ATIII activity ≤60% received postoperative prophylactic treatment with ATIII concentrates (ATIII[+] group), and the remaining 16 patients with preoperative ATIII activity >60% did not receive ATIII prophylaxis (ATIII[−] group). In addition to the difference in ATIII activity, Child-Pugh score was worse and splenomegaly, as shown by spleen weight and SVD, was more severe in the ATIII(+) than in the ATIII(−) group. A higher percentage of patients in the ATIII(+) group experienced surgical difficulties. Postoperative PVT was significantly less frequent in the ATIII(+) than in the ATIII(−) group (8.1% vs 43.8%; p = 0.005). Using the initial criteria based on ATIII activity alone, the overall prevalence of PVT was 19% (10 of 53 patients).

High frequency of portal vein thrombosis after splenectomy in the antithrombin III(−) group

Although we expected that patients in the ATIII(−) group would be at lower risk for PVT, the prevalence of PVT in this group was 43.8%. We had previously reported that large SVD was associated with PVT after splenectomy in patients with liver cirrhosis and portal hypertension.[19] We therefore evaluated the occurrence of PVT based on ATIII activity and SVD (Fig. 2). Portal vein thrombosis rates in patients with ATIII activity >60% but <70% and ≥70% (normal range) were 100% (3 of 3) and 30.8% (4 of 13), respectively. In the latter group, 66.7% (4 of 6) of patients with SVD ≥10 mm had PVT, compared with 0% (0 of 7) of patients with SVD <10 mm. Using ATIII activity <70% or SVD ≥10 mm as a threshold to predict the incidence of PVT had a sensitivity of 100% and a specificity of 77.8%; using ATIII activity <70% or SVD ≥9 mm, the sensitivity was 100% and the specificity was 55.6%.

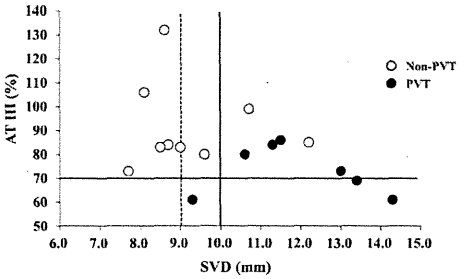

Figure 2. Relationship of portal vein thrombosis (PVT) with antithrombin III (AT III) activity and splenic vein diameter in 16 cirrhotic patients with ATIII activity >60% who received no prophylactic treatment with ATIII concentrates. Splenic vein diameter thresholds of 9 mm and 10 mm are indicated by dotted and solid lines, respectively. A threshold of 70% for ATIII activity is indicated by a solid line.

Prevention of portal vein thrombosis after splenectomy with antithrombin III concentrates and its therapeutic limitation

Although 37 patients with ATIII activity ≤60% and who received prophylactic ATIII concentrates were thought to be at higher risk for PVT after splenectomy, PVT developed in only 3 (8.1%) (Fig. 3). Of the first 21 patients, treated from April 2008 to December 2009, PVT developed in 3, despite prophylactic treatment with ATIII concentrates. Portal vein thrombosis was detected in these 3 patients by CT on POD 7, followed by immediate intravenous administration of danaparoid sodium (2,500 U/day) for 14 days followed by warfarin for 3 months, until there were no indications of PVT. These findings indicated that prophylactic ATIII cannot always prevent PVT after splenectomy. At a threshold of SVD ≥15 mm, the sensitivity and specificity for predicting PVT were 66.7% and 94.4%, respectively; at a threshold of SVD ≥13 mm, the sensitivity and specificity were 100% and 77.8%, respectively. These findings indicated that ATIII monotherapy was not suitable as a primary prophylaxis for patients with SVD ≥15 mm. We therefore modified treatment of these patients, starting with ATIII concentrates (1,500 U/day for 3 days), followed by danaparoid sodium (2,500 U/day for 14 days) and subsequent warfarin for 3 months or until PVT was completely eliminated. Of the 16 patients

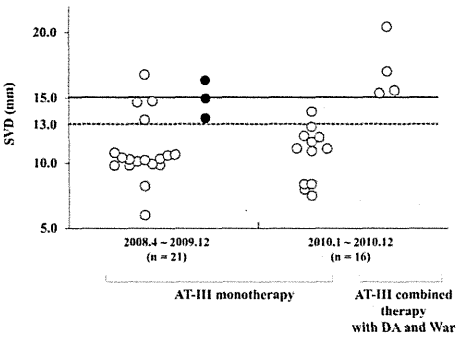

Figure 3. Relationship of portal vein thrombosis (PVT) to splenic vein diameter (SVD) in 37 cirrhotic patients with antithrombin III (ATIII) activity ≤60% who received prophylactic treatment with ATIII concentrates alone (ATIII monotherapy) or ATIII concentrates followed by danaparoid sodium (DA) and warfarin (War) (ATIII combined therapy with DA and War). Splenic vein diameter thresholds of 13.0 mm and 15.0 mm are indicated by dotted and solid lines, respectively. White dot, non-PVT; black dot, PVT.

treated from January to December 2010, four patients with SVD ≥15 mm received this schedule of prophylactic treatment, with none having PVT after the operation.

Risk stratification of portal vein thrombosis after splenectomy in patients with liver cirrhosis and portal hypertension

Based on the results of the testing cohort, we stratified the risk level of PVT after splenectomy in patients with liver cirrhosis and portal hypertension. Low risk was defined as ATIII activity ≥70% and SVD <10 mm; high risk as ATIII activity <70% and/or SVD ≥10 mm; and highest risk as SVD ≥15 mm (Table 2). Although patients at low risk received no prophylactic treatment for PVT, those at high risk received ATIII monotherapy, and those at highest risk received ATIII combined therapy, followed by danaparoid sodium and warfarin.

Validation cohort

Validation of risk stratification of portal vein thrombosis after splenectomy in patients with liver cirrhosis and portal hypertension

Table 3 shows the characteristics of the 57 patients in the validation group categorized by risk level of PVT after splenectomy. Only 2 (3.5%) of these patients had PVT under the new classification, a prevalence significantly lower than under the initial criteria ($p = 0.013$). None of the 14 patients classified as low risk experienced PVT, despite the lack of prophylaxis (Fig. 4). Of the 32 patients at high risk for PVT who received ATIII monotherapy, only 2 (6.3%) experienced PVT. Among the 11 patients at highest risk for PVT who received ATIII combined therapy followed by danaparoid sodium and warfarin, 8 showed partial and temporal PVT, extending from the splenic vein to the splenoportal confluence, on CT by POD 7, with PVT disappearing in all 8 by 3 months after splenectomy. None of the other 3 patients showed evidence of PVT after splenectomy, although they were maintained on warfarin for 3 months after the operation.

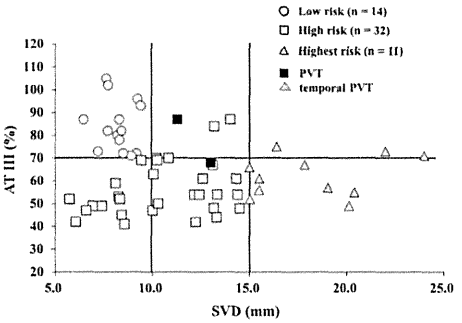

Figure 4. Relationship of portal vein thrombosis (PVT) with antithrombin III (AT III) activity and splenic vein diameter (SVD) in 57 cirrhotic patients who received prophylactic treatment of PVT according to the risk level of PVT after splenectomy. Fourteen patients at low risk received no prophylactic treatment, 32 patients at high risk received ATIII concentrates, and 11 patients at highest risk received ATIII concentrates followed by danaparoid sodium and warfarin. Thresholds of 70% for ATIII activity and 10.0 mm and 15.0 mm for SVD are indicated by solid lines.

DISCUSSION

Our previous study demonstrated that ATIII activity plays a crucial role in the development of PVT after laparoscopic splenectomy in cirrhotic patients.[18] Preoperative ATIII activity was found to be an independent predictor of PVT after laparoscopic splenectomy. The level of ATIII activity was significantly lower on PODs 1 and 4 than before surgery, but recovered to the preoperative level by POD 7, consistent with the observation that most PVTs developed within 7 days after splenectomy. Administration of ATIII concentrates (1,500 U/day) for 3 days corrected ATIII activity to near-normal range, as well as dramatically reduced the incidence of PVT after splenectomy. Therefore, the preoperative decrease in ATIII activity and its additional reduction during the early postoperative phase contribute to the development of PVT. In liver cirrhosis, hemostatic balance is fragile and easily tips to either a hypo- or hypercoagulable state.[7-9] After surgery, the decrease in hepatic synthesis of anticoagulants, such as ATIII, and their increased consumption due to intravascular coagulation can lead to a hypercoagulable state in the splanchnic and systemic circulation. Prophylactic administration of ATIII concentrates can return a hypercoagulable status to equilibrium between pro- and anticoagulants, but not to a hypocoagulable status. Therefore, despite being administered the day after surgery, ATIII concentrates do not contribute to bleeding complications.

In the testing cohort, we based the need for PVT prophylaxis on a cutoff level of preoperative ATIII activity. As ATIII activity decreases postoperatively, postoperative ATIII activity might be more accurate for predicting PVT than preoperative activity. However, ATIII activity could not be monitored on weekends in our hospital, and administration of ATIII concentrates should be started as soon as possible after splenectomy. Therefore, it might be more convenient to measure pre- than postoperative ATIII activity. Calculating ROC curves for the 25 patients included in the previous study and increasing the sensitivity to reduce the likelihood of false negatives resulted in our initial criterion, in which ATIII concentrates were administered to patients with ATIII activity ≤60%. Of the 53

patients in our testing cohort, 37 had ATIII ≤60% and received prophylactic treatment with ATIII concentrates, with PVT developing in only 3 (8.1%). However, of the 16 patients with ATIII >60%, considered at lower risk for PVT, PVT developed in 7 (43.8%) in the absence of prophylactic treatment. Of the 25 patients in the previous study, 12 had ATIII >60%, PVT developed without ATIII prophylaxis in only 1 (8.3%). The discrepancy between the current and previous studies might result from differences in potential risk levels, except for ATIII activity, in the 2 patient populations. Our other previous study showed that large SVD, which was associated with a decrease in portal venous flow after splenectomy, and low white cell counts (<2,000/μL) were risk factors for PVT after splenectomy.[19] In the current and previous studies, none of the patients with ATIII activity >60% had low white cell counts (<2,000/μL), and the mean spleen weight in patients with ATIII >60% was significantly greater in this study than in our previous study (473 ± 197 g vs 297 ± 160 g; p = 0.014), indicating that patients with ATIII >60% in this study had larger SVDs than in the previous study. Additionally, 6 of the 37 patients with ATIII ≤60% had low white cell counts (<2,000/μL), but PVT did not develop after ATIII prophylaxis in any of these patients. Therefore, this study assessed combinations of ATIII activity and SVD, but not white cell counts as risk factors for PVT. Using ATIII activity <70% or SVD ≥10 mm as a threshold to predict the incidence of PVT yielded a sensitivity of 100% and a specificity of 77.8%, and using ATIII activity <70% or SVD ≥9 mm had a sensitivity of 100% and a specificity of 55.6% (Fig. 2). These findings are consistent with our previous results, showing that SVD ≥9 mm is a risk factor for PVT after splenectomy,[19] although the cutoff level in this study is likely between 9 and 10 mm. We therefore defined high risk for PVT as ATIII activity <70% or SVD >10 mm.

