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ORIGINAL ARTICLE

Comparison of the Sixth and Seventh Editions of the AJCC TNM Classification for Gallbladder Cancer

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Abstract
Background This study aimed to compare the seventh edition of the tumor node metastasis (TNM) staging system to the sixth edition to validate its usefulness in predicting prognosis for gallbladder cancer.
Methods Gallbladder cancer patients were staged according to both the sixth and seventh editions of the American Joint Committee on Cancer (AJCC) staging system.
Results A total of 142 patients underwent cholecystectomy for gallbladder cancer. According to the seventh edition, the survival time of N1 and N2 was different (P=0.006), and the survival difference between N0 and N1 became significant after excluding cases with no lymph node dissection (P=0.035). The −2 log likelihoods of the sixth and seventh edition TNM stages were 216.282 and 217.460, respectively, suggesting non-superiority of the seventh edition. Excluding cases with no lymph node dissection resulted in a lower −2 log likelihood score for both editions (sixth, 157.002; seventh, 158.758).
Conclusions Sufficient lymph node dissection allows better prognostic stratification by application of the AJCC staging system. Even though the new N stage of the seventh edition showed some improvement in predicting prognosis, the overall performance of the seventh edition was not much better than the sixth. Further improvement is needed in the gallbladder cancer staging system.

Keywords Gallbladder cancer · TNM staging · Lymph nodes · Prognosis

Synopsis This study aimed to compare the seventh edition of the TNM staging system to the sixth edition to validate its usefulness in predicting prognosis for gallbladder cancer. The revised TNM staging was suggested.

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Introduction

Gallbladder cancer accounts for only about 2 % of all cancers, and its annual incidence is about 2,000 in South Korea. In the world, gallbladder cancer is the fifth most common cancer of the gastrointestinal tract and accounts for about 1 % of all cancers.[1] The survival rate has improved slowly with multimodality treatment and improvement of surgical methods.[2, 3] Due to its rarity and advanced stage at presentation, there have not been many studies aimed at identifying meaningful prognostic factors, and it is not easy to predict treatment outcomes of patients with gallbladder cancer.[4]

The American Joint Committee on Cancer (AJCC) staging system has been proven to be very important in the treatment and clinical trials of variety of cancers.[5] Accurate differentiation of the tumor node metastasis (TNM) stage is essential in predicting patient outcome and for planning post-surgery treatment.[6] Staging allows the selection of patients for clinical trials, their appropriate treatment, and

the evaluation of new therapies. For these reasons, TNM staging is continually modified and re-evaluated. As for most other cancers, the depth of tumor invasion and nodal metastasis are well-proven prognostic factors for gallbladder cancer.[7] The AJCC staging system differentiates gallbladder cancer based on the tumor depth of invasion (T stage), lymph node metastasis (N stage), and distant metastasis (M stage).[8] The latest edition of AJCC TNM staging for gallbladder cancer awaiting validation is the seventh edition published in 2009.

Modifying T, N, M, and overall stage is important, but it has to be followed by accurate assessment of these factors in gallbladder cancer.[7] Among various tumor factors, the accuracy of N stage evaluation is especially important in gallbladder cancer because it can be affected by the extent of lymph node sampling. Gallbladder cancer is commonly diagnosed incidentally, and re-exploration shows a high incidence of residual disease in lymph node.[9, 10] Inadequate lymph node sampling can lead to underestimation of N stage.

Here, we aimed to compare the seventh edition of the TNM system to the sixth edition to validate its usefulness in predicting prognosis. In addition, through the differentiation of staging, we aimed to determine the sufficient extent of lymph node dissection according to the stage of gallbladder cancer.

Patients and Methods

Study Population

Patients who underwent cholecystectomy for gallbladder cancer at Severance Hospital between November 2005 and March 2012 were enrolled. Patients who had a noncurative (R1 or R2) resection were excluded. Clinicopathologic data of all patients were analyzed retrospectively. Tumors were staged according to both the sixth and the seventh editions of the AJCC staging systems.

Data Analysis and Statistical Methods

The primary end points were overall survival (OS) and progression-free survival (PFS). OS was calculated from the date of diagnosis until death from any cause or the patient's last visit to the hospital. PFS was calculated from the date of operation until the date of recurrence or the day of the last radiological evaluation computed tomography or magnetic resonance imaging.

