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Is the tumor-stroma ratio a prognostic factor in gallbladder cancer?

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SUMMARY

OBJECTIVE: This study aimed to examine the prognostic effect of the tumor-stroma ratio, which has been shown to have prognostic value in various cancers, in patients with gallbladder cancer who have undergone curative resection.

METHODS: The records of gallbladder cancer patients who underwent surgical treatment in our clinic between December 2005 and March 2021 were analyzed retrospectively. The hematoxylin and eosin-stained sections representing the tumors were evaluated under light microscopy to determine tumor-stroma ratio, and based on the results, <50% was defined as the stroma-rich and ≥50% as the stroma-poor groups.

RESULTS: A total of 28 patients, including 20 females and 8 males, with a mean age of 64.6 years, were included in this study. Stroma-poor and stroma-rich tumors were detected in 15 and 13 patients, respectively. There was no statistically significant relationship identified between tumor-stroma ratio and advanced age, gender, serum levels of carbohydrate antigen 19-9 and carcinoembryonic antigen, incidental or nonincidental diagnosis, jaundice, adjacent organ or structure resection, tumor location, grades 1–2 or 3, T1/T2 or T3/T4, N0 or N1/N2, M stage, *American Joint Committee on Cancer* stage, lymphovascular invasion, and perineural invasion. The stroma-poor and stroma-rich groups had a 5-year survival rate of 30% and 19.2% and a median overall survival of 25.7 and 15.1 months, respectively, with no statistically significant difference between the groups (p=0.526).

CONCLUSIONS: A low tumor-stroma ratio tended to be a poor prognostic factor in gallbladder cancer, although not to a statistically significant degree. This can be considered one of the preliminary studies, as further studies involving larger groups are needed.

KEYWORDS: Gallbladder neoplasms. Prognosis. Tumor microenvironment.

INTRODUCTION

Gallbladder cancer (GBC) is generally considered to have a very poor prognosis. Surgical resection is the only treatment with curative potential and success that depends on the stage and biology of the tumor and the completeness of the resection[1]. The effect of many clinicopathological factors on prognosis, however, is still a matter of discussion.

Tumor tissue consists of carcinoma cells and the stroma that surrounds them. The tumor stroma, associated with tumor initiation, progression, and metastasis, has a prognostic value[2], with the tumor-stroma ratio (TSR) expressing the proportion of tumor cells to stroma in tumor tissue. A low TSR implies a high proportion of stroma and has recently been identified as a poor prognostic factor in many tumor types[3,4]. In contrast, the prognostic value of TSR in GBC has been examined in only two studies to date, and so further studies are needed[5,6].

This study aimed to examine the prognostic value of TSR through a retrospective review of GBC patients who underwent surgery for an R0 curative resection.

METHODS

A retrospective analysis was made of GBC patients who underwent surgery in our clinic between December 2005 and March 2021 and who met the inclusion criteria. The study was approved by the Institutional Ethics Committee of the University of Health Sciences Haydarpasa Numune Research and Training Hospital (2021/65-2).

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PATIENTS

The inclusion criteria were the achievement of R0 resection through surgical treatment and the availability of appropriate material for TSR assessment in the pathology laboratory. Patients who received neoadjuvant chemotherapy and/or radiotherapy, those who died within 30 days of surgery, those with insufficient records, and those were lost to follow-up were excluded from the study.

The American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition (AJCC, 8th Ed.) was used for clinical and pathological staging of the cases[7]. Standard radical cholecystectomy was sufficient to achieve R0 resection in most cases, while some advanced cases required extended radical resections. The patients were evaluated using a multidisciplinary approach, and cases were referred to postoperative adjuvant treatment where necessary.

DATA COLLECTION

The patient details were collected from the registered information in our hospital. The following patient data were extracted: age at the time of diagnosis, gender, complaints, preoperative and postoperative radiological and laboratory data, operative findings and surgical procedures, morbidity and mortality, TNM stage (AJCC, 8th Ed.), tumor location, histopathological type, and grade, lymphovascular invasion (LVI), perineural invasion (PNI), and long-term follow-up data.

