Original Article

Clinical Significance of Serum Antithrombin III Activity After Hepatectomy for Hepatocellular Carcinoma

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ABSTRACT

Background   As antithrombin III (AT-III) is produced in the hepatocytes, its serum activity decreases at the time of liver failure, in addition to ischemia reperfusion injury, vascular endothelial dysfunction, and disseminated intravascular coagulation (DIC). Here, we examined whether the serum AT-III value after hepatectomy could be a prognostic factor for hepatocellular carcinoma (HCC).

Methods   Of 141 patients who underwent hepatectomy for HCC, data for 101 patients in whom serum AT-III activity was measured on the first postoperative day were extracted. Patients with serum AT-III activity > 50% and ≤ 50% were assigned to high value (72 cases) and low value (29 cases) groups, respectively. We examined the clinical and prognostic differences between these two groups.

Results   The average age of enrolled patients (83 men and 18 women) was 68.0 years. The 5-year overall survival rate was 88% and 60% in the high and low value groups, respectively ($P < 0.01$). Furthermore, the 2-year relapse-free survival rate was 71% and 54% in the high and low value groups, respectively ($P = 0.03$).

Conclusion   This is the first study to demonstrate that serum AT-III levels on the first postoperative day may serve as a prognostic factor in HCC patients.

Key words   Antithrombin III; Hepatectomy; Hepatocellular carcinoma; Prognostic factor

Hepatic resection is considered a curative treatment for early stage hepatocellular carcinoma (HCC).[1] Advances in surgical modalities have improved the prognosis of HCC patients, but many patients show cancer recurrence after surgery. It is imperative to identify patients with high risk of recurrence and poor prognosis, as careful surveillance of the poor prognosis group may allow early detection of recurrent disease and immediate administration of treatment. Furthermore, some positive interventions such as adjuvant chemotherapy may be possible for the high-risk group for cancer recurrence in the future.

The prognostic factors after surgery for HCC are extensively studied. As indicated in the Japan Society of Hepatology–HCC Guidelines 2017, the major prognostic factors are tumor size, tumor number, vascular invasion, and liver function. Furthermore, the guidelines state that the Glasgow Prognostic Score (GPS), neutrophil-to-lymphocyte ratio (NLR), and sarcopenia are also prognostic factors after hepatectomy.[2] Taken together, various factors such as tumor stage, liver function, inflammation, immunity, and nutrition are involved as prognostic factors.

Further, cancer and coagulation are known to be closely related. For example, the presence of malignant tumors is a possible cause of thrombotic tendency.[3] The hemostatic mechanism of blood is maintained by balance between coagulation and activities of fibrinolysis factors in the blood. Among them, antithrombin III (AT-III) accounts for about 80% of the thrombin inactivation in the blood, and is also known to inactivate other coagulation factors (FX IIa, FX Ia, FIXa, FXa), plasmin, and kallikrein.[4] AT-III is synthesized in the liver, and its levels are reduced in disseminated intravascular coagulation (DIC).[5–9] Moreover, end-stage malignancies, severe infection, liver failure, ischemia reperfusion injury,[10, 11] and vascular endothelial dysfunction decrease the activity of AT-III.

However, whether levels of AT-III post-surgery correlate with prognosis remained to be studied. This study aimed to determine the clinical and prognostic significance of serum AT-III values in patients who underwent hepatectomy for hepatocellular carcinoma.

Patients who underwent curative liver resection of HCC (*n* = 141)

Exclusion
- HCC ＋ Intrahepatic cholangiocarcinoma
- extrahepatic lesions
- liver transplantation
- combined with ablation therapy

