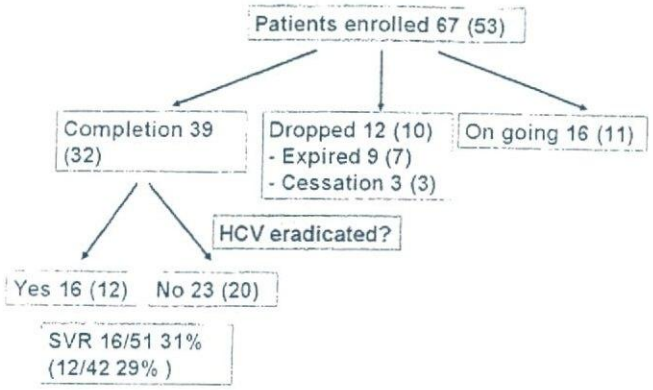

Figure 1. Results after preemptive antiviral therapy in University of Tokyo Hospital. *Numbers in parentheses* indicate those of genotype 1b. *SVR*, sustained viral response ratio. HCV eradicated? = Was the patient negative for HCV (<1000 copies/mL)?

spite application of granulocyte colony-stimulating factor (Gran; Sankyo Co Ltd, Tokyo, Japan), hemolytic anemia (hemoglobin <8 g/L), renal dysfunction (serum creatinine >2 mg/dL), depressive psychologic status, or general fatigue. The subjects were removed from the protocol if they could not continue the therapy for 12 months because of adverse effects or could not start the therapy as a result of early death.

Blood counts and liver function test results were checked every 2 weeks for the first month and at 4-week intervals thereafter. Serum samples were collected once a month for quantitative HCV RNA detection. Protocol liver biopsy was not performed. The log-rank test was used to compare the survival rate of the HCV-positive patients with the HCV-negative patients who underwent transplantation during the same period (n = 168).

Results

A total of 28 patients were excluded from the analysis (Figure 1). Twelve patients were removed from the protocol because of early death (n = 9) or because of drug cessation (n = 3). Another 16 patients are currently on the protocol and were therefore excluded from the analysis. Of the remaining 39 patients, 16 (41%) obtained a sustained virologic response. The cumulative 5-year survival of the HCV-positive patients was 84%, comparable with that of patients negative for HCV (n = 168, 86%).

Discussion

Because interferon is more effective in patients with a lower viral load,[6] initiating preemptive therapy before peak viral loads are reached is a rational approach. There is, however, a theoretical risk of increasing cellular rejection, as observed in kidney and liver transplantation.[7] Preemptive therapy during the early post-transplantation period with interferon in combination with ribavirin has been attempted in DDLT.

In a case series by Mazzaferro et al,[8] 36 recipients were treated with interferon alfa-2b (3 MU ×3 per week) and ribavirin (10 mg · kg^{-1} · day^{-1}). Treatment was started a median of 18 days after the operation and continued for 11 months. After a median follow-up of 52 months, the 5-year patient survival was 88%. Serum HCV RNA clearance was obtained in 12 patients (33%). No further antiviral treatment was required because of negative HCV RNA in serum and normal liver histology for a median of an additional 36 months. In another study,[9] 63 patients (<50% of screened cases) were randomized within 4 weeks after DDLT and treated for 48 weeks: 20 control subjects, 21 interferon alone, and 22 interferon and ribavirin. At 2 years, HCV RNA was negative in 13%, 13%, and 33%, respectively. Remarkably, there was no histologic recurrence in patients with a sustained viral response.

The association between LDLT and early HCV recurrence remains to be determined,[10] although most of the recent reports suggest that living donor graft has no effect on short-term outcome or severity of virus recurrence. Reports from New York-Presbyterian Hospital[11] indicate that the time to diagnosis of recurrent HCV is significantly shorter in LDLT. Other data indicate that the 5-year survival of HCV patients (n = 69) who undergo LDLT is 64%, which is comparable with that of DDLT patients (n = 202, 69%). The multicenter adult to adult LDLT cohort study (A2ALL) might soon provide answers to questions about recurrent HCV after LDLT and DDLT.[12]

In areas where the cavaderic organ source is almost negligible, LDLT must be selected as a therapeutic option, regardless of any potential additional risk. The results of LDLT for HCV cirrhosis in our hospital were comparable with those for non-HCV patients. If living donor graft is associated with early HCV recurrence and consequently poorer graft survival, an aggressive antiviral protocol should be performed to improve the outcome of LDLT for HCV. The present data indicate that the protocol after LDLT is safe and warrants a controlled study to confirm its benefit for graft survival.

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ORIGINAL ARTICLE

Is blood eosinophilia an effective predictor of acute rejection in living donor liver transplantation?*

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Keywords
acute cellular rejection, eosinophilia, liver transplantation, steroid.

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Summary

The association of blood eosinophilia with acute cellular rejection (ACR) after living donor liver transplantation has not been examined yet. The subjects were the 167 recipients who underwent liver biopsy (314 times). The blood eosinophil counts in the preoperative period ($n = 167$), 3 days before ($n = 314$) and on the day of biopsy ($n = 314$) were compared among the groups stratified by severity of ACR. Among 314 biopsy specimens, the 140 biopsy specimens were diagnosed with ACR. In the 140 ACR episodes, eosinophil counts before and after therapy was compared between the episodes that responded to therapy ($n = 80$) and those not ($n = 60$). The sensitivity and specificity of preoperative eosinophilia (eosinophil counts >130 mm^3) to predict ACR was 33% and 65%, respectively. The eosinophil counts >400 mm^3 3 days before and on the day of biopsy was associated with the severity of ACR ($P < 0.0001$). The sensitivity to predict ACR was 26% and 33%, and the specificity, 94% and 93%, respectively. There was no significant difference in changes of eosinophil counts between the steroid-responders versus the nonresponders. The present results suggested the limited role of eosinophilia as a predictor of ACR after living donor liver transplantation.

Introduction

In liver transplantation, acute cellular rejection (ACR) is still a major complication that can lead to mortality. Early diagnosis is necessary for prompt treatment, which must be based on liver biopsy. Several reports indicate a relationship between blood eosinophilia and acute rejection in liver transplantation [1–4]. Infiltration of eosinophils into the graft and peripheral blood eosinophilia might relate to ACR. In most studies, eosinophilia preceded ACR by 2–4 days [1,5]. One report demonstrated a close relationship between pretransplantation peripheral blood eosinophilia and postoperative ACR [6]. All of these reports, however, were based on data from deceased donor liver transplantation. In living donor liver transplantation (LDLT), the relation between eosinophilia and ACR has not been examined.

It is controversial that whether there is a difference in the frequency of ACR rejection between LDLT and deceased donor liver transplantation [7,8]. Some authors

reported lower incidence of steroid resistant [9] or late onset ACR [10] after LDLT. This might be due in part to the length of graft cold ischemic time [7] or the HLA haplotype matching in living-related donor cases [9]. The difference in the frequency and severity of ACR between deceased donor liver transplantation and LDLT led us to examine whether blood eosinophilia can predict ACR after LDLT.

Patients and methods

Patients

Subjects were 305 consecutive patients that underwent LDLT at our hospital. Two patients complicated by chronic rejection and eight patients who underwent emergent transplantation were excluded. Of the remaining 299 patients, biopsies were performed in 167 patients consisting 131 adults [47 ± 1.0 (mean ± SE) in age] and 36 children (6.3 ± 1.0 years old). The indications for LDLT included HCV related cirrhosis ($n = 39$), hepatitis B virus related cirrhosis ($n = 14$), cirrhosis of other etiologies ($n = 7$), biliary atresia ($n = 37$), primary biliary cirrhosis ($n = 33$), primary sclerosing cholangitis ($n = 4$), autoimmune hepatitis ($n = 5$), fulminant hepatic failure ($n = 15$), metabolic diseases ($n = 7$) and others ($n = 6$).

Acute cellular rejection was diagnosed based on biopsy and graded into four classes according to the Banff scheme [11] [Grade 0 (G0): no evidence of rejection, Grade 1 (G1): mild rejection, Grade 2 (G2): moderate rejection, Grade 3 (G3): severe rejection; Fig. 1]. Postoperative immunosuppression was achieved with tacrolimus and methylprednisolone [12]. Tacrolimus was administered to control the trough level at approximately 16–18 ng/ml for the first week, and gradually tapered to 5–8 ng/ml over 6 months. Steroids were also tapered day by day from 3 mg/kg on the first postoperative day to 0.3 mg/kg on the fifteenth postoperative day. The dose was then decreased slowly to 0.06 mg/kg over 6 months. When the diagnosis of ACR was confirmed, 20 mg/kg of methylprednisolone was administered, which was then tapered by reducing the dose by half each day until the same dose as before therapy was achieved.