The 3-year survival rate and OS were calculated using the Kaplan–Meier method. Differences between OS curves were assessed by the log rank test. The likelihood ratio chi-square test related to the Cox regression model was used for measuring goodness of fit. The −2 log likelihood (which was the parameter in the Cox regression) of the sixth edition was compared to that of the seventh edition—the smaller the value of this statistic, the better the model. All analyses were performed with the SPSS statistical program (version 18.0; SPSS Inc.). $P<0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 174 patients underwent cholecystectomy for gallbladder cancer. Thirty two patients who did not have R0 resection were excluded, and 142 patients were enrolled. The median follow-up time was 26.5 months. The 3-year overall survival rate was 69 %. All patients underwent radical or simple cholecystectomy for gallbladder cancer. The mean patient age was 62.3 years (range, 38.0–90.0), and the sex ratio (male/female) was 63:79. Twenty one of the 142 patients had D0 dissection (no lymph node dissection), 19 patients had D1 dissection (with N1 lymph node dissection and without N2 lymph node dissection), and 102 patients had D2 dissection (with both N1 and N2 lymph node dissection). Even though the role of adjuvant chemotherapy was not definitely established in gallbladder cancer, we advised all the patients with lymph node metastasis to receive 5-fluorouracil-based adjuvant chemotherapy.

Stratification of Prognosis According to the Sixth and Seventh Editions of TNM Staging Systems

In the seventh edition, the survival difference between N1 and N2 was significant (median OS; N1 (56.1 months) vs. N2 (27.6 months), $P=0.006$; Fig. 1), and the survival difference between N0 and N1 became significant after excluding cases with no lymph node dissection (median OS; N0 (67.6 months) vs. N1 (56.1 months), $P=0.035$). Even though it was not statistically significant, patients without lymph node dissection (Nx) showed a trend of poor outcome compared to the N1 group (median OS; N1 (56.1 months) vs. Nx (36.7 months), $P=0.263$) (Fig. 1).

Generally, the reassignment of disease stage occurred to a higher tier (Table 1). The stage IB and IIA patients of the sixth edition were reclassified to a higher tier in the seventh edition (stages II and IIIA, respectively). All of the stage IIB patients in the sixth edition had a 58 % 3-year survival rate and were restaged due to N stage reclassification (N0, N1, N2). These patients were reclassified to a higher tier in the seventh edition: 22 of 36 were staged as stage IIIB and 14 as stage IVB. The 22 patients of stage IIB according to the sixth edition, who were reclassified to stage IIIB in the seventh edition, had a 74.0 % 3-year survival rate. The other

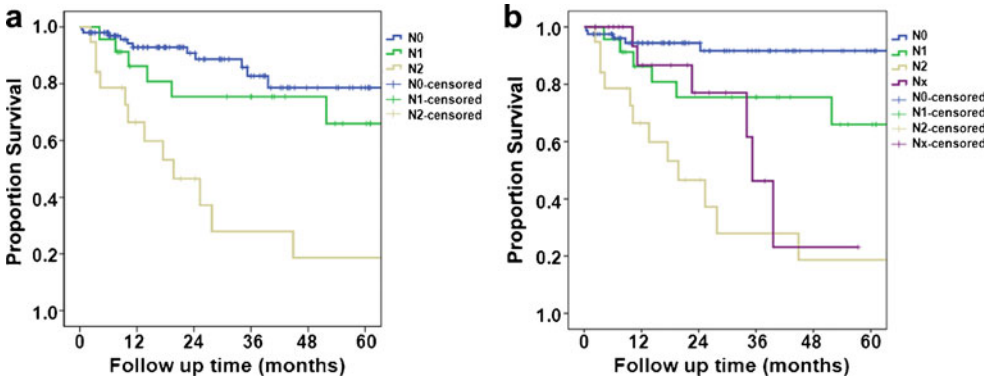

Fig. 1 Kaplan–Meier overall survival curves for 142 patients **a** stratified by N stage according to the seventh edition of AJCC TNM staging system, **b** after differentiating Nx group (overall log rank $P<0.001$)

14 patients of stage IIB according to the sixth edition, who were reclassified to stage IVB in the seventh edition, had a 35.7 % 3-year survival rate. In addition, all of the stage III patients in the sixth edition were staged due to N stage reclassification (N0, N1, N2). These patients were reclassified to a higher tier in the seventh edition: six of the ten patients were staged as stage IVA and four as stage IVB.