HISTOPATHOLOGICAL SCORING

All hematoxylin and eosin (H&E)-stained sections from the tumors that were used to diagnose the resection pieces of the patients were retrieved from the archive of the pathology laboratory and reevaluated under light microscope. Sections that were unsuitable for evaluation were resectioned with a 4-micron section thickness from paraffin blocks and stained with H&E. Multiple H&E-stained sections representing the tumors were examined under a light microscope using a 4× objective, and the most invasive sections of the tumors were determined. These sections were examined with a 10× objective according to the TSR assessment criteria recommended in the study by Van Pelt et al.[8]. A stroma-rich microscopic area surrounded by tumor cells at four corners was determined in the most invasive tumor area, which is deemed most suitable for evaluation. The proportion of stroma in this area was assessed by two independent pathologists in a blinded manner and scored per 10-fold percentage. Following the assessment, inconsistent results were determined by consensus. A 50% cutoff value was accepted as described by Mesker et al.[9]. Accordingly, TSR was defined as follows: TSR-low <50% and TSR-high ≥50%. The TSR-low cases were defined as the stroma-rich group, and the TSR-high cases were defined as the stroma-poor group.

STATISTICAL ANALYSIS

A Shapiro-Wilk test was used to analyze whether the normal distribution assumption was met. Categorical data were expressed as numbers (n) and percentages (%), while quantitative data were presented as median (25th–75th) percentiles. The kappa coefficient was calculated to determine the level of agreement between the TSRs established by two independent pathologists. A Kaplan-Meier survival analysis with a log-rank test was used to determine whether the TSR had a statistically significant effect on overall survival (OS). Cumulative 1-, 3-, 5-, and 10-year survival rates; median life expectancy; and 95% confidence intervals were also calculated. The differences in continuous variables between the groups were compared with a Mann-Whitney U test. A continuity-corrected χ^2 test was used for all 2×2 contingency tables to compare categorical variables when one or more of the cells had an expected frequency of 5–25, and a Fisher's exact test was applied when one or more of the cells had an expected frequency of ≤5. For all R×C contingency tables to compare categorical variables, the Fisher-Freeman-Halton test was used when 25% or more of the cells had an expected frequency of ≤5. Data analysis was performed using IBM SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA). A $p \leq 0.05$ was considered statistically significant.

RESULTS

The 28 eligible GBC patients had a mean age of 64.6 years and included 20 (71.4%) females and 8 (28.6%) males. Of the total, 10 (35.7%) patients were diagnosed incidentally by cholecystectomy for cholelithiasis or polyps, while 18 (64.3%) had a nonincidental diagnosis. All patients underwent R0 curative resection, with a standard radical cholecystectomy in 24 (85.7%) and extended radical resection in 4 (14.3%) (hepatopancreatoduodenectomy in 3 and right hepatic trisectionectomy in 1) patients. Of the total, 14 (50.0%) patients required en-bloc adjacent organ or structure resection to achieve R0 resection. Histological assessment

Table 1. Demographic and clinicopathological characteristics of cases by tumor-stroma ratio value.

	Stroma-poor n: 15 (53.6%) n (%) or mean (95%CI)	Stroma-rich n: 13 (46.4%) n (%) or mean (95%CI)	p
Age ≥60 years	10 (66.7)	10 (76.9)	0.686†
Gender			0.686†
Male	5 (33.3)	3 (23.1)	
Female	10 (66.7)	10 (76.9)	
CA 19-9 (U/mL)	10.03 (2.04–26.73)	30.54 (6.75–320.25)	0.126‡
CEA (U/mL)	3.20 (1.67–7.52)	3.09 (1.96–9.56)	0.755‡
Nonincidental	10 (66.7)	8 (61.5)	>0.999†
Jaundice	3 (20.0)	3 (23.1)	>0.999†
Adjacent organ or structure resection	8 (53.3)	10 (76.9)	0.254†
Location			0.751¥
Fundus	3 (20)	3 (23.1)	
Corpus	9 (60)	5 (38.5)	
Neck	1 (6.7)	2 (15.4)	
Multiple	0 (0.0)	1 (7.7)	
Diffuse	2 (13.3)	2 (15.4)	
Grade			>0.999†
1–2	11 (73.3)	10 (76.9)	
3	4 (26.7)	3 (23.1)	
AJCC, 8th Ed. Stage			0.316¥
I	2 (13.3)	0 (0.0)	
II	3 (20.0)	1 (7.7)	
III	3 (20.0)	6 (46.2)	
IV	7 (46.7)	6 (46.2)	
T stage			0.114†
T1/T2	7 (46.7)	2 (15.4)	
T3/T4	8 (53.3)	11 (84.6)	
N stage			0.322¶
N0	9 (60.0)	4 (33.3)	
N1–N2	6 (40.0)	8 (66.7)	
M stage			>0.999†
M0	12 (80.0)	11 (84.6)	
M1	3 (20.0)	2 (15.4)	
LVI	9 (60.0)	8 (61.5)	>0.999¶
PNI	7 (46.7)	10 (76.9)	0.212¶