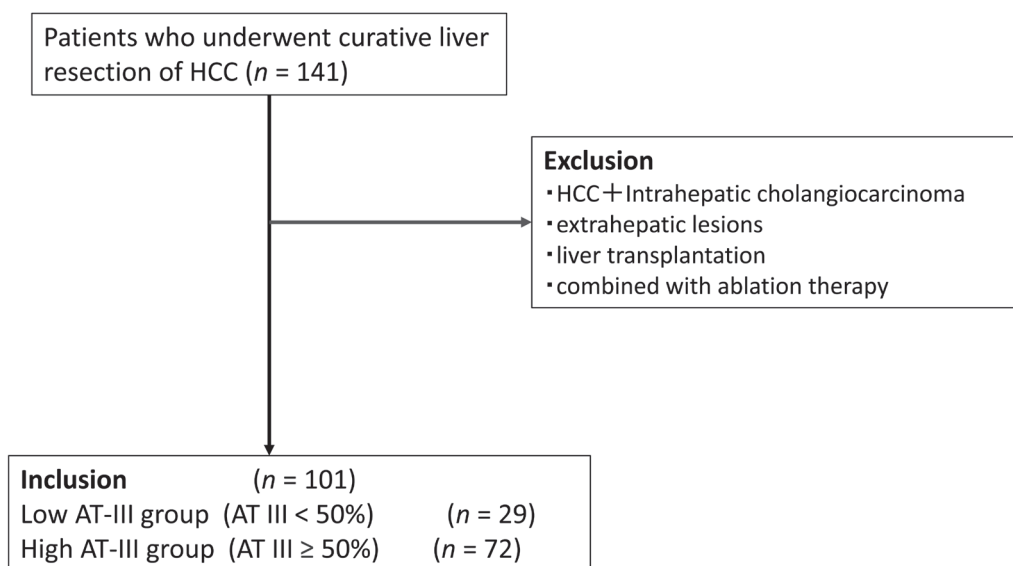|	
Inclusion	(*n* = 101)
Low AT-III group (AT III < 50%)	(*n* = 29)
High AT-III group (AT III ≥ 50%)	(*n* = 72)

Fig. 1. Patient inclusion, flow chart demonstrating patient inclusion.

MATERIALS AND METHODS
Patients
We examined the medical records of consecutive patients who underwent hepatic resection for HCC at the Tottori University Hospital between 2004 and 2013. A total of 211 patients with histologically diagnosed HCC underwent hepatectomy, and 141 patients underwent initial and curative hepatectomy. All patients were diagnosed based on contrast-enhanced computed tomography (CT) or Gd ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging (MRI). We excluded patients diagnosed with: 1) intrahepatic cholangiocarcinoma tumors based on the histological findings of the resected specimens; 2) extrahepatic lesions, preoperative vascular invasion, positive surgical margins, or macroscopic residual tumor; and 3) underwent liver transplantation or surgical resection combined with ablation therapy. Operative indications included following factors: performance status < 3, preserved liver function estimated by both indocyanine green test and volumetric CT, and good general condition without serious organ failure. Non-anatomical hepatectomy was performed in patients with insufficient volume of remnant liver or those with tumors located peripherally. Intraoperative temporary inflow clump and transfusion were performed on demand from the operator and anesthesiologists in charge, respectively. Finally, a total of 101 patients were enrolled in the study. Patients were divided into two groups based on their postoperative (day 1) AT-III activity as: High AT-III (> 50%, *n* = 72) and Low AT-III (≤ 50%, *n* = 29) group

(Fig. 1). A cutoff value of 50% was obtained based on the receiver operating characteristic (ROC) curve for overall mortality.

Written informed consent was obtained from all patients prior to surgery. Medical records were reviewed retrospectively after approval by the Institutional Review Board of the Tottori University Faculty of Medicine in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (1606A029).

Variables
The data included patient characteristics [age, sex, body mass index (BMI), cause of hepatitis, Child-Pugh score, and Model for End-Stage Liver Disease (MELD) score], tumor characteristics [number of tumors, maximum tumor diameter, and alpha-fetoprotein (AFP) level], intraoperative data (extent of resection, usage of intermittent total hepatic inflow occlusion, duration of surgery, intraoperative hemorrhage volume, and amount of intraoperative blood transfusion), and tumor pathological findings [capsule formation, microscopic vascular invasion, tumor differentiation, and stage of fibrosis of non-tumor-bearing liver according to the hepatitis activity index (HAI)].[12] Patients were followed up every 3–6 months with liver function tests, tumor marker measurements, and imaging examination with CT and/or MRI. Data for disease recurrence and survival duration were collected from our patients' database. Major resection was defined as surgery that included more than two Couinaud segments. Tumor recurrence was diagnosed

