Alpha fetoprotein: A biomarker for the recruitment of progenitor cells in the liver in patients with acute liver injury or failure

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Key words: hepatic progenitor cell, LPC, donor, liver tissue damage

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Running title: AFP as a marker for LPC recruitment

Number of:

Figures - 5

Table - 2

Supplemental table – 1

References - 29

Word count - 4286

Abbreviations: alpha fetoprotein (AFP), acute liver failure (ALF), acute liver injury (ALI), alanine aminotransferase (ALT), auxiliary partial orthotopic liver transplantation (APOLT), body mass index (BMI), hepatic growth factor (HGF), living donor liver transplantation (LDLT), liver

progenitor cells (LPCs), partial hepatectomy (PH), postoperative day (POD), prothrombin time-international normalized ratio (PT-INR)

Abstract

Background & Aims: The optimal conditions for hepatocyte proliferation should be clarified in an attempt to improve the impaired liver regeneration observed in patients with acute liver failure (ALF). In order to evaluate the significance of the serum AFP level and PT-INR as possible biomarkers of the proliferation of liver stem/progenitor cells (LPCs) and mature hepatocytes (MHs), respectively, we focused on donors of living donor liver transplantation (LDLT) and patients with acute liver injury (ALI), including ALF. **Methods**: Seventy-three patients with ALI/ALF and 11 donors for LDLT were evaluated. LPC induction was histologically evaluated using cytokeratin (CK)-7 staining in 45 ALI/ALF patients. **Results:** The AFP level was not apparently elevated during the observation period in any of the LDLT donors, whereas the serum AFP levels were substantially increased in the patients with ALI/ALF and significantly correlated with the number of CK-7 positive LPCs in the liver**, except for very severe damaged liver.** All patients exhibiting an early peak in the AFP level prior to PT-INR elevation died. **Conclusions:** The serum AFP level may reflect the induction of LPCs in ALI/ALF patients. The substantial and persistent induction of LPCs until sufficient regeneration of MHs may be needed for a recovery from ALF. We herein demonstrate that the serum AFP level may be a serum marker of LPCs in patients with ALI/ALF. A comparison of the serial changes in the AFP levels and PT-INR in our study patients showed impaired proliferation of LPCs and delayed recovery of MHs in the patients who died.

Introduction

Acute liver failure (ALF) affects approximately 200 patients per year in Japan [1]. ALF patients can be divided into two groups: those with and without encephalopathy. ALF patients with encephalopathy are further classified based on the duration of precoma into those with acute type or subacute type disease. Impaired liver regeneration appears to be the main factor accounting for the poor prognosis of patients with the subacute type of ALF with encephalopathy, although the underlying mechanism has not been sufficiently investigated [2]. The development of a new modality to promote liver regeneration is therefore needed in order to improve the prognosis of ALF patients treated without liver transplantation.

Two cell types, namely mature hepatocytes (MHs) and liver stem/progenitor cells (LPCs), such as oval cells, are considered to be potential cell sources for regeneration of the liver parenchyma [3]. Katoonizadeh et al. showed that the number of LPCs significantly increases according to the severity of hepatocyte loss [4]. Hence, MH and LPCs are not generally increased under the same conditions[3, 5]. This finding indicates that these cell types have different optimal microenvironments for proliferation. These cells also show different cell-signaling reactions to the same stimuli. Fujita et al. evaluated serial changes in the microscopic findings of the graft and native liver in an ALF patient who underwent auxiliary partial orthotopic liver transplantation (APOLT)[6]. That was a unique report in that it included sequential observations of the regeneration of the native liver. Consequently, a ductular reaction was observed on POD7, followed by proliferation of small round cells approximately two months after APOLT. The patient's complete recovery from massive liver injury took 14 months after the APOLT procedure. These findings indicate that LPCs require a long period for liver tissue repair. In addition, serial changes due to liver regeneration have been observed in models of partial

hepatectomy. In a rodent model, two-thirds hepatectomy resulted in the rapid generation of 93% of the original volume between seven and 14 days after the operation [7]. In the clinical setting, it has been demonstrated that partial hepatectomy repairs 86% of the original liver volume by 12 months [8]. A rapid recovery in the liver volume is observed if a sufficient number of hepatocytes remain in the injured liver, such as that observed after partial hepatectomy.

Intriguingly, patients with ALF usually present with increased serum AFP levels during hospitalization [9-11]. AFP is thought to be secreted from LPCs, as LPCs, but not MHs, express AFP, and are the major cells involved in regeneration [5, 12]. Furthermore, an increase in the AFP level from the time of administration to three days after the start of therapy is associated with the prognosis of patients with ALF [9], while a decrease in the AFP level during hospitalization is a predictive marker of a poor prognosis in such patients [13]. These results suggest that the recruitment of LPCs is associated with both the severity of liver damage and the regenerative activity induced by the LPCs. Meanwhile, the histological findings observed during liver regeneration are well described in patients with acute-onset autoimmune hepatitis and chronic hepatitis [14, 15]. Accordingly, LPCs with an oval nucleus for recruitment in liver regeneration express cytokeratin (CK)-7 [14, 15]. Intriguingly, the severity of hepatocyte loss positively correlates with the number of LPCs in cases of mild to severe hepatocyte loss [4]. Moreover, the severity of liver damage appears to be associated with the serum AFP level and number of LPCs in the damaged liver. However, the relationships between laboratory parameters and the number of LPCs in the liver have not yet been clarified.

