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# Development of a Prognostic Score for Cholangiocarcinoma Patients Using a Combination of Biochemical Parameters

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**Abstract.** *Background/Aim: Prognosis of cholangiocarcinoma (CCA), especially of intrahepatic CCA (iCCA), is poor primarily due to difficulties in earlier diagnosis. Since the majority of iCCA patients are elders, their prognosis cannot be correctly predicted by pathological features and/or resection status alone. Consideration for comorbidity and/or risks of subclinical diseases at diagnosis is critically necessary for the prediction of prognosis of iCCA patients. This study aimed to develop a simple but reliable scoring system for prognosis of iCCA patients at the time of diagnosis. Patients and Methods: Serum samples from 152 iCCA patients were collected, and four commonly used biochemical markers, serum aspartate aminotransferase, alkaline phosphatase, cystatin C and creatinine-based estimated glomerular filtration rate were measured. Then, the values of individual patients were scored as 0, 1, and 2 (low, medium, and high) by tertiles or clinically relevant cut-off points and summed to construct a prognostic score with a range between 0 to 8. Results: Patients with high scores of 2-4 and 5-8 exhibited significantly shorter survival times compared to those with low scores of 0-1 (Chi-square: 15.75, p<0.001). Cox regression analysis suggested that the score could be an independent predictor for the survival of iCCA patients. The odds of advanced tumor stage in high score iCCA patients (2-4 and 5-8) were 12.310 (95%CI=2.241-67.605) and 23.964 (95%CI=3.296-174.216), respectively. This scoring system allowed further stratification of death rates per 100 person-years of iCCA patients. Conclusion: The ability of such a simple scoring system to discriminate risk might be helpful for iCCA patients to determine therapeutic programs at the time of diagnosis.*

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*Key Words:* Cholangiocarcinoma, biochemical parameters, scoring system, survival prognosis.



Cholangiocarcinoma (CCA) is the second most prevalent type of primary liver cancer, and its mortality tends to increase globally (1, 2). In general, intrahepatic CCA (iCCA) accounts for approximately 10% of CCA. In our study area of the Northeast Thailand, however, the proportion of iCCA is extremely high due to the association of infection with carcinogenic liver fluke, *Opisthorchis viverrini*, which mainly parasitizes the intrahepatic bile ducts (3). Most iCCAs are asymptomatic and difficult to diagnose at the early stage and are usually lethal due to delayed diagnosis and the lack of appropriate non-surgical treatment modalities (1, 4). At the time of diagnosis, most patients have unresectable diseases that are difficult to treat (5). In fact, the median survival of iCCA patients is approximately 28 months after supportive treatment, and the 5-year overall survival is approximately 30% (6).

Numerous prognostic scores based on clinical features and histological status have been developed to predict the survival of iCCA patients, which are helpful to a certain degree to improve the prognosis after hepatectomy. Recently, several prognostic scores were developed in iCCA studies; (i) the Fudan score based on serum alkaline phosphatase (ALP), carbohydrate antigen 19-9 (CA19-9),

Table I. *The score values of the components.*

| Parameters | Score 0 | Score 1 | Score 2 |
|---|---|---|---|
| AST, U/l | ≤30 | 31-52 | >52 |
| ALP, U/l | ≤136 | 137-253 | >253 |
| Cys C, mg/l | ≤1.65 | 1.66-2.27 | >2.27 |
| eGFRcr, ml/min/1.73 $m^2$ | ≥90 | 60-89 | <60 |

AST: Aspartate transaminase; ALP: alkaline phosphatase; Cys C: cystatin C; eGFRcr: creatinine-based estimated glomerular filtration rate. Tertile cut points were used for scores of AST, ALP, and Cys C. Clinically relevant cut points were used for eGFRcr score.

the tumor boundary type, the number and diameter of intrahepatic tumors (7), (ii) the MEGNA score based on age>60 years, tumor grading, lymph node metastasis, multifocality and extrahepatic tumor extension (8) and (iii) preoperative risk model based on tumor size, CA19-9, neutrophil-lymphocyte ratio (NLR) and serum albumin (9). Nevertheless, none of these scoring methods performed well enough to allow accurate clinical decisions. In addition, some pathological features used in those scoring systems are not available from most of iCCA patients in Thailand at the time of diagnosis. These prognostic systems are, thus, not routinely used at an early stage and their prognostic values are still controversial. Moreover, there may be other confounding factors that influence the survival of iCCA patients. For example, Qu *et al*. (2020) reported that the age adjusted Charlson Comorbidity Index developed on clinically recognized diseases could predict survival in iCCA patients after curative resection (10). However, it is questionable whether iCCA patients who do not have recognizable diseases can have risks of substantial subclinical diseases, which impact mortality and survival.

