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# Macroscopic Inflammation Status of Resected Gallbladder Predicts Therapeutic Outcome After Radical Resection for Gallbladder Carcinoma

Ryota Iwase, Hiroaki Shiba, Koichiro Haruki, Yuki Fujiwara, Kenei Furukawa, Yasuro Futagawa, Shigeki Wakiyama, Takeyuki Misawa, Katsuhiko Yanaga

*Department of Surgery, The Jikei University School of Medicine, Tokyo, Japan*

**Objective:** Gallbladder carcinoma (GBC) is one of the digestive cancers with poor prognosis, for which surgical resection is the only potentially curative therapy. Prognostic value of macroscopic inflammatory status of the resected gallbladder in patient with GBC has not been fully investigated. We retrospectively investigated the relation between macroscopic inflammatory status and disease-free as well as overall survival after radical resection for GBC.

**Method:** The subjects were 44 patients who underwent radical resection for GBC between January 2004 and April 2011 at Jikei University Hospital. We retrospectively investigated the relationship between clinicopathologic variables, including macroscopic inflammatory status and disease-free as well as overall survival.

**Results:** In univariate analysis, disease-free survival was poor in patients with Tumor-Nodes-Metastasis (TNM) stage ≥III ($P < 0.0001$) and positive vascular invasion ($P = 0.0001$). Patients with macroscopic chronic inflammation tended to have poor disease-free survival than those with normal type ($P = 0.0930$). Overall survival was poor in patients with TNM stage ≥III ($P < 0.0001$), presence of intraoperative blood transfusion ($P = 0.0125$), positive vascular invasion ($P = 0.0055$), and macroscopic chronic inflammation ($P = 0.0281$). In multivariate analysis, TNM stage ≥III ($P < 0.0114$) and macroscopic chronic inflammation ($P = 0.0350$) were independent predictors of disease-free survival. For overall survival, TNM stage ≥III ($P = 0.0054$) and macroscopic chronic inflammation ($P = 0.0124$)

Corresponding author: Ryota Iwase, MD, Department of Surgery, The Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan.
Tel: +81 3 34331111 ext. 3401; Fax: +81 3 54724140; E-mail: ryotaiwa@jikei.ac.jp

were the independent predictors. Moreover, macroscopic chronic inflammation correlated with the presence of gallstones.

**Conclusion:** The macroscopic Inflammation status of resected gallbladder cancer correlates with oncologic outcome in patients with GBC treated by radical resection.

*Key words:*

Gallbladder carcinoma (GBC) is a cancer of the digestive tract with poor prognosis and is the most common cancer of the biliary tract. The prognosis of advanced GBC remains poor, even with recent advances in diagnostic modalities and surgical techniques.[1–3] It is well known that surgical resection is the only treatment that can achieve long-term survival in patients with GBC. The prognosis of patients with early GBC is associated with a 5-year survival rate ranging from 90% to 100%.[4–6] On the other hand, advanced GBC is characterized by a very poor prognosis, with the 5-year survival rate below 20%.[7] Therefore, assessment of prognostic predictors is important for the management of patients with GBC.

Recent studies reported that perioperative findings of immunologic and inflammatory responses correlate with tumor recurrence and prognosis in various types of malignant tumor, including GBC.[8–11] Several investigators have suggested that inflammatory cells and cytokines found in tumors are likely to contribute to tumor growth, progression, and immunosuppression.[12,13] Preoperative C-reactive protein (CRP) concentrations may be related to poor tumor-specific survival in patients undergoing curative resection for colorectal cancer[14] and pancreatic cancer.[15]

Macroscopic chronic inflammation (MCI) is frequently observed in patients charring GBC. This condition reflects both acute and chronic inflammation of gallbladder. However, the association between MCI and prognosis in patients with GBC remains unclear. In this study, therefore, we retrospectively investigated the relation between macroscopic inflammation status of resected gallbladder and disease-free as well as overall survival in patients with GBC after radical resection.

## Materials and Methods

Between January 2004 and December 2011, 56 patients underwent radical resection for GBC at the Department of Surgery, Jikei University Hospital, Tokyo, Japan. Of these, 12 patients were excluded, 3 patients for insufficient data, and 9 patients who were lost to follow-up, leaving the remaining 44 patients (20 male and 24 female; mean age: 68.0 years; range: 38–88 years) for this study. All patients underwent macroscopically curative resection for GBC. When the patient was diagnosed as GBC preoperatively, we performed open cholecystectomy or cholecystectomy with extended hepatectomy, liver bed resection, hepatic resection of segment 4a and segment 5 of Couinaud's classification, or right lobectomy according to depth of liver invasion in preoperative imaging. Lymph node and bile duct resection were performed when the regional lymph node metastasis was suspected by intraoperative histologic examination or preoperative diagnostic imaging. When the patient diagnosed as GBC incidentally after noncurative cholecystectomy, we performed additional hepatic resection with or without bile duct and lymph node resection. Of these, 3 patients underwent liver bed resection, one patient hepatic resection of segment 4a and segment 5 of Couinaud's classification, leaving 4 patients who could not undergo additional resection mainly for poor general condition or advanced age.