Before the start of this study, we did not consider administering ATIII concentrates to all splenectomized patients, regardless of their potential risks of PVT, but wanted to identify patients who did not require ATIII prophylaxis, because of the potential biologic hazards and expense of ATIII concentrates. Although we failed to detect patients at lower risk for PVT in the testing cohort, none of the 14 patients in the validation cohort considered as at low risk for PVT according to risk stratification had PVT. Risk stratification showed that 7 (13%) of the 53 patients in the testing cohort, compared with 14 (25%) of the 57 patients in the validation cohort, were at low risk for PVT. Even in different patient populations, risk stratification of PVT can accurately identify patients who do or do not require ATIII prophylaxis.

Of the 37 patients in our testing cohort with ATIII ≤60% who received ATIII concentrates, PVT developed after splenectomy in only 3 (8.1%). In addition, PVT developed in only 1 of 30 (3.3%) patients with SVD <15 mm, compared with 2 of 3 patients with SVD ≥15 mm (Fig. 3). This result indicated that, in patients with SVD <15 mm, correction of a hypercoagulable state with ATIII concentrates can overcome the effects of decreased portal venous flow. Of the 3 patients with postoperative PVT despite prophylactic ATIII monotherapy, 2 had supermassive splenomegaly with SVD ≥15 mm, and 1 had an SVD of 13.5 mm and huge hepatofugal collateral vessels of 14 mm in diameter. Doppler US showed that postoperative portal venous flow was <50% of preoperative flow in these patients, suggesting that patients with SVD ≥15 mm or huge hepatofugal collateral vessels are at highest risk for PVT because ATIII monotherapy cannot overcome the great decrease in portal venous flow (Table 2). However, the diameter of collateral vessels predictive of PVT can be difficult to determine because the extent of portal venous flow through collateral vessels can depend on their location, number, size, and/or intrahepatic portal vascular resistance. Patients with huge collateral vessels, ≥10 mm in diameter, require systematic screening with repeated Doppler US and/or CT (Table 2). In the validation cohort, PVT developed in only 2 of 32 (6.3%) patients classified as at high risk for PVT (Fig. 4). Stratification of PVT risk level using 2 indicators, ATIII activity, which is associated with a hypercoagulable state, and SVD, which is related to decreased portal venous flow, was more specific and more correct than stratification by ATIII activity alone. In addition, ATIII monotherapy can prevent PVT in most patients at high risk.

We found that 3 patients in the testing cohort who were treated early, from April 2008 to December 2009, had PVT develop despite ATIII monotherapy (Fig. 3). These patients were started on intravenous danaparoid sodium (2,500 U/day) just after detection of PVT. Treatment for 14 days abolished PVT in the portal trunk, although thrombosis remained in the splenic vein. Warfarin was subsequently administered for 3 months until the thrombosis in the splenic vein was eliminated, preventing the recurrent extension of splenic vein thrombosis to the portal trunk. Patients with SVD ≥15 mm were considered at highest risk for PVT, with ATIII monotherapy not always sufficient to prevent PVT. The 15 patients at highest risk for PVT, who were treated from January 2010 to September 2013, received ATIII combined therapy, followed by danaparoid sodium and warfarin (Figs. 3 and 4). Eight of these patients showed partial PVT transiently extending from the splenic vein thrombosis to the splenoportal confluence by CT on POD 7, but disappearing within 3 months after splenectomy. Portal vein thrombosis never developed in the

remaining 7 patients. These findings suggest that prophylaxis for patients at highest risk for PVT is more therapeutic than prophylactic. Although better methods might prevent PVT, our prophylactic regimen for highest risk for PVT might be acceptable, eradicating PVT without any bleeding complications. Additionally, this combined regimen successfully eradicated PVT in 7 patients who received no prophylactic treatment and in 5 who received ATIII prophylaxis. Antithrombin III combined therapy with danaparoid sodium and warfarin is promising for the prevention or treatment of PVT after splenectomy, with an excellent safety profile.

Danaparoid sodium, rather than a continuation of ATIII concentrates, was used for prophylaxis of patients at highest risk for PVT or to treat PVT. Our previous study showed that administration of ATIII concentrates for 3 days maintained ATIII activity at a near-normal level (70%) up to POD 7.[18] Higher-dose ATIII concentrates and/or continued treatment, resulting in supranormal activity, did not have beneficial effects in patients with sepsis or disseminated intravascular coagulation.[21,22] As administration of ATIII concentrates to supranormal levels is associated with a potential bleeding risk and is very costly, we used the low-molecular weight heparinoid danaparoid sodium as an anticoagulant. Three classes of heparins were also available: unfractionated heparin (UFH), low-molecular weight heparins (LMWHs), such as enoxaparin and the synthetic pentasaccharide fondaparinux. The anticoagulant effects of these drugs depend on anti-Xa and anti-thrombin activities. The ratios of anti-Xa to anti-thrombin activity are about 1 for UFH; about 4 for enoxaparin; ≥22 for danaparoid sodium; and about 7,400 for fondaparinux. The anticoagulation effects of danaparoid sodium and fondaparinux are characterized by higher selectivity for factor Xa compared with UFH and enoxaparin. Direct inhibition of thrombin by UFH and enoxaparin inhibits thrombus formation and can increase bleeding risk. Low-molecular weight heparins and especially UFH have potential risks of heparin-induced thrombocytopenia, and danaparoid sodium and fondaparinux do not. Although there is limited evidence on the use of these drugs in patients with liver cirrhosis and PVT, LMWHs such as enoxaparin appear to be safe and effective in prophylactic or therapeutic treatment of PVT, even in patients with liver cirrhosis.[23-25] However, these heparins require ATIII to exert their anticoagulant effects and their efficacy can be unpredictable in cirrhotic patients with decreased ATIII activity.[7,9] Using in vitro thrombin generation assays of plasma from patients with liver cirrhosis, LMWHs were found to amplify anticoagulant effects, despite reductions in ATIII activity, especially in patients with Child-Pugh class C.[26,27] Additionally, a reduced anticoagulant response to fondaparinux was observed in plasma from patients with live cirrhosis.[26] The ratios of anti-Xa to anti-thrombin activity and the results presented here suggest that danaparoid sodium can be as effective as LMWHs, as well as safer, in preventing or treating PVT in patients with liver cirrhosis.

Patients with highest risk for PVT or those with substantial PVT require long-term treatment with anticoagulants until the thrombosis in the splenic vein disappears. Because long-term intravenous administration of danaparoid sodium is inconvenient, patients were switched to oral warfarin for up to 3 months. Although long-term administration of warfarin eradicated PVT without bleeding complications, warfarin has a narrow therapeutic window and requires frequent monitoring and dose adjustments. In addition, warfarin has been associated with a higher bleeding risk than LMWHs in patients with liver cirrhosis, especially in those with thrombocytopenia (platelet counts $<50 \times 10^3/\mu L$).[24] The optimal warfarin dose in patients with high prothrombin time and INR is unclear.[9] Warfarin can reduce the concentrations of vitamin K-dependent anticoagulant factors, such as protein C, potentially increasing the risk for thrombosis.[8] Fortunately, in our patient population, platelet counts were $>100 \times 10^3/\mu L$ on POD 14,[28] and end-stage liver cirrhosis did not develop in any patient, as indicated by prothrombin time and INR >2.0. Two novel oral anticoagulants have recently been approved for clinical use: rivaroxaban, a direct inhibitor of factor Xa; and dabigatran, a direct inhibitor of factor IIa.[9] Both drugs are independent of ATIII, do not require dose adjustment, and have a wider therapeutic range in the general population. To date, only 2 case reports have described the successful treatment of PVT with rivaroxaban of 1 cirrhotic and 1 noncirrhotic patient.[29,30] In vitro thrombin generation assays using plasma from cirrhotic patients showed a reduced anticoagulant response to rivaroxaban and a substantially increased anticoagulant response to dabigatran.[26] Although both agents are promising pharmacologic drugs, large cohorts are necessary to assess their efficacy and safety in cirrhotic patients with coagulation disorders.

CONCLUSIONS

We optimized risk stratification of PVT after splenectomy in patients with liver cirrhosis and portal hypertension and developed prophylactic treatments of PVT centered on ATIII concentrates. Classification of risk level can reduce the incidence of PVT after splenectomy and accurately identifies patients who do not require prophylaxis of PVT. Antithrombin III, as monotherapy or combined therapy, followed by danaparoid sodium and warfarin, is

safe and effective for both primary prophylaxis of PVT and treatment of PVT after splenectomy. These regimens warrant clinical trials for prevention of PVT after splenectomy or treatment of de novo PVT in patients with liver cirrhosis.

Author Contributions

Study conception and design: Kawanaka, Akahoshi, Shirabe, Takenaka, Maehara

Acquisition of data: Kawanaka, Akahoshi, Itoh, Iguchi, Harimoto, Uchiyama, Yoshizumi, Shirabe

Analysis and interpretation of data: Kawanaka, Akahoshi, Itoh, Iguchi, Harimoto, Uchiyama, Yoshizumi, Shirabe

Drafting of manuscript: Kawanaka, Akahoshi, Itoh, Iguchi, Harimoto, Uchiyama, Yosizumi, Shirabe, Takenaka, Maehara