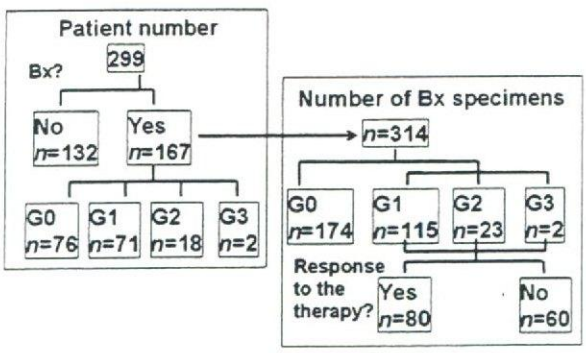

Figure 1 The numbers of the patients and liver specimens studied. Bx, liver biopsy.

Biopsy was performed when levels of all blood liver function tests, including transaminases, bilirubin, gamma-glutamyl transpeptidase and alkaline phosphatase, elevated. No protocol biopsy was performed.

Analysis

The relationship between preoperative eosinophilia and ACR stratified by grade was examined. Preoperative eosinophilia was defined as absolute eosinophil count (AEC) >130 mm^3 [6]. The relationship of eosinophilia 3 days before or on the day of biopsy and ACR grouped by grade was examined. Here, the number of eosinophils was evaluated as AEC or relative eosinophil count (REC: AEC × 100/total leukocyte count). Postoperative eosinophilia was defined as AEC more than 400/mm^3 and/or REC more than 4% [3].

Pre- or post-treatment AEC, REC, and eosinophil count changes were compared between patients that responded to the treatment and those that did not. Treatment was judged successful when transaminase and bilirubin levels improved to normal levels and did not increase again during the following month. If liver dysfunction recurred again within 1 month, followed by biopsy-proven ACR, the treatment was defined as failed.

Statistics

Data were expressed as mean ± SE. Sensitivity and specificity of eosinophilia was calculated for the prediction of ACR or improvement of ACR. AEC and REC were compared between groups using an unpaired t-test or one-way ANOVA. A P-value of <0.05 was considered statistically significant.

Results

Preoperative eosinophilia

An average of 2.2 biopsies were performed per patient. The interval between transplantation and biopsy was on 32 ± 2.0 days. The degree of ACR included G1 in 71, G2 in 18 and G3 in two patients. Other 76 patients showed only indeterminate evidence of ACR in every biopsy samples and were classified to G0. Preoperative AEC of the patients with and without postoperative ACR was 168 ± 27/mm^3 and 114 ± 16/mm^3, respectively ($P = 0.78$). There was no significant difference in REC (G0,

2.6 ± 0.34%; G1, 2.9 ± 0.52%; G2, 3.7 ± 0.98%; $P = 0.54$) or AEC (G0, 114 ± 18/mm^3; G1, 159 ± 27/mm^3; G2, 217 ± 51/mm^3; $P = 0.10$) among the G0–G2 grades of ACR (Fig. 2a). Two G3 specimens were excluded from the analysis. Preoperative eosinophilia predicted ACR with a sensitivity of 33% and a specificity of 65%, respectively (Table 1).

Eosinophilia 3 days before the biopsy

Eosinophil counts 3 days before the biopsy were available for 314 biopsy samples (Fig. 1), graded as G1 ($n = 115$) and G2 ($n = 25$). The other 174 samples showed indeterminate evidence of ACR and were classified to G0. The major findings the samples included nonspecific hepatitis with or without cholestasis ($n = 122$), congestion ($n = 15$), recurrent hepatitis C ($n = 15$) only mild lymphocyte infiltration or endothelialitis ($n = 5$), cholangitis ($n = 3$) and no abnormal findings ($n = 14$). REC and AEC 3 days before biopsy in patients complicated with ACR were 2.5 ± 0.3% and 234 ± 33/mm^3, respectively. REC and AEC in patients without ACR were 0.8 ± 0.1% and 77 ± 12/mm^3, respectively. When the biopsy samples were grouped according to the severity of ACR, there was a significant difference between the groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2b). Eosinophilia (REC > 4%) 3 days before the biopsy predicted ACR with a sensitivity of 26% and a specificity of 94%, respectively (Table 1).

Eosinophilia on the day of biopsy

Eosinophil counts on the day of the biopsy were available for 314 biopsy samples. The REC and AEC on the day of the biopsy with findings of ACR were 3.3 ± 0.3% and 312 ± 35/mm^3, respectively, being significantly higher than those without ACR ($n = 174$, 0.8 ± 0.1%, $P < 0.0001$ and 78 ± 13/mm^3, $P < 0.0001$). When biopsy episodes were grouped according to the severity of ACR, there was a significant difference between groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2c). Eosinophilia (REC > 4%) on the day of biopsy predicted ACR with a sensitivity of 33% and a specificity of 93%, respectively (Table 1).

Eosinophil count in response to treatment

Eosinophil count changes (count 1 week after treatment minus that just before treatment) could be calculated in the 140 biopsy episodes. Of these, 80 were responsive to steroid recycling therapy and 60 were resistant. Pretreatment REC and AEC were 2.8 ± 0.4% and 226 ± 35/mm^3 in the responding group and 4.0 ± 0% and 426 ± 65/mm^3 in the nonresponding group, respectively. Post-treatment REC and AEC were 2.3 ± 0.5% and 176 ± 32/mm^3 in the responding group and 2.6 ± 0.6% and 202 ± 55/mm^3 in the nonresponding group, respectively. There was a significant difference between groups in the pretreatment AEC ($P = 0.04$), but not in pretreatment REC ($P = 0.07$), post-treatment REC ($P = 0.49$), or post-treatment AEC ($P = 0.48$).

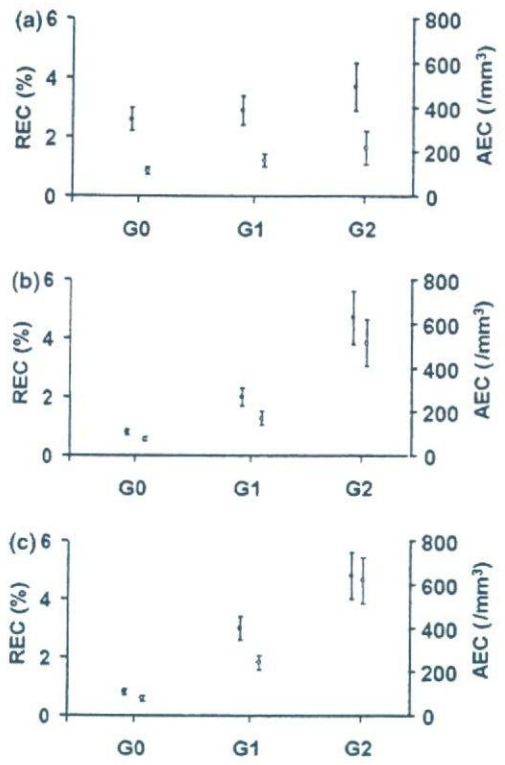

Figure 2 Relative (REC, thick bar and closed circle) and absolute eosinophil counts (AEC, thin bar and open circle) stratified by grade of rejection at preoperative (a) $n = 197$; 3 days before the biopsy (b) $n = 314$; and on the day of biopsy (c) $n = 314$. $P < 0.0001$ after comparison among the groups in the analyses of (b) and (c).

Table 1. Significance of eosinophil counts to predict acute cellular rejection.

Conditions	Events	Results	Sensitivity (%)	Specificity (%)
Pre-Tx	AEC > 130	ACR	33	65
Before Bx	REC > 4	ACR	26	94
	AEC > 400		20	95
On Bx	REC > 4	ACR	33	93
	AEC > 400		28	97
Before and after SRT	Decreased REC	Improvement of ACR	45	50
	Decreased AEC		50	43

Tx, transplantation; Bx, biopsy; SRT, steroid recycle therapy; ACR, acute cellular rejection; AEC, absolute eosinophil count; REC, relative eosinophil count.

Relative eosinophil count decreased in 36 and 30 treatments in the responding and nonresponding groups, respectively, whereas AEC decreased in 40 and 34 treatments. A decrease in REC or AEC predicted successful treatment of ACR with a sensitivity of 45% or 50% and a specificity of 50% or 43% (Table 1).

Discussion

Few studies have evaluated whether preoperative eosinophilia predicts ACR [6]. Nagral *et al.* [2] reviewed 129 biopsy cases. They demonstrated that there was no association between preoperative eosinophil count and the severity of ACR. They also demonstrated that AEC 1 or 2 days before or on the day of biopsy predicted ACR with low sensitivity (30.3–37.5%) and high specificity (83.3–91.8%). In our study also, eosinophilia both 3 days before and on the day of biopsy predicted ACR with low sensitivity and high specificity.

In contrast, Hughes *et al.* [13] emphasized that monitoring blood eosinophil count and serum eosinophil cationic protein was useful for early ACR diagnosis because they increase 2–3 days earlier than serum transaminase or alkaline phosphatase levels. Foster *et al.* [14] reported high sensitivity and specificity of blood eosinophilia in predicting ACR when they combined elevated serum transaminase or alkaline phosphatase levels. The exact reason for the discrepancy remains unclear, but might be due to a different dose of methylprednisolone for basal immunosuppression in our protocol: 3.0 mg/kg on the first postoperative day versus 1.5 mg/kg in Foster's report. The baseline eosinophil numbers might be decreased because of higher doses of steroid [15].