Table 1. Comparison of clinicopathological features between the low AT-III and high AT-III groups

		Low AT-III (n = 29)	High AT-III (n = 72)	P
Demographic data				
Age (yr)	≥ 75 / < 75	9/20	26/46	0.80
Gender	Male / Female	21/8	63/9	0.12
BMI (kg/m^2)	≥ 25 / < 25	6/23	20/52	0.63
Cause of hepatitis	HBV / HCV	9/11	18/30	0.84
Child-Pugh grade	A/ ≥ B	3/26	1/71	0.07
MELD score	≥ 10/ < 10	6/23	4/68	0.03*
AFP value (IU/L)	≥ 20/ < 20	12/17	24/48	0.59
Operative data				
Extent of hepatic resection				
	Non-anatomical	15/14	27/45	0.28
	Major resection	8/21	21/51	1.00
Blood loss (mL)	≥ 500/ < 500	13/14	29/34	0.25
Blood transfusion	Yes	9	17	0.63
Pringle maneuver	Yes	18	44	1.00
Pathological data				
Tumor number	Mt/St	12/17	10/62	< 0.01**
Tumor size (cm)	≥ 5 / < 5	9/20	16/56	0.50
Vascular invasion	Yes	12/17	21/51	0.34
TNM stage	≥ II / IA, IB	19/10	30/42	0.05
Liver cirrhosis (HAI = IV)	Yes	11/18	22/50	0.63

*$P < 0.05$, **$P < 0.01$. AFP, alpha-fetoprotein; BMI, body mass index; HAI, hepatitis activity index; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD score, Model for End-Stage Liver Disease score; Mt, multiple tumor; St, solitary tumor; TNM, tumor (T) nodes (N) and metastases (M); yr, year(s).

based on the findings of either CT or MRI that was routinely performed every 6 months. Extrahepatic recurrence was defined as recurrence that included only extrahepatic metastasis without intrahepatic recurrence. Early phase recurrence was defined as recurrence diagnosed within two years after the operation, and late phase recurrence was defined as recurrence diagnosed more than two years after the operation.

Statistical analysis

The primary endpoint of this study was overall survival rate, and the secondary endpoints were disease-free survival rate and early phase recurrence rate in the high AT-III and low AT-III groups. Overall survival (OS) and recurrence-free survival (RFS) were determined by Kaplan-Meier analysis. Predictive factors of overall survival and early recurrence were investigated using multivariate analysis based on the variables selected by each univariate analysis using a logistic regression model and Cox proportional hazards regression model,

respectively. All continuous values are presented as mean ± standard deviation (SD). Statistical analysis was performed using the Chi-square test and Fisher's exact test (when less than five variables) for categorical variables. Welch's two-sample t-test was used for continuous variables. A $P < 0.05$ was considered statistically significant. R software version 3.1.3. (www.r-project.org/) was used for comparative statistical analysis. The optimal cutoff values of AT-III for survival and recurrence were determined by ROC curve analysis. A cutoff value for overall mortality was 50% of AT-III with a sensitivity and specificity of 42% and 77%, respectively. An AT-III activity of 50% was considered as the cutoff level in this study.