The number of LDLT procedures has recently increased in Japan [16]. LDLT donors have a “unique status” in that their participation makes it possible to observe the features of typical liver regeneration after partial hepatectomy in humans [8]. Therefore, assessing serial changes in

various blood markers in patients undergoing LDLT after hepatectomy may provide helpful information for evaluating the regenerative process in the “pathological liver” in the setting of ALI/ALF. Furthermore, confirming whether ALI/ALF induces LPC production and/or the AFP expression may help to clarify the significance of elevation of the serum AFP levels in ALI/ALF patients.

The aims of this study were to (1) determine serial serum data regarding liver regeneration in living donors during the early perioperative period, (2) compare various serum markers and the prognosis of ALF/ALI patients and (3) confirm the relationships between LPC recruitment on immunohistochemistry and serum parameters, including the AFP level, in patients with ALI/ALF.

Materials and Methods

Subjects: Eleven healthy donors who underwent liver transplantation with partial hepatectomy (PH) from 2008 to 2012 at our institute were enrolled in this study, as subjects treated with PH exhibit a rapid recovery of the liver volume after PH. Serum samples were collected from the day before the operation until the seventh postoperative day (POD) and stored at -20°C until the analysis. The residual liver volume of the donors was calculated as the actual liver graft weight divided by the liver volume estimated on computed tomography prior to surgery (Table 1).

A total of 192 patients who consulted our department for the treatment of acute liver injury between 2004 and 2013 were retrospectively evaluated in this study. One hundred and nineteen of these subjects were excluded from the study for various reasons, including complications associated with disseminated intravascular coagulopathy or a lack of serum data or blood samples. The eligible 73 patients were further classified into subjects with ALI and those with ALF. ALI was defined as liver injury in a patient with no known previous liver disease, a PT-INR of < 1.5 during hospitalization and a duration of illness of < 26 weeks. ALF was defined as ALI with a PT-INR of >1.5 during hospitalization. Cases of ALF with coma were subdivided into acute and subacute types; the acute type was defined as the development of hepatic encephalopathy within 10 days from date of onset of symptoms, and the subacute type was defined as the development of hepatic encephalopathy more than 11 days after the date of onset of symptoms[1, 17]. In order to evaluate the clinical course of the disease, the 73 patients were divided into two groups based on outcome: namely, death or survival (Table 2).

For the assessment of serial changes in the serum AFP levels and PT-INR values, these parameters were measured on the date of admission, three to five days after admission and then

at least every seven days after admission. In order to compare these data between the surviving and deceased patients, the date for each parameter was determined based on the period: at admission, three to seven days after admission and 10 days around discharge, defined as "admission," "early" and "late," respectively.

Informed consent for a liver biopsy was obtained from 45 of the 73 patients with ALI/ALF. The liver specimens were further evaluated with respect to the presence of CK-7 positive LPCs in the liver using immunohistochemistry.

All protocols reported in this paper were approved by the Institutional Review Board of Iwate Medical University (approval number: H20-36), and informed consent was obtained from all participants.

Measurements and calculations: Body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/ height $(m)^2$. In order to calculate the residual liver volume, the total liver volume was determined using sets of axial images obtained preoperatively using computed tomography. The residual liver volume was calculated by subtracting the actual weight of the liver specimen from the total liver volume divided by the total liver volume.

Laboratory data: The plasma PT-INR value and serum levels of AFP, alanine transaminase (ALT) and hepatocyte growth factor (HGF) were analyzed using an autoanalyzer (JCA-BM2250, JEOL, Tokyo, Japan).

Immunohistochemistry of CK-7 positive LPCs in the liver: Liver specimens were obtained from 45 of the 73 patients with ALI/ALF. In order to evaluate the number of hepatic progenitor cells, CK-7 positive cells were identified using immunohistochemistry and then distinguished based on morphological findings, such as a small cell size and oval nucleus[4, 15]. The small CK-7 positive cells with oval nucleus was counted as LPCs in three high-power fields

and is presented as the total number. Immunohistochemical staining was performed using a Ventana HX System Discovery device with a DAB Map kit (Ventana, Tucson, AZ, USA) and an anti-CK7 antibody (Dako, Glostrup, Denmark).

Evaluation of the severity of liver tissue damage: The liver specimens used in the above analyses were assessed using H&E-stained sections according to a classification previously reported by others [4]. Briefly, the severity of liver tissue damage was expressed as a percentage of the overall parenchymal area: mild= less than 30% hepatocyte loss, moderate= 30% to 50% hepatocyte loss, severe= 50% to 75% hepatocyte loss and very severe= more than 75% hepatocyte loss.

Statistical analysis: The results are expressed as the mean and standard deviation. All statistical analyses were performed using the SPSS 17.0 software program (SPSS Inc., Chicago, IL, United States). Non-parametric tests (Kruskal-Wallis followed by Dunn's multiple comparisons) were used to evaluate the statistical significance of the results. A two-sided p value of <0.05 was considered to be statistically significant. Spearman’s correlation was used to assess the correlations.

Results

Clinical characteristics of the liver donors: The characteristics and resected liver volume of the 11 donors are summarized in Table 1. None of the patients exhibited any complications prior to surgery. All donors underwent laparoscopic and hand-associated hepatectomy, began to consume an oral diet on the second day after the operation and were discharged within 10 days without major complications. The residual liver volume for all donors was over 41.5% of the total liver volume. The gallbladder was resected in all cases.