In our study area and neighboring Great Mekong Subregions, iCCA patients are exclusively senior persons because CCA is caused by chronic infection with carcinogenic liver fluke, *Opisthorchis viverrini*. Thus, not only comorbidity, but also general health status including subclinical diseases should be considered for the prediction of prognosis. Also, in general, cancers including CCA affect the metabolism and of not only the targeted organs (11) but also of the whole-body including vital organs such as the heart, liver, kidney, and central nervous systems. Thus, for the prediction of prognosis of iCCA patients at the time of diagnosis, consideration for the risks of pre-existing health conditions or subclinical diseases related to these organs is critically necessary before starting treatment.

In this study, therefore, we aimed to develop a new scoring system that provides a simple and effective prediction of the prognosis of iCCA patients at the time of CCA diagnosis. For this purpose, we selected four routinely

Table II. *Clinicopathological characteristics of the intrahepatic cholangiocarcinoma patients.*

| Characteristics | No. | Percentage | Median±Q.D (Minimum-Maximum) |
|---|---|---|---|
| Sex | | | |
| Female | 46 | 30.3 | |
| Male | 106 | 69.7 | |
| Age (Years) | | | |
| ≤64 | 80 | 52.6 | 64.0±5.0 |
| >64 | 72 | 47.4 | (31-83) |
| Total bilirubin (mg/dl) | | | |
| ≤1.5 | 95 | 62.9 | 0.7±3.3 |
| >1.5 | 56 | 37.1 | (0.2-20.1) |
| Direct bilirubin (mg/dl) | | | |
| ≤0.5 | 85 | 56.3 | 0.4±1.0 |
| >0.5 | 66 | 43.7 | (0.1-18.7) |
| ALT (U/l) | | | |
| ≤36 | 70 | 46.1 | 38.0±22.3 |
| >36 | 82 | 53.9 | (9-993) |
| ALP (U/l) | | | |
| ≤121 | 40 | 26.3 | 187.5±88.3 |
| >121 | 112 | 73.7 | (35-1,409) |
| AST (U/l) | | | |
| ≤32 | 56 | 36.8 | 43.5±17.8 |
| >32 | 96 | 63.2 | (4-454) |
| Creatinine (mg/dl) | | | |
| ≤1.2 | 135 | 88.8 | 0.89±0.15 |
| >1.2 | 17 | 11.2 | (0.4-1.8) |
| Cys C (mg/l) | | | |
| ≤1.21 | 22 | 14.5 | 1.94±0.48 |
| >1.21 | 130 | 85.5 | (0.72-4.97) |
| eGFRcr (ml/min/1.73 $m^2$) | | | |
| ≥90 | 78 | 51.3 | 85.0±10.6 |
| <90 | 74 | 48.7 | (33-120) |
| Albumin (g/dl) | | | |
| ≥3.8 | 101 | 68.2 | 4.0.±0.35 |
| <3.8 | 47 | 31.8 | (2.4-5.0) |
| CA 19-9 (U/ml) | | | |
| ≤37 | 59 | 40.7 | 111.3±419.9 |
| >37 | 86 | 59.3 | (0.59-1,001) |
| CEA (ng/ml) | | | |
| ≤2.5 | 33 | 24.6 | 5.2±5.5 |
| >2.5 | 101 | 75.4 | (1.0-917.6) |
| Vascular invasion | | | |
| No | 25 | 25 | |
| Yes | 75 | 75 | |
| Lymph node metastasis | | | |
| No | 58 | 43.9 | |
| Yes | 74 | 56.1 | |
| Tumor stage | | | |
| I-III | 58 | 40.6 | |
| IVA-IVB | 85 | 59.4 | |
| Survival (days) | | | |
| ≥463 | 76 | 50 | 463±408.5 |
| <463 | 76 | 50 | (15-2565) |

Values represent Q.D: quartile deviation. A total of 152 patients were not fully determined due to the absence of the corresponding clinical data. ALT: Alanine transaminase; ALP: alkaline phosphatase; AST: aspartate transaminase; Cys C: cystatin C; eGFRcr: creatinine-based estimated glomerular filtration rate; CA 19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen.