At first, we investigated the relationship between clinicopathologic variables and disease-free and overall survival after radical resection for GBC by univariate and multivariate analyses. The factors consisted of the following 11 criteria: age, gender, duration of operation, operative procedure, simple cholecystectomy or not, intraoperative blood loss, presence or absence of intraoperative blood transfusion, presence or absence of gallstones, tumor stage, vascular and or perineural invasion based on tumor pathology, maximum tumor diameter, and the macroscopic appearance of the resected gallbladder. Clinicopathologic continuous variables were classified into 2 groups for the log-rank test and the Cox proportional hazard regression model as follows: age $<$ 60 or $\geq$ 60 years, duration of operation $<$ 300 or $\geq$ 300 min, blood loss $<$ 1,000 or $\geq$ 1,000 g, and tumor diameter $<$ 50 or $\geq$ 50 mm. Tumor stage was classified into 2 groups: Tumor-Nodes-Metastasis (TNM) stage $\leq$ II or $\geq$ III.

Vascular and or perineural invasion was defined by positive venous invasion, perineural invasion, or angiolymphatic invasion. The macroscopic appearance was classified into 2 groups: MCI and normal type (N). MCI was defined as wall thickening of the gallbladder and or adhesion with other organs including the duodenum, colon, or omentum. These classifications of tumor stage, vascular invasion, and the macroscopic appearance were based on the 5th Japanese edition of the *General Rules for Surgical and Pathological Studies on Cancer of the Biliary Tract*.[16]

Next, to assess the risk factor for MCI, we analyzed the relationship between the patients' characteristics and the macroscopic appearance, using the following 9 factors: age, gender, duration of operation, intraoperative blood loss, intraoperative blood transfusion, presence or absence of gallstone, tumor diameter, vascular and or perineural invasion, and TNM stage.

Recurrence of GBC was defined as newly detected abdominal or extra-abdominal tumors by computed tomography or magnetic resonance image, with or without an increase in serum carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA 19-9). For recurrence of GBC, chemotherapy or conversion to other chemotherapy were chosen based on performance status. For patients with poor performance status or refusal, best supportive care was given.

This retrospective study was approved by the Ethics Committee of the Jikei University School of Medicine (#21-121).

## Statistical Analysis

Data are expressed as a mean ± standard deviation (SD). Univariate analysis was performed using the non-paired Student's *t* test and chi-square test. Disease-free and overall survivals were analyzed using the log-rank test. Factors where *P* value was less than 0.1 were then used in the Cox proportional hazard model for multivariate analysis. These analyses were performed using STATA version 14. All *P* values were considered statistically significant when the associated probability was $<0.05$.

## Results

### *Patient characteristics and clinicopathologic variables*

Patient characteristics and clinicopathologic variables are outlined in Table 1. Among the study population, the mean age was 67.4 years, with a range from 38 to 88 years, and 20 of them were male. Twenty-eight out of the 44 patients had TNM stage ≤II disease on tumor pathology. In this study, the 5-year disease-free and overall survival rates after elective resection of GBC were 51.4% (Fig. 1A) and 61.2% (Fig. 1B), respectively.

*Table 1 Patients' characteristics*

| Factor | Mean ± SD or rate | Range |
|---|---|---|
| Age (years) | 67.4 ± 9.8 | 38–88 |
| Gender (M : F) | | |
| M | 20 | |
| F | 24 | |
| Procedures | | |
| LC | 11 | |
| OC | 7 | |
| CBR | 1 | |
| EC | 16 | |
| CSH | 8 | |
| ERL | 1 | |
| Operation time (min) | 306.9 ± 201.8 | 45–1005 |
| Intraoperative blood loss (g) | 846.0 ± 1273.3 | 0–7230 |
| Intraoperative blood transfusion | | |
| Yes | 17 | |
| No | 27 | |
| Gallstones | | |
| Present | 21 | |
| Absent | 23 | |
| TNM stage | | |
| I or II | 28 | |
| III or IV | 16 | |
| Curability | | |
| R0 | 36 | |
| R1 | 7 | |

C, extended cholecystectomy; CBR, cholecystectomy with bile duct resection; CSH, cholecystectomy with subsegmental hepatectomy; ELC, laparoscopic cholecystectomy; ERL, extended right lobectomy; OC, open cholecystectomy.