The prothrombin time immediately recovered after partial hepatectomy without drastic elevation of the alpha fetoprotein level: The ALT and PT-INR values in the living donors were significantly higher on POD2 than on the day before the procedure ($p<0.01$, Figures 1A and B). However, both the PT-INR and ALT values quickly recovered by POD7. The PT-INR on POD2 was not associated with age, SLV, ALT or maximum AFP (data not shown). The AFP levels were not apparently elevated during the observation period in the LDLT donors (Figure 1C), whereas the HGF levels were significantly higher on POD1 than the baseline values ($p<0.05$, Figure 1d).

The number of CK-7 positive LPCs in the liver tissue was increased among mild to severe grade, but not very severe grade, of liver damage in the ALI/ALF patients: In order to assess the recruitment of LPCs in the liver tissue, the degree of CK7 positivity in the liver tissue was determined using immunohistochemistry in hepatocytes. Pathological examinations of the liver tissue were performed in 45 of the 73 patients with ALI/ALF, including both survivors and non-survivors (Supplemental Table 1). In one patient with ALF caused by drug-induced liver

injury, single CK7-positive small cells with oval nuclei appeared in the liver (Figures 2A). CK-7 positive LPCs were also detected in a patient with ALI of unknown origin (Figures 2B). The subjects were classified into four groups based on the severity of liver tissue damage: mild (<30%), moderate (30–50%), severe (50-75%) and very severe (>75%). The number of CK-7 positive LPCs in the liver increased in the moderate and severe cases**,** but not in the very severe cases (Figure 3), and significantly correlated with the serum AFP level (Figure 4; ρ=0.403, $p<0.01$).

Clinical characteristics of the patients with acute liver injury or failure: The prognosis, type of disease, etiology of ALI/ALF and AFP levels in the 73 patients evaluated in this study are summarized in Table 2. Because we focused on the optimal microenvironment for liver regeneration, the patients with ALI/ALF were divided into groups based on their prognosis. The patients with a poor prognosis were further stratified based on whether they had ALF without coma or ALF with a coma subacute type. The AFP levels in the non-surviving patients were not significantly different from those observed in the surviving patients (Table 2 and Figure 5A). The HGF levels were also not significantly different between the non-surviving patients and the surviving patients at the time of admission (Table 2).

The ALI/ALF patients whose AFP level peaked before the PT-INR exhibited a poorer prognosis: Eight of the 11 non-surviving patients exhibited a peak in the AFP level prior to the peak in the PT-INR (Figure 5B). In contrast, three of the 11 non-surviving patients and all of the surviving patients displayed an earlier peak in the PT-INR than the AFP level (Figure 5B). The serial changes in the serum AFP levels and PT-INR values in all patients with ALI/ALF are

summarized in Figure 5C. Seven of the eight patients presented with a PT-INR of > 1.5 in the late phase during hospitalization; the AFP levels also consistently decreased over the disease course in all eight patients. The serial changes in these parameters in a representative patient are presented in Figure 5D. The 76-year-old Japanese female, who had been diagnosed with ALF of unknown origin, was treated with steroid pulse therapy for three days. Although the PT-INR decreased around the 14th hospital day, an increase in the PT-INR and decrease in the AFP level were noted around the 28th hospital day (Figure 5D). The patient ultimately died due to liver failure.

Discussion

The present study demonstrated the following findings: (1) the serial changes in principal liver parameters after large volume hepatectomy for living donor liver transplantation showed that the liver function of the living donors quickly recovered after hepatectomy; (2) the number of LPCs in the liver was associated with the severity of liver tissue damage and positively correlated with the serum AFP levels in the patients with ALI/ALF; (3) the serum AFP levels were elevated in the patients with ALI/ALF, and the non-surviving patients exhibited a delayed recovery in the synthetic function of the liver and a decrease in the serum AFP level. Based on the present results, a sustained functional decline in the liver associated with ALI/ALF may lead to the suppression of LPC proliferation and/or inhibition of MH replication, thus resulting in a delayed recovery of the synthetic function. Although the detailed conditions required to promote LPC proliferation remain unclear, providing an optimal environment for LPC proliferation may yield a better prognosis for patients with ALF.

The poor prognosis of ALI/ALF is characterized by a collapse of the liver function, progressively induced by liver atrophy due to impaired liver regeneration [18-20]. Therefore, liver regeneration is considered to play an important role in the recovery of the liver function and increasing the liver volume. For the purpose of promoting liver regeneration, the development of a method to identify LPC recruitment to liver tissue is needed. Based on data showing a correlation between the serum AFP level and histological positivity of CK-7 positive LPCs in cases of ALI/ALF, we consider the serum AFP level to be a marker of the induction and proliferation of LPCs[14, 15].