Critical revision: Kawanaka, Akahoshi, Shirabe, Takenaka, Maehara

REFERENCES

1. Kawanaka H, Akahoshi T, Kinjo N, et al. Technical standardization of laparoscopic splenectomy harmonized with hand-assisted laparoscopic surgery for patients with liver cirrhosis and hypersplenism. J Hepatobiliary Pancreat Surg 2009;16:749–757.
2. Ikegami T, Shimada M, Imura S. Recent role of splenectomy in chronic hepatic disorders. Hepatol Res 2008;38:1159–1171.
3. Akahoshi T, Tomikawa M, Kawanaka H, et al. Laparoscopic splenectomy with interferon therapy in 100 hepatitis-C-virus-cirrhotic patients with hypersplenism and thrombocytopenia. J Gastroenterol Hepatol 2012;27:286–290.
4. Sugawara Y, Yamamoto J, Shimada K, et al. Splenectomy in patients with hepatocellular carcinoma and hypersplenism. J Am Coll Surg 2000;190:446–450.
5. Yoshizumi T, Taketomi A, Soejima Y, et al. The beneficial role of simultaneous splenectomy in living donor liver transplantation in patients with small-for-size graft. Transpl Int 2008;21:833–842.
6. Uehara H, Kawanaka H, Akahoshi T, et al. The feasibility and effectiveness of a hand-assisted laparoscopic splenectomy for hypersplenism in patients after living-donor liver transplantation. Surg Laparosc Endosc Percutan Tech 2009;19:484–487.
7. Senzolo M, Sartori MT, Lisman T. Should we give thromboprophylaxis to patients with liver cirrhosis and coagulopathy? HPB (Oxford) 2009;11:459–464.
8. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med 2011;365:147–156.
9. Lisman T, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis—possibilities and caveats. J Hepatol 2013;59:358–366.
10. Tripodi A, Salerno F, Chantarangkul V, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology 2005;41:553–558.
11. Tripodi A, Primignani M, Chantarangkul V, et al. Thrombin generation in patients with cirrhosis: the role of platelets. Hepatology 2006;44:440–445.
12. Potze W, Arshad F, Adelmeijer J, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. Br J Haematol 2013;163:666–673.
13. Tripodi A, Primignani M, Chantarangkul V, et al. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. Gastroenterology 2009;137:2105–2111.
14. Shahani T, Covens K, Lavend'homme R, et al. Human liver sinusoidal endothelial cells but not hepatocytes contain factor VIII. J Thromb Haemost 2014;12:36–42.
15. Amitrano L, Brancaccio V, Guardascione MA, et al. Inherited coagulation disorders in cirrhotic patients with portal vein thrombosis. Hepatology 2000;31:345–348.
16. Amitrano L, Guardascione MA, Brancaccio V, et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. J Hepatol 2004;40:736–741.
17. Francoz C, Belghiti J, Vilgrain V, et al. Splanchnic vein thrombosis in candidates for liver transplantation: usefulness of screening and anticoagulation. Gut 2005;54:691–697.
18. Kawanaka H, Akahoshi T, Kinjo N, et al. Impact of antithrombin III concentrates on portal vein thrombosis after splenectomy in patients with liver cirrhosis and hypersplenism. Ann Surg 2010;251:76–83.
19. Kinjo N, Kawanaka H, Akahoshi T, et al. Risk factors for portal venous thrombosis after splenectomy in patients with cirrhosis and portal hypertension. Br J Surg 2010;97:910–916.
20. Gando S, Sawamura A, Hayakawa M, et al. First day dynamic changes in antithrombin III activity after supplementation have a predictive value in critically ill patients. Am J Hematol 2006;81:907–914.
21. Scherer R, Kabatnik M, Erhard J, Peters J. The influence of antithrombin III (AT III) substitution to supranormal activities on systemic procoagulant turnover in patients with end-stage chronic liver disease. Intensive Care Med 1997;23:1150–1158.
22. Warren BL, Eid A, Singer P, et al. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. JAMA 2001;286:1869–1878.
23. Amitrano L, Guardascione MA, Menchise A, et al. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. J Clin Gastroenterol 2010;44:448–451.
24. Delgado MG, Seijo S, Yepes I, et al. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. Clin Gastroenterol Hepatol 2012;10:776–783.
25. Villa E, Camma C, Marietta M, et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology 2012;143:1253–1260 e1–4.
26. Potze W, Arshad F, Adelmeijer J, et al. Differential in vitro inhibition of thrombin generation by anticoagulant drugs in plasma from patients with cirrhosis. PLoS One 2014;9:e88390.
27. Senzolo M, Rodriguez-Castro KI, Rossetto V, et al. Increased anticoagulant response to low-molecular-weight heparin in plasma from patients with advanced cirrhosis. J Thromb Haemost 2012;10:1823–1829.
28. Yoshida D, Nagao Y, Tomikawa M, et al. Predictive factors for platelet count after laparoscopic splenectomy in cirrhotic patients. Hepatol Int 2012;6:657–661.
29. **Martinez M, Tandra A, Vuppalanchi R. Treatment of acute portal vein thrombosis by non-traditional anticoagulation. Hepatology 2014 Jan 7.** http://dx.doi.org/10.1002/hep.26998 **[Epub ahead of print].**
30. Pannach S, Babatz J, Beyer-Westendorf J. Successful treatment of acute portal vein thrombosis with rivaroxaban. Thromb Haemost 2013;110:626–627.

Clin Transplant 2014: 28: 1256–1262 DOI: 10.1111/ctr.12457

Clinical Transplantation

Evaluation of graft stiffness using acoustic radiation force impulse imaging after living donor liver transplantation

Ijichi H, Shirabe K, Matsumoto Y, Yoshizumi T, Ikegami T, Kayashima H, Morita K, Toshima T, Mano Y, Maehara Y. Evaluation of graft stiffness using acoustic radiation force impulse imaging after living donor liver transplantation.

Abstract: Acoustic radiation force impulse (ARFI) imaging is an ultrasound-based modality to evaluate tissue stiffness using short-duration acoustic pulses in the region of interest. Virtual touch tissue quantification (VTTQ), which is an implementation of ARFI, allows quantitative assessment of tissue stiffness. Twenty recipients who underwent living donor liver transplantation (LDLT) for chronic liver diseases were enrolled. Graft types included left lobes with the middle hepatic vein and caudate lobes (n = 11), right lobes (n = 7), and right posterior segments (n = 2). They underwent measurement of graft VTTQ during the early post-LDLT period. The VTTQ value level rose after LDLT, reaching a maximum level on postoperative day 4. There were no significant differences in the VTTQ values between the left and right lobe graft types. Significant correlations were observed between the postoperative maximum value of VTTQ and graft volume-to-recipient standard liver volume ratio, portal venous flow to graft volume ratio, and post-LDLT portal venous pressure. The postoperative maximum serum alanine aminotransferase level and ascites fluid production were also significantly correlated with VTTQ. ARFI may be a useful diagnostic tool for the noninvasive and quantitative evaluation of the severity of graft dysfunction after LDLT.

Hideki Ijichi, Ken Shirabe, Yoshihiro Matsumoto, Tomoharu Yoshizumi, Toru Ikegami, Hiroto Kayashima, Kazutoyo Morita, Takeo Toshima, Yohei Mano and Yoshihiko Maehara

Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Key words: acoustic radiation force impulse – graft function – graft stiffness – living donor liver transplantation

Corresponding author: Hideki Ijichi, MD, PhD, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
Tel.: 81-92-642-5466;
fax: 81-92-642-5482;
e-mail h_iditi@yahoo.co.jp

Conflict of interest: None.

Accepted for publication 2 September 2014

Living donor liver transplantation (LDLT) has emerged as a critical surgical option for patients with end-stage liver disease of various etiologies (1). However, problems related to graft size have been found, and small-for-size (SFS) syndrome remains a major complication after adult-to-adult LDLT (2). Previous reports have demonstrated that SFS syndrome can lead to severe graft dysfunction and decrease recipient survival rates after transplantation (3, 4). The use of SFS grafts (graft-to-recipient weight ratio [GRWR] of <0.8–1.0% or graft volume-to-recipient standard liver volume [GV/SLV] ratio of <30–40%) has been a risk factor for SFS syndrome (5). Moreover, multiple factors related to both donors and recipients influence the occurrence of this syndrome (6). The mechanisms of SFS syndrome have been investigated, and it has been found that increased portal venous flow (PVF) and hypertension are the important etiologic factors leading to the development of SFS syndrome (7, 8). Shear stress caused by transient portal hypertension is thought to induce damage to sinusoidal endothelial cells, which leads to the subsequent process of hepatocyte injury (9).

Acoustic radiation force impulse (ARFI) imaging is a new ultrasound-based modality to evaluate tissue stiffness using short-duration acoustic pulses in the region of interest selected on a conventional B-mode image (10). An acoustic push pulse transmitted by the transducer toward the tissue produces shear waves that propagate into the tissue. Virtual touch tissue quantification (VTTQ) measures the velocity of the shear wave propagation, which allows quantitative assessment of tissue stiffness. VTTQ is the first available implementation of ARFI (11). ARFI has been applied to determine the elasticity of various tissues such as liver,

kidney, spleen, and pancreas (11, 12). Recently, several studies have shown that ARFI is a useful modality for noninvasive evaluation of liver diseases (13, 14). Moreover, our data have also shown that liver stiffness evaluated by ARFI is significantly correlated with the elevation of serum total bilirubin levels after donor hepatectomy (15).

SFS syndrome is characterized by impairments of graft function such as prolonged cholestasis and increased ascites output (2). Although there are several noninvasive methods for the evaluation of graft function after LDLT, the value of ARFI in the assessment of graft stiffness has not been reported so far. The purpose of this study was to investigate the clinical utility of measuring graft stiffness using ARFI after LDLT.

Patients and methods

Patients

Between April 2010 and March 2011, a total of 27 cases of adult LDLT were performed at Kyushu University Hospital. Among these, seven recipients were excluded because of the difficulty in obtaining their consent, and consequently, 20 recipients were enrolled in the study. LDLTs were performed as a result of liver cirrhosis resulting from hepatitis C (n = 11), alcoholic cirrhosis (n = 4), primary biliary cirrhosis (n = 1), primary sclerosing cholangitis (n = 1), and others (n = 3). Graft types included left lobes (LL) with the middle hepatic vein (MHV) and caudate lobes (n = 11), right lobes (RL) without the MHV (n = 7), and right posterior segments (n = 2). All the LDLTs were performed after obtaining full informed consent from the patients and approval by the Liver Transplantation Committee of Kyushu University Hospital. The study protocol was conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Donor and graft selection

Donors were selected from among the candidates who offered to be living donors (16, 17). They were limited to within the third degree of consanguinity with recipients or spouses and were aged between 20 and 65 yr. For a donor beyond the third degree of consanguinity with the recipient, individual approval was obtained from the Ethics Committee of Kyushu University Hospital. Three-dimensional computed tomography was used for volumetric analysis and delineation of vascular anatomy. Our decision on the type of liver graft was based on the preoperatively predicted GV/SLV ratio. LL grafts were selected when the predicted GV/SLV ratio was >35%. We decided to use RL grafts for recipients whose GV/SLV ratio was going to be <35% if LL grafts were selected, or for recipients with a high model for end-stage liver disease (MELD) score. Moreover, our selection criteria for RL grafts included the requirement that the estimated remnant liver volume was >35% in the donor. However, graft selection is still carried out on a case-by-case basis, considering various factors such as anatomical variations and recipient and donor conditions.

Operative procedure

The surgical procedures for both donors and recipients have been described elsewhere (17, 18). Briefly, parenchymal transection of the donor liver was performed using the Cavitron Ultrasonic Surgical Aspirator (Valleylab Inc, Boulder, CO, USA) and electrocautery. The recipient total hepatectomy was usually performed while preserving the vena cava. After venous and portal anastomoses, hepatic arteries were reconstructed under the operative microscope. MHV tributaries in the anterior segment of RL graft were reconstructed according to a previously described algorithm (19). Biliary reconstruction was performed using duct-to-duct anastomosis (n = 19) or hepatico-jejunostomy (n = 1). Simultaneous splenectomy (n = 14) was performed to decrease the portal pressure or alleviate persistent thrombocytopenia (20), and three cases had undergone splenectomy prior to LDLT.