The 3-year survival rates measured at each overall stage were as follows: 100 % (IA), 80 % (IB), 60 % (IIA), 58 % (IIB), 22 % (III), and 0 % (IV) (sixth edition); and 100 % (I), 80 % (II), 60 % (IIA), 74 % (IIIB), 45 % (IVA), and 19 % (IVB) (seventh edition) (Table 2). Survival curves of the sixth and seventh editions are described in Fig. 2a, b. Since the analysis of N stage showed the extent of lymph node dissection could affect overall survival, we also analyzed survival according to overall stage after excluding cases with no lymph node dissection. The 3-year survival rates of each overall stage measured according to the seventh edition were 100, 93, 88, 74, 40, and 19 % (Table 1). At the time of last follow-up, the percentages of survivor according to seventh edition were as follows: 100, 91, 69, 73, 50, and 37 %.

Table 1 Cross-table of gallbladder cancer patients according to sixth and seventh editions of AJCC staging

	6th edition						Total
	IA	IB	IIA	IIB	III	IV	
7th edition							
I	38						38
II		44					44
IIIA			13				13
IIIB				22			22
IVA					6		6
IVB				14	4	1	19
Total	38	44	13	36	10	1	142

AJCC American Joint Committee on Cancer

Subgroup Analysis in the Seventh Edition TNM Staging System

Positive N2 lymph nodes were found more frequently in the T3 or T4 stage compared to the T1 or T2 stage (Table 3). Furthermore, even in T1B, there was tumor involvement of the N2 lymph nodes, suggesting that D2 dissection was needed even in early gallbladder cancer. Among stage IIIB, a trend existed of differences in the 3-year survival rate between T1-2N1M0 (93 %) and T3N1M0 (35 %) subgroups. The T1-2N1M0 (93 %) subgroup also tended to have a better outcome than T3N0M0 (88 %).

Rearrangement of the Staging System for Gallbladder Cancer

We tried grouping T3N1M0 and T4N0-1 M0 together. In addition, we moved the T1-2N1M0 subgroup to a lower tier. Taken together, we suggest a new staging system as follows: I (T1N0M0), IIA (T2N0M0), IIB (T1-2N1M0), IIIA (T3N0M0), IIIB (T3-4N1M0, T4N0M0), IVA (T1-4N2M0), and IVB (T1-4 N0-2 M1). The 3-year survival rates of each overall stage measured according to our suggestion would be as follows: 100, 93, 93, 88, 36, 21, and 0 %. Survival curves for our suggested staging are described in Fig. 2c.

Verification of the Seventh Edition of TNM Staging System

The performances of the sixth and seventh edition staging systems were quantified by the −2 log likelihood. The −2 log likelihoods of sixth and seventh edition TNM stages were 216.282 and 217.460, respectively. After excluding cases with no lymph node dissection, the −2 log likelihood score of the sixth and seventh editions were 157.002 and 158.758, respectively, suggesting non-superiority of the seventh edition and the importance of sufficient lymph node dissection. The predictive ability of both editions was improved with sufficient lymph node dissection. We also compared our suggested staging to the sixth and seventh

Table 2 Three-year survival rate of gallbladder cancer patients according to the seventh edition of AJCC staging and our suggestion

7th edition	Number	3-year survival rate	Number[a]	3-year survival rate[a]	Our suggestion	Number[a]	3-year survival rate[a]
I T1N0M0	38	100	30	100	I T1N0M0	30	100
II T2N0M0	44	80	36	93	IIA T2N0M0	36	93
IIIA T3N0M0	13	60	9	88	IIB T1-2N1M0	15	93
IIIB T1-3N1M0	22	74	22	74	IIIA T3N0M0	9	88
IVA T4N0-1M0	6	45	5	40	IIIB T4N0M0 T3-4N1M0	12	36
IVB T1-4N2M0 T1-4N0-1 M1	19	19	19	19	IVA T1-4N2M0	18	21
					IVB T1-4N0 M1	1	0

AJCC American Joint Committee on Cancer

[a]After excluding cases with no lymph node dissection

editions, which showed that, with lymph node dissection, the −2 log likelihood score of our suggested system was 153.742.

Discussion

The AJCC cancer staging system has been revised every 6–8 years since the first edition was introduced in 1977.[5] The seventh edition was published in 2009 and has been used since January 2010. According to the seventh edition, the lymph nodes of gallbladder cancer are divided into the hilar lymph nodes and other regional lymph nodes. This has been reclassified in terms of the possibility of surgical resection and patient outcome.[8] In addition, there are some changes in overall stages reflecting reclassification of N stage. Here, we aimed to validate the usefulness of AJCC staging of the seventh edition in predicting prognosis, and we also determined what the sufficient extent of lymph node dissection according to the stages of gallbladder cancers was.