In the present study, we evaluated living liver donors, as the participation of living donors allows the normal recovery process after partial hepatectomy to be monitored in healthy

humans. Precise monitoring of the results of liver tests in this study revealed several new insights regarding the recovery of a "healthy liver." Of the investigated liver function parameters, the serum ALT level is considered to reflect the degree of hepatocyte damage, while the PT-INR represents the functional mature hepatocyte mass. The early peak and subsequent rapid recovery of the ALT level after the operation suggest that hepatocyte damage subsides in the very early stage after PH (Figure 1A). In contrast, the uniformly fixed peak of PT-INR observed on POD 3 and ensuing gradual decrease in this parameter indicate that hepatocytes do not begin to function sufficiently until three days after surgery, at which time they rapidly recover their abilities (Figure 1B). Although PH resulted in the loss of functional hepatocytes in this study, liver damage from PH did not induce AFP elevation, which suggests the induction of LPCs in the liver. Although the serum AFP levels in the living donors were significantly elevated during hospitalization, the degree of elevation was not drastic (Figure 1C). A previous report also indicated that the AFP level is not dramatically increased following partial hepatectomy in humans [21]. Intriguingly, in the current study, the serum HGF levels were elevated on POD1 and then returned to the normal range within a few days (Figure 1D). Elevation of the HGF level around the early stage has also been reported in an animal model of partial hepatectomy [22]. These data suggest that mature hepatocytes, not progenitor hepatocytes, respond to the regeneration of PH and that HGF possibly contributes to this response during the early stage after PH in healthy humans as well as rodent models [7, 22, 23].

In the present analysis, the patients with ALF presented with hepatocyte necrosis in the liver. Considering the deteriorated liver function of these patients, including coagulopathy, they lacked a sufficient functional hepatocyte mass the liver. Against this background, the liver induced the AFP expression via LPCs through several signaling pathways [24-28]. Hence, the serum

AFP levels were substantially higher in the patients with ALI/ALF than in the donors who underwent PH (Figure 5A) and positively correlated with the number of LPCs in the liver in the patients with ALI/ALF (Figure 4). Importantly, there were no significant differences in the serum AFP levels between the surviving and non-surviving patients (Figure 5A). Therefore, LPC induction occurred in almost all of the ALI/ALF patients. In addition, both the donors and patients with ALI/ALF showed severe elevation of PT-INR due to the loss of the functional hepatocyte mass. However, the PT-INR values in the donors and surviving patients with ALI/ALF displayed a rapid recovery to the normal range, whereas the PT-INR recovery was delayed or absent in the non-surviving patients with ALI/ALF (Figures 5B and 5C). Taken together, these findings indicate that the liver damage associated with ALI/ALF promotes the induction of LPCs and that the number of LPCs decreases in association with the recovery of the liver function. It's important to note that histological severity does not always accord with survival although the association among histological severity, AFP and CK7 expression is presented. Thus, future studies would be needed for clarification about microenvironments of the liver with ALI/ALF.

According to the present results, we speculate that the decreased number of LPCs observed in the surviving patients was preceded by LPC differentiation to MHs and/or MH proliferation from recovered MHs. On the other hand, as the recovery of the liver function was delayed in the non-surviving patients, these individuals lacked sufficient MHs originating from LPC proliferation and/or MH replication of residual MHs. Therefore, the induction of sufficient MHs is a critical step in the recovery from massive hepatocyte necrosis resulting from ALI/ALF. Indeed, the progression of LPC proliferation in an *in vivo* model of liver injury has been shown to inhibit hepatocyte demise and improve patient outcomes [26, 29]. Therefore, agents promoting the

differentiation of LPCs to MHs may be good candidates as therapeutic targets in ALI/ALF patients with a poor prognosis.

In conclusion, the current data suggest that the residual liver in donors rapidly regains its function without deploying progenitor cells for tissue repair, thus suggesting that the serum of the donor after surgery possesses features associated with optimal conditions for mature hepatocyte proliferation. Liver damage, such as that observed in cases of ALI/ALF, promotes LPC induction, and a sustained functional decline in the liver may lead to the suppression of LPC proliferation or inhibition of MH replication, thereby resulting in a delayed recovery of the synthetic function and a consequent poor prognosis in patients with ALI/ALF. In order to appropriately treat such patients, the optimal conditions for a functional recovery of the liver based on LPC proliferation must be understood. The present study provides preliminary evidence regarding these conditions, and further studies should thus be performed to validate our findings.

Acknowledgments

The study was supported in part by Grants-in-Aid from the Ministry of Health, Labour and Welfare of Japan to the Intractable Hepatobiliary Diseases Study Group (#25461008). There are no conflicts of interest with regard to this work.

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Figure Legends

Figure 1. Serial changes in the laboratory data of the living donors for liver transplantation during the perioperative period

A, B, C and D: The serial changes in the alanine aminotransferase (ALT), prothrombin (PT-INR), alpha-fetoprotein (AFP) and hepatocyte growth factor (HGF) levels are presented in the indicated line graphs. The data were collected preoperatively until postoperative day 7. The open circles indicate outliers. All data are expressed as the mean ± SD; *P < 0.05, vs Pre; **P < 0.05, vs POD6; † P<0.05, vs POD3; ‡ P<0.05, vs POD1.

Figure 2. Evaluation of cytokeratin 7-positive cells in the patients with acute liver injury or failure

A and B: Liver specimens were obtained from the patients with drug-induced acute liver failure (A) and patients with acute liver injury of unknown origin (B). A and B: Cytokeratin 7 (CK7) staining, used as a marker of liver stem/progenitor cells, was evaluated using immunohistochemistry.