Assessment of portal hemodynamics and postoperative graft function

Intra-operative portal venous pressure (PVP) was monitored via a branch of the mesenteric vein. PVF was measured by an electromagnetic blood flowmeter (MVF-3100; Nihon Koden Corp., Tokyo, Japan) during surgery. The serum levels of alanine aminotransferase (ALT), total bilirubin, and the amount of daily production of ascites were recorded daily for seven d after surgery to assess postoperative graft function. Ascites production was defined as the loss of the fluid through indwelling drains and surgical wounds.

Assessment of tissue stiffness by VTTQ

The stiffness of the graft was measured by ARFI, using the ACUSON S2000 ultrasound system (Mochida Siemens Medical Systems, Tokyo, Japan) with the VTTQ software package. Measurements were performed daily for seven d after surgery. B-mode images were obtained throughout

the liver graft, after which ARFI was performed in all patients. Each measurement was triggered at the end of an expiratory phase, and its timing was intended to avoid the effect of the heartbeat to reduce the motion artifacts (21). The region of interest was placed at a depth between 2 and 5 cm below the liver capsule and kept away from the large blood vessels (11). To ensure the quality of the data, a total of 10 valid measurements per liver segment (S2, 3, 4, 5, 6 and 8) were performed, and their mean values were recorded (i.e., the VTTQ value of the right liver graft was the mean of S5, 6, and 8, and that of the left liver graft was the mean of S2, 3, and 4). The VTTQ value of the donor liver before surgery was also measured (S2, 3, 4, 5, 6, and 8). The results of the VTTQ measurement were expressed as meters per second (m/s).

Statistical analysis

All values were expressed as mean ± standard error of the mean. All statistical analyses were performed using the StatView® 5.0 software package (Abacus Concepts, Berkeley, CA, USA). Statistical significance was determined by the Student's *t*-test or the Mann–Whitney *U*-test. Correlation between VTTQ value and variable parameters was determined by linear regression analysis. The differences were considered to be significant if $p < 0.05$.

Results

Patients characteristics

The clinical parameters of patients are summarized in Table 1. The mean age of the recipients was 56.2 ± 2.0 yr (range, 40–72), and the sex ratio (M:F) was 8:12. The mean Child–Pugh score and MELD score were 9.8 ± 0.5 and 16.3 ± 1.3. The mean GV/SLV ratio and GRWR were 40.4 ± 2.0% and 0.76 ± 0.04%, respectively. Post-LDLT PVP measured at the end of the operation was 15.0 ± 0.8 mmHg, which was significantly lower than that of pre-LDLT (24.4 ± 1.2 mmHg, $p < 0.0001$). The mean PVF measured at the end of the operation was 1744.5 ± 133.1 mL/min, and portal venous flow to graft volume (PVF/GV) ratio was 3.8 ± 0.3 mL/min/g. The mean age of the donors was 35.0 ± 2.3 yr (range, 20–55), and the sex ratio (M:F) was 14:6.

Changes in the stiffness of the graft

Figure 1A shows the serial changes in the VTTQ values in all patients after LDLT. The baseline value before the operation in the donor liver was 1.21 ± 0.03 m/s. The VTTQ value level rose after LDLT, reaching a maximum level on postoperative day (POD) 4, and the level declined thereafter. The patients were divided into three groups according to graft type: LL graft (n = 11), RL graft (n = 7), and right posterior segment graft (n = 2). There were no significant differences in the VTTQ values between the LL graft and RL graft after LDLT (Fig. 1B).

Table 1. Clinical features of patients

Variables	Number of patients
Age (yr)	
Mean (range)	56.2 (40–72)
Gender	
Male:female	8:12
Indications	
Liver cirrhosis	
Hepatitis C (HCC)	11 (11)
Alcohol (HCC)	4 (2)
Primary biliary cirrhosis	1
Primary sclerosing cholangitis	1
Others	3
Child–Pugh score	9.8 ± 0.5
MELD score	16.3 ± 1.3
Donor age (yr)	
Mean (range)	35.0 (20–55)
Donor gender	
Male:female	14:6
Blood combination	
Identical: compatible: imcompatible	13:4:3
GV (g)	473.8 ± 28.8
GV/SLV (%)	40.4 ± 2.0
GRWR (%)	0.76 ± 0.04
Operative time (min)	757.5 ± 28.0
Cold ischemic time (min)	83.5 ± 9.8
Warm ischemic time (min)	36.5 ± 1.9
Blood loss (g)	2348.8 ± 480.5
Pre-LDLT PVP (mmHg)	24.4 ± 1.2
Post-LDLT PVP (mmHg)	15.0 ± 0.8
PVF (mL/min)	1744.5 ± 133.1
PVF/GV (mL/min/g)	3.8 ± 0.3
Peak ALT (U/L)	489.9 ± 162.2
Peak T-Bil (mg/dL)	7.8 ± 0.9
Peak ascites production (mL/d)	1226.5 ± 249.3

HOC, hepatocellular carcinoma; MELD, model for end-stage liver disease; GV, graft volume; SLV, standard liver volume; GV/SLV, graft volume-to-recipient standard liver volume; GRWR, graft-to-recipient weight ratio; LDLT, living donor liver transplantation; PVP, portal venous pressure; PVF, portal venous flow; PVF/GV, portal venous flow to graft volume; ALT, alanine aminotransferase; T-Bil, total bilirubin.

Correlation between the value of VTTQ and clinical parameters

Correlations between VTTQ and clinical parameters were analyzed. Significant correlations were observed between the postoperative maximum value of VTTQ and GV/SLV ratio ($R^2 = 0.233$,

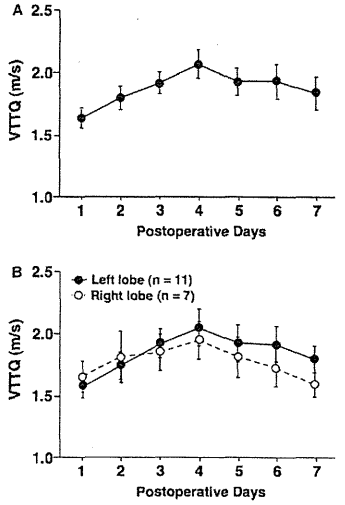

Fig. 1. Serial changes in the virtual touch tissue quantification (VTTQ) of the graft after LDLT. (A) The VTTQ values in all patients increased after LDLT until postoperative day (POD) 4 and then tended to decrease. (B) There were no significant differences in the VTTQ values between the left lobe (LL) graft and right lobe (RL) graft types.

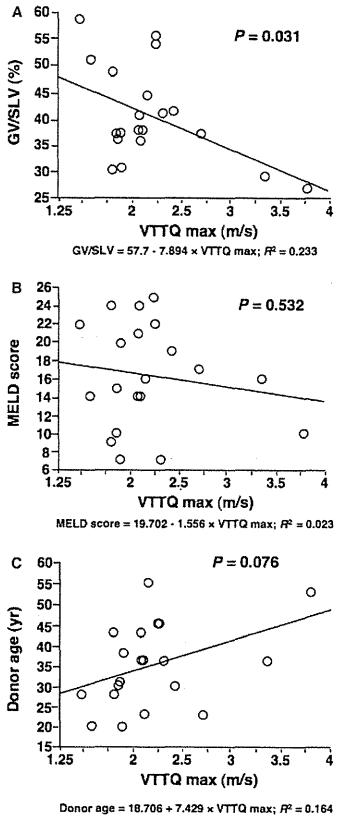

Fig. 2. Correlation between the values of virtual touch tissue quantification (VTTQ) and graft volume-to-recipient standard liver volume (GV/SLV) ratio, model for end-stage liver disease (MELD) score, and donor age. Although a significant correlation was observed between the postoperative maximum value of VTTQ and GV/SLV ratio (R^2 = 0.233, $p < 0.05$) (A), MELD score (B), and donor age (C) were not correlated with VTTQ.

$p < 0.05$) (Fig. 2A), and GRWR (R^2 = 0.248, $p < 0.05$). However, MELD score and donor age were not correlated with VTTQ (Fig. 2B,C). Although PVF was not correlated with VTTQ, there were significant correlations between VTTQ and PVF/GV (R^2 = 0.401, $p < 0.005$), and post-LDLT PVP (R^2 = 0.403, $p < 0.005$) (Fig. 3A,B). The postoperative maximum serum ALT level was significantly correlated with VTTQ (R^2 = 0.349, $p < 0.01$) (Fig. 4A); in contrast, serum total bilirubin level was not correlated. There was a very strong correlation between VTTQ and the maximum ascites fluid production (R^2 = 0.705, $p < 0.001$) (Fig. 4B).

Impact of the value of VTTQ on clinical course

The patients were divided into two groups based on the median postoperative maximum value of VTTQ (2.08 m/s): group L (low VTTQ value ≤2.08, n = 10) and group H (high VTTQ value >2.08, n = 10). The mean VTTQ values were 1.83 ± 0.06 in group L and 2.54 ± 0.18 in group H. There were no significant differences in post-transplant hospital stay and graft survival between both groups. However, the patients in group L had a significantly shorter time to extubation (1.2 ± 0.2 vs. 3.8 ± 1.0 d, $p < 0.05$) and intensive care unit (ICU) length of stay (4.7 ± 1.3 vs. 6.6 ± 1.0 d, $p < 0.05$) compared with those in group H after LDLT.

Discussion

In the present study, the graft stiffness measured by ARFI increased after LDLT until POD 4, and tended to decrease thereafter. The highest degree of graft stiffness during the early post-LDLT period was significantly correlated with perioperative

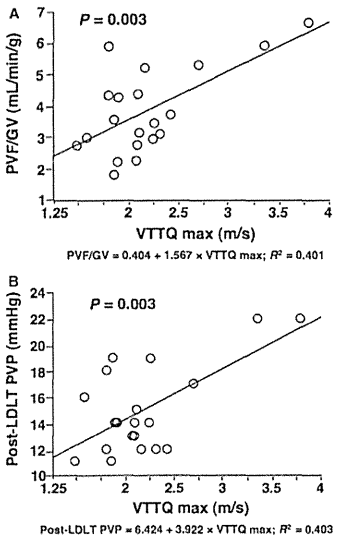

Fig. 3. Correlation between the values of virtual touch tissue quantification (VTTQ) and portal venous flow to graft volume (PVF/GV) ratio, and post-LDLT portal venous pressure (PVP). The postoperative maximum value of VTTQ had strong correlations with both PVF/GV ratio (R^2 = 0.401, p < 0.005) (A) and post-LDLT PVP (R^2 = 0.403, p < 0.005) (B).