RESULTS
Postoperative AT-III and patient characteristics

The mean follow-up period of the 101 HCC patients enrolled in this study was 65.1 months. Table 1 summarizes patients' clinicopathological characteristics,
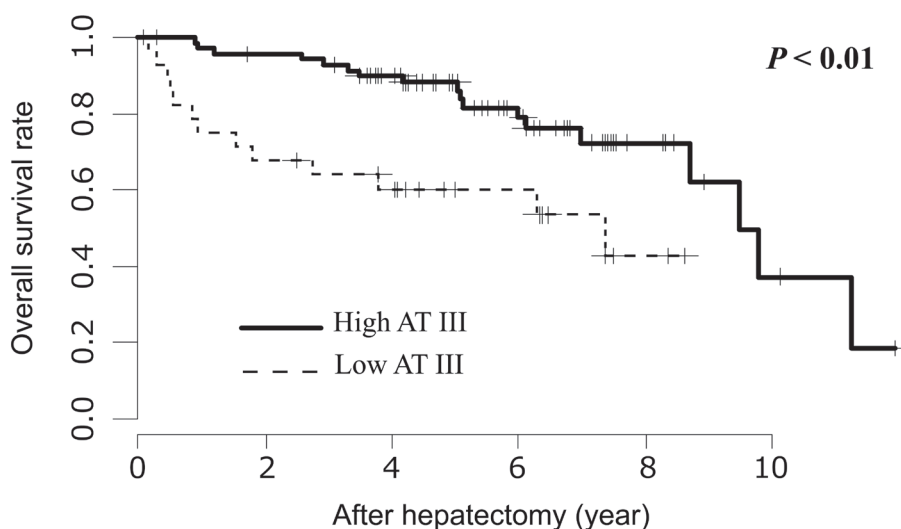
Fig. 2. Overall survival curves of the low AT-III and high AT-III groups. Patients in the low AT-III group showed significantly poor overall survival than those in high AT-III group ($P < 0.01$).

Table 2. Univariate and multivariate analysis for overall survival

		Univariate	Multivariate		
		P	HR	95% CI	P
Child-Pugh	≥ B	< 0.01	2.2	0.6–7.9	0.24
AFP	≥ 20 IU/L	< 0.01	2.7	1.3–5.8	< 0.01**
Blood transfusion	Yes	< 0.01	1.4	0.6–3.3	0.42
Tumor number	Multiple	0.07	3.9	1.3–12.0	0.02*
Vascular invasion	Yes	< 0.01	4.1	1.1–14.8	0.03*
TNM stage	≥ II	0.04	0.3	0.1–1.5	0.14
AT-III	Low	< 0.01	2.6	1.1–6.0	0.03*

*$P < 0.05$, **$P < 0.01$. AFP, alpha-fetoprotein; AT-III, antithrombin III; CI, cumulative index; HR, hazards ratio; TNM, tumor (T) nodes (N) and metastases (M).

and operative and postoperative data in the low AT-III and high AT-III groups. AT-III activity significantly correlated with Child-Pugh score, MELD score, tumor number, and TNM stage. However, AT-III activity showed no correlation with operative factors, such as hepatic resection type, blood loss, operating time, pringle maneuver, and blood transfusion.

Overall survival and relapse-free survival analysis
Figure 2 shows the prognosis of patients with HCC after surgery. Patients in the low AT-III group showed significantly poor overall survival than those in high AT-III group. Moreover, multivariate analysis identified AT-III activity as an independent prognostic factor for overall survival in addition to AFP, tumor number, and vascular invasion (Table 2). The overall relapse-free

survival between the low AT-III and high AT-III groups tended to be different, although they were not significant (Fig. 3). Among the fatal cases, HCC-related deaths were 50.0% in the high AT-III group and 61.5% in the low AT-III group. On the other hand, death from hepatic failure was 18.1% in the high AT-III group and not in the low AT-III group.