Figure 3. Comparison of the number of cytokeratin 7-positive cells based on the severity of hepatocyte necrosis

The patients evaluated in the immunohistochemical study (n=45) were classified into subgroups according to the degree of hepatocyte necrosis. The number of cytokeratin 7 (CK7)-positive cells per high-power field was significantly higher in the livers of the patients with moderate and severe hepatocyte necrosis than in the livers of those with mild or moderate hepatocyte necrosis. All data are expressed as the mean ± SD; **P*<0.05 vs Mild.

Figure 4. Correlation between the serum alpha fetoprotein level and the number of cytokeratin 7-positive LPCs in the patients with acute liver injury or failure

The horizontal axis shows the number of cytokeratin 7 (CK7)-positive LPCs per high-power field. The vertical axis shows the serum AFP levels on or around the day of the liver biopsy. The relationship between the serum AFP level and the number of CK-7 positive LPCs in the liver was investigated using Spearman's correlation coefficient test.

Figure 5. Comparison of the peaks in the alpha fetoprotein level and prothrombin time in the patients with acute liver injury or failure

A: The peak serum alpha-fetoprotein (AFP) levels were compared between the living donors and all patients with acute liver injury or failure (ALI/ALF) and the surviving and non-surviving patients with ALI/ALF. The open circles indicate outliers. B: The deceased and surviving patients with ALI/ALF were classified into two groups: “peak AFP after peak PT-INR” and “peak AFP before peak PT-INR.” C: Serial changes in the AFP levels (upper panel) and PT-INR values (lower panel) in the deceased and surviving patients. The open circles indicate outliers. D: Serial changes of the serum AFP level and plasma PT-INR in the 76-year-old Japanese female with ALF of unknown origin were presented. Right vertical axis indicated the level of AFP. Left vertical axis indicated the PT-INR value.

Supplemental table 1. The Characteristics of the Patients for Evaluated in the Immunohistochemical Study of the Liver

	Deceased (n=4)	Survived (n=41)
M:F	2:2	15:26
Types of Disease		
Acute liver injury (ALI)	0	14
Acute liver failure (ALF)	4	27
ALF without coma	(1)	(26)
ALF with coma, acute	(0)	(1)
ALF with coma, subacute	(3)	(0)
Cause of Disease		
HBV	0	5
Drug	2	8
Autoimmune	1	9
Other virus	0	2
Unknown	1	17

Forty five of the patients with acute liver failure or acute liver injury were divided into groups based on their prognosis; died or survived. The definition of each type of disease was described in the “Subjects” subsection of the “Materials & Methods” section.

Tables

Table 1. The Characteristics of the Donors for Liver Transplantation

Sex (M: F)	Age	Body mass index	Graft (right: left)	Residual liver volume (%)
4: 7	36.4 ± 3.4	22.4 ± 1.0	9: 2	41.5 ± 3.1

The residual liver volume was calculated as the actual liver graft weight divided by the liver volume estimated by computed tomography before surgery. All data are expressed as the means ± SD.

Table 2. The Characteristics of the Patients with Acute Liver Injury or Acute Liver Failure

		Deceased (n=11)	Survived (n=62)
M:F		8:3	33:29
Types of Disease			
Acute liver injury (ALI)		0	41
Acute liver failure (ALF)		11	21
ALF without coma		(4)	(19)
ALF with coma, acute		(0)	(2)
ALF with coma, subacute		(7)	(0)
Cause of Disease			
HBV		3	11
Drug		3	11
Autoimmune		1	11
Other virus		0	5
Unknown		4	24
alpha-fetoprotein	(ng/mL)	171.4 ± 65.0	215.4 ± 56.9
HGF	(ng/mL)	3.7 ± 0.6	2.5 ± 0.8

The 73 patients with acute liver failure or acute liver injury were divided into two groups based on their prognosis; died or survived. The definition of each type of disease was described in the “Subjects” subsection of “Materials & methods” section. All data are expressed as the means ± SD.