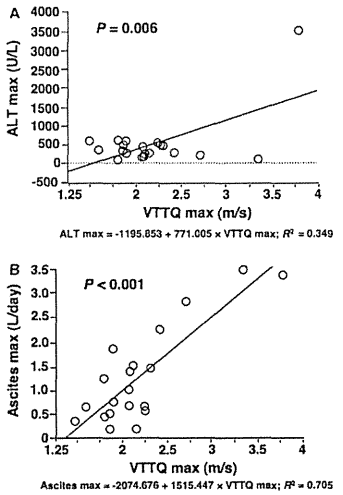

Fig. 4. Correlation between the values of virtual touch tissue quantification (VTTQ) and the postoperative maximum serum alanine aminotransferase (ALT) level, and ascites fluid production. The postoperative maximum serum ALT level was significantly correlated with VTTQ (R^2 = 0.349, p < 0.01) (A), and there was a very strong correlation between VTTQ and the maximum ascites fluid production (R^2 = 0.705, p < 0.001) (B).

variables, including graft size, portal hypertension, graft injury, and graft dysfunction. However, there were no significant differences in stiffness between LL graft and RL graft types.

SFS syndrome, which is characterized clinically by prolonged cholestasis, ascites, and coagulopathy, is one of the major causes of graft failure after LDLT (3, 4). Previous reports have suggested that multiple risk factors affect the development of SFS syndrome (6). These factors can be divided into graft-related factors such as graft size and donor age and recipient-related factors such as severity of cirrhosis and portal hypertension (6, 22, 23). In the setting of adult-to-adult LDLT, small grafts are exposed to relatively excessive portal perfusion and pressure, as cirrhotic recipients demonstrate higher portal flow than non-cirrhotic patients (24). It is thought that shear stress resulting from portal hypertension might lead to sinusoidal endothelial cell injury and the release of deleterious mediators, which is ultimately a cause of serious graft injury (9). Our data show that graft stiffness was negatively correlated with GV/SLV ratio and GRWR and had a strong positive correlation with PVF/GV and post-LDLT PVP. Moreover, graft stiffness was positively correlated with the postoperative maximum level of serum ALT. These data suggest that graft injury induced by the excessive shear stress might play an important role in the elevation of graft stiffness during the early post-LDLT period.

Ascites production also had a strong positive correlation with graft stiffness. Shirouzu et al. (25) suggest that sinusoidal endothelial injury caused by relative portal hyperperfusion into the graft may be a mechanism of ascites production. From the data presented, we assume that increased graft stiffness may also be an important factor involved in the pathogenesis of ascites production after LDLT. During the early post-LDLT period, excessive portal perfusion and pressure can increase graft stiffness, which generates an altered physiological state, including additional portal hypertension, which may contribute to an increase in ascites production. Notably, Cirera et al. (26) have reported that post-sinusoidal portal hypertension due to difficulty in hepatic venous outflow is one of the major causes of massive ascites production after liver transplantation. Although we have no data regarding hepatic venous pressure after LDLT, we have speculated that there are no hepatic outflow complications in our patients, because

the hepatic venous flow evaluated by daily Doppler ultrasound examination demonstrates no significant abnormalities.

In contrast, graft stiffness was not significantly correlated with MELD score and donor age. Moreover, the choice of graft type (LL or RL) had no significant influence on graft stiffness during the early post-LDLT period. Previously, we have reported a predictive model, which has indicated that graft size, donor age, and patient status (MELD score and presence of shunts) are important factors relating to early graft function after LDLT (6). In the present study, the choice of graft type was also made in consideration of this predictive model. Although LL grafts were mainly selected in cases of younger donors and low MELD score recipients, RL grafts were used in cases of high MELD score recipients to maximize the safety of both donors and recipients. Therefore, it seems that our patients would have almost the same level of graft function regardless of graft type, donor age, and MELD score during the early post-LDLT period. Consequently, we could also find no significant correlation between graft stiffness and these factors, because our data suggested that graft stiffness might reflect the graft function.

In the present study, the elevation of graft stiffness during the early post-LDLT period was significantly associated with a longer time to extubation and ICU stay. Based on these results, we also assume that graft stiffness reliably reflects the graft function. Although our data did not show that the elevation of graft stiffness could predict post-transplant survival, further evaluation during the post-LDLT period might reveal the relationship between graft stiffness and survival. Therefore, it is possible that the serial assessment of graft stiffness can play an important role in the management of patients after LDLT.

Although several studies have shown that the measurement of liver stiffness is useful for the assessment of liver diseases, including liver fibrosis and non-alcoholic fatty liver disease (13, 14, 27), there are few reports concerning changes in liver stiffness evaluated by ARFI during the early post-operative period. Recently, we have demonstrated that ARFI can be an efficient modality for the assessment of the remnant liver after a living donor hepatectomy (15). Distinct from the recipient data presented in this study, the type of donor remnant liver had a significant influence on the elevation of the stiffness. It is possible that the patient status may be the cause of the difference. Exposure of the graft-to-portal hyperperfusion in cirrhotic recipients can result in the elevation of graft stiffness. In contrast, the remnant liver volume seems to have a great influence on the change of stiffness in healthy donors with normal PVF, because our data have shown that PVF/GV has a strong positive correlation with the graft stiffness.

The question of whether liver regeneration influences the changes of graft stiffness after LDLT is not resolved by our data. After donor hepatectomy, the hepatocyte proliferation and subsequent formation of hepatic islands may reflect the increases in the stiffness of the remnant liver. However, it is not clear whether the transplanted liver, which is damaged by the portal hyperperfusion and ischemia-reperfusion injury, can go through the normal regeneration process. The evaluation of graft stiffness for longer during the post-LDLT period may be necessary to provide clues to the answers to these questions.

In summary, our studies demonstrate that the elevation of graft stiffness measured by ARFI is associated with the factors that influence the development of graft dysfunction, especially in SFS grafts, during the early post-LDLT period. Further studies may reveal the usefulness of the measurement of graft stiffness for assessment of other forms of graft injury, including acute cellular rejection and the recurrence of hepatitis C. Therefore, ARFI seems to be an effective diagnostic tool for the noninvasive and quantitative evaluation of severity of graft dysfunction after LDLT.

References

1. Florman S, Miller CM. Live donor liver transplantation. Liver Transpl 2006: 12: 499.
2. Dahm F, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. Am J Transplant 2005: 5: 2605.
3. Kiuchi T, Kasahara M, Uryuhara K et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation 1999: 67: 321.
4. Ikegami T, Shimada M, Imura S et al. Current concept of small-for-size grafts in living donor liver transplantation. Surg Today 2008: 38: 971.
5. Kiuchi T, Tanaka K, Ito T et al. Small-for-size graft in living donor liver transplantation: how far should we go? Liver Transpl 2003: 9: S29.
6. Yoshizumi T, Taketomi A, Uchiyama H et al. Graft size, donor age, and patients status are the indicators of early graft function after living donor liver transplantation. Liver Transpl 2008: 14: 1007.
7. Man K, Fan ST, Lo CM et al. Graft injury in relation to graft size in right lobe live donor liver transplantation: a study of hepatic sinusoidal injury in correlation with portal hemodynamics and intragraft gene expression. Ann Surg 2003: 237: 256.
8. Masetti M, Siniscalchi A, De Pietri L et al. Living donor liver transplantation with left liver graft. Am J Transplant 2004: 4: 1713.

9. Xu X, Man K, Zheng SS et al. Attenuation of acute phase shear stress by somatostatin improves small-for-size liver graft survival. Liver Transpl 2006: 12: 621.
10. Nightingale K, Soo MS, Nightingale R, Trahey G. Acoustic radiation force impulse imaging: in vivo demonstration of clinical feasibility. Ultrasound Med Biol 2002: 28: 227.
11. Gallotti A, D'Onofrio M, Pozzi Mucelli R. Acoustic radiation force impulse (ARFI) technique in ultrasound with virtual touch tissue quantification of the upper abdomen. Radiol Med 2010: 115: 889.
12. Palmeri ML, Frinkley KD, Zhai L et al. Acoustic radiation force impulse (ARFI) imaging of the gastrointestinal tract. Ultrason Imaging 2005: 27: 75.
13. Carrion JA, Torres F, Crespo G et al. Liver stiffness identifies two different patterns of fibrosis progression in patients with hepatitis C virus recurrence after liver transplantation. Hepatology 2010: 51: 23.
14. Toshima T, Shirabe K, Takeishi K et al. New method for assessing liver fibrosis based on acoustic radiation force impulse: a special reference to the difference between right and left liver. J Gastroenterol 2011: 46: 705.
15. Ninomiya M, Shirabe K, Ijichi H et al. Temporal changes in the stiffness of the remnant liver and spleen after donor hepatectomy as assessed by acoustic radiation force impulse: a preliminary study. Hepatol Res 2011: 41: 579.
16. Shimada M, Shiotani S, Ninomiya M et al. Characteristics of liver grafts in living-donor adult liver transplantation: comparison between right- and left-lobe grafts. Arch Surg 2002: 137: 1174.
17. Yoshizumi T, Shirabe K, Soejima Y et al. Living donor liver transplantation in patients older than 60 years. Transplantation 2010: 90: 433.
18. Soejima Y, Taketomi A, Yoshizumi T et al. Feasibility of left lobe living donor liver transplantation between adults: an 8-year, single center experience of 107 cases. Am J Transpl 2006: 6: 1004.
19. Toshima T, Taketomi A, Ikegami T et al. V5-drainage-preserved right lobe grafts improve graft congestion for living donor liver transplantation. Transplantation 2012: 93: 929.
20. Yoshizumi T, Taketomi A, Soejima Y et al. The beneficial role of simultaneous splenectomy in living donor liver transplantation in patients with small-for-size graft. Transpl Int 2008b: 21: 833.
21. Fahey BJ, Palmeri ML, Trahey GE. The impact of physiological motion on tissue tracking during acoustic force imaging. Ultrasound Med Biol 2007: 33: 1149.
22. Sanefuji K, Iguchi T, Ueda S et al. New prediction factors of small-for-size syndrome in living donor adult liver transplantation for chronic liver disease. Transpl Int 2010: 23: 350.
23. Ben-Haim M, Emre S, Fishbein TM et al. Critical graft size in adult-to-adult living donor liver transplantation: impact of the recipient's disease. Liver Transpl 2001: 7: 948.
24. Eguchi S, Yanaga K, Sugiyama N, Okudaira S, Furui J, Kanematsu T. Relationship between portal venous flow and liver regeneration in patients after living donor right-lobe liver transplantation. Liver Transpl 2003: 9: 547.
25. Shirouzu Y, Ohya Y, Suda H, Asonuma K, Inomata Y. Massive ascites after living donor liver transplantation with a right lobe graft larger than 0.8% of the recipient's body weight. Clin Transplant 2010: 24: 520.
26. Cirera I, Navasa M, Rimola A et al. Ascites after liver transplantation. Liver Transpl 2000: 6: 157.
27. Palmeri ML, Wang MH, Rouze NC et al. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. J Hepatol 2011: 55: 666.