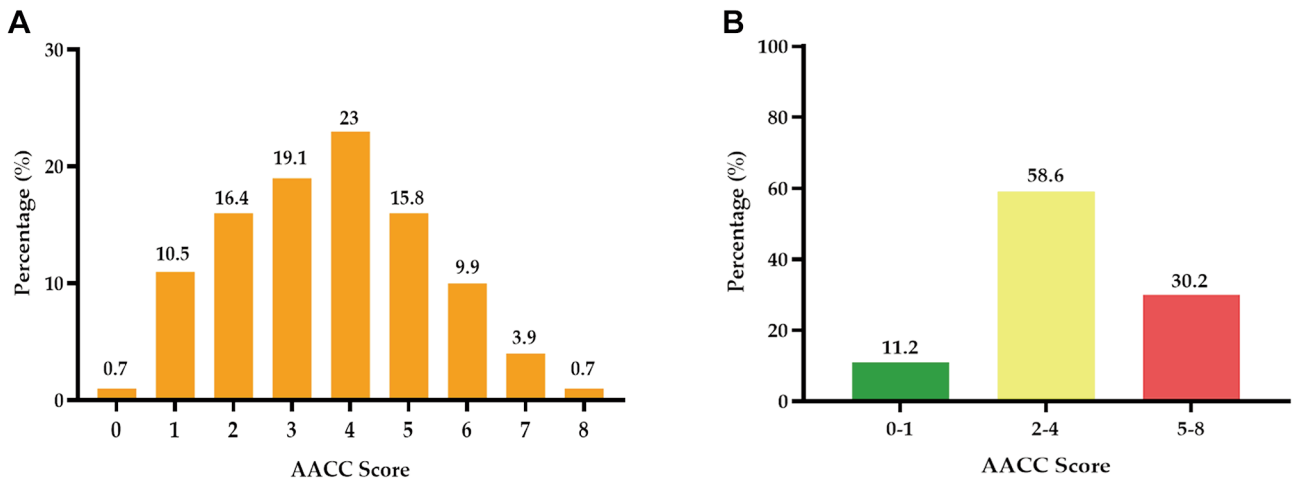


Figure 1. *Distribution patterns of the AACC Score of intrahepatic cholangiocarcinoma (iCCA) patients (n=152). (A) According to score 0 to 8, (B) according to grouped scores of 0-1, 2-4, and 5-8.*

used biochemical parameters in the sera of iCCA patients at the time of diagnosis: **A**spartate aminotransaminase (AST), **A**lkaline phosphatase (ALP), **C**ystatin C (Cys C) and **C**reatinine-based estimated glomerular filtration rate (eGFRcr) (AACC). These biochemical markers potentially indicate underlying subclinical diseases across four organ systems such as cardiovascular (12-14), liver (15, 16), nervous system (17-19), and kidneys (20, 21). The values of these four markers of each iCCA patient were converted to tertile score and the sum of the scores (ranging from 0 to 8) was taken as the prognostic score, termed as the AACC score. Then, we collected the retrospective clinical data of iCCA patients and examined the association of the prognostic score and clinicopathological features using Kaplan-Meier test, log-rank test, Cox and logistic regression analysis. The results show that this simple and easy-to-use prognostic scoring system could help clinicians to predict prognosis and to stratify the risk of outcomes regardless of histopathological features of iCCA patients at the time of diagnosis.

## Patients and Methods

*Sample size calculation*. To determine the sample size required to establish the prognostic score, the preliminary study was performed using iCCA patients' data (n=82) from our previous study (22). The sample size was calculated using the G*Power program (version 3.1.9.7, the G*Power team, Heinrich Heine University, Düsseldorf, Germany) (23). With the assumption of correlation coefficient=0.32, alpha=0.05 and power=0.8 (beta=0.2), the minimum sample size was calculated to be 75 samples with the actual power of 0.95 in this study.

*Data and sample collection for iCCA patients*. Serum samples from 152 iCCA patients at the time of diagnosis (median±quartile deviation, 64±8.3 years, range=31-83 years) were kindly provided by the Cholangiocarcinoma Research Institute (CARI), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. All the patients were positive for *O. viverrini* infection either by coproparasitological or serological tests. The inclusion criteria were diagnosis of iCCA by clinicopathological examinations either by biopsy or intraoperative rapid diagnosis including immunohistochemistry for CD56. Classification and staging of iCCA followed the AJCC/UICC 8$^{th}$ edition (24). Exclusion criteria included previous history of surgical treatment (including preoperative biliary drainage) or chemotherapy, and other cancers including concurrent double cancers or extrahepatic CCA. All selected patients had clinical background information including vital status, survival days and spreading status for retrospective analysis. This retrospective study was approved by the Khon Kaen University Ethics Committee for Human Research and performed in accordance with the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (HE641324). Informed consent was obtained from all subjects involved in the study.

*Serum Cystatin C (Cys C) measurement*. Serum Cys C was measured using the Latex-Particles enhanced immunoturbidimetric assay kit (Diazyme, Poway, CA, USA). Cys C in the sample binds to the specific anti-Cys C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination was measured optically and the Cys C concentration of patient's specimens was calculated using a calibration curve constructed from 6-point concentration ranges of 0.52 to 8.15 mg/l. The standard high and low concentrations of Cys C provided by the manufacturer were used as internal quality controls.