Figure 1

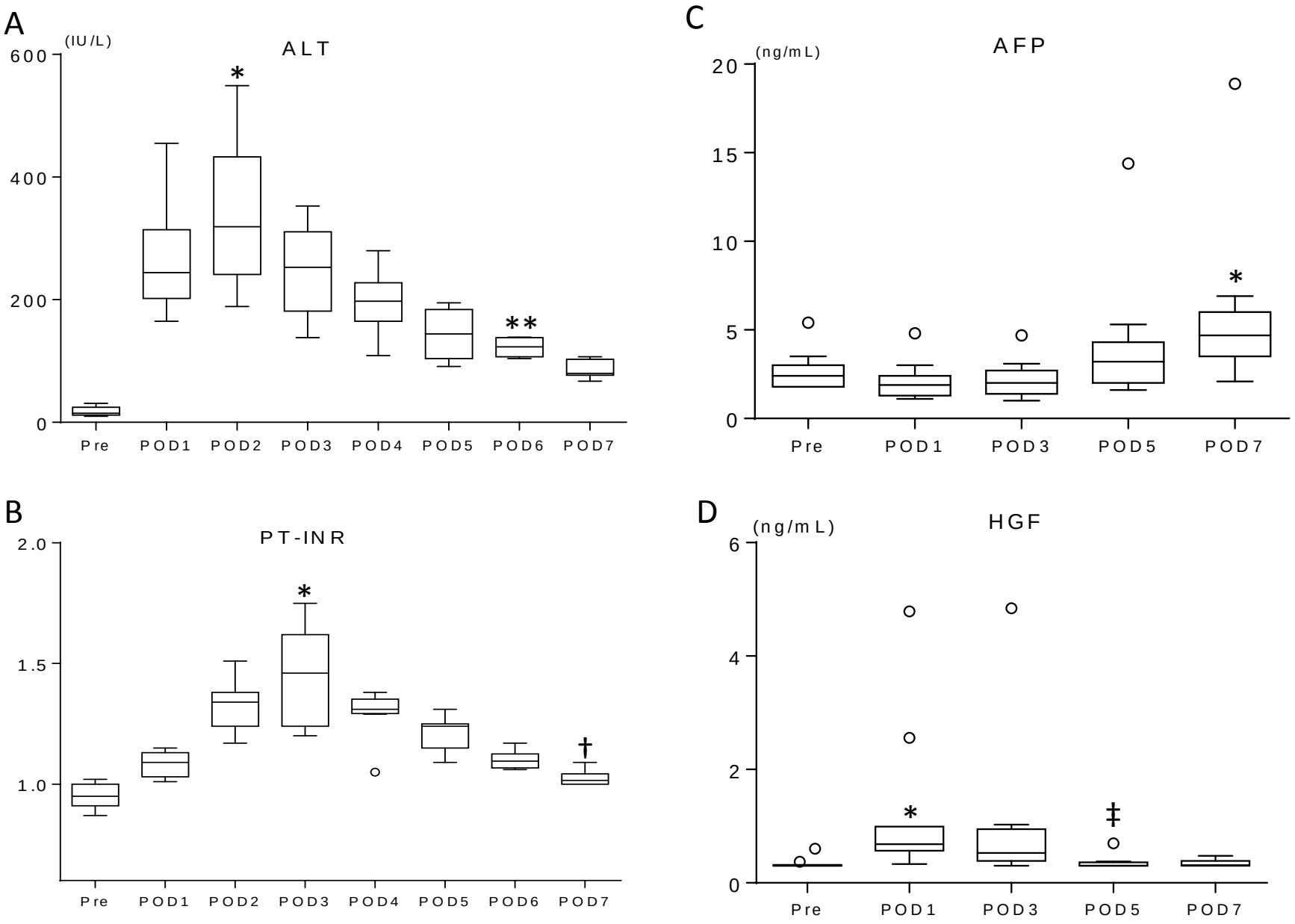
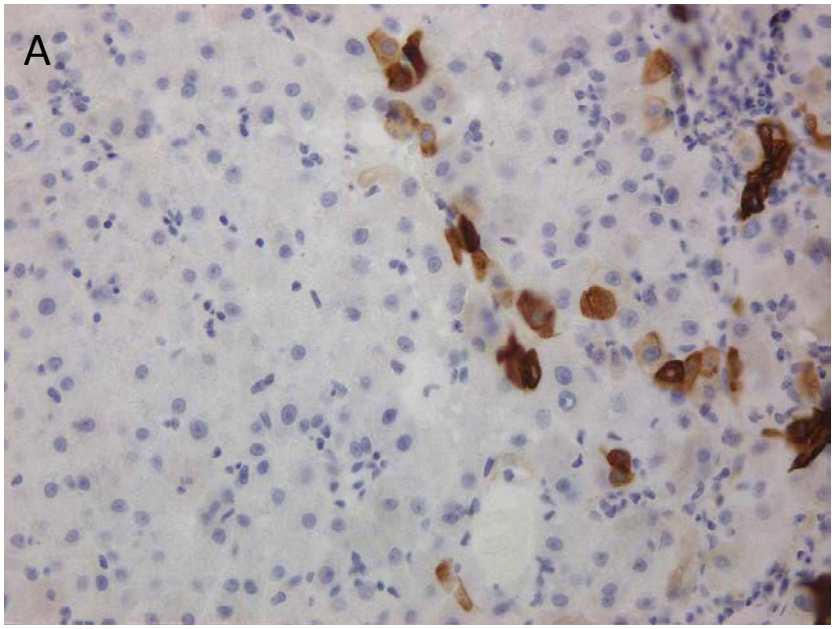

A
(IU/L)
ALT
600
400
200
0
*
**
Pre
POD1
POD2
POD3
POD4
POD5
POD6
POD7
B
PT-INR
2.0
1.5
1.0
*
†
Pre
POD1
POD2
POD3
POD4
POD5
POD6
POD7
C
(ng/mL)
AFP
20
15
10
5
0
*
Pre
POD1
POD3
POD5
POD7
D
(ng/mL)
HGF
6
4
2
0
*
‡
Pre
POD1
POD3
POD5
POD7