Our results indicated a higher pretreatment AEC in the steroid nonresponding ($426 \pm 65/mm^3$) compared with that of the responding group ($226 \pm 35/mm^3$, $P = 0.04$). They may support the phenomenon that the eosinophil count before or on the day of biopsy correlated well with the grade of ACR. A similar association was also reported by Barnes *et al.* [3] in liver transplantation and Trull *et al.* [15] in cardiac and lung transplantation. However REC was not a predictor of the response to the steroids, indicating the association between eosinophil counts before the treatment and the response to the treatment was not to be firm. Additionally the decrease in REC and AEC was not useful for predicting the effect of steroids on ACR in our series. Our results revealed a significant decrease in REC and AEC after steroid recycle therapy irrespective of the response to therapy. The finding might be explained by the hypothesis that steroids downregulate eosinophilia [16].

In summary, eosinophilia in the preoperative period, 3 days before and on the day of biopsy, predicted consequent ACR with high specificity, but low sensitivity. The present results suggested the limited role of eosinophilia as a predictor of ACR after LDLT.

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Should living donor liver transplantation be offered to patients with hepatitis C virus cirrhosis?

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Cirrhosis secondary to hepatitis C virus (HCV) infection is a leading indication for living donor liver transplantation (LDLT) among adults in the United States, Europe, and Japan [1,2]. LDLT is now a common alternative procedure to deceased donor liver transplantation (DDLT), which reduces waiting-time mortality in an era of deceased donor shortage. By June 2003, 1275 LDLT cases were recorded in the European Liver Transplantation Registry [4]. The 3-year graft survival rates were 71%, although the survival rates of the HCV-positive patients are unknown. In the United States [5], 1526 adult LDLT cases were performed by May 2004. HCV is the most common indication for LDLT and the number of HCV-positive patients is stable, approximately 100 per year between 2000 and 2002. According to the Japanese Liver Transplantation Society [6], the number of LDLT patients has increased over time and 1063 adult LDLT procedures were performed in Japan by the end of 2002.

One of the hottest debates is the possibility of increased severity of recurrent HCV in LDLT patients. The benefit of LDLT might be offset if the outcome of LDLT for HCV patients is worse than that of DDLT. In the present manuscript, we review studies of LDLT and DDLT for HCV. Our results of LDLT for HCV are also reported herein.

1. Live donor as a risk factor for early recurrence?

1.1. Pros

At the American Transplant Congress held in Chicago in 2002, the UCLA group [7] reported that the time interval to HCV recurrence in LDLT ($n=11$) was significantly shorter than in DDLT patients ($n=510$) (Table 1). Another group from the University of Colorado [8] reported that serum alanine aminotransferase and total bilirubin levels increased more rapidly after LDLT ($n=24$) than after DDLT ($n=41$). Those preliminary reports indicate that more intensive antiviral therapy might be necessary for recipients of living donor grafts. At the American Transplant Congress in 2004, two reports indicated a disadvantage of LDLT for HCV. One report from Barcelona [9,10] compared the results of LLDT ($n=22$) and DDLT ($n=95$). LDLT patients had younger donors, less graft steatosis, more frequent biliary complications, and earlier and more severe recurrent acute hepatitis. The other report from Cedars Sinai Medical Center [11] indicated that the time to diagnosis of recurrent HCV was significantly shorter in LDLT. Data from Colombia University [12,13] indicated that cholestatic hepatitis or severe recurrence occurs more frequently in LDLT.

A possible cause includes better HLA matching between donor and recipient. Because cellular immune reactions restricted by both HLA class I and II antigens are involved in the recognition of HCV peptides [14], HLA matching between donor and recipient could potentially increase damage to the graft from recurrent viral infections by facilitating host recognition of viral antigens [3]. Recently, a beneficial effect of a complete HLA-DQ mismatch was reported in 14 patients after transplantation for HCV cirrhosis [15]. Another possible cause might be related to liver regeneration [16]. In vitro, HCV internal ribosome entry site activity and replication were higher in actively dividing cells, and it is possible that viral translation is enhanced by factors that stimulate the regeneration of hepatocytes. Moreover, there are experimental data suggesting that liver regeneration induces LDL receptor

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Abbreviations: DDLT, deceased donor liver transplantation; HCV, hepatitis C virus; LDLT, living donor liver transplantation.

Table 1
Comparison between living and deceased donor liver transplantation (LDLT and DDLT) for hepatitis C virus cirrhosis

Study			N		Dif[a]	Protocol biopsy	Findings
Author	Year	Institution	LDLT	DDLT			
Ghobrial [7]	2002	UCLA	11	510	Yes	No	Rec in 86% of LDLT and 30% of DDLT during 1 year
Taniguchi [8]	2002	U. of Colorado	24	41	Yes	No	90 days (LDLT) and 168 days (DDLT) until Rec ($P<0.05$) Higher AST level in LDLT until 30POD
Gaglio [12]	2003	Colombia U.	23	45	Yes	No	Cholestatic hepatitis in 17% of LDLT and 0% of DDLT ($P=0.001$). No significant difference in incidence of Rec.
Garcia-Retorillo M [9,10]	2004	Hopital Clinic, Barcelona	22	95	Yes	Yes	Severe Rec in 41% of LDLT and 18% of DDLT. Higher ALT levels after 1 and 3 months
Pan [11]	2004	Cedars Sinai Medical C.	30	37	Yes	No	Earlier Rec in LDLT (161 days) than DDLT (295 days, $P<0.006$). No significant difference in 1 year survival
Gaglio [13]	2004	Colombia U.	31	72	Yes	No	More frequent Rec in 87% of LDLT and 70% of DDLT ($P=0.008$). No significant difference in time to Rec.
Trotter [17]	2001	Mount Sinai Medica C.	41	39	No	No	No significant difference in graft survival.
Schiffman [18]	2003	Virginia commonwealth U.	22	53	No	Yes	79% patient survival in LDLT and 91% in DDLT during 3 year (NS). No significant difference in inflammation score in liver specimen after 3 years
Vlierberghe [19]	2003	Ghent U.	17	26	No	No	No significant difference in frequency of (35% vs 38%), time (227 days vs 158 days) to Rec or 1 year survival.
Rodriguez-Luna [23,24]	2003, 2004	Mayo Clinic	9	20	No	Yes	No significant difference in frequency and severity of Rec after 4 and 12 months.
Vlierberghe [25]	2004	Ghent U.	17	26	No	No	Rec in 35% of LDLT and 38% of DDLT during 1 year ($P=0.1$)
Gordon [20]	2004	Lahey Clinic Medical C.	19	47	No	No	No difference in severity of Rec and 3-year patient survival.
Fahmy [21]	2004	NYU Medical C.	33	52	No	No	No significant difference in incidence of Rec or cholestatic hepatitis.
Russo [22]	2004	UNOS data	279	3955	No	No	87% 1-year patient survival in both.
Bozorgzadeh [27]	2004	Rochester U.	35	65	No	No	Rec in 77% of LDLT and 72% of DDLT during 1 year (NS), 89% patient survival in LDLT and 75% in DDLT during 39 months (NS)

Abbreviations: Rec, Virus recurrence; AST, asparate aminotransferase; ALT, alanine aminotransferase; C, Center; U, University: UNOS, United network for organ sharing; NS, not significant.

[a] Difference in short-term outcomes or severity of virus recurrence between living and deceased donor liver transplantation.

expression, which might facilitate HCV entrance into the hepatocytes.

1.2. Cons

Some groups reported comparable data between LDLT and DDLT for HCV [17–21]. Russo and colleagues [22] compared patient and graft survival in recipients transplanted for chronic HCV who received a living donor organ ($n=279$) and deceased donor organ ($n=3955$) using the United Network for Organ Sharing liver transplant database. One-year patient survival was 87% in both groups and 2-year patient survival was 83 and 81% in the living donor group and deceased donor group ($P=0.68$), respectively. Analyses from the Mayo Clinic [23,24] and Gent University [25] failed to show a negative impact of live liver on the results of liver transplantation for HCV-related cirrhosis.

The data indicating poorer outcome with LRLT should be interpreted with caution because of the important clinical distinction between LDLT and DDLT recipients. At the time of transplantation, the LDLT group recipients are far less sick than their DDLT group counterparts [26]. The LDLT ($n=35$) and DDLT ($n=65$) data from a single institution, Rochester University, were examined [27]. Patient survival, graft survival, rate of HCV recurrence, severity of HCV recurrence, graft loss from HCV, and interval for recurrence in DDLT and LDLT were similar.

