; Cr, creatinine; PT, prothrombin time.

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### Table 3. Liver function and model for End-Stage Liver Disease (MELD) score before artificial liver support system (ALSS) and before liver transplantation (LT).

| Parameters                  | Pre-first ALSS | Pre-LT |
|-----------------------------|----------------|--------|
| TB (μmol/L)                 | 557±195        | 576±169|
| ALT (U/L)                   | 127±113        | 105±80 |
| AST (U/L)                   | 182±152        | 165±207|
| TBA (μmol/L)                | 246±98         | 144±108|
| MELD score                  | 31±6           | 29±9   |

Pre-treatment TB, ALT, AST, TBA, and MELD score.

Abbreviations: TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBA, total bile acid.

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As a promising liver assist system, ALSS can perform partial functions of liver, with important therapeutic potentials in various patients with hepatitis, liver cirrhosis, and acute liver failure [17,18]. ALSS treatment seemed to reduce the mortality in patients with ACLF [19]. As an important part of ALSS, PE has been long applied in fulminant hepatic failure with aim of removing overabundant toxic substances and correcting the severe coagulopathy [20]. For removal of the hepatic encephalopathic substances such as aromatic amino acids, ammonia and middle molecules, plasma perfusion or continuous hemodiafiltration are often used with PE simultaneously [21,22]. MARS is actually combining hemodialysis/filtration and plasma perfusion, and serves to remove albumin-bound toxins and water-soluble toxins [23]. To patients complicated with hepatorenal syndrome, PE plus continuous hemodiafiltration and MARS also helped to improve renal function. In this study, PE-centered ALSS including PE alone, PE plus continuous hemodiafiltration, PE plus plasma perfusion and MARS were applied based on individuals’ conditions.

Our early study has found that non-biological artificial liver techniques can efficiently decrease the mortality of patients with severe hepatitis of early and middle stages [3]. Our results supported the favorable effects of PE-centered ALSS. Before ALSS treatment, all the patients developed pre-terminal or terminal clinical manifestations, such as hepatic encephalopathy, hepato-renal syndrome, disturbance of water and electrolytes, and other severe complications. The initial serum bilirubin level of 115 patients with ACLF in the ALSS-LT group was as high as 557 ± 195 μmol/L and still in an increasing tendency. After treatment of ALSS, liver and renal function and coagulopathy improved evidently. Neurological improvements were found in patients with encephalopathy following repeated sessions of ALSS treatment. Disorders of the internal environment prior to LT were also corrected to a certain extent, thus facilitating improvement of patients’ general condition. The result of this study suggests that for patients with ACLF who are in the waiting list for LT, ALSS should be considered as an important part of preoperative management. When a donor liver is not available, salvaging ALSS should be carried out timely to support liver function and

|                          | Blood loss (ml) | Intubation time (days) | ICU staying time (days) |
|--------------------------|-----------------|------------------------|-------------------------|
| ALSS-LT group            | 3941.8 ± 1997.4 | 3.3 ± 2.6              | 9.8 ± 4.5               |
| LT group                 | 5058.3 ± 2193.6 | 4.5 ± 3.6              | 10.5 ± 4.7              |
| P value                  | <0.05           | <0.05                  | >0.05                   |

Figure 1. Comparison of patient cumulative survival between ALSS-LT group and LT group. (P=0.406) (ALSS, artificial liver support system; LT, liver transplantation).

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win precious waiting time till a donor liver is available. In the present study, patients after each ALSS treatment showed marked improvement in liver function and stabilized general condition, sustaining patients’ lives with a median time of nearly 2 weeks and the longest time of 226 days.

The beneficial influences of ALSS on intraoperative blood loss and ICU staying were also observed. Furthermore, it was demonstrated from our results that the combined treatment of ALSS and LT achieved the same 1-year survival rate as emergency LT which was applied to those critical patients in 72 hours. Although a prospective, randomized, and controlled trial is needed to confirm the beneficial effects of ALSS, our study has undoubtedly demonstrated the efficacy and safety of ALSS in supporting liver function and extending the waiting time for donor livers. In this regard, ALSS for ACLF patients will broaden the indications of LT, and more patients in the waiting list of LT will achieve a new life.

For ACLF patients, however, what ALSS may provide is still a transient liver function support [3,24]. The biochemical manifestation of liver failure may relapse and approach or even exceed the level before the previous ALSS treatment [25,26]. Therefore, for the patients who developed massive necrosis of hepatocytes and lost ability of liver regeneration, several times of ALSS and sequential timely LT were needed. Although further prospective and randomized studies should be performed, we believe that ALSS is beneficial in salvaging patients with ACLF when a donor liver is not available and LT is the fundamental treatment modality to rescue these patients.

Author Contributions
Conceived and designed the experiments: XX SZ LL. Performed the experiments: XI QL QW. Analyzed the data: ZL XX LZ. Contributed reagents/materials/analysis tools: MZ JW JH JS. Wrote the paper: XX.