In the seventh edition of TNM staging, N differentiation is probably the most notable point. Our study showed that prognosis worsened as N stage increased. Even in the T3 and T4 stages, if there was no lymph node metastasis, a good prognosis could be expected. The 3-year survival rates of T3N0M0 and T4N0M0 were much higher than the same T stage gallbladder cancers with N1 or N2 stage. Regarding the extent of lymph node sampling, we found that the survival curve of the Nx group overlapped the N1 and N2 groups, suggesting that the Nx group (without lymph node dissection) probably included regional lymph node metastasis (N1) and even distant lymph node metastasis (N2). If the Nx group was assumed as the N0 group, the staging determination could result in underestimation. These results clearly demonstrated that the new N stage was good at predicting prognosis and that sufficient node sampling was required to accurately predict prognosis.

The accurate analysis of the prognosis of gallbladder cancer is difficult partly because of persistent confusion over the adequate extent of resection and the fact that

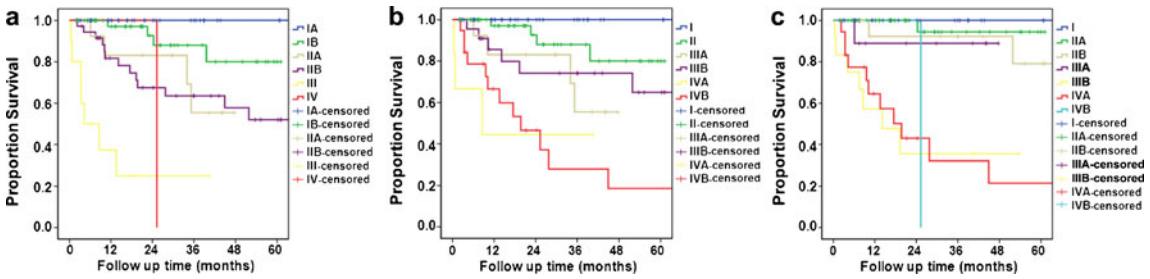

Fig. 2 Kaplan–Meier overall survival curves for 142 patients stratified by stage according to **a** the sixth edition, **b** the seventh edition, and **c** our suggestion of staging system (overall log rank $P<0.001$)

Table 3 Cross-table between T and N stages in the seventh edition of TNM staging system

	N stage (%)				Total
	Nx[a]	N0	N1	N2	
T stage (%)					
T1a	6 (33.3)	12 (66.7)	0 (0)	0 (0)	18
T1b	2 (8.3)	18 (75.0)	2 (8.3)	2 (8.3)	24
T2	8 (12.5)	36 (56.3)	13 (20.3)	7 (10.9)	64
T3	4 (15.4)	9 (34.6)	7 (26.9)	6 (23.1)	26
T4	1 (10.0)	4 (40.0)	1 (10.0)	4 (40.0)	10
Total	26 (14.9)	84 (48.3)	28 (16.1)	36 (20.7)	142

[a] The number of patients without lymph node dissection

staging of gallbladder cancer is often confused by incomplete removal of the tumor.[11, 12] In particular, lymph node evaluation is very important for the radical resection of gallbladder cancer.[13] In the absence of lymph node evaluation, as we have demonstrated above, radical resection might not provide benefits over simple cholecystectomy.[14, 15] Even though the importance of lymph node evaluation is clear, the extent of lymph node dissection at each T stage remains controversial.[16] We did not find lymph node metastasis in any patients at the T1A stage. Regional or systemic spreading of T1B gallbladder cancer at presentation has been debated.[17] In the case of T1B gallbladder cancer, we showed that 4 of 24 patients (16.7 %) had lymph node metastasis (N1, two patients; N2, two patients). Pawlik et al.[18] also reported recently that 24 % of patients with T1B and 45 % of patients with T2 cancers have lymph node metastasis. Radically extended surgical resection is needed for better survival outcome.[19, 20] Considering the difficulty of differentiating the T1A stage from more advanced T stages, we recommend extended lymph node sampling even when early gallbladder cancer is suspected in preoperative evaluation to ensure the accurate evaluation of lymph nodes and removal of hidden metastasis.[21, 22]