CA 19-9: serum carbohydrate antigen 19-9; CEA: serum carcinoembryonic antigen; AJCC, 8th Ed.: *The American Joint Committee on Cancer (AJCC) Cancer Staging Manual*, 8th Edition; LVI: lymphovascular invasion; PNI: perineural invasion. † Fisher's exact test; ‡ Mann-Whitney U test; ¥ Fisher-Freeman-Halton test; ¶ continuity-corrected χ^2 test.

revealed adenocarcinoma in 25 (89.3%) patients, squamous cell carcinoma in 2 (7.1%) patients, and neuroendocrine carcinoma in 1 (3.6%) patient.

The results of the histopathological TSR scoring showed an almost-perfect agreement between the two independent pathologists, with a kappa of 0.929. A high TSR (stroma-poor) and a low TSR (stroma-rich) were detected in 15 (53.6%) and 13 (46.4%) patients, respectively. The other demographic and clinicopathological characteristics of the cases related to TSR are presented in Table 1. A comparison of the stroma-poor and stroma-rich groups revealed no statistically significant difference in advanced age (≥60 years), gender distribution, serum levels of carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA), incidental or nonincidental diagnosis, jaundice, adjacent organ or structure resection, tumor location, a grade 3 rather than grades 1–2, a T stage of T1/T2 or T3/T4, an N stage of N0 or N1/N2, M stage, AJCC stage, LVI, and PNI.

The median follow-up of the patients was 15.6 (range, 2.3–145.6) months. The 1-, 3-, 5-, and 10-year survival rates and the expected median OS are presented in Table 2 for the stroma-poor, stroma-rich, and overall patient groups. As can be seen, the stroma-rich group tended to have lower expected survival rates and a shorter median OS, although the difference was not statistically significant (p=0.526). Kaplan-Meier survival curves of the patient groups are presented in Figure 1.

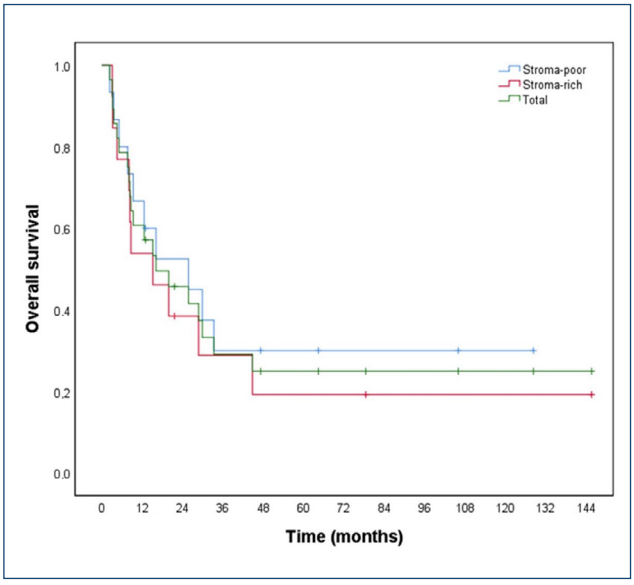

Figure 1. Kaplan-Meier survival curves for overall survival according to tumor-stroma ratio (stroma-poor versus stroma-rich) and the total number of patients.

Table 2. Kaplan-Meier survival analyses of overall survival with the log-rank test.

	N	Cumulative survival rates				Life expectancy	Log-rank	p
		1 year	3 years	5 years	10 years	Median (95%CI)		
TSR							0.402	0.526
Stroma-poor	15	66.7	30.0	30.0	30.0	25.7 (3.0–48.4)		
Stroma-rich	13	53.8	28.8	19.2	19.2	15.1 (1.6–28.6)		
Total	28	60.7	29.1	24.9	24.9	16.1 (0.3–31.8)		

CI: confidence interval; TSR: tumor-stroma ratio.