### *Comparison of clinical variables in relation to disease-free survival after radical resection of GBC*

Table 2 lists the relationship between the clinicopathologic variables and disease-free survival after resection. In univariate analysis, disease-free survival was poor in patients who did not undergo simple cholecystectomy ($P = 0.0319$) and patients with TNM stage ≥III ($P < 0.0001$), and positive vascular and or perineural invasion ($P = 0.0001$). Patients with MCI tended to have poor disease-free survival than those with N, which did not achieve significant difference ($P = 0.0930$; Fig. 2A). In multivariate analysis, TNM stage ≥III ($P < 0.0114$) and MCI ($P = 0.0350$) were independent and significant predictors of poor disease-free survival.

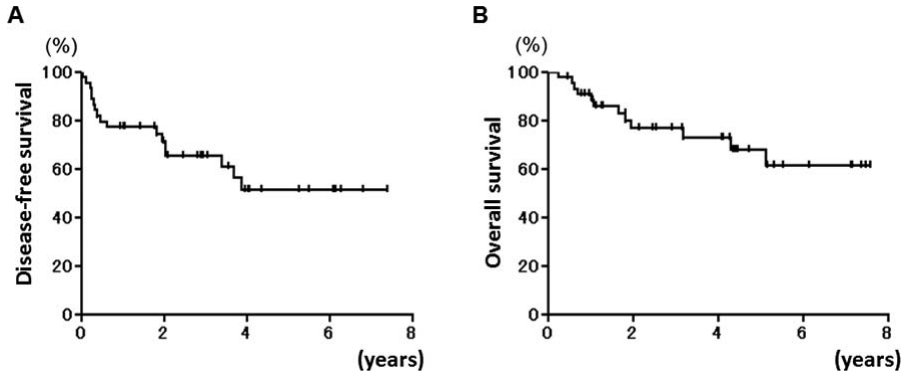


**Fig. 1** The 5-year disease-free and overall survival rates after radical resection for GBC are 51.4% (A) and 61.2% (B), respectively.

*Table 2 Comparison of clinical variables in relation to disease-free survival after radical resection for gallbladder carcinoma*

| | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| Factor | N | Hazard ratio (95% CI) | *P* value | Hazard ratio (95% CI) | *P* value |
| Age (years) | | | | | |
| ≥60 | 33 | 1.560 | 0.4243 | | |
| <60 | 11 | (0.5240–4.644) | | | |
| Gender | | | | | |
| Female | 24 | 1.856 | 0.2506 | | |
| Male | 20 | (0.7124–4.836) | | | |
| Operation time (min) | | | | | |
| ≥300 | 21 | 1.878 | 0.2005 | | |
| <300 | 23 | (0.7155–4.932) | | | |
| Simple cholecystectomy | | | | | |
| Yes | 18 | 0.309 | 0.0319 | 0.592 | 0.455 |
| No | 26 | (0.088–1.078) | | (0.150–2.339) | |
| Intraoperative blood loss (g) | | | | | |
| ≥1000 | 14 | 1.129 | 0.8172 | | |
| <1000 | 30 | (0.4032–3.161) | | | |
| Blood transfusion | | | | | |
| Yes | 17 | 2.252 | 0.1129 | | |
| No | 27 | (0.8253–6.147) | | | |
| Gallstones | | | | | |
| Absent | 23 | 1.311 | 0.5783 | | |
| Present | 21 | (0.5048–3.404) | | | |
| Maximum tumor size (mm) | | | | | |
| ≥50 | 16 | 1.294 | 0.6139 | | |
| 50 | 28 | (0.4756–3.520) | | | |
| TNM stage | | | | | |
| III or IV | 16 | 14.02 | <0.0001 | 6.150 | 0.034 |
| I or II | 28 | (4.517–43.52) | | (1.506–25.119) | |
| Vascular and or perineural invasion | | | | | |
| Yes | 19 | 7.350 | 0.0001 | 2.864 | 0.139 |
| No | 25 | (2.652–20.36) | | (0.696–11.787) | |
| Macroscopic appearance | | | | | |
| MCI | 24 | 2.266 | 0.0930 | 3.304 | 0.047 |
| N | 20 | (0.8724–5.883) | | (1.087–10.041) | |

CI, confidence interval; MCI, macroscopic chronic inflammation; N, normal type; TNM, Tumor-Nodes-Metastasis.