Risk factors contributing to early phase recurrence
Of 59 patients (58%) who developed HCC recurrence after curative resection, early phase recurrence (within two years after surgery) was observed in 35 patients (59%). Cox regression analysis identified four variables as risk factors contributing to early phase recurrence: serum AFP level, multiple tumors, and histological type. The low AT-III group showed a significantly
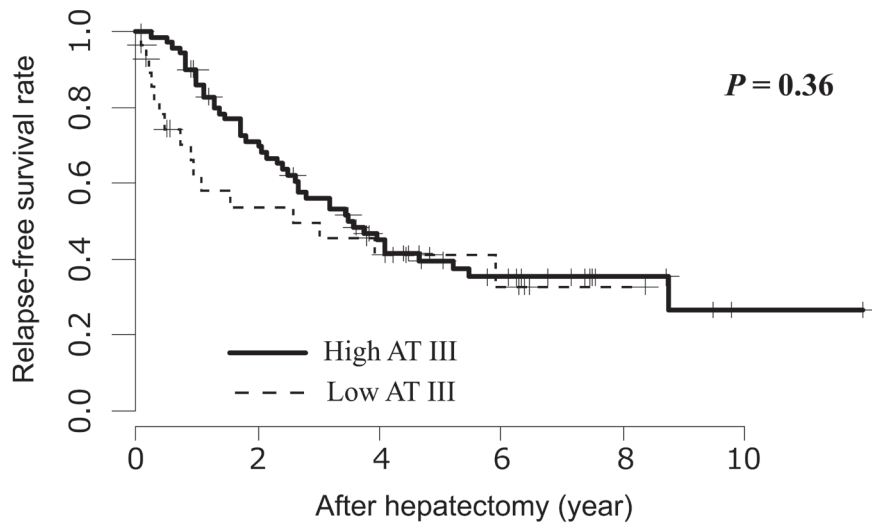
Fig. 3. Recurrence-free survival curves of the low AT-III and high AT-III groups. Between the low AT-III and high AT-III groups tended to be different, although they were not significant ($P = 0.36$).
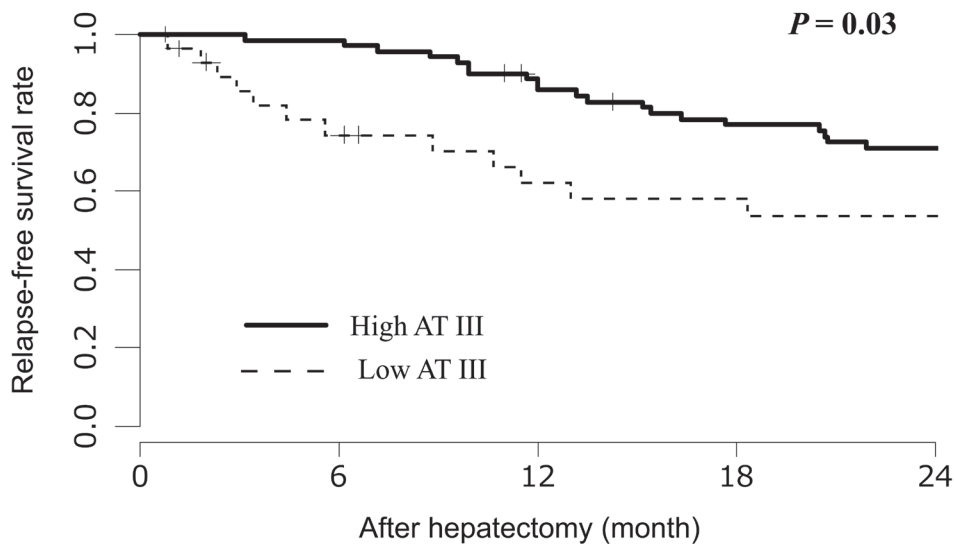

Fig. 4. Recurrence-free survival curves of the low AT-III and high AT-III groups within 2 years after surgery. The low AT-III group showed a significantly higher recurrence rate than that in high AT-III group ($P = 0.03$).

higher recurrence rate than that in high AT-III group (Fig. 4). Furthermore, multivariate analysis showed that low AT-III level is an independent risk factor for early phase recurrence (Table 3).

DISCUSSION

This study investigated the prognostic significance of the serum AT-III levels on the first day after hepatectomy for primary liver cancer. The results suggested that low AT-III activity on day 1 was a useful predictive factor for poor prognosis and early recurrence.