*Estimated glomerular filtration rate (eGFR) calculation*. eGFRcr was calculated based on the serum creatinine level using a modified version of the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI), 2021 (25).

*Components of the sore*. The values of AST, ALP, Cys C, and eGFRcr in the serum of 152 iCCA patients were used to create our

Table III. *Association of AACC score with clinicopathological characteristics of intrahepatic cholangiocarcinoma patients.*

| Characteristics | AACC Score | | | | |
|---|---|---|---|---|---|
| | Overall | 0-1 | 2-4 | 5-8 | *p*-Value |
| Sex | | | | | |
| Female | 46 (30.3%) | 9 (52.9%) | 27 (30.3%) | 10 (21.7%) | 0.057 |
| Male | 106 (69.7%) | 8 (47.1%) | 62 (69.7%) | 36 (78.3%) | |
| Age (Years) | 62.8±8.3 | 59.4±9.5 | 62.9±8.2 | 63.8±7.8 | 0.173 |
| | (61.5-64.0) | (54.0-63.6) | (61.2-64.6) | (61.4-65.9) | |
| Total bilirubin (mg/dl) | 2.0±3.1 | 0.6±0.6 | 1.6±2.3 | 3.4±4.4 | <0.001* |
| | (1.6-2.6) | (0.3-1.0) | (1.1-2.1) | (2.2-4.8) | |
| Direct bilirubin (mg/dl) | 1.6±2.8 | 0.4±0.6 | 1.3±2.1 | 2.9±3.9 | <0.001* |
| | (1.2-2.2) | (0.1-0.7) | (0.8-1.8) | (1.8-4.1) | |
| ALT (U/l) | 61.2±97.0 | 32.2±20.4 | 48.3±51.5 | 96.7±155.9 | 0.009* |
| | (47.7-79.2) | (23.6-42.7) | (38.7-59.9) | (61.3-149.2) | |
| ALP (U/l) | 237.3±181.0 | 121.7±36.2 | 243.2±209.9 | 268.6±131.1 | 0.014* |
| | (210.2-267.3) | (106.4-139.8) | (199.8-288.6) | (233.4-307.3) | |
| AST (U/l) | 58.4±59.1 | 24.2±11.3 | 50.7±44.3 | 86.2±80.6 | <0.001* |
| | (49.6-68.6) | (19.0-30.1) | (42.3-60.6) | (65.2-110.8) | |
| Creatinine (mg/dl) | 0.9±0.2 | 0.8±0.1 | 0.8±0.2 | 1.0±0.2 | <0.001* |
| | (0.8-0.9) | (0.7-0.8) | (0.7-0.9) | (0.9-1.1) | |
| eGFRcr (ml/min/1.73 $m^2$) | 87.7±18.8 | 94.5±16.4 | 91.6±16.8 | 77.7±19.8 | <0.001* |
| | (84.6-90.6) | (86.8-102.5) | (88.0-95.1) | (72.0-83.2) | |
| Cys C (mg/l) | 2.0±0.7 | 1.4±0.3 | 1.8±0.7 | 2.5±0.6 | <0.001* |
| | (1.9-2.1) | (1.2-1.5) | (1.7-2.0) | (2.3-2.7) | |
| Albumin (g/dl) | 3.9±0.6 | 4.2±0.5 | 3.9±0.6 | 3.7±0.6 | 0.036* |
| | (3.8-4.0) | (3.9-4.4) | (3.8-4.0) | (3.5-3.9) | |
| CA19-9 (U/ml) | 351.1±410.8 | 272.4±398.9 | 390.3±428.0 | 299.6±375.5 | 0.358 |
| | (282.7-420.3) | (98.2-477.7) | (303.1-482.3) | (184.6-428.4) | |
| CEA (ng/ml) | 26.4±102.2 | 28.9±47.5 | 21.9±82.0 | 35.3±152.1 | 0.805 |
| | (12.7-45.5) | (804-53.3) | (9.8-43.6) | (5.9-95.2) | |
| Vascular invasion | | | | | |
| No | 25 (25%) | 3 (23.1%) | 14 (24.6%) | 8 (26.7%) | 0.963 |
| Yes | 75 (75%) | 10 (76.9%) | 43 (75.4%) | 22 (73.3%) | |
| Lymph node metastasis | | | | | |
| No | 58 (43.9%) | 9 (75%) | 35 (45.5%) | 14 (32.6%) | 0.030* |
| Yes | 74 (56.1%) | 3 (25%) | 42 (54.5%) | 29 (67.4%) | |
| Tumor stage | | | | | |
| I-III | 58 (40.6%) | 12 (70.6%) | 34 (41.5%) | 12 (27.3%) | 0.008* |
| IVA-IVB | 85 (59.4%) | 5 (29.4%) | 45 (58.5%) | 32 (72.7%) | |
| Survival (Days) | 655.0±550.6 | 1137.9±598.6 | 649.0±534.4 | 488.3±463.5 | <0.001* |
| | (569.9-743.8) | (877.5-1449.2) | (541.3-761.3) | (362.9-627.7) | |