References
1. Sarin SK, Kumar A, Almeida JA, Chawda YK, Fan ST, et al. (2009) Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). Hepatol Int 3: 269–292.
2. Santoro A, Mancini E, Battiglieri S, Krause A, Yakubovich M, et al. (2004) Extracorporeal support of liver function (II Part). Int J Artif Organs 27:176–185.
3. Li Lj, Zhang YM, Liu XL, Du WB, Huang JR, et al. (2006) Artificial liver support system in China: a review over the last 30 years. Ther Apher Dial 10:160–167.
4. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, et al. (2003) Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology 124:91–96.
5. Lu AW, Zheng SS, Wu MP, Shen Y, Yang RW. (2008) Reevaluation of the effect of lamivudine therapy preoperative to prevent HBV recurrence after liver transplantation. Hepatobiliary Pancreat Dis Int 7:357–361.
6. Xu X, Ling Q, He ZL, Guo F, Zheng SS. (2008) Post-transplant diabetes mellitus in liver transplantation: Hangzhou experience. Hepatobiliary Pancreat Dis Int 7:465–470.
7. Adam R, Hori E. (2009) Liver transplantation: the current situation. Semin Liver Dis 29:3–18.
8. Matsui Y, Sugawara Y, Yamashita N, Kaneko J, Tamura S, et al. (2008) Living donor liver transplantation for fulminant hepatic failure. Hepatol Res 38: 987–996.
9. Wu J, Zheng SS. (2004) Liver transplantation in China: problems and their solutions. Hepatobiliary Pancreat Dis Int 3: 170–174.
10. Chan C, Berhiaume F, Nath BD, Tilles AW, Toner M, et al. (2004) Hepatic tissue engineering for adjunct and temporary liver support: critical technologies. Liver Transpl 10: 1331–42.
11. Borra M, Galavotti D, Bellini C, Fumi L, Moriani E, et al. (2002) Advanced technology for extracorporeal liver support system devices. Int J Artif Organs 25: 939–949.
12. Palme D, Qayumi AK, Spiegel HU. (2000) Liver bridging techniques in the treatment of acute liver failure. J Invest Surg 13: 299–311.
13. Ichida T. (2003) Artificial liver support system for fulminant hepatic failure as bridge-use to living donor liver transplantation. Intern Med 42: 920–921.
14. Ueda Y, Ivata H, Park HJ, Ko IK, Shimooka Y, et al. (2003) Biosartificial liver with whole blood perfusion. ASAIO J 49: 401–406.
15. Faenza S, Mancini E, Petrimi F, Santoro A, Zanoni A, et al. (2004) Role of intensive therapy in liver transplant recipients: experience in blood purification and biosynthetic techniques. Transplant Proc 36: 555–575.
16. Demetriou AA, Brown RS Jr, Bussinell RW, Fair J, McGuire BM, et al. (2004) Prospective, randomized, multicenter, controlled trial of a biosartificial liver in treating acute liver failure. Ann Surg 239: 660–667.
17. Nitta M, Hirasa H, Oda S, Shiga H, Nakamichi K, et al. (2002) Long-term survivors with artificial liver support in fulminant hepatic failure. Ther Apher 6: 201–211.
18. Patzer II JF, Lopez RC, Zhu Y, Wang ZF, Mazzarelos GV, et al. (2002) Biosartificial liver assist devices in support of patients with liver failure. Hepatobiliary Pancreat Dis Int 1: 18–25.
19. Kjaergaard LL, Liu J, Als-Nielson B, Guld C. (2003) Artificial and bioartificial support for acute and acute-on-chronic liver failure: a systematic review. JAMA 289: 217–222.
20. De Silvestro G, Marson P, Brandesde R, Pitoni G, Ongaro G. (2000) A single institution’s experience (1962–1999) with plasma-exchange therapy in patients with fulminant hepatic failure. Int J Artif Organs 23: 454–461.
21. Secher A, Ossorio J, Frieze C, Ossorio RW. (2001) Artificial liver support devices for fulminant liver failure. Clin Liver Dis 5: 415–430.
22. Koizaki K, Fukatsu A, Kasabara M, Ogura Y, Egawa H, et al. (2004) The role of apheresis therapy in living donor liver transplantation. Ther Apher Dial 8: 174–179.
23. Feldlin M, Friman S, Backman L, Siewert-Delle A, Henriksson BA, et al. (2003) Treatment with the molecular adsorbent recirculating system in patients with acute liver failure. Transplant Proc 35: 822–823.
24. Pascher A, Sauer DM, Hammer C, Gestrich JC, Neuhaus P. (2002) Extracorporeal liver perfusion as hepatic assist in acute liver failure: a review of world experience. Xenotransplantation 9: 309–324.
25. Chambacou RA. (2003) Artificial liver support in the third millennium. Artif Cells Blood Substit Immobil Biotechnol 31: 117–126.
26. Onodera K, Sakata H, Yonekawa M, Kawamura A. (2006) Artificial liver support at present and in the future. J Artif Organs 9: 17–28.