DISCUSSION

Recent studies have analyzed tumor cells and tumor microenvironments to identify additional biomarkers with a high prognostic and/or predictive value, investigating molecular mechanisms, tumor cell structure, genetic mutations, tumor immune response, and gene expression, although the transcriptomic and genetic data collection in these methods leads to high costs[10-12]. The traditional pathological approach to analysis using a microscope is simple, inexpensive, and effective and so a microscopic analysis-based biomarker is desirable. TSR was first reported in 2007 to have potential in meeting this need due to its prognostic effect on colon cancers[9]. Subsequent studies put forward TSR as a promising outcome prediction tool, which demonstrates its prognostic effect on other cancer types, such as rectal cancer, breast cancer, hepatocellular carcinoma, and esophageal cancer[3,4]. While studies of TSR are increasing day by day, only two studies have been published to date for GBC[5,6]. Among these studies, Li et al.[5] reported median OS of 6 and 17 months for the stroma-rich and stroma-poor groups, respectively (p=0.004), and suggested TSR as an important prognostic factor in GBC. However, the authors also reported that TSR was not an independent prognostic factor for OS, with only the operative technique being an independent prognostic factor. The said study evaluated 51 patients, of which 37.3% underwent palliative resection. It should not be ignored that patients undergoing palliative resection, who should have been excluded from the study in our opinion, might have affected the results. Goyal et al.[6], in turn, examined the associations among TSR, tumor budding (TBd), and desmoplastic stromal reaction (DSR) with conventional prognostic factors and OS, in 96 patients, all of whom underwent curative resection. The authors, using the mean value instead of the median for OS, reported 18.9 months for the stroma-rich group and 89.5 months for the stroma-poor group (p<0.001) and showed TSR to be a prognostic factor for OS. The multivariate analysis also identified a low TSR along with the presence of metastases and positive surgical margins as independent poor prognostic factors for OS. Our study included only patients undergoing R0 curative resection, and despite the tendency for lower survival rates and shorter median OS in the stroma-rich group, the difference was not statistically significant. That said, the low number of patients in our study might have prevented our results from reaching statistical significance.

A meta-analysis study evaluating the effect of TSR on OS in various solid tumor patient groups established that a low TSR resulted in significantly poorer OS in patients with colorectal cancer, non-small cell lung cancer, hepatocellular carcinoma, breast cancer, and esophagus cancer, while no such effect was identified in cervical cancer patients[3]. The same study evaluated TSR according to the clinical stage subgroups and found a high TSR to be a positive predictor of OS in the stages I–IV, I–III, and II–III groups, while no such effect was identified in the stages I–II group[3]. The stage-specific effect of TSR was not assessed in this study due to the small sample size, and no such assessment was made also in the other two studies[5,6].

In this study, an analysis of the relationships between TSR and demographic and clinicopathological characteristics revealed no statistically significant relationship. In contrast, Li et al.[5] examined the relationships between TSR and gender, age, pathology type, differentiation grade, pTNM stage, surgical margins, and operative techniques and found only the stroma-rich group to be statistically significantly associated with higher T stages. Goyal et al.[6], in turn, reported TSR to be significantly associated with T stage, AJCC stage, LVI, PNI, resection margins, TBd score and category, and the type of DSR. The stroma-rich group was significantly associated with immature DSR, and the stroma-poor group with fibrotic

DSR[6]. No evaluation of the TBd score or the category or type of DSR was made in this study.

The underlying mechanism of the prognostic effect of TSR has yet to be clarified. The components of tumor-related stroma are complex, including the extracellular matrix (ECM), various cell types, and different secreted factors. While ECM helps cancer cells to communicate with stromal cells, it has been shown that the abnormal expression of some secreted protein factors that activate ECM may promote tumorigenesis[13]. Factors such as matrix metalloproteinases that degrade the ECM also facilitate tumor initiation and invasion[14]. In several types of cancer, activated fibroblasts, known also as cancer-associated fibroblasts (CAFs), are the predominant cell type within the tumor tissue rather than cancer cells. In the early stages of tumor progression, CAFs act as suppressors of contact inhibition in cancer cells by increasing the formation of gap junctions among activated fibroblasts. In later stages, CAFs function as promoters of tumor growth and progression after activation by several tumor-secreted factors[15]. Stromal cells also promote angiogenesis and metastasis and thus have a significant negative impact on prognosis[16]. Clarifying the relationship between the stromal component and cancer cells and the impact of this relationship on cancer progression may also be beneficial to the development of new therapeutic approaches in the future and should be evaluated from this perspective[3].

This study has some limitations, primarily including its retrospective design and the associated risk of selection bias. Second, the number of eligible patients was relatively low, which did not allow for subgroup assessments.

CONCLUSION

In this study, a low TSR (stroma-rich) tended to be a poor prognostic factor in GBC, although not to a statistically significant degree. Further studies should be conducted with larger patient groups. If the prognostic effect of TSR is strongly proven, its inclusion in routine pathological assessment as a simple, inexpensive, and useful method may be recommended.