*Statistically significant ($p<0.05$). Values represent mean±standard deviation (95% confidence interval), number (percentages within score group); A total of 152 patients were not fully determined due to the absence of the corresponding clinical data; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; eGFRcr: creatinine-based estimated glomerular filtration rate; Cys C: cystatin C; CEA: carcinoembryonic antigen; CA 19-9: carbohydrate antigen 19-9.

AACC score system. The components of the scoring system were chosen based on previous reports where each component was shown to have the potential to be used clinically for risk prediction of mortality or subclinical diseases (please, see the last paragraph of the introduction). Although these noninvasive tests may not represent identical phenotypes of the organ system, they can be general indicators or disease related variables.

To construct the score, each of the components except for eGFRcr was classified into three categories using tertile cut points of iCCA patients; score 0 (low), score 1 (medium), and score 2 (high). Clinically relevant cut-off points were used to categorize eGFRcr. The detailed score values are shown in (Table I). The AACC score was constructed as the sum of the scores of 4 components, ranging from 0 to 8.

*Statistical analysis*. The median±quartile deviation (minimum to maximum) was used for the description of non-normally distributed data. We estimated the median AACC scores of iCCA patients and the relative frequencies of the patients in each score of 0 to 8. In iCCA patients, the association between the three groups of AACC score (0-1, 2-4, and 5-8) and clinicopathological characteristics was analyzed using the Chi-square test. The overall survival curves were

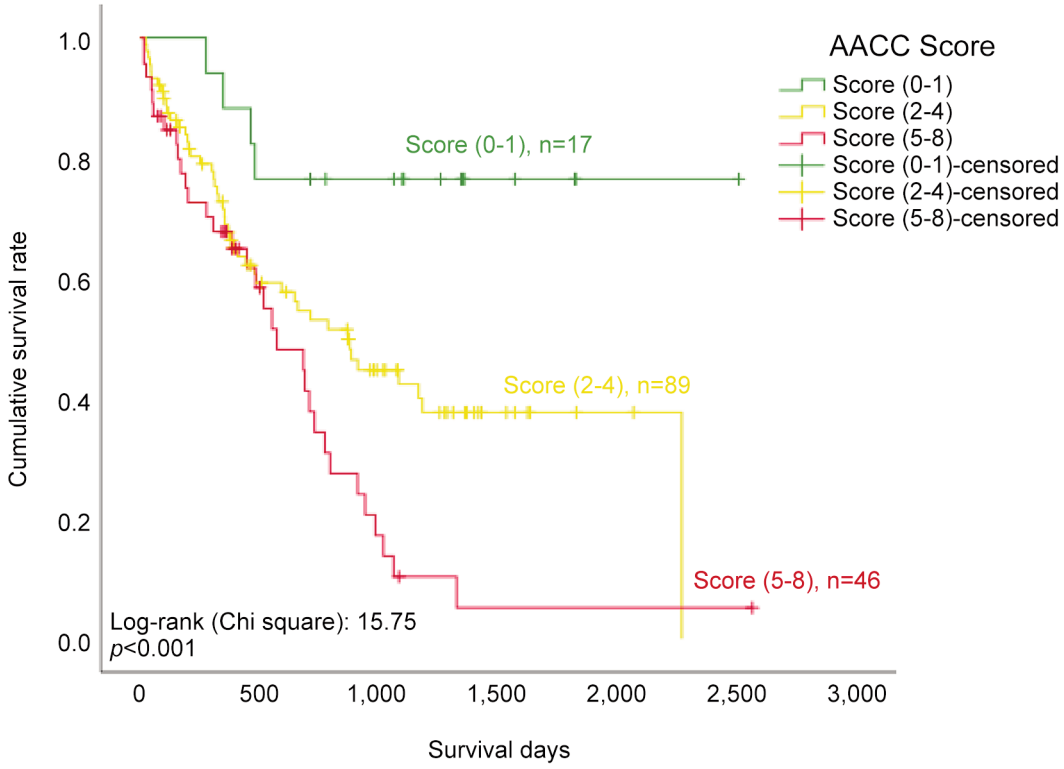


Figure 2. *Kaplan-Meier analysis of overall survival time of intrahepatic cholangiocarcinoma (iCCA) patients. The cases were assigned to the AACC Score (0-1; green line), (2-4; yellow line) and (5- 8; red line) groups. There was a significant difference in the survival time between each score group (p<0.001), 0-1 and 2-4 (p=0.012), 2-4 and 5-8 (p=0.017), 0-1 and 5-8 (p<0.001) by log-rank test.*