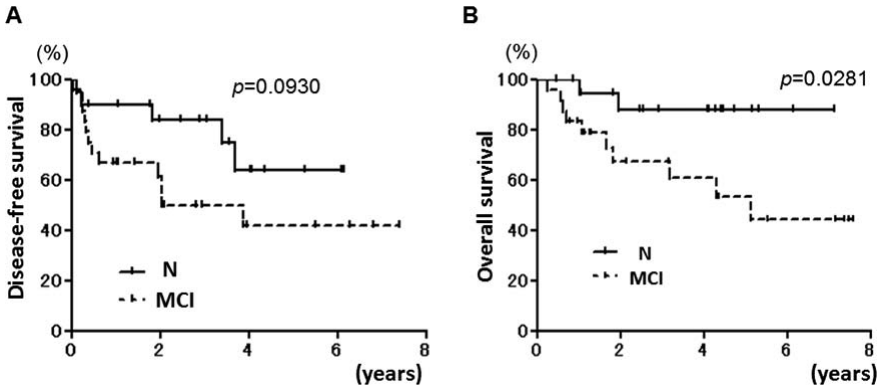


**Fig. 2** Patients with MCI tended to have worse disease-free survival ($P = 0.0930$, A) and had significantly poor overall survival ($P = 0.0281$, B).

*Comparison of clinical variables in relation to overall survival after radical resection of GBC*

Table 3 lists the relationship between the clinicopathologic variables and overall survival after resection. In univariate analysis, overall survival was poor in patients with TNM stage ≥III ($P < 0.0001$), intraoperative blood transfusion ($P = 0.0125$), positive vascular and or perineural invasion ($P = 0.0055$), and MCI ($P = 0.0281$; Fig. 2B). In multivariate analysis, TNM stage ≥III ($P = 0.0054$) and MCI ($P = 0.0124$) were independent and significant predictors of poor survival.

*Univariate analysis of clinicopathologic variables in relation to the macroscopic appearance after radical resection of GBC*

Table 4 lists the relationship between clinicopathologic variables and the macroscopic appearance. In univariate analysis, only the presence of gallstone correlated with MCI ($P = 0.0316$).

## Discussion

According to the Japanese registry of GBC, the resection rate and the curative resection rate in Japan are reported as 69.8% and 37.7%, respectively.[7] Recently, reported prognostic factors associated with good outcome in patients with GBC included early TNM stage, extent of surgical resection, microscopic curative resection, negative perineural invasion, well-differentiation, adenocarcinoma, age <70 years, and female gender.[17–19] The presence of preoperative inflammation has also been reported as an independent prognostic factor for poor survival in patients with GBC.[20]

MCI is characterized by features such as wall-thickening of the gallbladder, adhesions with other organs and so on,[16] and the appearance reflects long-term inflammatory response of the gallbladder. In the present study, the macroscopic appearance was shown to be an independent factor of disease-free survival and a significant and independent prognostic factor of overall survival. Moreover, the macroscopic appearance of resected gallbladder correlated with the presence of gallstone, but not with the other factors including TNM stage. These results suggest that GBC with long-term inflammation have poor prognosis after resection, compared to that without inflammation. Chronic inflammation and cholelithiasis are correlated with cancer generation in GBC.[21–23] It has been presumed that longstanding chronic inflammation caused by cholelithiasis plays a role in the tumor progression, and that gallstones are regarded as a co-morbid condition in 54%–97% of the patients of GBC.[24] Coussens *et al* reported that the host inflammatory response in the form of cytokines and other chemical messengers plays an important role in cancer growth and metastasis.[25] At the molecular level, it has been shown that chronic inflammation of the gallbladder may lead to an allele-specific mutation, particularly loss of heterozygosity of the p53 gene and excessive expression of p53 protein, which may result in malignant transformation.[26,27] However, there are a lot of cases in which GBC was generated without inflammation. In this study, a remarkable new finding is that the GBC developed in the condition with chronic cholecystitis has worse prognosis than those developed in the normal gallbladder. This result means consecutive inflammation or past inflammation in gallbladder generates relatively