Low AT-III activity is reported to promote progression in several cancers, including those originating in the colon, lung, prostate, and ovary.[13, 14] Additionally, a previous association study, based on propensity scores and multivariate analysis, showed that preoperative low AT-III levels may be a risk factor for tumor recurrence and prognosis in patients with HCC.[15] It has been reported that the β-phase half-life of AT-III is reduced to 52.7 hours for cirrhosis and dramatically reduced to 23.9 hours for liver cancer compared to the normal 58.6 hours.[16] Therefore, AT-III is prone to change, especially

Table 3. Univariate and multivariate analysis for early recurrence

		Univariate	Multivariate		
		P	HR	95% CI	P
AFP	≥ 20 IU/L	< 0.01	3.6	1.7–7.6	< 0.01**
Blood transfusion	Yes	< 0.01	1.7	0.8–3.8	0.2
Tumor number	Multiple	< 0.01	4.0	1.4–11.4	< 0.01**
Vascular invasion	Yes	< 0.01	3.0	1.1–8.9	0.04*
TNM stage	≥ II	< 0.01	0.6	0.2–2.0	0.38
AT-III	Low	0.03	2.2	1.0–4.9	0.04*

*$P < 0.05$, **$P < 0.01$. AFP, alpha fetoprotein; AT-III antithrombin III; CI, cumulative index; HR, hazards ratio; TNM, tumor (T) nodes (N) and metastases (M).

after liver cancer treatment.

In this study, we focused on postoperative, but not preoperative, AT-III activity. AT-III is a marker reflecting liver function, ischemia reperfusion, vascular endothelial dysfunction, DIC, sepsis, and so on.[6, 17] As postoperative AT-III activity is associated with multiple factors, including preoperative, operative and postoperative indicators, we hypothesized that AT-III activity might associate with prognosis of HCC patients. We focused on AT-III levels on postoperative day-1, as AT-III activity on the first day was affected by surgical and infectious invasion, which might indicate cancer prognosis.[18–20] Pereyra D et al. shows that AT-III activity on POD1 significantly predicted postoperative liver dysfunction in patients undergoing liver resection for colorectal cancer patients with liver metastasis.[21]

Postoperative complications are known to adversely affect oncological outcomes in various cancers.[22, 23] In case of HCC, postoperative complications were an independent prognostic factor for early phase recurrence.[24] In other words early phase postoperative status may affect cancer prognosis. The duration from surgery to the appearance of secondary tumors has been recognized as an indicator to distinguish intrahepatic metastasis from de novo tumors. Liver tumors developing within two years after surgery are believed to be intrahepatic metastases.[25] This study also examined the impact of postoperative status, including AT-III, on intrahepatic metastases for HCC.

AT exerts anticoagulant action by binding to thrombin and other coagulation factors. Additionally, AT exerts direct anti-inflammatory and glycocalyx protecting actions of the vascular endothelium that controls vascular permeability.[26] Iwako et al. reported that inadequate levels of AT-III increase the susceptibility of AT-insufficient mice to liver tumorigenesis via increased inflammation.[27] Recent studies have shown that AT-III can suppress the proliferation and migration of HCC cells by inhibiting thrombin-induced tumor growth and angiogenesis.[28–32] However, further basic and clinical studies are required to determine the antitumor mechanisms of AT-III.

This study has some limitations. First, the study was retrospective in design, meaning that there were missing values for several variables. Second, the sample size may have affected the statistical robustness of the results to certain extent. Third, as preoperative AT-III levels were not measured, we could not determine which was a more significant prognostic factor, pre- or post AT-III. Fourth, we did not assess the administration of antiviral drugs or interferon. In patients with hepatitis B-related HCC, adefovir antiviral therapy reduced late HCC recurrence and significantly improved overall survival after R0 hepatic resection.[33] Finally, this was a single-institution cohort study. Therefore, further studies would be required to validate our results. Furthermore, the study on the clinical significance of AT administration for HCC surgery is another topic for future research.

In conclusion, this is the first study to demonstrate that serum AT-III levels on the first postoperative day may serve as a prognostic factor in HCC patients. Additionally, low AT-III activity can also serve as a predictive marker for early recurrence after HCC surgery. These results demonstrate that close observation and intense treatment may be required for HCC patients with low AT-III levels after hepatectomy.

The authors declare no conflict of interest.

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