analyzed using the Kaplan-Meier test and log-rank test. The Cox proportional hazard regression model was used to determine the association of the AACC score with overall survival. We calculated the death rates per 100 person-years across score groups. Logistic regression analysis was performed to determine the odds of outcomes. $p<0.05$ was considered as statistical significance. All analyses were conducted using IBM SPSS version 28 Statistics (The International Business Machines Corporation, Charles Randlett Flint, Armonk, NY, USA) and GraphPad Prism version 5 software (GraphPad Software, San Diego, CA, USA).

## Results

*Clinicopathological characteristics of iCCA patients*. The clinicopathological characteristics of 152 iCCA patients are shown in Table II. A total of 106 patients were male and 46 females: with a median age of 64 years. The median value of serum ALT, ALP, AST, Cys C, CA19-9, and CEA of iCCA patients were significantly higher than the upper limit of the normal value. The eGFRcr median value was lower than the upper limit of the normal value.

*Distribution of the AACC scores*. The distribution pattern of the AACC score was quite broad with slight skewing toward lower values. The frequency of patients having a score of 0 and 8 was very low (Figure 1A). The median±quartile deviation of the AACC score of iCCA patients was 4.0±1.5 (score range 0 to 8). Then, we categorized the AACC score into three groups as 0-1, 2-4, and 5-8. Among iCCA patients, 11.2% of them are in the AACC score of 0-1, 58.6% in the score of 2-4, and 30.2% in the score of 5-8 (Figure 1B).

*Association of the AACC score with clinicopathological characteristics of iCCA patients*. To determine the clinical importance of the AACC score, we analyzed the association between three AACC score groups (0-1, 2-4, and 5-8) and clinicopathological characteristics of iCCA patients. The results showed that significant differences were observed between different AACC score groups and the preoperative levels of total bilirubin ($p<0.001$), direct bilirubin ($p<0.001$), ALT ($p=0.009$), ALP ($p=0.014$), AST ($p<0.001$), creatinine ($p<0.001$), eGFRcr ($p<0.001$), Cys C ($p<0.001$) and albumin ($p=0.036$). Patients in the higher score group (2-8) had significantly shorter survival, compared with those of the low score group (0-1) ($p<0.001$). Also, lymph node metastasis and advanced tumor stage were more frequently presented in the higher score groups (2-4 and 5-8). However, there were no significant differences between

Table IV. *Univariate and multivariate Cox regression analysis of clinical predictors for overall survival of intrahepatic cholangiocarcinoma patients.*

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| Variables | HR (95%CI) | *p*-Value | HR (95%CI) | *p*-Value |
| Age (years) | | | | |
| ≤64 | 1 | | 1 | |
| >64 | 1.345 (0.872-2.075) | 0.181 | 1.364 (0.681-2.734) | 0.381 |
| Sex | | | | |
| Female | 1 | | 1 | |
| Male | 1.339 (0.821-2.813) | 0.242 | 0.542 (0.151-1.947) | 0.348 |
| CA19-9 (U/ml) | | | | |
| ≤37 | 1 | | 1 | |
| >37 | 1.517 (1.137-2.874) | 0.077 | 1.114 (0.654-1.898) | 0.692 |
| CEA (ng/ml) | | | | |
| ≤2.5 | 1 | | 1 | |
| >2.5 | 1.925 (1.193-3.105) | 0.007* | 1.162 (0.660-2.047) | 0.602 |
| AST score | | | | |
| 0-1 | 1 | | 1 | |
| 2 | 1.210 (0.771-1.899) | 0.406 | 1.135 (0.859-1.507) | 0.401 |
| ALP score | | | | |
| 0-1 | 1 | | 1 | |
| 2 | 1.120 (0.976-1.438) | 0.687 | 1.195 (0.882-2.093) | 0.534 |
| Cys C score | | | | |
| 0-1 | 1 | | 1 | |
| 2 | 2.019 (1.30-3.136) | 0.002* | 1.849 (1.092-3.130) | 0.022* |
| eGFRcr score | | | | |
| 0-1 | 1 | | 1 | |
| 2 | 3.116 (1.731-5.608) | <0.001* | 2.870 (1.629-6.408) | <0.001* |
| AACC score group | | | | |
| 0-1 | 1 | | 1 | |
| 2-4 | 3.596 (1.291-10.015) | 0.014* | 3.672 (1.105-12.202) | 0.034* |
| 5-8 | 6.157 (2.161-17.542) | <0.001* | 5.080 (1.381-18.684) | 0.014* |

*Statistically significant ($p<0.05$). CI: Confidence interval; HR: hazard ratio; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; eGFRcr: creatinine-based estimated glomerular filtration rate; Cys C: cystatin C; CEA: carcinoembryonic antigen; CA 19-9: carbohydrate antigen 19-9.

score groups in terms of sex, age, serum CA19-9, serum CEA and vascular invasion (Table III).