J Hepatobiliary Pancreat Sci (2014) 21:809–817
DOI: 10.1002/jhbp.137

ORIGINAL ARTICLE

Characteristics of printing company workers newly diagnosed with occupational cholangiocarcinoma

Shoji Kubo · Masahiko Kinoshita · Shigekazu Takemura · Shogo Tanaka · Hiroji Shinkawa · Takayoshi Nishioka · Genya Hamano · Tokuji Ito · Makoto Abue · Masaru Aoki · Kei Nakagawa · Michiaki Unno · Susumu Hijioka · Toshihisa Fujiyoshi · Yasuhiro Shimizu · Toru Mizuguchi · Ken Shirabe · Akihiro Nishie · Yoshinao Oda · Kenji Takenaka · Tomonari Kobarai · Terumasa Hisano · Akio Saiura · Hiroshi Numao · Mayura Toda · Yuko Kuwae · Yasuni Nakanuma · Ginji Endo

Published online: 3 August 2014

S. Kubo (✉) · M. Kinoshita · S. Takemura · S. Tanaka · H. Shinkawa · T. Nishioka · G. Hamano · T. Ito
Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
e-mail: m7696493@msic.med.osaka-cu.ac.jp

M. Abue
Department of Gastroenterology, Miyagi Cancer Center, Natori, Japan

M. Aoki
Department of Digestive Surgery, Nippon University School of Medicine, Tokyo, Japan

K. Nakagawa · M. Unno
Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan

S. Hijioka · T. Fujiyoshi
Department of Gastroenterology, Aichi Cancer Center Hospital, Nagoya, Japan

Y. Shimizu
Department of Surgery, Aichi Cancer Center Hospital, Nagoya, Japan

T. Mizuguchi
Department of Surgery 1, Sapporo Medical University School of Medicine, Sapporo, Japan

K. Shirabe
Department of Surgery and Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

A. Nishie
Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Y. Oda
Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

K. Takenaka
Department of Surgery, Fukuoka City Hospital, Fukuoka, Japan

T. Kobarai
Department of Surgery, HaraSanshin Hospital, Fukuoka, Japan

T. Hisano
Department of Hepato-Biliary-Pancreatology, National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan

A. Saiura
Department of Surgery, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan

H. Numao
Department of Gastroenterology, Aomori Prefectural Central Hospital, Aomori, Japan

M. Toda
Department of Gastroenterology and Hepatology, Kansai Rosai Hospital, Amagasaki, Japan

Y. Kuwae
Department of Diagnostic Pathology, Osaka City University Graduate School of Medicine, Osaka, Japan

Y. Nakanuma
Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

G. Endo
Department of Preventive Medicine and Environmental Health, Osaka City University Graduate School of Medicine, Osaka, Japan

Abstract
Background Cholangiocarcinoma has been reported in workers exposed to chlorinated organic solvents and has consequently been classified as an occupational disease (occupational cholangiocarcinoma) by the Japanese Ministry of Health, Labour and Welfare. This study aimed to identify the characteristics of nine workers newly diagnosed with occupational cholangiocarcinoma.
Methods This study was a retrospective study conducted in 13 hospitals and three universities. Clinicopathological findings of nine occupational cholangiocarcinoma patients from seven printing companies in Japan were investigated and compared with 17 cholangiocarcinoma patients clustered in a single printing company in Osaka.
Results Patient age at diagnosis was 31–57 years. Patients were exposed to 1,2-dichloropropane and/or dichloromethane. Serum γ-glutamyl transpeptidase activity was elevated in all patients. Regional dilatation of the intrahepatic bile ducts without tumor-induced obstruction was observed in two patients. Four patients developed intrahepatic cholangiocarcinoma and five developed hilar cholangiocarcinoma. Biliary intraepithelial neoplasia and/or intraductal papillary neoplasm of the bile duct was observed in four patients with available operative or autopsy specimens.
Conclusions Most of these patients with occupational cholangiocarcinoma exhibited typical findings, including high serum γ-glutamyl transpeptidase activity, regional dilatation of the bile ducts, and precancerous lesions, similar to findings previously reported in 17 occupational cholangiocarcinoma patients in Osaka.

Keywords γ-glutamyl transpeptidase · Biliary intraepithelial neoplasia · Intraductal papillary neoplasm of the bile duct · Occupational cholangiocarcinoma · Organic solvent

Introduction

Recently, an outbreak of cholangiocarcinoma was reported among workers in an offset color proof-printing department at a printing company in Osaka, Japan [1, 2]. These 17 patients with cholangiocarcinoma had been exposed to chemicals, including 1,2-dichloropropane (DCP) and/or dichloromethane (DCM), at the company. This type of cholangiocarcinoma was recently recognized as an occupational disease by the Japanese Ministry of Health, Labour and Welfare, on 1 October 2013 [3]. Until the end of 2013, the Ministry confirmed "occupational cholangiocarcinoma" in the 17 former or current workers of the company [2], along with nine workers of seven other printing companies. We have previously reported the clinicopathological findings of the first 17 workers [2]. The aim of this study was to investigate the clinical and pathological findings of the nine patients employed at the other printing companies who were also diagnosed with occupational cholangiocarcinoma.

Subjects and methods

The subjects of this study were nine patients with occupational cholangiocarcinoma, which was newly recognized as an occupational disease by the Japanese Ministry of Health, Labour and Welfare until the end of 2013. Patients with cholangiocarcinoma diagnosed between March 1988 and November 2011 were enrolled in this study. The nine patients were former or current workers at seven printing companies in Hokkaido, Aomori, Miyagi, Saitama, Aichi, Osaka, and Fukuoka. All nine had been exposed to chemicals, including a high concentration of chlorinated organic solvents, such as 1,1,1-trichloroethane (TCE), DCM, and/or DCP, over a long period of time. These organic solvents are used to remove ink residues. We investigated the clinical findings, laboratory test results, diagnostic imaging results, pathologic findings, treatments, and prognoses of the nine patients. The clinicopathological findings of nine occupational cholangiocarcinoma patients from seven printing companies were compared with those in our previous study, which examined 17 cholangiocarcinoma patients clustered in a single printing company in Osaka.

Patient data, including history of alcohol intake and smoking, and clinical findings were obtained from the medical records from each hospital and/or interviews with the patients or their family members. The results of laboratory tests and diagnostic imaging were obtained from the medical records and/or films from each hospital. For all nine patients, the diagnosis of cholangiocarcinoma was confirmed at the individual hospitals by pathologists who analyzed surgically resected specimens in four patients and biopsy specimens in five patients.

The pathological findings were recorded and described according to the World Health Organization classification of intrahepatic and extrahepatic cholangiocarcinoma [4]. Intrahepatic cholangiocarcinoma was grossly classified as a mass-forming, periductal infiltrating, or intraductal growth. Extrahepatic cholangiocarcinoma was grossly classified as a papillary, nodular, scirrhous constricting, or diffusely infiltrating tumor. Preneoplastic or early preinvasive neoplastic lesions of the biliary tree were classified as flat dysplastic epithelial tumors (biliary intraepithelial neoplasia [BilIN]) or grossly visible papillary tumors (intraductal papillary neoplasm of the bile duct [IPNB]) [4–7]. BilIN lesions were histologically classified according to their cellular and structural features as BilIN-1 (mild atypia), BilIN-2 (moderate atypia), or BilIN-3 (severe atypia corresponding to *in situ*

Table 1 Clinical findings in patients with cholangiocarcinoma

Patient no.	Clinical findings		Exposure to chlorinated organic solvents
	Age	Symptom(s) or health examination	Organic solvents and periods of exposure[a]
1	37	Liver dysfunction	DCP (16 years), TCE, DCM[b]
2	42	Jaundice	DCP (16 years), TCE, DCM[b]
3	49	Liver dysfunction	DCM (12 years), TCE
4	57	Jaundice	DCP (11 years), DCM (11 years)
5	31	Abdominal pain	DCP (3 years 10 months), DCM (3 years 10 months)
6	47	Abdominal pain, back pain	DCP (12 years), DCM (12 years)
7	47	Nausea	DCP (16 years), DCM (6 years)
8	46	Epigastralgia, nausea	DCP (13 years), DCM (19 years)
9	41	Jaundice, abdominal pain	DCM (11 years), TCE

DCM dichloromethane, *DCP* 1,2-dichloropropane, *TCE* 1,1,1-trichloroethane

[a] The period of exposure to TCE was not evaluated because TCE is not considered to be a main causative agent

[b] The period of exposure to DCM was not evaluated because the amount of DCM used was small

carcinoma). BilIN-1 lesions presented with mild atypical cellular and nuclear features, such as nuclear membrane irregularities or nuclear enlargements with only minimal disturbances to cellular polarity. BilIN-2 lesions had evident aberrant cellular and nuclear features not sufficient to suggest overt carcinoma; these lesions also had focal disturbances in cellular polarity. BilIN-3 lesions presented with diffuse disturbances in cellular polarity with or without distinct atypical cellular and nuclear features that corresponded to carcinoma *in situ*. In this study, primarily BilIN-2 and BilIN-3 lesions were examined because it is controversial whether BilIN-1 lesions contain any reactive hyperplastic changes. Other pathological terms used in this study were characterized or defined as follows [2]. "Chronic bile duct injury" was used as a collective term to describe duct injuries such as epithelial damage, fibrosis of the duct wall and periductal tissue, and chronic inflammatory cell infiltration, in various combinations. "Proliferative changes of bile ducts" was used to describe bile ducts with non-neoplastic biliary epithelial proliferation. "Bile duct sclerosis" was used to describe fibrous thickening of the duct wall with or without additional periductal fibrosis.

This study was approved by the ethics committee of Osaka City University, and all subjects or their legally authorized representatives (for deceased patients) provided written informed consent. This multicenter occupational cholangiocarcinoma study group consisted of investigators at 13 hospitals (including five university hospitals) and three universities.

Statistical analysis

Student's *t*-test or the Mann–Whitney *U*-test were used to determine significant difference in age and results of laboratory tests. The χ^2 test or Fisher's exact tests was used to evaluate significant differences in the categorical data between groups. Differences with $P < 0.05$ were considered to be statistically significant. Statistical analysis was performed with JMP 9.0 (SAS Institute, Cary, NC, USA).

Results

Clinical findings

The age of the patients at diagnosis was between 31 and 57 years (mean, 44 years), and all patients were men (Table 1). Of the nine patients, five (patients 4–8) had been exposed to DCP and DCM; two (patients 1, 2) had been exposed to DCP, DCM, and TCE; and two patients (patients 3, 9) had been exposed to DCM and TCE. The period of exposure to chlorinated organic solvents ranged from 3 years and 10 months to 19 years. No patient was a habitual alcohol user (≥80 g of ethanol consumed daily), and six patients (patients 1, 5–9) were smokers. Seven patients experienced abdominal pain, back pain, nausea, and/or jaundice. Abnormal liver function was noted in two patients (patients 1, 3) during regular health examinations.