AUTHORS' CONTRIBUTIONS

MAU: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **MT**: Conceptualization, Data curation, Formal Analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **AG**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **FA**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **SAK**: Conceptualization, Data curation, Formal Analysis, Investigation, Validation, Writing – review & editing. **GÇO**: Conceptualization, Data curation, Formal Analysis, Investigation, Validation, Writing – review & editing.

REFERENCES

1. Hickman L, Contreras C. Gallbladder cancer: diagnosis, surgical management, and adjuvant therapies. Surg Clin North Am. 2019;99(2):337-55. https://doi.org/10.1016/j.suc.2018.12.008
2. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol. 2003;200(4):429-47. https://doi.org/oi:0.1002/path.1398
3. Wu J, Liang C, Chen M, Su W. Association between tumor-stroma ratio and prognosis in solid tumor patients: a systematic review and meta-analysis. Oncotarget. 2016;7(42):68954-65. https://doi.org/10.18632/oncotarget.12135
4. Zhang R, Song W, Wang K, Zou S. Tumor-stroma ratio (TSR) as a potential novel predictor of prognosis in digestive system cancers: a meta-analysis. Clin Chim Acta. 2017;472:64-8. https://doi.org/10.1016/j.cca.2017.07.003
5. Li H, Yuan SL, Han ZZ, Huang J, Cui L, Jiang CQ, et al. Prognostic significance of the tumor-stroma ratio in gallbladder cancer. Neoplasma. 2017;64(4):588-93. https://doi.org/10.4149/neo_2017_413
6. Goyal S, Banga P, Meena N, Chauhan G, Sakhuja P, Agarwal AK. Prognostic significance of tumour budding, tumour-stroma ratio and desmoplastic stromal reaction in gall bladder carcinoma. J Clin Pathol. 2021;1. https://doi.org/10.1136/jclinpath-2021-207957
7. Zhu AX, Pawlik TM, Kooby DA, Schefter TE, Vauthey J-N. Gallbladder. In: Amin MB, et al. (editors) AJCC Cancer Staging Manual. 8th ed. New York: Springer International Publishing; 2017. p. 303-9.
8. van Pelt GW, Kjær-Frifeldt S, van Krieken JHJM, Al Dieri R, Morreau H, Tollenaar RAEM, et al. Scoring the tumor-stroma ratio in colon cancer: procedure and recommendations. Virchows Arch. 2018;473(4):405-12. https://doi.org/10.1007/s00428-018-2408-z
9. Mesker WE, Junggeburt JM, Szuhai K, de Heer P, Morreau H, Tanke HJ, et al. The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. Cell Oncol. 2007;29(5):387-98. https://doi.org/10.1155/2007/175276
10. Carrasco C, Tittarelli A, Paillaleve N, Pozo MD, Rojas-Sepúlveda D, Barría O, et al. The evaluation of 17 gastrointestinal tumor markers reveals prognosis value for MUC6, CK17, and CD10 in gallbladder-cancer patients. Diagnostics (Basel). 2021;11(2):153. https://doi.org/10.3390/diagnostics11020153
11. Wu Y, Meng D, Xu X, Bao J, You Y, Sun Y, et al. Expression and functional characterization of INPP4B in gallbladder cancer patients

and gallbladder cancer cells. BMC Cancer. 2021;21(1):433. https://doi.org/10.1186/s12885-021-08143-6

12. Wang FT, Li XP, Pan MS, Hassan M, Sun W, Fan YZ. Identification of the prognostic value of elevated ANGPTL4 expression in gallbladder cancer-associated fibroblasts. Cancer Med. 2021;10(17):6035-47. https://doi.org/10.1002/cam4.4150

13. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9(4):274-84. https://doi.org/10.1038/nrc2622

14. Luo H, Tu G, Liu Z, Liu M. Cancer-associated fibroblasts: a multifaceted driver of breast cancer progression. Cancer Lett. 2015;361(2):155-63. https://doi.org/10.1016/j.canlet.2015.02.018

15. Cirri P, Chiarugi P. Cancer-associated fibroblasts and tumor cells: a diabolic liaison driving cancer progression. Cancer Metastasis Rev. 2012;31(1-2):195-208. https://doi.org/10.1007/s10555-011-9340-x

16. Krishna Priya S, Nagare RP, Sneha VS, Sidhanth C, Bindhya S, Manasa P, et al. Tumour angionesis-Origin of blood vessels. Int J Cancer. 2016;139(4):729-35. https://doi.org/10.1002/ijc.30067