*Potential predictivity of the AACC score for overall survival of iCCA patients.* To examine whether the AACC score can predict the prognosis of iCCA patients, we analyzed the overall survival of patients of each score group using the Kaplan-Meier curve with a log-rank test. The results indicated that the survival time of iCCA patients in the higher AACC score groups (2-4 and 5-8) was significantly shorter than that of patients in the low score group (0-1) (Chi-square: 15.75, $p<0.001$) (Figure 2). Moreover, significant difference in the survival time was observed between patients with a AACC score of 0-1 and 2-4 ($p=0.012$), 2-4 and 5-8 ($p=0.017$) and 0-1 and 5-8 ($p<0.001$) (Figure 2). Our results showed that the higher the levels of the AACC score, the shorter the overall survival time. Hence, the AACC score can well predict the overall survival time of iCCA patients without additional information.

To identify the independent predictors that were correlated with overall survival of iCCA patients, we determined the hazard ratio (HR) of each score group using Cox's univariate and multivariate hazard models. On univariate analysis, patients with AACC score 2-4 (HR=3.596, 95%CI=1.291-10.015) and score 5-8 (HR=6.157, 95%CI=2.161-17.542) were independently associated with overall survival. Moreover, multivariable analysis revealed that the patients in the highest score group (5-8) have an almost five-fold risk of shorter survival compared to those in the low score group (0-1). Among AACC components, Cys C and eGFRcr scores have significant prognostic values for overall survival compared with AST and ALP scores of iCCA patients (Table IV).

*Association between the AACC score and the outcomes of iCCA patients.* To evaluate further the potential of the AACC score to predict prognosis of iCCA patients, we examined the correlation of the AACC score with the risk of outcomes

Table V. *Association between the AACC score and the outcomes of intrahepatic cholangiocarcinoma patients.*

| Comparison groups | Univariate OR (95%CI) | *p*-Value | Multivariate OR (95%CI) | *p*-Value |
|---|---|---|---|---|
| Vascular invasion | | | | |
| No *vs*. Yes | | | | |
| Score group | | | | |
| 0-1 | 1 | | 1 | |
| 2-4 | 0.921 (0.222-3.828) | 0.910 | 0.581 (0.074-4.567) | 0.605 |
| 5-8 | 0.825 (0.180-3.783) | 0.804 | 0.581 (0.048-7.037) | 0.670 |
| Lymph node metastasis | | | | |
| No *vs*. Yes | | | | |
| Score group | | | | |
| 0-1 | 1 | | 1 | |
| 2-4 | 3.600 (0.904-14.331) | 0.069 | 4.982 (0.753-32.956) | 0.096 |
| 5-8 | 6.214 (1.452-26.599) | 0.014* | 7.108 (0.843-59.952) | 0.071 |
| Tumor stage | | | | |
| Early *vs*. Advanced | | | | |
| Score group | | | | |
| 0-1 | 1 | | 1 | |
| 2-4 | 3.388 (1.092-10.510) | 0.035* | 12.310 (2.241-67.605) | 0.004* |
| 5-8 | 6.400 (1.859-22.036) | 0.003* | 23.964 (3.296-174.216) | 0.002* |

*Statistically significant ($p<0.05$). CI: Confidence interval; OR: Odds ratio; Early: stage I, II, III; Advanced: stage IVA, IVB.

(vascular invasion, lymph node metastasis and tumor stage) of iCCA patients (Table V). The results showed that the relationship between the score and tumor stage persisted in multivariable analysis. Patients in the score group of 2-4 and 5-8 had 12.31 times and 23.964 times, respectively, higher odds of advanced tumor stage compared with those in the low score group (0-1). The score had no association with vascular invasion and lymph node metastasis of iCCA patients. Moreover, within the AACC score group, death rates associated with a score of 0-1, 2-4, and 5-8 were 7.55, 29.08, and 52.03 (per 100 person-years), respectively (Figure 3).