Laboratory test results

At the time of cholangiocarcinoma diagnosis, the serum concentrations of total bilirubin were elevated in six patients (Table 2). Serum aspartate aminotransferase (AST) activity was elevated in seven patients, and alanine aminotransferase (ALT) activity was elevated in eight patients. Serum γ-glutamyl transpeptidase (γ-GTP) activity was elevated in all nine patients. In two (patients 1, 3) of the three patients for whom laboratory test results from regular health

Table 2 Results of laboratory test and diagnostic imaging

Patient no.	T-Bil (mg/dL)	Laboratory tests at diagnosis					Diagnostic imaging			
		AST (IU/L)	ALT (IU/L)	γ-GTP (U/L)	CEA (ng/mg)	CA19-9 (U/ml)	Space-occupying lesion or mass lesion	Bile duct stenosis or obstruction	Dilated bile ducts due to tumor-induced obstruction	Dilated bile ducts without tumor-induced obstruction
1	1.0	114 (h)	212 (h)	526 (h)	2.5	2418 (h)	Yes	Yes	Yes	Yes
2	10.0 (h)	188 (h)	321 (h)	1404 (h)	3.1	23253 (h)	Yes	Yes	Yes	No
3	1.2	84 (h)	137 (h)	2034 (h)	0.7	565.3 (h)	Yes	Yes	Yes	No
4	22.8 (h)	34	27	105 (h)	1.4	1283 (h)	Yes	Yes	Yes	No
5	5.2 (h)	96 (h)	145 (h)	270 (h)	49.2 (h)	2392 (h)	Yes	No	Yes	Yes
6	0.6	26	36 (h)	346 (h)	11.2 (h)	3.3	Yes	Yes	No	No
7	1.8 (h)	167 (h)	391 (h)	1109 (h)	1.5	16	No	Yes	Yes	No
8	1.8 (h)	261 (h)	301 (h)	1120 (h)	1.4	8.6	Yes	Yes	Yes	No
9	9.2 (h)	55 (h)	133 (h)	437 (h)	1.6	144.6 (h)	Yes	Yes	Yes	No

γ-GTP γ-glutamyl transpeptidase, *ALT* alanine aminotrasferase, *AST* aspartate aminotrasferase, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *h* higher than the reference range, *T-Bil* total bilirubin

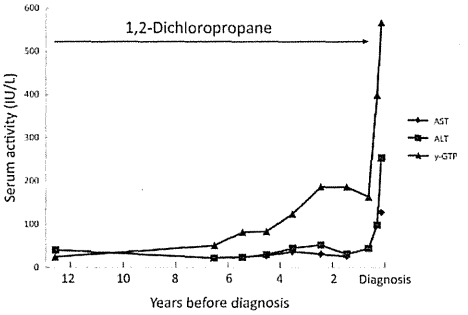

Fig. 1 Changes in laboratory test results before cholangiocarcinoma diagnosis (patient no. 1)

examinations for several years prior to the diagnosis of cholangiocarcinoma were available, the serum γ-GTP activity was observed to have increased gradually (Fig. 1). The serum concentration of carcinoembryonic antigen was elevated in two patients, and the serum concentration of carbohydrate antigen 19-9 (CA19-9) was elevated in six patients. The serum concentration of Dupan-2 was elevated in three of four patients examined, and the serum concentration of Span-1 was elevated in all three patients examined. All nine patients were negative for serum hepatitis B surface antigen and hepatitis C virus antibodies.

Diagnostic imaging results

Space-occupying lesions were observed in eight patients on computed tomography, magnetic resonance imaging, and/or ultrasonography (Table 2 and Fig. 2a). Stenosis or obstruction of the bile duct was observed in eight patients (Fig. 2b) on magnetic resonance cholangiopancreatography and/or direct cholangiography. Dilatation of peripheral bile ducts due to tumor-induced bile duct obstruction was observed in eight patients on ultrasonography, computed tomography, and/or magnetic resonance imaging (Fig. 2b). Regional dilatation of intrahepatic bile ducts without tumor-induced obstruction, a characteristic finding in the previous study [2], was observed in two patients (patients 1, 5) through magnetic resonance cholangiopancreatography (Fig. 2c).

Diagnosis and pathological findings of cholangiocarcinoma

Considering the radiological and pathological findings, four patients (patients 1, 4–6) were diagnosed with intrahepatic cholangiocarcinoma, and the remaining five patients (patients 2, 3, 7–9) were diagnosed with extrahepatic

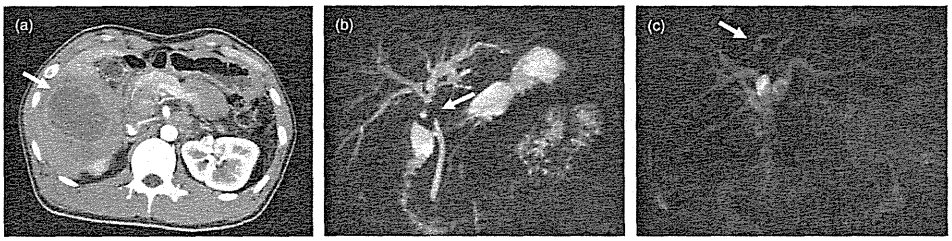

Fig. 2 Diagnostic imaging results of patients with cholangiocarcinoma. (**a**) Intrahepatic cholangiocarcinoma of the mass-forming type (arrow) in patient 5. (**b**) Extrahepatic (hilar) cholangiocarcinoma in patient 7; stenosis of the bile ducts in the hepatic hilum is observed (arrow). (**c**) Dilated intrahepatic bile ducts without tumor-induced obstruction (arrow) in patient 5

Table 3 Pathological findings of cholangiocarcinoma

Patient no.	Location	Type	Pathological examination		Stage by TNM classification
			Specimens	Histology of tumor and BilIN or IPNB in non-cancerous tissue	
1	Intrahepatic	Mass-forming	Biopsy during ERCP; autopsy	Adenocarcinoma with papillary component; adenocarcinoma, BilIN and IPNB	IVB
2	Extrahepatic (hilar)	Scirrhous constricting	Surgically resected	Tubular adenocarcinoma and BilIN	IIIB
3	Extrahepatic (hilar)	Unknown	Biopsy during ERCP	Papillary adenocarcinoma with compact pattern	I
4	Intrahepatic	Mass-forming	Surgically resected	Well to moderately differentiated adenocarcinoma and BilIN	IVB
5	Intrahepatic	Mass-forming	Needle liver biopsy	Adenocarcinoma	IVA
6	Intrahepatic	Mass-forming	Surgically resected (2 nodules)	Well to moderately differentiated adenocarcinoma with tubular or papillary component, poorly differentiated adenocarcinoma and BilIN	IVA
7	Extrahepatic (hilar)	Unknown	Biopsy during ERCP	Well differentiated adenocarcinoma with papillary component	II
8	Extrahepatic (hilar)	Diffusely infiltrating	Surgically resected	Moderately differentiated tubular adenocarcinoma	IIIA
9	Extrahepatic (hilar)	Nodular	Biopsy during ERCP	Adenocarcinoma	II

BilIN biliary intraepithelial neoplasia, *ERCP* endoscopic retrograde cholangiopancreatography, *IPNB* intraductal papillary neoplasm of the bile duct

cholangiocarcinoma (hilar cholangiocarcinoma, Table 3). The four cases of intrahepatic cholangiocarcinoma were classified as the mass-forming type. Comprehensive imaging studies and gross analyses demonstrated that the primary and most invasive cholangiocarcinoma lesions were located in the common hepatic duct, the left or right hepatic duct, or the first to third branches of the intrahepatic bile duct (also known as the large bile duct) [8]. Lymph node metastsis in three patients (patients 1, 5, 6) was observed on diagnostic imaging scans at the time of cholangiocarcinoma diagnosis.

In four (patients 1, 3, 7, 9) of the nine patients, adenocarcinoma was identified in biopsy specimens obtained during endoscopic retrograde cholangiopancreatography. Three (patients 1, 3, 7) of these specimens exhibited papillary proliferation (Fig. 3a). In one patient (patient 5),

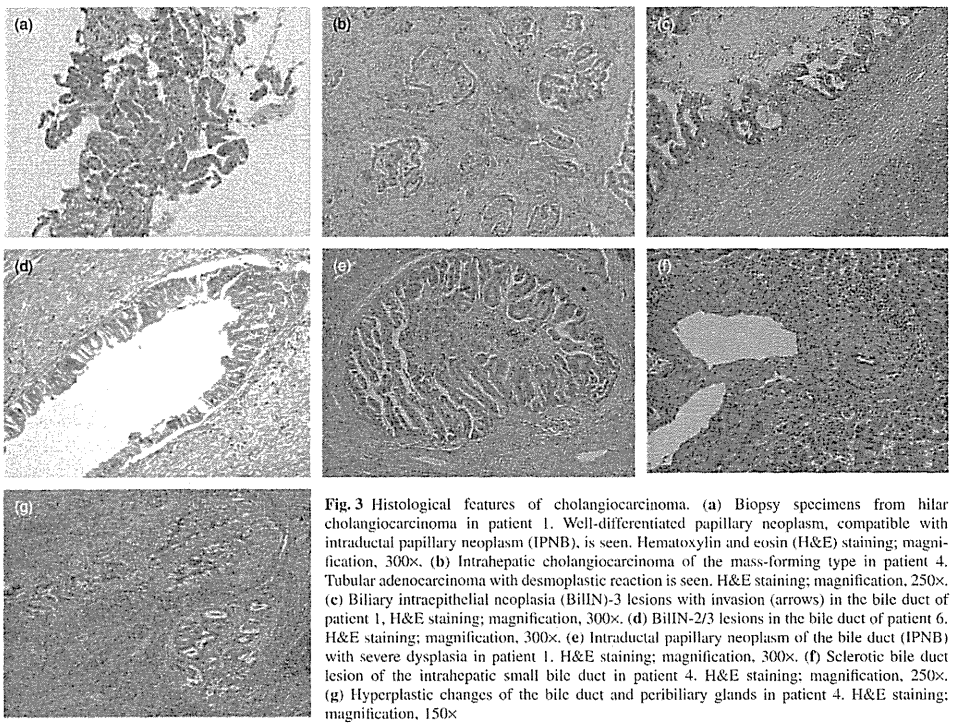

Fig. 3 Histological features of cholangiocarcinoma. (**a**) Biopsy specimens from hilar cholangiocarcinoma in patient 1. Well-differentiated papillary neoplasm, compatible with intraductal papillary neoplasm (IPNB), is seen. Hematoxylin and eosin (H&E) staining; magnification, 300×. (**b**) Intrahepatic cholangiocarcinoma of the mass-forming type in patient 4. Tubular adenocarcinoma with desmoplastic reaction is seen. H&E staining; magnification, 250×. (**c**) Biliary intraepithelial neoplasia (BilIN)-3 lesions with invasion (arrows) in the bile duct of patient 1, H&E staining; magnification, 300×. (**d**) BilIN-2/3 lesions in the bile duct of patient 6. H&E staining; magnification, 300×. (**e**) Intraductal papillary neoplasm of the bile duct (IPNB) with severe dysplasia in patient 1. H&E staining; magnification, 300×. (**f**) Sclerotic bile duct lesion of the intrahepatic small bile duct in patient 4. H&E staining; magnification, 250×. (**g**) Hyperplastic changes of the bile duct and peribiliary glands in patient 4. H&E staining; magnification, 150×

adenocarcinoma was confirmed by needle biopsy of the liver. Of the remaining four patients (patients 2, 4, 6, and 8), adenocarcinoma was confirmed by surgical specimens. An autopsy was performed on one patient (patient 1).