Figure 2



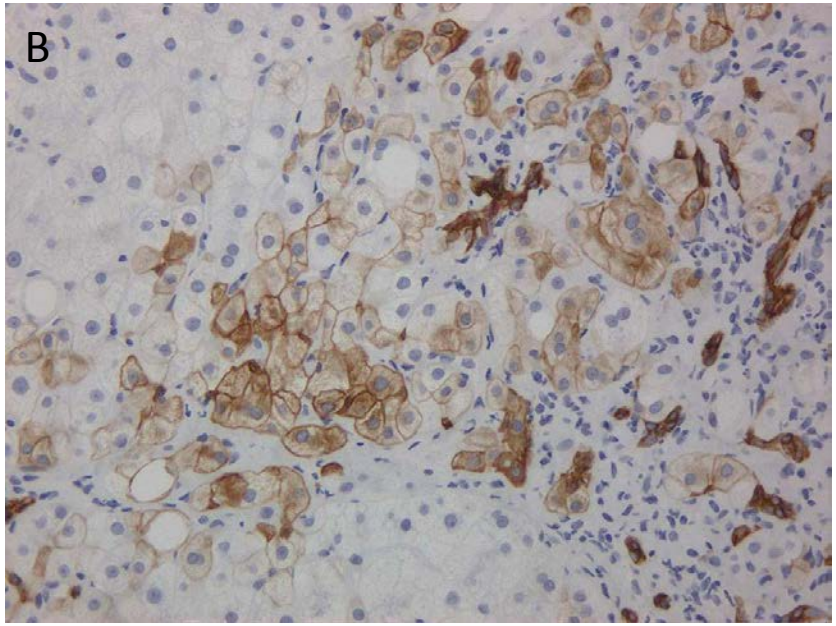


Figure 3

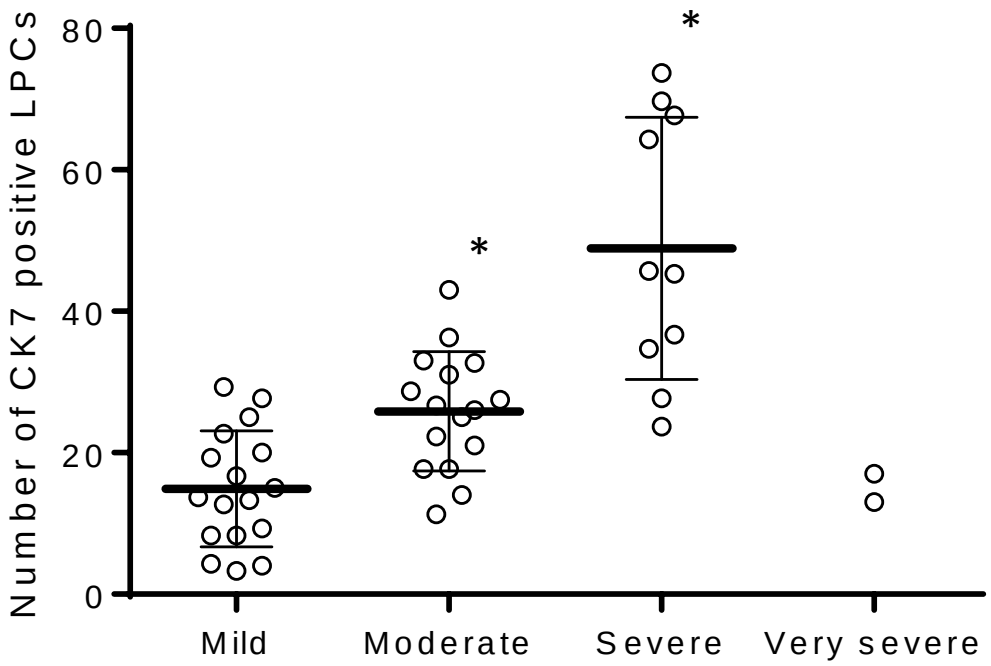

Number of CK7 positive LPCs
80
60
40
20
0
*
*
Mild
Moderate
Severe
Very severe

Figure 4

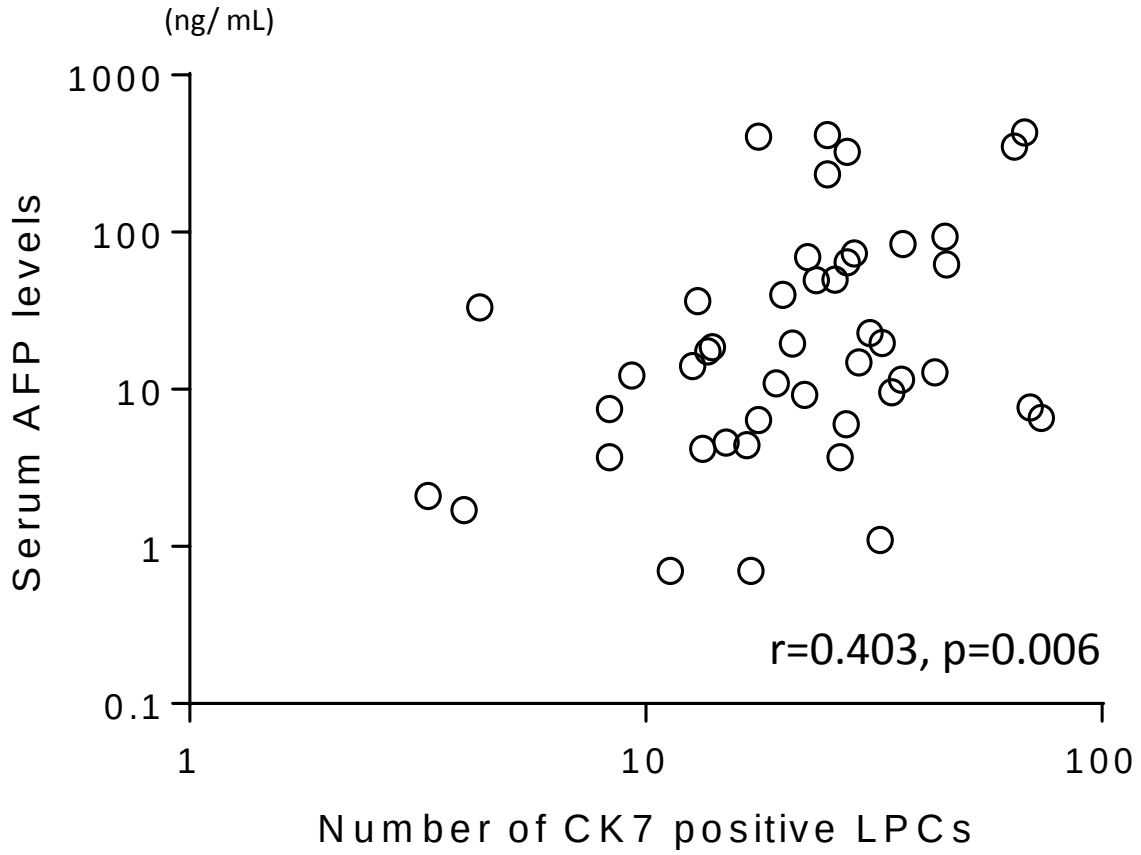
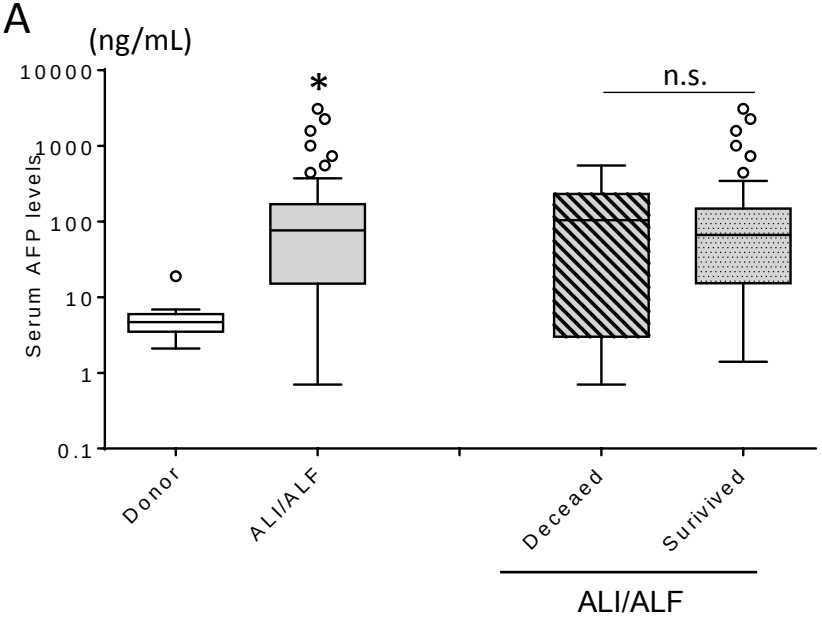