With sufficient lymph node sampling, both the sixth and seventh edition staging systems performed better for predicting prognosis. However, the seventh edition was not superior to the sixth edition. Overlapping of survival curves among stages and a higher −2 likelihood score of the seventh edition suggested that further improvement was needed. We investigated the supplemental point of the seventh edition of TNM staging, by first trying to differentiate TanyN2M0 from TanyNanyM1 in stage IVB. In our study, the 3-year survival rate of stage IVB was 19 % with D2 dissection. However, all survivors at this stage were in the TanyN2M0 group, and no patients in the TanyNanyM1 group survived. We assumed that the TanyNanyM1 group had a tendency of worse survival rate compared to the TanyN2M0 group. Second, due to differences of survival between the T1-2N1M0 and T3N1M0 subgroups, we tried to move the T3N1M0 group to a higher stage. Third, stage T1 and T2 subgroups with sufficient lymph node dissection were expected to show a better outcome. In contrast, the stage T3 and T4 subgroups had a tendency of poor outcome, even if lymph node dissection was carried out. Indeed, the T1-2N1M0 subgroup showed a trend of better outcome than T3N0M0. We, therefore, moved the T1-2N1M0 subgroup to a lower stage. Taken together, our suggestions are as follows: I (T1N0M0), IIA (T2N0M0), IIB (T1-2N1M0), IIIA (T3N0M0), IIIB (T3-4N1M0, T4N0M0), IVA (T1-4N2M0), IVB (T1-4 N0-2 M1). These suggestions resulted in a much lower −2 log likelihood score (153.742) compared to the existing sixth and seventh edition staging systems. It seems that the gallbladder cancer staging system can be further improved, and further validation is required.

We note that our study had some limitations. First, minimal overlap remained in the graph of survival analysis even after our modifications, probably due to the limited number of patients and heterogeneity in surgical methods. After the standardization of surgical protocols, further validation with large population will be needed. Second, as we suggested above, extended cholecystectomy (D2 dissection) was required for gallbladder cancer to allow accurate prediction of prognosis, but the survival benefit of extending lymph node dissection and the extent of lymph node dissection that was required remain unknown.[23–26] Even though relatively large portion of gallbladder cancer patients in our institution had D2 dissection compared to other study,[27] some of them (13.4 %) still had only D0 dissection. If all of gallbladder cancer patients had adequate lymph node dissection, more precise validation for TNM staging could be possible, and also, survival benefit of extended lymph node dissection could be validated. Third, due to poor outcome of gallbladder cancer and short inclusion time, the median follow-up time was not so long, and we only showed 3-year survival rate. It could limit making any significant conclusions about long-term survival. Finally, heterogeneity existed not only in the extent of lymph node dissection but also in surgical methods. Future analysis of long-term survival with a large number of patients and standardized surgical methods will be required to validate a new staging system.

Our study is the first to validate the seventh edition of TNM staging in gallbladder cancer. Although the seventh edition of TNM staging for gallbladder cancer did not show better performance in predicting prognosis than the sixth edition, we were able to add some supplemental points to the seventh edition. In addition, we showed that the extent of lymph node dissection greatly affected the prognostic predictive ability of TNM staging. N2 metastasis may occur even in early stage gallbladder cancer, and adequate lymph node dissection, possibly D2 dissection, is, therefore,

required for almost all cases of gallbladder cancer staging. After standardizing surgical techniques, especially the extent of lymph node dissection, large population-based studies are needed to establish better staging system for gallbladder cancer.

Conflict of interest The authors have no financial conflicts of interest.