With regard to the histological subtypes, well- to poorly differentiated adenocarcinoma (Fig. 3b) was identified in the four patients diagnosed with the mass-forming type of intrahepatic cholangiocarcinoma, and tubular adenocarcinoma was identified in two patients with hilar cholangiocarcinoma (patients 2 and 8).

BilIN-2/3 lesions with or without invasion were detected at various sites of the large intrahepatic bile ducts and/or hilar bile ducts and the peribiliary glands in three available surgical specimens (patients 2, 4, 6) and in one autopsy specimen (patient 1, Fig. 3c,d). IPNB lesions with severe dysplasia were detected in one patient (patient 1; Fig. 3e). In these same four patients (patients 1, 2, 4, 6), sclerosis of the bile duct with variable inflammatory cell proliferation, biliary epithelial injuries/focal bile duct loss, and biliary epithelial hyperplasia were also observed at various sites of the bile ducts in the noncancerous hepatic tissues (Fig. 3f,g). With regard to the pathology of the non-neoplastic liver tissue, we observed non-specific reactive changes or cholestatic changes secondary to obstruction or stenosis of the bile ducts affected by cholangiocarcinoma or radiation.

No cirrhotic changes or other hepatobiliary diseases were detected in the noncancerous hepatic tissues of the four patients (patients 1, 2, 4, 6).

Risk factors for cholangiocarcinoma

The laboratory test results, diagnostic imaging results, and/or pathological findings indicated that the nine patients did not have any known risk factors for developing cholangiocarcinoma, such as primary sclerosing cholangitis, hepatolithiasis, pancreaticobiliary maljunction, or infection with liver flukes (e.g., *Clonorchis sinensis* or *Opisthorchis viverrini*) [4, 9–13].

Table 4 Treatments and prognoses after diagnosis

Patient no.	Treatments	Prognosis
1	Chemotherapy, stenting, hyperthermia, radiotherapy, proton beam radiotherapy	3 years 3 months, dead
2	Extended right lobectomy, resection of extrahepatic duct, resection and reconstruction of portal vein, chemotherapy, radiation	3 years 5 months, alive
3	Proton beam radiotherapy, resection of metastatic lymph node (recurrence), chemotherapy	6 years 10 months, alive
4	Extended left lobectomy, resection of extrahepatic ducts	1 year 4 months, dead
5	Chemotherapy, stenting	8 months, dead
6	Partial resection of the liver	2 years, dead
7	Chemotherapy, stenting	5 years 7 months, alive
8	Extended left lobectomy, resection of extrahepatic ducts, resection and reconstruction of portal vein, chemotherapy	4 years 7 months, alive
9	Chemotherapy, stenting	1 year 6 months, dead

Treatment and prognosis

Surgical resection was performed in four patients (Table 4). Dissection or sampling of lymph nodes was performed in three patients (patients 2, 4, 8); one (patient 2) of the three patients exhibited metastasis to the lymph nodes around the common bile duct or the common hepatic artery. Portal invasion was diagnosed in two patients (patients 2, 8) by pathological examination. Neoadjuvant chemoradiotherapy with gemcitabine and adjuvant chemotherapy with gemcitabine and S-1 (tegafur/gimeracil/oteracil potassium) was administered to one patient (patient 2). In one patient (patient 6), chemotherapy with cisplatin and 5-fluorouracil was administered before surgery. Another patient (patient 3) received proton beam radiotherapy followed by chemotherapy (gemcitabine and cisplatin), according to his wishes. In the remaining four (patients 1, 5, 7, and 9) of the nine patients, chemotherapy (S-1, gemcitabine, and/or cisplatin) with stenting to treat the obstruction of the bile duct was performed because of the advanced stage of the disease, as indicated by multiple tumors in the liver and metastasis to the lymph nodes. One patient (patient 1) received hyperthermia, radiation, and proton beam radiation therapy in addition to chemotherapy.

Among the four patients who underwent surgical treatment, intrahepatic recurrence or local recurrence occurred in three patients (patients 4, 6, and 8). In these three patients, radiation (patient 4), second liver resection (patient 6), and chemotherapy (patient 8) was performed, respectively. In one patient who underwent proton beam radiotherapy and chemotherapy, surgical resection of the lymph node recurrence was performed (patient 3).

Two (patients 4, 6) of the four patients who underwent surgical treatment died of cholangiocarcinoma recurrence. Three (patients 1, 5, 9) of the remaining five patients died of cholangiocarcinoma. The survival time from the diagnosis of cholangiocarcinoma to death or the end of the study (February 2014) ranged from 255 to 2517 days (median, 825 days).

Comparison in clinicopathlogical findings between 17 patients at a printing company in Osaka and in nine patients at other printing companies

The mean age was significantly higher in the nine patients at other printing companies than in the 17 patients at a printing company in Osaka (Table 5). The proportions of patients with elevated serum activity of γ-GTP and elevated serum concentration of CA19-9 were not different between the groups. When the regional dilatation of intrahepatic bile ducts without tumor-induced obstruction were investigated in patients without hilar cholangiocarcioa or upper bile duct cancer (because the dilatation of intrahepatic bile duct due to tumor-induced obstruction tends to obscure this characteristic), the proportion of patients with the regional dilatation of intrahepatic bile ducts without tumor-induced obstruction were not different between the groups. The proportion of patients with intrahepatic cholangiocarcinoma and/or extrahepatic cholangiocarcinoma was not different between the groups. The preneoplastic or early preinvasive neoplastic lesions such as BilIN or IPNB, and chronic bile duct injury were detected in all patients examined in both groups.

Discussion

We previously reported on 17 patients with occupational cholangiocarcinoma who were former or current workers in an offset color proof-printing department at a printing company in Osaka, Japan [2]. These patients had been exposed to a high concentration of chlorinated organic

Table 5 Characteristic findings in 17 patients with occupational cholangiocarcinoma at a printing company in Osaka and in nine patients at other printing companies

Findings	Company in Osaka ($n = 17$)	Other companies ($n = 9$)	*P*
Age (years old, mean)	25–45 (36)	31–57 (44)	0.0046
Elevated γ-GTP	17	9	>0.999
Elevated CA 19-9	13	6	0.661
Regional dilated bile ducts[a]	5/10	2/4	>0.999
ICC:ECC:ICC+ECC	10:5:2	4:5:0	0.312
BilIN and/or IPNB[b]	8/8	4/4	>0.999
Chronic bile duct injury[b]	8/8	4/4	>0.999

γ-GTP γ-glutamyl transpeptidase, *BilIN* biliary intraepithelial neoplasia, *CA 19-9* carbohydrate antigen 19-9, *ECC* extrahepatic cholangiocarcinoma, *ICC* intrahepatic cholangiocarcinoma, *IPNB* intraductal papillary neoplasm of the bile duct

[a] The lesions were examined in patients without hilar cholangiocarcinoma or upper bile duct carcinoma

[b] The pathological findings were revealed upon examination of the operative or autopsy specimens

solvents, including TCE, DCM, and DCP, over a long period of time (from 6 years and 1 month to 19 years and 9 months). DCM is classified under group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer [14]. As a result of the meticulous analysis of patients with this type of cholangiocarcinoma who have had a long history of the exposure to DCP and/or DCM, cholangiocarcinoma was recognized as a new occupational disease by the Japanese Ministry of Health, Labour and Welfare as of 1 October 2013 [3]. Until the end of 2013, the cholangiocarcinoma cases identified in 26 patients (17 original patients from one company and nine patients who worked at seven other printing companies) were classified as this new type of occupational cholangiocarcinoma.

The 17 original patients with occupational cholangiocarcinoma had the following characteristics: in addition to being relatively young, they had elevated serum activity of γ-GTP and serum concentration of CA19-9, regional dilatation of intrahepatic bile ducts without tumor-induced obstruction, precancerous or early-cancerous lesions at various parts of the bile ducts (BilIN and IPNB), and chronic bile duct injury (Table 5). Typically, cholangiocarcinoma occurs in the sixth or seventh decade of life, and the disease is rarely seen in younger patients. In the current patient series, patient ages ranged from 31 to 57 years (mean, 44 years). Although the mean age was significantly higher in the current series than in the previous series, the mean age in the current series also seemed to be young, similar to the reports in previous series (mean, 36 years). At cholangiocarcinoma diagnosis, the serum activity of γ-GTP was elevated in all 26 patients in both the previous and current studies (Table 5). In one patient in the previous series and two patients in the current series, we observed that the serum activity of γ-GTP increased gradually, with or without elevated AST and ALT activity, during regular health examination before the cholangiocarcinoma detection [15, 16]. These findings suggest that the observed liver dysfunction might be related to the patient's exposure to chlorinated organic solvents. The presence of regional dilatation of intrahepatic bile ducts without tumor-induced obstruction is a characteristic finding on diagnostic imaging [2]. The regional dilatation of intrahepatic bile ducts without tumor-induced obstruction was observed in five of the 10 patients in the previous series and in two of the four patients in the current series. In the current series, the primary and most invasive cholangiocarcinoma lesions were located in the large bile ducts, similar to the previous series. In the previous series, pathological examination revealed that BilIN-2/3 lesions and/or IPNB lesions with or without invasive portions were observed at various sites of the large intrahepatic bile ducts and/or hilar bile ducts and peribiliary glands. In addition, chronic bile duct injury, proliferative changes of the bile ducts, and bile duct sclerosis, those are characteristics of the pathological findings of the patients of the previous series [2, 17], were observed at various sites of the bile ducts in the noncancerous hepatic tissue. In the current series, operative or autopsy specimens were available for four patients. BilIN-2/3 and/or IPNB lesions with or without invasion, and chronic bile duct injury were observed at various sites of the bile ducts in all four patients. Thus, the pathological findings in the current series were similar to those of the previous series, although pathological examination could not be performed for all patients in either series. Taken together, most of the patients in the current study had characteristic findings of cholangiocarcinoma patients who had been exposed to chlorinated organic solvents.

Although five of the nine patients died of cholangiocarcinoma, four patients survived for more than 3 years after diagnosis. In all four of these patients, surgery, chemo-