According to the data from Russo et al. [22], from 1999 to 2000 the 1-year patient survival in the LDLT group increased from 69 to 90% ($P=0.04$), and 1-year graft survival increased from 63 to 79% ($P=0.16$). In contrast, in the DDLT group, 1-year patient and graft survival did not substantially change from 1999 to 2000. As a result, 1-year survival rates became similar between the LDLT and DDLT groups in 2000. The results indicated an experience effect and learning curve on outcomes after LDLT for HCV. Therefore, initial reports indicating poorer results of LDLT might be due to technical problems from a lack of experience. Thus, recent data indicating similar results between LDLT and DDLT might be due to the increased experience with LDLT.

2. Management of HCV after LDLT

2.1. Therapy for reccurrence in DDLT

If HCV recurs earlier and more severely after LDLT, a specific strategy for preventing the detrimental effects of HCV on living donor grafts must be developed. One strategy might be aggressive treatment for HCV. Treatment of recurrent HCV disease with interferon and ribavirin after DDLT is used in some centers [28–30] (see reviews from Berenguer and Negro in this FORUM). One standard regimen includes interferon-alpha 2b (3 MU×3 per week) and ribavirin (1000 mg/day) for 6 months. In a recent trial, polyethylene glycol-conjugated interferon therapy was used [31–33], with a sustained viral response rate ranging from 13 to 31%.

2.2. University of Tokyo experience [32]

We have performed preemptive therapy for LDLT patients for HCV. From July 1996 to July 2002, 23 patients underwent LDLT for HCV cirrhosis at the University of Tokyo Hospital. All the patients preemptively received antiviral therapy consisting of interferon-alpha 2b and ribavirin, which was started approximately 1 month after the operation. The therapy was given for 12 months after the first negative HCV-RNA test. The patients were then observed without the therapy for 6 months (Group 1). The therapy was continued for at least 12 months even when the HCV-RNA test remained positive (Group 2). The subjects were removed from the protocol if they could not continue the therapy for 12 months due to adverse effects or could not start the therapy due to early death.

Eight patients were removed from the protocol. Nine patients were assigned to Group 1 and the other six to Group 2. The sustained virologic response ratio was 39% (9/23). There was a significant difference between the groups in the histologic activity score 1 year after the therapy. The cumulative 3-year survival of the HCV-positive patients was 85%, comparable with that of patients negative for HCV ($n=93$, 90%). The present study is preliminary, but the results warrant a randomized protocol to examine the feasibility of preemptive therapy for LDLT.

In conclusion the association between LDLT and early HCV recurrence remains to be determined, although most of the recent papers suggest that live donor graft had no effect on short-term outcome or severity of virus recurrence. The multicenter adult to adult LDLT cohort study (A2ALL) might soon provide some answers to the questions about recurrent HCV after LDLT and DDLT [33].

In areas where the cavaderic organ source is almost negligible, LDLT must be selected as a therapeutic option regardless of any potential additional risk. The results of LDLT for HCV cirrhosis in our hospital were comparable with those for non-HCV patients. If living donor graft is associated with early HCV recurrence and consequently poorer graft survival, an aggressive antiviral protocol could be performed to improve the outcome of LDLT for HCV.

In Western countries, the benefits of LDLT should not be overlooked and include reduced waiting-time mortality and a potential cure for some patients with hepatocellular carcinoma who might otherwise develop an incurable disease awaiting DDLT [41]. These real benefits of LDLT should be considered before making a decision about the risk of recurrent HCV with LDLT [33].

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Forum Feedback

MELD is not enough—enough of MELD?

To the Editor:

The recent review [1] on MELD confirms that interest in assessing prognosis in cirrhotic patients has been revived. However, several drawbacks of MELD, which deserve some comment, have not been considered.

Firstly, use of MELD for allocation is a 'justice' and not a 'utility' score, as it does not consider outcome after liver transplantation (LT). One reason is that donor factors are not considered [2]. As a result, both pre-LT MELD and change in MELD [3] do not correlate with post-LT survival, with only a *c*-statistic of 0.58 in the UK [4]. In the USA, survival after transplantation was unchanged [1]. Secondly, the *c*-statistic for 3-month survival on the waiting list is as low as 0.75 [5]. Use of MELD outside the USA, has also given poor predictive accuracy in individual patients and poor generalisability [6].

Thirdly, the component variables of MELD may be difficult to assess, which may be one reason for poor

Clinical utility of quantitative RT-PCR targeted to α1,4-*N*-acetylglucosaminyltransferase mRNA for detection of pancreatic cancer

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α1,4-*N*-Acetylglucosaminyltransferase (α4GnT) is a glycosyltransferase responsible for the biosynthesis of α1,4-GlcNAc-capped *O*-glycans, and is frequently expressed in pancreatic cancer cells but not peripheral blood cells. In the present study, we tested the clinical utility of α4GnT mRNA expressed in the mononuclear cell fraction of peripheral blood as a biomarker of pancreatic cancer. Total RNA isolated from the peripheral blood mononuclear cells from 55 pancreatic cancer patients, 10 chronic pancreatitis patients, and 70 cancer-free volunteers was analyzed quantitatively by reverse transcription-polymerase chain reaction with primers specific for α4GnT, and the expression level of α4GnT mRNA relative to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured. When the ratio of α4GnT to GAPDH transcripts exceeded a defined cut-off value, patients were considered to have pancreatic cancer. By these standards, 76.4% of the pancreatic cancer patients were detected by this assay. A strong correlation was obtained between positivity in this assay and the expression of α4GnT protein detected immunohistochemically in pancreatic cancer tissues resected subsequently, suggesting that α4GnT mRNA detected in the peripheral blood is derived from circulating pancreatic cancer cells. Although increased levels of α4GnT mRNA was detected in 40.0% of chronic pancreatitis patients and 17.1% of cancer-free volunteers, the expression levels were significantly lower than those seen in pancreatic cancer patients. These results suggest that quantitative analysis of α4GnT mRNA expressed in the mononuclear cell fraction of peripheral blood will contribute to the detection of pancreatic cancer. (*Cancer Sci* 2006; 97: 119–126)

Pancreatic cancer is one of the most intractable malignancies.[1,2] In particular, the 5-year survival rate of this neoplasm is the lowest of all types of cancer, and it is the fifth leading cause of cancer death in Japan.[3] The poor prognosis of pancreatic cancer is largely attributable to the difficulty in diagnosis of the disease at relatively early stages as well as the highly invasive character of the cancer cells, regardless of the tumor size. In fact, the vast majority of pancreatic cancer patients are diagnosed at advanced stages associated with clinical manifestations such as jaundice and back pain, likely due to the limitation of tumor markers available for the diagnosis of pancreatic cancer at potentially curable stages.[4] Several well-established biomarkers, including CEA,[5] CA19-9,[6] DU-PAN-2[7,8] and Span-1,[9] are available for the detection of pancreatic cancer, but it is also true that these biomarkers are not elevated in certain numbers of pancreatic cancer patients. Thus, in order to detect pancreatic cancer more efficiently, it is necessary to identify novel biomarkers that will be useful for its diagnosis.[10,11]

Mucous glycoproteins secreted from the gastroduodenal mucosa are heavily glycosylated and protect the mucosa against various pathogens and physical stresses. Among the oligosaccharides found in human gastrointestinal mucins, α1,4-GlcNAc-capped *O*-glycan is unique because its expression in normal tissues is limited to gastric gland mucous cells, Brunner's gland of the duodenal mucosa and accessory gland of the pancreaticobiliary tract.[12] Interestingly, this unique *O*-glycan is expressed frequently in neoplastic cells such as carcinomas of the stomach, bile duct and pancreas, as well as pancreatic intraepithelial neoplasia (PanIN-I, PanIN-II and PanIN-III),[13] thus it is regarded as a tumor-associated carbohydrate antigen for these tumors.[12] Recently we isolated a cDNA encoding human α4GnT, which is responsible for the biosynthesis of α1,4-GlcNAc-capped *O*-glycans, by expression cloning from a gastric mucosa cDNA library.[14] We subsequently demonstrated that α4GnT is expressed in the Golgi of gastric gland mucous cells and Brunner's glands in normal gastroduodenal mucosa as well as the Golgi of adenocarcinoma cells such as gastric, pancreatic and biliary tract cancers expressing α1,4-GlcNAc-capped *O*-glycans.[15–17]

Our previous study demonstrated that neither α4GnT RNA nor protein is detectable in the normal peripheral blood cells.[17] Thus, we quantitatively measured the expression levels of α4GnT mRNA in the mononuclear cell fraction of peripheral blood obtained from gastric cancer patients using RT-PCR and demonstrated that this assay is useful to detect, as well as monitor, gastric cancer.[17] The present study extends this assay for detection of pancreatic cancer using a technically

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Abbreviations: α4GnT, α1,4-*N*-Acetylglucosaminyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H. pylori, Helicobacter pylori; MTC, multiple tissue cDNA; ROC, receiver operating characteristic; RT-PCR, reverse transcription–polymerase chain reaction; TAMURA, 3′-6-carboxy-*N*, *N*,*N*′,*N*′-tetramethylrhodamine; TBS, Tris buffered saline.