*Table 3 Comparison of clinical variables in relation to overall survival after radical resection for gallbladder carcinoma*

| | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| Factor | N | Hazard ratio (95% CI) | *P* value | Hazard ratio (95% CI) | *P* value |
| Age (years) | | | | | |
| ≥60 | 33 | 1.137 | 0.8428 | | |
| <60 | 11 | (0.3195–4.047) | | | |
| Gender | | | | | |
| Female | 24 | 1.455 | 0.5224 | | |
| Male | 20 | (0.4612–4.589) | | | |
| Operation time (min) | | | | | |
| ≥300 | 21 | 1.529 | 0.4645 | | |
| <300 | 23 | (0.4902–4.768) | | | |
| Simple cholecystectomy | | | | | |
| Yes | 18 | 0.301 | 0.0701 | 0.851 | 0.866 |
| No | 26 | (0.066–1.377) | | (0.130–5.580) | |
| Intraoperative blood loss (g) | | | | | |
| ≥1000 | 14 | 2.248 | 0.1921 | | |
| <1000 | 30 | (0.6655–7.594) | | | |
| Intraoperative blood transfusion | | | | | |
| Yes | 17 | 4.680 | 0.0125 | 1.129 | 0.869 |
| No | 27 | (1.395–15.70) | | (0.265–4.806) | |
| Gallstones | | | | | |
| Absent | 23 | 0.9356 | 0.9086 | | |
| Present | 21 | (0.3004–2.914) | | | |
| Maximum tumor size (mm) | | | | | |
| ≥50 | 16 | 1.058 | 0.9276 | | |
| <50 | 28 | (0.3139–3.565) | | | |
| TNM stage | | | | | |
| III or IV | 16 | 24.52 | <0.0001 | 37.557 | 0.006 |
| I or II | 28 | (6.067–99.14) | | (2.836–497.406) | |
| Vascular and or perineural invasion | | | | | |
| Yes | 19 | 5.930 | 0.0055 | 0.721 | 0.759 |
| No | 25 | (1.688–20.82) | | (0.089–5.856) | |
| Macroscopic appearance | | | | | |
| MCI | 24 | 3.571 | 0.0281 | 10.498 | 0.014 |
| N | 20 | (1.147–11.12) | | (1.624–67.884) | |

CI, confidence interval; MCI, macroscopic chronic inflammation, N, normal type; TNM, Tumor-Nodes-Metastasis.

aggressive cancer with high metastatic potential. However, the reason for that is unclear.

Recent studies showed preoperative inflammation is associated with poor prognosis in GBC.[11,20,28] Glasgow Prognostic Score (GPS), neutrophil to lymphocyte ratio (NLR), or CRP were shown to be one of prognostic predictors in the patient with GBC. However, MCI reflects not only preoperative acute inflammation but also chronic or past inflammation. Therefore, MCI in pathologic finding may be a new possible marker that predicts the prognosis of the GBC patient.

In the present study, the number of patients was 44, which was a small sample size. Therefore, we could not perform further analysis according to each pathologic stage or include details of an operative procedure such as hepatectomy, bile duct resection, or lymph node dissection. That is a major limitation of this study. Because survival benefit of adjuvant chemotherapy has not been established on gallbladder cancer after surgery,[29] further assessments of correlation between inflammation and cancer recurrence by multicenter trial may improve therapeutic outcome of GBC after curative resection.

## Conclusion

The macroscopic appearance of the resected gallbladder positively correlates with tumor recurrence and survival in patients with GBC after resection. Macroscopic inflammatory status of the resected gallbladder may help risk stratification and decision-making in the management of patients with GBC after radical resection.

*Table 4 Univariate analysis of clinicopathologic variables in relation to the macroscopic appearance after radical resection for gallbladder carcinoma*

| Factor | Macroscopic appearance | | *P* value |
|---|---|---|---|
| | N (n = 20) | MCI (n = 24) | |
| Age (years) | 67.9[a] ± 11.5 | 67.0 ± 8.4 | 0.755 |
| Gender | | | 0.246 |
| Male | 11 | 9 | |
| Female | 9 | 15 | |
| Operation time (min) | 272.6 ± 178.5 | 335.6 ± 219.0 | 0.308 |
| Simple cholecystectomy | | | 0.614 |
| Yes | 11 | 15 | |
| No | 9 | 9 | |
| Intraoperative blood loss (g) | 661.8 ± 965.1 | 999.6 ± 1485.8 | 0.397 |
| Intraoperative blood transfusion | | | 0.090 |
| Yes | 5 | 12 | |
| No | 15 | 12 | |
| Gallstones | | | 0.032 |
| Present | 6 | 15 | |
| Absent | 14 | 9 | |
| Maximum tumor size | | | 0.153 |
| ≥50 | 5 | 11 | |
| <50 | 15 | 13 | |
| TNM stage | | | 0.864 |
| I or II | 13 | 15 | |
| III or IV | 7 | 9 | |
| Vascular and or perineural invasion | | | 0.697 |
| Yes | 8 | 11 | |
| No | 12 | 13 | |

MCI, macroscopic chronic inflammation; N, normal type; TNM, Tumor-Nodes-Metastasis.
[a]Mean ± SD.

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