References

1. Lai CH, Lau WY. Gallbladder cancer—a comprehensive review. Surgeon 2008;6(2):101–110.
2. Witjes CD, van den Akker SA, Visser O, et al. Gallbladder cancer in the Netherlands: incidence, treatment and survival patterns since 1989. Dig Surg 2012;29(2):92–98.
3. Kiran RP, Pokala N, Dudrick SJ. Incidence pattern and survival for gallbladder cancer over three decades—an analysis of 10301 patients. Ann Surg Oncol 2007;14(2):827–832.
4. Ito H, Ito K, D'Angelica M, et al. Accurate staging for gallbladder cancer: implications for surgical therapy and pathological assessment. Ann Surg 2011;254(2):320–325.
5. Greene FL, American Joint Committee on Cancer, American Cancer Society. AJCC cancer staging handbook: from the AJCC cancer staging manual. 6th ed. New York: Springer, 2002.
6. Tsukada K, Hatakeyama K, Kurosaki I, et al. Outcome of radical surgery for carcinoma of the gallbladder according to the TNM stage. Surgery 1996;120(5):816–821.
7. Ito H, Matros E, Brooks DC, et al. Treatment outcomes associated with surgery for gallbladder cancer: a 20-year experience. J Gastrointest Surg 2004;8(2):183–190.
8. Edge SB, American Joint Committee on Cancer, American Cancer Society. AJCC cancer staging handbook: from the AJCC cancer staging manual. 7th ed. New York: Springer, 2010.
9. Lendoire JC, Gil L, Duek F, et al. Relevance of residual disease after liver resection for incidental gallbladder cancer. HPB (Oxford) 2012;14(8):548–553.
10. Duffy A, Capanu M, Abou-Alfa GK, et al. Gallbladder cancer (GBC): 10-year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). J Surg Oncol 2008;98(7):485–489.
11. Kang CM, Choi GH, Park SH, et al. Laparoscopic cholecystectomy only could be an appropriate treatment for selected clinical R0 gallbladder carcinoma. Surg Endosc 2007;21(9):1582–1587.
12. Dixon E, Vollmer CM, Jr., Sahajpal A, et al. An aggressive surgical approach leads to improved survival in patients with gallbladder cancer: a 12-year study at a North American Center. Ann Surg 2005;241(3):385–394.
13. Choi SB, Han HJ, Kim CY, et al. Fourteen year surgical experience of gallbladder cancer: validity of curative resection affecting survival. Hepatogastroenterology 2012;59(113):36–41.
14. Cziupka K, Partecke LI, Mirow L, et al. Outcomes and prognostic factors in gallbladder cancer: a single-centre experience. Langenbecks Arch Surg 2012;397(6):899–907.
15. Jensen EH, Abraham A, Jarosek S, et al. Lymph node evaluation is associated with improved survival after surgery for early stage gallbladder cancer. Surgery 2009;146(4):706–711; discussion 711–703.
16. Shirai Y, Sakata J, Wakai T, Hatakeyama K. Full-thickness cholecystectomy with limited lymphadenectomy for gallbladder cancer. Hepatogastroenterology 2012;59(117):1338–1340.
17. Lee SE, Jang JY, Lim CS, Kang MJ, Kim SW. Systematic review on the surgical treatment for T1 gallbladder cancer. World J Gastroenterol 2011;17(2):174–180.
18. Pawlik TM, Gleisner AL, Vigano L, et al. Incidence of finding residual disease for incidental gallbladder carcinoma: implications for re-resection. J Gastrointest Surg 2007;11(11):1478–1486; discussion 1486–1477.
19. Kang CM, Lee WJ, Choi GH, et al. Does "clinical" R0 have validity in the choice of simple cholecystectomy for gallbladder carcinoma? J Gastrointest Surg 2007;11(10):1309–1316.
20. Nanashima A, Tobinaga S, Abo T, et al. Evaluation of surgical resection for gallbladder carcinoma at a Japanese cancer institute. Hepatogastroenterology 2012;59(118):1717–1721.
21. Jensen EH, Abraham A, Habermann EB, et al. A critical analysis of the surgical management of early-stage gallbladder cancer in the United States. J Gastrointest Surg 2009;13(4):722–727.
22. Fetzner UK, Holscher AH, Stippel DL. Regional lymphadenectomy strongly recommended in T1b gallbladder cancer. World J Gastroenterol 2011;17(38):4347–4348.
23. Shirai Y, Wakai T, Sakata J, Hatakeyama K. Regional lymphadenectomy for gallbladder cancer: Rational extent, technical details, and patient outcomes. World J Gastroenterol 2012;18(22):2775–2783.
24. Sakata J, Shirai Y, Wakai T, Ajioka Y, Hatakeyama K. Number of positive lymph nodes independently determines the prognosis after resection in patients with gallbladder carcinoma. Ann Surg Oncol 2010;17(7):1831–1840.
25. Negi SS, Singh A, Chaudhary A. Lymph nodal involvement as prognostic factor in gallbladder cancer: location, count or ratio? J Gastrointest Surg 2011;15(6):1017–1025.
26. Shirai Y, Sakata J, Wakai T, Ohashi T, Ajioka Y, Hatakeyama K. Assessment of lymph node status in gallbladder cancer: location, number, or ratio of positive nodes. World J Surg Oncol 2012;10(1):87.
27. De Jong MC, Nathan H, Sotiropoulos GC, et al. Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. J Clin Oncol 2011;29(23):3140–3145