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improved modification. Specifically, levels of α4GnT mRNA in the mononuclear cell fraction of peripheral blood from pancreatic cancer patients were determined quantitatively using multiplex PCR employed to detect simultaneously both α4GnT and an internal standard gene in a single reaction tube.

Materials and Methods

Clinical samples

The present study involved 55 pancreatic cancer patients (34 men and 21 women; age range 45–92 years [mean ± SE, 68.5 ± 9.8 years]). For the reduction of jaundice, a drainage tube was placed in the common bile duct of 16 of 32 patients whose tumors were located in the pancreatic head, whereas none of the 23 patients whose tumor was located in the body or tail of the pancreas received such drainage. In addition to the pancreatic cancer patients, samples from 10 chronic pancreatitis patients (10 men; ages ranging from 55 to 75 years [65.8 ± 7.6]) and 70 volunteers (70 men; ages ranging from 31 to 90 years [69.4 ± 1.4]) were analyzed. These volunteers underwent a health screening and were verified to be cancer-free by routine examinations including abdominal ultrasonography. Written informed consent was obtained from all patients and volunteers prior to the study. Peripheral blood samples were taken from patients and volunteers. In pancreatic cancer patients, blood samples were collected before surgical resection of the primary tumor. When patients underwent endoscopic biopsy of the gastric mucosa, blood samples were taken minimally at 2-week intervals after biopsy.

In addition to the patients' samples, the Human Blood Fractions MTC Panel of the first-strand cDNA (Clontech, Palo Alto, CA, USA) was analyzed. This panel is composed of mononuclear cells (B cells, T cells and monocytes) pooled from 50 male or female Caucasians, resting $CD8^+$ cells pooled from 33 male or female Caucasians, resting $CD4^+$ cells pooled from 20 male or female Caucasians, resting $CD14^+$ cells pooled from 36 male or female Caucasians, resting $CD19^+$ cells pooled from 34 male or female Caucasians, $CD19^+$ cells activated with pokeweed mitogen pooled from four male or female Caucasians, mononuclear cells activated with pokeweed mitogen and concanavalin A pooled from four male or female Caucasians, $CD4^+$ cells activated with concanavalin A pooled from 12 male or female Caucasians, and $CD8^+$ cells activated with phytohemagglutinin pooled from eight male or female Caucasians. These samples were analyzed using a real-time quantitative RT-PCR assay. In parallel, tissue specimens of pancreatic cancer obtained from 23 patients who subsequently underwent surgical operation for removal of primary tumors were examined by immunohistochemistry, and the tumor stage was classified according to the tumor node metastasis classification system.[18] In addition, pancreatic tissue specimens of two cases operated for chronic pancreatitis were archived from the pathology files of Shinshu University Hospital, Matsumoto, Japan. The study protocol was approved by the Institutional Review Board of Shinshu University School of Medicine.

RNA extraction and cDNA synthesis

Five milliliters of peripheral blood was collected, treated with ethylene diamine tetraacetic acid to prevent coagulation, and layered on 3 mL of Lymphprep (Nycomed Pharma, Oslo, Norway) in a 15-mL polypropylene tube. The tube was centrifuged at 2000*g* for 30 min at 20°C. The mononuclear cell fraction was transferred to a new tube, resuspended in 5 mL phosphate-buffered saline, and then centrifuged at 3000*g* for 5 min. Total RNA was isolated from the pellet using a RNeasy Mini kit (Qiagen, Valencia, CA, USA), followed by DNaseI treatment. After inactivation of DNaseI, 11 μL of the DNaseI-treated RNA was incubated with 1 μL of 10 mM dNTPs and 1 μL of 0.5 mg/mL oligo$(dT)_{15}$ primer (Promega, Madison, WI, USA) at 65°C for 5 min. After chilling on ice, these mixed samples were then incubated with 4 μL of 5× first strand buffer, 1 μL of 0.1 M dithiothreitol, 1 μL of RNase inhibitor (Promega), and 1 μL of the reverse transcriptase SuperScript 2 (Invitrogen, Carlsbad, CA, USA) at 42°C for 1 h. The reaction was terminated by heating at 70°C for 15 min, and samples were then kept at −20°C until real-time quantitative RT-PCR analysis.

Real-time RT-PCR

Quantitation of α4GnT mRNA expressed in peripheral blood mononuclear cells as well as the Human Blood Fractions MTC Panel was carried out using an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA) as described previously, with minor modifications.[17] On the basis of the published human *α4GnT* sequence,[14] specific primer pairs and probes were designed using the Primer Express program (PE Applied Biosystems). Forward and reverse primers for human *α4GnT* were 5′-GTTTTCCTCTTCCC-TTTGGATATGA-3′ (nucleotides +340 to +364; the first nucleotide of the initiation methionine codon is +1) and 5′-AGCTGATGTGGAGCCAGTTTCT-3′ (nucleotides +427 to +448), respectively. These primers were designed to hybridize to different exons of the *α4GnT* gene to avoid amplifying genomic DNA. The TaqMan probe was synthesized as 5′-TGGTACAATCAAATCAACGCCAGCGC-3′ (nucleotides +397 to +422) by PE Applied Biosystems, and it carried a 5′-6-carboxyfluorescein reporter label and a TAMURA quencher group. To normalize α4GnT mRNA expression levels, a housekeeping gene, *GAPDH*, was quantitatively analyzed simultaneously as a control. To construct a standard curve, 10-fold dilutions of the plasmid cDNA harboring *α4GnT* (pcDNAI-α4GnT) ranging from 3×10^{-2} to 3×10^{-10} μg/mL, corresponding to 5×10^9 to 5×10^1 copies/mL were prepared. Similarly, a 10-fold dilution of the plasmid cDNA harboring a partial cDNA sequence of *GAPDH* (pCR2.1-GAPDH), which was constructed as described previously,[17] was prepared from 2.3×10^{-2} to 2.3×10^{-10} μg/mL, corresponding to 5×10^9 to 5×10^1 copies/mL.

Multiplex PCR was carried out in 50 μL of reaction mixture containing 3 μL of cDNA sample, 25 μL of 1× Universal PCR Master Mix (PE Applied Biosystems), 800 nM of the primer set for α4GnT, 80 nM of the primer for *GAPDH*, 125 nM of the TaqMan probe for *α4GnT*, and 100 nM of the TaqMan probe for *GAPDH* that carries the 5′-VIC reporter label and 3′-TAMURA quencher group (PE Applied Biosystems). Reaction tubes were placed in the ABI PRISM 7700 Sequence Analyzer, preheated at 95°C for 10 min and amplified for 50 cycles of 95°C for 15 s, followed by 60°C for 1 min. The abundance of α4GnT mRNA and GAPDH mRNA was determined by comparison with the standard curves for

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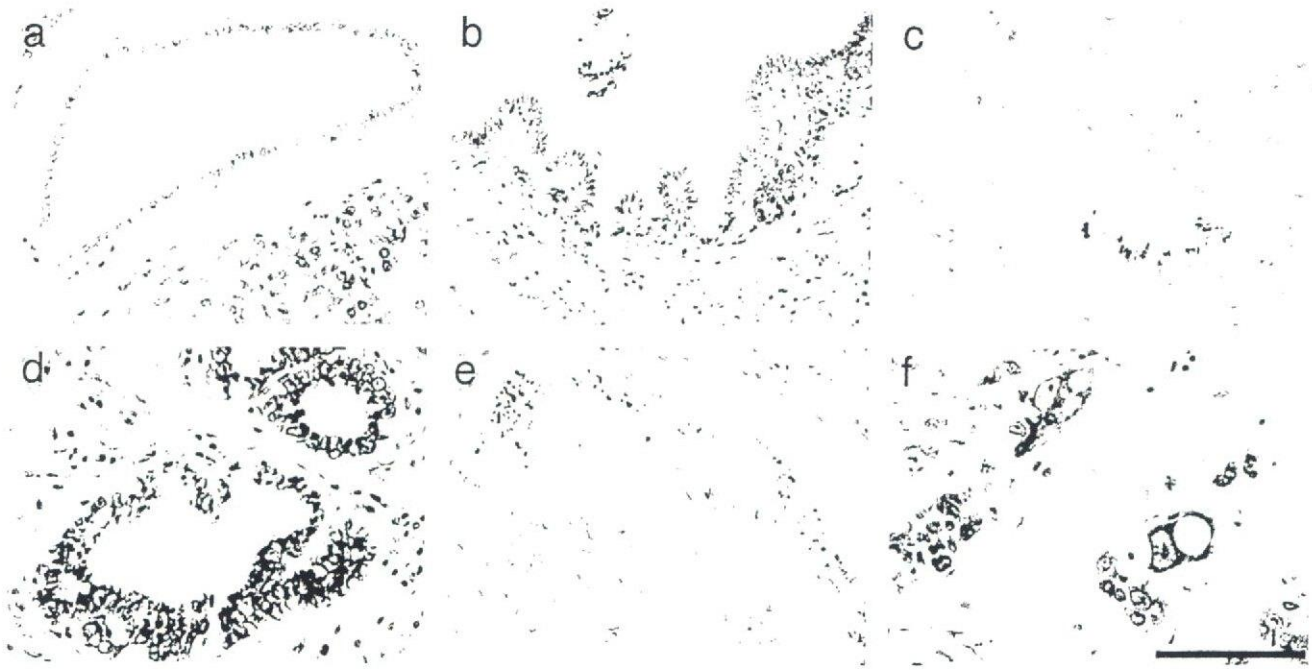

Fig. 1. Expression of α4GnT protein in the normal and neoplastic pancreatic tissues. α4GnT was detected by immunohistochemistry using the antiα4GnT antibody I17K. α4GnT is not expressed in the normal pancreatic duct (a), whereas it is expressed in the Golgi region of pancreatic ducts exhibiting PanIN-IB (b). The α4GnT protein is also expressed in the pancreatic ducts with PanIN-II found in chronic pancreatitis (c). In the pancreatic carcinoma, α4GnT protein is detected in well differentiated (d), moderately differentiated (e), and poorly differentiated (f) adenocarcinomas. Scale bar = 100 μM.