(ng/ mL)
1000
100
10
1
0.1
Serum AFP levels
r=0.403, p=0.006
1
10
100
Number of CK7 positive LPCs

Figure 5

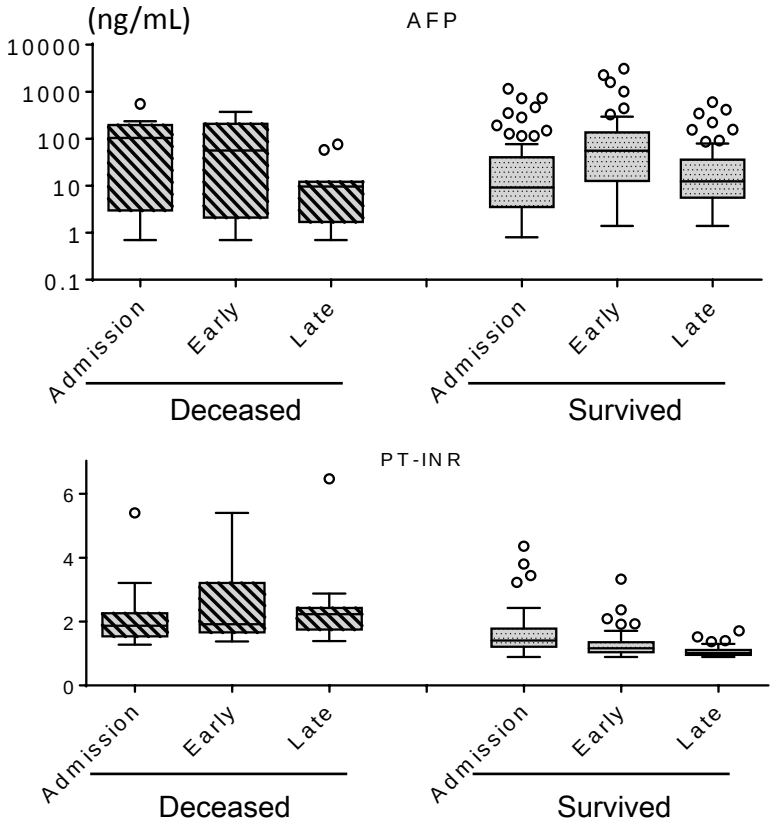
A



B

	AFP $_{peak}$ after PT-INR $_{peak}$	AFP $_{peak}$ before PT-INR $_{peak}$
Deceased	3	8
Survived	62	0

C



D