α4GnT and GAPDH, respectively, and the relative expression level of α4GnT mRNA was defined by multiplying the α4GnT : GAPDH mRNA ratio by 1.0×10^7. The assays were carried out in duplicate, and mean values of the two experiments were indicated.

Immunohistochemistry

To detect α4GnT protein in pancreatic cancer cells, 23 cases of the resected pancreatic cancer tissues were subjected to immunohistochemistry with the monospecific antiα4GnT polyclonal antibody, I17K, as described previously.[16] Briefly, 3 μM-thick sections were deparaffinized and treated with 0.3% H_2O_2 in methanol and then blocked with 1% normal goat serum in TBS. The sections were incubated with the antibody for 1.5 h. After washing with TBS, sections were incubated with biotinylated antirabbit IgG and then horseradish peroxidase-labeled streptavidin. The peroxidase reaction was developed with a diaminobenzidine/H_2O_2 solution, and counterstained with hematoxylin. In control experiments carried out by replacing the primary antibody with preimmune serum or omitting the primary antibody from the staining procedure, no specific staining was seen. Tissue specimens containing > 5% positively stained cancer cells were defined as positive, and the others were classified as negative according to previously described criteria.[19]

Enzyme immunoassay of biomarkers in patients' serum

Various biomarkers, including CEA, CA19-9, DU-PAN-2 and Span-1, in pancreatic cancer patients' serum were evaluated by enzyme immunoassay before surgery. CEA (cut-off value, 2.5 ng/mL) was measured using a CEA•Dainapack kit (Dainabot, Tokyo, Japan), and CA19-9 (cut-off value, 37 U/mL) was measured using an AxSYM CA19-9•Dainapack kit (Dainabot). DU-PAN-2 (cutoff value, 150 U/mL) and Span-1 (cutoff value, 30 U/mL) were measured by SRL at Tokyo, Japan.

Statistics

Statistical analyses comparing two independent groups categorized by the clinicopathological variables of pancreatic cancer were carried out using the Mann–Whitney *U*-test. Similarly, comparisons among more than three groups were carried out using the Kruskal–Wallis test. These analyses were performed using StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA). In addition, a cut-off value was determined by constructing a ROC curve using StatMate III (ATMS, Tokyo, Japan). Statistical association between the expression of α4GnT protein in the resected pancreatic cancer tissues and the expression level of α4GnT mRNA determined in the mononuclear cell fraction of peripheral blood was evaluated using Fisher's test (Abacus Concepts). In these analyses, *P*-values < 0.05 were considered to be statistically significant.

Results

Expression of α4GnT protein in pancreatic cancer cells

In order to determine the expression of α4GnT protein in pancreatic ductal adenocarcinoma cells, immunohistochemistry using the antiα4GnT antibody I17K was undertaken with normal and neoplastic tissues of the pancreas, which were resected surgically at the time of operation. In the normal pancreas, α4GnT was not detected in the main or interlobular pancreatic ducts (Fig. 1a). By contrast, α4GnT protein was

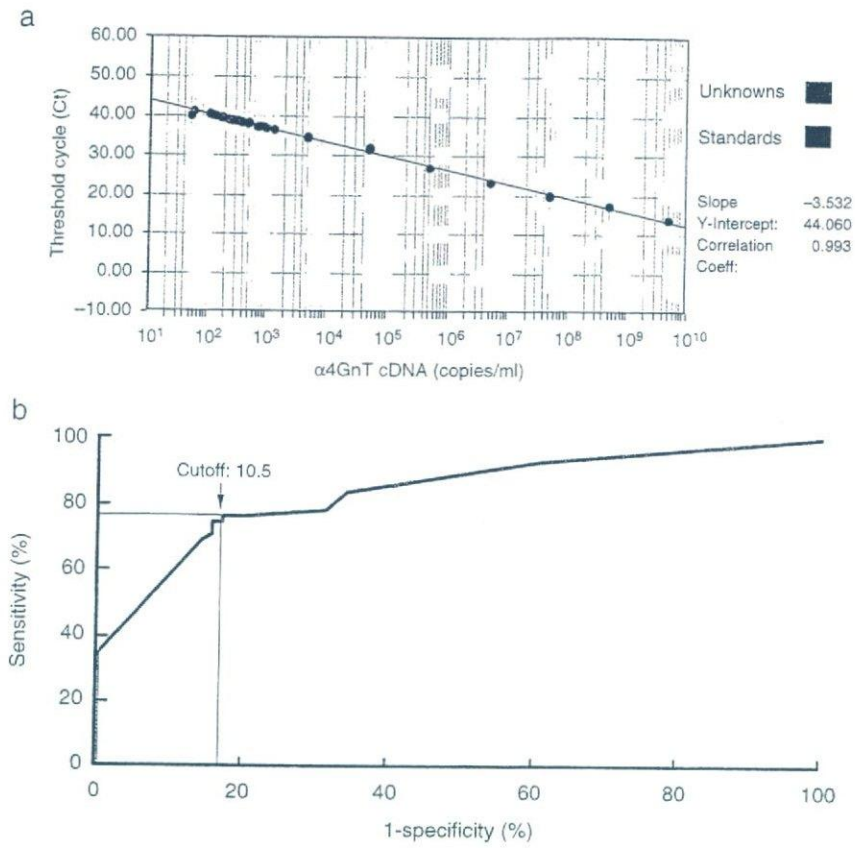

Fig. 2. Quantitative RT-PCR assay targeting α4GnT mRNA. (a) A standard curve for α4GnT was constructed by plotting serially diluted α4GnT cDNA, pcDNAI-α4GnT (black dots), where unknown samples from patients or cancer-free volunteers are indicated as red dots. (b) ROC curve was created by plotting the expression level of α4GnT mRNA in the peripheral blood from 55 pancreatic cancer patients and 70 cancer-free volunteers. Arrow denotes the cutoff value of 10.5, which best discriminates pancreatic cancer patients from cancer-free volunteers with 76.4% sensitivity and 82.9% specificity.

associated with the Golgi region of pancreatic ducts with PanIN-IB (Fig. 1b). α4GnT protein was also expressed in the pancreatic ducts with PanIN-II found in the inflammatory lesions of chronic pancreatitis in both of the two cases examined (Fig. 1c). In pancreatic cancer, α4GnT was detected in the Golgi of adenocarcinoma cells in 73.9% of 23 patients, irrespective of histological tumor type; that is, five of nine patients with well-differentiated adenocarcinoma (Fig. 1d), seven of eight patients with moderately differentiated adenocarcinoma (Fig. 1e) and five of six patients with poorly differentiated adenocarcinoma (Fig. 1f) were positive for α4GnT protein in cancer tissues.

Construction of a standard curve for the quantitative RT-PCR assay

The standard curve for α4GnT mRNA was constructed using 10-fold dilutions of α4GnT cDNA, pcDNAI-α4GnT (Fig. 2a). By defining the cycle number where fluorescence reached a detection threshold as Ct, we obtained a strong linear relationship between Ct and the log of the cDNA concentration. Based on the standard curve, levels of α4GnT mDNA ranging from 5×10^1 to 5×10^9 copies/mL were detected in a reaction tube. Similarly, GAPDH mRNA was detected ranging from 5×10^1 to 5×10^9 copies/mL based on the standard curve for GAPDH constructed using 10-fold dilutions of pCR2.1-GAPDH. Using these standard curves, the expression level of α4GnT mRNA relative to that of GAPDH mRNA was determined.

Determination of a cut-off value distinguishing pancreatic cancer patients from cancer-free volunteers

To most efficiently discriminate pancreatic cancer patients from cancer-free volunteers, a ROC curve was constructed (Fig. 2b). Thus, the α4GnT:GAPDH mRNA ratios multiplied by 1.0×10^7 were defined as the expression level of α4GnT, and the values determined in the mononuclear cell fraction of the peripheral blood from 55 patients with pancreatic cancer versus 70 cancer-free volunteers were plotted. By defining the cut-off value as 10.5, the optimal combination of 76.4% for sensitivity and 82.9% for specificity was obtained. Thus, we regarded a value as positive when expression levels of α4GnT mRNA greater than 10.5 were obtained in this assay.

Determination of the expression levels of α4GnT mRNA in peripheral blood samples from pancreatic cancer patients and cancer-free volunteers

Based on the criterion that the expression level of α4GnT mRNA should exceed the cut-off value of 10.5 for a positive result in this assay, we determined the expression levels of α4GnT mRNA in the mononuclear cell fraction of peripheral blood isolated from 55 pancreatic cancer patients and 70 cancer-free volunteers (Fig. 3).

In pancreatic cancer, 42 (76.4%) of 55 patients examined were positive for this assay, and the expression level of α4GnT mRNA was 37.50 ± 5.44 (mean ± SE). The expression level of α4GnT transcripts was then evaluated by clinicopathological variables including tumor location, stage, venous invasion,

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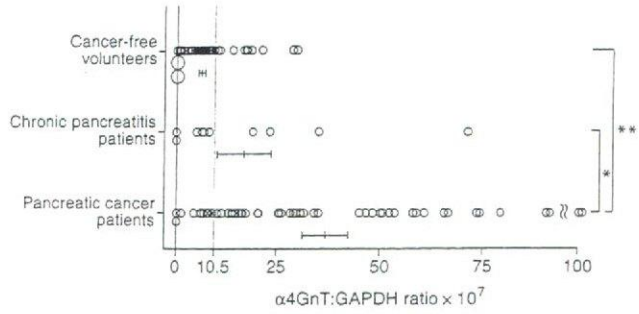

Fig. 3. Scatter plots indicating the expression level of α4GnT mRNA in the mononuclear cell fraction of peripheral blood measured by the quantitative RT-PCR. Small and large circles represent one and 10 individuals, respectively. Horizontal bars indicate mean ± SE. $*P < 0.05$, $**P < 0.001$.

lymphatic invasion and lymph node metastasis determined at subsequent surgical operation. Although the frequency of the positive patients and the expression level of α4GnT seemed to be associated with tumor progression, no significant statistical differences were seen in any clinicopathological variables examined (Table 1).

In addition, the expression level of α4GnT mRNA in the mononuclear cell fraction of blood samples from cancer-free volunteers was determined. Of the 70 cancer-free volunteers examined, 12 (17.1%) volunteers were found to be positive for this assay, but the expression level of α4GnT transcripts was 7.2 ± 0.9, which was significantly lower than that seen in pancreatic cancer patients ($P < 0.001$).

We then tested whether activated lymphocytes express α4GnT mRNA aberrantly by using the Human Blood Fractions MTC Panel, and it was shown that α4GnT mRNA was not detectable in any of the blood fractions examined, including activated lymphocytes.

Detection of α4GnT mRNA in peripheral blood samples from chronic pancreatitis patients

We next measured the expression level of α4GnT in the mononuclear cell fraction of peripheral blood isolated from chronic pancreatitis patients (Fig. 3). Of the 10 patients examined, four (40.0%) were classed as positive by exceeding the defined cutoff of 10.5. However the expression level of α4GnT mRNA was found to be 17.87 ± 6.98, which was again significantly lower than that seen in pancreatic cancer patients ($P < 0.05$). Statistically significant differences between expression levels were not seen between cancer-free volunteers and chronic pancreatitis patients.

Comparison of α4GnT mRNA with well-characterized biomarkers in pancreatic cancer patients

The results of the real-time PCR analysis of α4GnT mRNA expressed in the mononuclear cell fraction of peripheral blood from the pancreatic cancer patients were then compared with the results of enzyme immunoassays for well-characterized biomarkers including CEA, CA19-9, DU-PAN-2 and Span-1. As shown in Table 2, more than 74% of pancreatic patients were positive for either α4GnT or CA19-9, and CEA and DU-PAN-1 were found to less frequently detect pancreatic cancer compared with α4GnT and CA19-9.

Table 1. Frequency of pancreatic cancer patients positive for the α4GnT assay and correlation between expression levels of α4GnT mRNA in peripheral blood mononuclear cells and clinicopathological variables

Variable	Frequency of positive patients†		α4GnT mRNA‡ (mean ± SE)	*P*-value
	n	%		
Tumor location				
Head	25/32	78.1	37.95 ± 7.03	0.9728§
Body and tail	17/23	73.9	36.87 ± 8.78	
Tumor stage				
0	0/1	0	5.290	0.4571¶
II	2/3	66.7	29.44 ± 11.67	
III	7/8	87.5	32.85 ± 8.55	
IV	33/43	76.7	39.68 ± 6.72	
Venous invasion				
Negative	3/5	60.0	29.29 ± 11.20	0.6954§
Positive	17/19	89.5	36.97 ± 10.36	
Lymphatic invasion				
Negative	1/3	33.3	11.81 ± 4.82	0.0606§
Positive	19/21	90.5	38.74 ± 9.44	
Lymph node metastasis				
Negative	5/7	71.4	28.78 ± 9.93	0.8241§
Positive	15/17	88.2	38.09 ± 11.32	

†Expression levels greater than 10.5 were defined as positive.
‡α4GnT:GAPDH mRNA ratios multiplied by 1.0×10^7 are indicated.
§Analyzed using the Mann–Whitney *U*-test. ¶Analyzed using the Kruskal–Wallis test.

We then tested whether a combined assay with α4GnT and another biomarker would detect pancreatic cancer patients more efficiently than the enzyme immunoassays targeting single biomarkers (Table 3). Notably, it was found that more than 86% of pancreatic cancer patients were detected when the α4GnT assay was combined with enzyme immunoassay for CEA, CA19-9, DU-PAN-2 or Span-1. In particular, 96.4% of pancreatic cancer patients were positive for either α4GnT mRNA or Span-1 or both, whereas 71.4% of the patients were positive for Span-1 alone.

Detection of α4GnT protein in resected pancreatic cancer tissues

Transcripts of α4GnT are not detectable in peripheral blood cells, including leukocytes, lymphocytes and monocytes.[17] Thus, it is possible that α4GnT mRNA detected in the mononuclear cell fraction of peripheral blood from pancreatic cancer patients is derived from circulating pancreatic cancer cells expressing α4GnT mRNA. To test this hypothesis, the results of real-time RT-PCR of α4GnT mRNA expressed in the peripheral blood were compared with those of α4GnT protein expressed in 23 cases of the subsequently resected pancreatic cancer tissues by immunohistochemistry with the antiα4GnT antibody I17K. In 19 patients positive for α4GnT transcripts in the peripheral blood, 17 were also positive for α4GnT protein in the resected pancreatic cancer tissues. By contrast, α4GnT protein was not detected in pancreatic cancer tissues of three of four patients who were also negative for the α4GnT mRNA assay. These results indicate a significant association between α4GnT mRNA in the peripheral blood

Table 2. Frequency of pancreatic cancer patients detected using assays for α4GnT mRNA, CEA, CA19-9, DU-PAN-2 and SPan-1

Tumor stage	α4GnT mRNA (> 10.5)		CEA (> 2.5 ng/mL)		CA19-9 (> 37 U/mL)		DU-PAN-2 (> 150 U/mL)		SPan-1 (> 30 U/mL)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
0	0/1	0	0/1	0	0/1	0	NE	NE	NE	NE
II	2/3	66.7	0/3	0	0/3	0	0/2	0	0/2	0
III	7/8	87.5	3/8	37.5	6/8	75.0	1/8	12.5	2/7	28.6
IV	33/43	76.7	23/41	56.1	34/42	82.9	16/22	72.7	18/19	94.7
Total	42/55	76.4	26/53	49.1	40/54	74.1	17/32	53.1	20/28	71.4

NE, not evaluated.

Table 3. Frequency of pancreatic cancer patients detected using combined assays†

Biomarker	CEA (> 2.5 ng/mL)		CA19-9 (> 37 U/mL)		DU-PAN-2 (> 150 U/mL)		SPan-1 (> 30 U/mL)	
	n	%	*n*	%	*n*	%	*n*	%
α4GnT mRNA	46/53	86.8	48/54	88.9	30/32	93.8	27/28	96.4
CEA	–	–	43/53	81.1	24/32	75.0	21/28	75.0
CA19-9	–	–	–	–	27/32	84.4	23/28	82.1
DU-PAN-2	–	–	–	–	–	–	21/28	75.0

†Frequency of the patients positive for either or both biomarkers combined is indicated.

and α4GnT protein in pancreatic cancer tissues ($P = 0.0209$), suggesting that α4GnT mRNA detected in patients' peripheral blood is derived from circulating pancreatic cancer cells.

Discussion

α1,4-*N*-Acetylglucosaminyltransferase is a glycosyltransferase that mediates the transfer of GlcNAc with an α1,4-linkage from UDP-GlcNAc to βGal residues, forming α1,4-GlcNAc-capped *O*-glycans.[14] As shown in our previous studies and confirmed here, α4GnT is expressed frequently in pancreatic cancer cells as well as in gastric cancer cells, but not in peripheral blood cells.[15,17] Therefore, we used quantitative RT-PCR to determine the expression level of α4GnT mRNA in tumor cells circulating in the peripheral blood of pancreatic cancer patients. We primarily defined the cut-off value as 10.5 for this assay, based on the ROC curve, and could detect 76.4% of 55 pancreatic cancer patients. The significant correlation between the expression level of α4GnT mRNA in the peripheral blood detected by the RT-PCR assay and α4GnT protein detected in resected pancreatic cancer tissues by immunohistochemistry strongly suggests that α4GnT mRNA detected in the peripheral blood is derived from circulating pancreatic cancer cells. Although 40% of 10 chronic pancreatitis patients and 17.1% of 70 cancer-free volunteers were also positive by this assay, the expression levels of α4GnT mRNA in both groups were significantly lower than those seen in pancreatic cancer patients. These results indicate the clinical utility of real-time RT-PCR targeted to α4GnT mRNA for detection of pancreatic cancer.

The present study also revealed that the location of the pancreatic tumor does not alter the results of the assay (Table 1). It is known that early detection of pancreatic cancer occurring in the tail and body of the pancreas can be particularly difficult because jaundice, which is frequently associated with pancreatic head cancer, is not evident unless the common bile duct is affected by the tumor.[1] Thus, the assay demonstrated here will likely contribute to early detection of pancreatic body and tail cancers that are not associated with jaundice.

In the present study, we have also shown that the expression level of α4GnT mRNA in the peripheral blood from pancreatic cancer patients is elevated in a manner correlated with tumor stage (Table 1), suggesting that the number of cancer cells entering the peripheral blood is increased as the tumor progresses. Most recently, we have shown that α1,4-GlcNAc-capped *O*-glycans secreted from gastric gland mucous cells function as an antibiotic against *H. pylori* infection.[20] The role of these unique *O*-glycans expressed on pancreatic cancer cells remains unknown, and thus further study will be required to address this problem.

There are several biomarkers for pancreatic cancer, including CEA,[5] CA19-9,[6] DU-PAN-2[7,8] and Span-1.[9] Among them, CA19-9 is the most widely used in screening and monitoring of the disease.[21–24] We compared α4GnT with other biomarkers (including CA19-9) and found that the frequency of pancreatic cancer patients detected by α4GnT was much the same as that detected by CA19-9 (Table 2). The same analysis also revealed that DU-PAN-2 and CEA detected pancreatic cancer patients less frequently than α4GnT, CA19-9 and Span-1. It is noteworthy that two of three patients at stage II were positive for α4GnT mRNA, suggesting the possible usefulness of α4GnT mRNA for the early detection of pancreatic cancer. Further study on a larger number of patients with stages 0, I and II will be required to prove this possibility.

The present study demonstrated that the frequency of pancreatic cancer patients detected using enzyme immunoassays for CEA, CA19-9, DU-PAN-2 and Span-1 was increased substantially when combined with the α4GnT assay (Table 3). It is generally accepted that the quantitative RT-PCR assay requires much time and cost compared with enzyme immunoassay.

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However, the combined assay with α4GnT mRNA can detect pancreatic cancer patients more efficiently when compared with the enzyme immunoassays targeting single biomarkers.

The present study also demonstrated that the expression levels of α4GnT mRNA were elevated in 40% of chronic pancreatitis patients and 17% of cancer-free volunteers, albeit at much lower levels than those of pancreatic cancer patients. Previously, we showed that significant amounts α4GnT mRNA were detected in patients with *H. pylori* infection or chronic gastroduodenal ulcers.[17] It has been reported that unexpected genes such as α-fetoprotein are transcribed in lymphocytes when they are activated,[25] suggesting the possibility that α4GnT mRNA might be induced in the activated lymphocytes circulating in the *H. pylori*-infected patients. However, as shown previously[17] and further confirmed here, we have demonstrated that α4GnT mRNA is not detectable in activated lymphocytes or resting lymphocytes. By contrast, we have also reported that extensive biopsy of the gastric mucosa results in elevation of the α4GnT mRNA level in peripheral blood.[26] These results combined suggest that the gastric gland mucous cells expressing α4GnT mRNA enter the bloodstream through the injured sites of gastric mucosa caused by inflammation or biopsy. Considering the high incidence of *H. pylori* infection in individuals over 40 years of age in Japan,[27,28] α4GnT mRNA detected in the cancer-free volunteers is most likely derived from gastric gland mucous cells that have entered the peripheral blood through injured sites of the gastric mucosa caused by *H. pylori* infection. We previously demonstrated that α4GnT mRNA is not detected in the peripheral blood of healthy volunteers without *H. pylori* infection.[17] Similary, it may also be possible that α4GnT mRNA detected in the chronic pancreatitis patients originated from the α4GnT-positive pancreatic duct epithelia entering the blood circulation, because the disruption of pancreatic ducts could occur in chronic pancreatitis (Fig. 1c).[29] Further studies will be of significance to identify the cells that elevate the α4GnT mRNA level in the peripheral blood of these non-cancerous patients.

Recently we demonstrated that α4GnT is expressed not only in pancreatic carcinoma cells but also in biliary tract carcinoma cells that produce α1,4-GlcNAc-capped *O*-glycans.[15] Thus, the α4GnT assay will also be applicable to the detection of patients with biliary tract cancers. We have shown that α4GnT mRNA was detected in three of five patients with biliary tract cancer.[17] It is of great significance to determine the clinical utility of the α4GnT assay for diagnosis of biliary tract cancer as well.

Collectively, our results obtained in the present study indicate that quantitative analysis of α4GnT mRNA expressed in the peripheral blood allowed us to detect pancreatic cancer cells expressing α1,4-GlcNAc-capped *O*-glycans. In order to clarify the clinical contribution of this assay system, prospective controlled trials are needed in the screening, diagnosis and monitoring of pancreatic cancer patients.

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□ ORIGINAL ARTICLE □

Ten Years of Experience with Liver Transplantation for Familial Amyloid Polyneuropathy in Japan: Outcomes of Living Donor Liver Transplantations

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Abstract

Object **We summarize 10 years of experience with liver transplantation for FAP patients in Japan and review the current opinions regarding this treatment for FAP.**

Methods and Patients **All basic report data on patients at the time of transplantation were registered with the Japanese Liver Transplantation Society (JLTS). Based on the JLST report data, more detailed information on FAP patients was requested from each center.**

Results **Living donor liver transplantation (LDLT) for FAP patients was first performed in Japan in 1993. LDLT has since been performed in 41 FAP patients, including nine cases of temporary auxiliary partial orthotopic liver transplantation (APOLT). Orthotopic liver transplantation (OLT) from cadaveric donors for FAP patients began in 1999, but only one FAP patient has subsequently undergone this procedure. Of these total of 43 FAP patients, 36 are currently alive: the one-year survival rate of patients after transplantation was 93%, and the five-year survival rate of these cases was 77%. Preoperative clinical severity and the nutritional status of patients are correlated with their outcome after liver transplantation. Domino (sequential) liver transplantation has been carried out in 20 domino recipients with end-stage liver diseases. Of the 20 domino recipients, 12 are currently alive.**

Conclusion **For FAP patients, these outcomes after the operation were very similar to those of OLT from cadaveric donors reported in other countries. Therefore, we concluded that for the treatment of FAP, LDLT from a living donor is equally effective as OLT from a cadaveric donor.**

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Key words: familial amyloid polyneuropathy, living donor liver transplantation, Japan

Introduction

Familial amyloid polyneuropathy (FAP) is one type of hereditary generalized amyloidosis, initially showing polyneuropathy and autonomic dysfunction but with later involvement of many visceral organs (1). Four endemic areas of this disease are known to exist in Portugal (2), Sweden (3), and Japan (1). The precursor protein of amyloid fibrils is a variant form of transthyretin (TTR) in serum (4). The vast majority of patients show the typical clinical picture known as type I FAP: the disease is caused by a mutation in the TTR gene resulting in the substitution of methionine for valine at position 30 (Val30Met) (5). Polyneuropathy begins in the legs and progresses in an ascending fashion. As TTR is produced mainly in the liver, liver transplantation for FAP patients was first performed in 1990 in Sweden (6), which was shown to result in the disappearance of variant TTR with Val30Met from the sera of transplant recipients. Since then, more than 800 patients in 16 countries have undergone orthotopic liver transplantation (OLT) (7). Moreover, domino (sequential) liver transplantation using a graft from the FAP patient was first performed in Portugal (8) in 1995, and more than 200 domino transplant procedures reutilizing explanted FAP livers had been performed by the end of 2002 (7, 9).

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