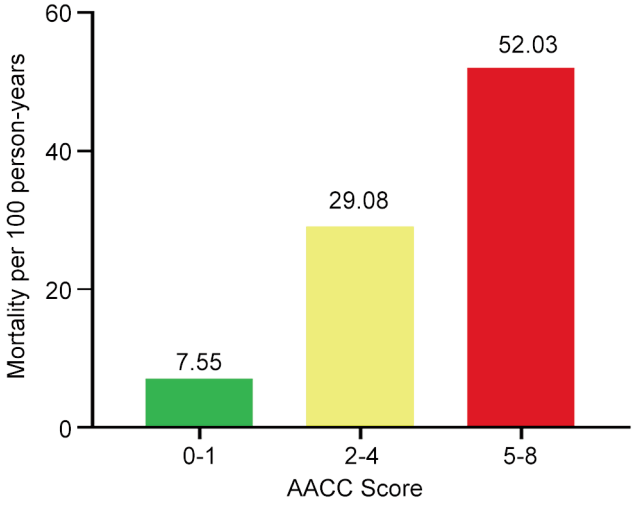


Figure 3. *Death events across the AACC score among intrahepatic cholangiocarcinoma (iCCA) patients (n=152). 95% confidence interval was 2.83-20.12 for patients with a score of 0-1, 21.78-38.83 for patients with a score of 2-4, 36.79-73.58 for patients with a score of 5-8.*

## Discussion

In this study, we constructed and evaluated a simple and clinically applicable prognostic scoring system for iCCA patients using the combined score of biochemical markers, namely AST, ALP, Cys C, and eGFRcr at the time of diagnosis. The results showed that this simple scoring system, named AACC score, can predict overall survival and discriminate risks at the time of diagnosis of iCCA patients.

Prediction of the long-term survival benefit is crucial for treatment options and is potentially associated with subclinical diseases that generally have poor prognosis. Noticeably, cancer affects the entire metabolism of the body rather than just the targeted organs, and therefore, cancer can have an impact on important organs including the heart, liver, and kidneys, and the nervous system and other organ

systems (11). Also, it is possible that the assessment of cancer consequences at the time of diagnosis could be extended to blood biochemical tests to distinguish between low risk and very high-risk treatment options. Therefore, we chose simple biochemical parameters that reflect deterioration of other vital organ systems rather than the targeted organ, which can be applicable to all iCCA patients at diagnosis. We also hypothesized that the simple AACC scoring system might provide an effective prediction of prognosis and discriminate the risk level of iCCA patients before treatment. Each component used to construct AACC score has the potential for risk prediction of mortality, comorbidities or subclinical diseases and has been studied for prognostic prediction in cancer and in CCA (21, 26-32). For example, serum AST was the first biochemical parameter used for the diagnosis of acute myocardial infarction (AMI) in the past, although this enzyme is mostly found in the liver (33). When using the De Ritis ratio (AST/ALT ratio), high AST level is a significant predictor for long-term risk compared with ALT (34). Regarding ALP, the elevation of its serum levels is the most common comorbid condition associated with not only iCCA but also hepatobiliary diseases such as hepatitis, bile ducts obstruction (obstructive jaundice), primary sclerosis cholangitis, choledocholithiasis, gall stones, cirrhosis and even a prognostic risk factor for cancer patients other than CCA (15, 16, 35, 36). The component Cys C belongs to the cystatin superfamily and is a cystine protease inhibitor. Besides being recognized as a novel renal function biomarker, Cys C is considered an independent prediction marker of common comorbid conditions such as neurological disorders including Alzheimer's disease and other dementias (17, 18, 37). In addition, increase of circulating Cys levels are generally associated with poor clinical outcomes and poor prognosis in various malignancies such as breast (38, 39), ovarian (40), prostate (41), head and neck (30, 42), CCA (31), renal (32) and esophageal (43) cancers. The eGFRcr component is useful in clinical practice to determine the degree of kidney failure and to monitor the progression of the disease (21). In accordance with these previous findings, our score components represented reasonably the possibility of comorbid complications from distinct organ systems among iCCA patients. Therefore, we hypothesized that the combination of the scores of these components (AACC score) may be of interest to iCCA researchers and might provide an opportunity for a better understanding of optimal health and comorbid complications as risk factors of prognosis when evaluating the prognosis of iCCA patients. In the present study, we found that only 11.2% of iCCA patients were classified in the low AACC score group (0-1, low risk) with long survival and low prognosis risk. Our results showed that higher the score due to higher level of preoperative AST, ALT, eGFRcr, and Cys C values may indirectly associate with the poor survival and high prognosis risk in iCCA patients.

Recently, researchers developed several prognostic scores such as the Fudan score (7) and the MEGNA score (8) based on pathological features to predict the survival of iCCA patients. They showed that their score had prognostic value for prediction of overall survival and outcomes of iCCA patients. However, they could not distinguish between those who were at high or low risks. Our study revealed that the score using a combination of biochemical parameters at the time of diagnosis has a strong association with overall survival and advanced tumor stage of iCCA patients.

This simple and easy scoring system using the combination of the four clinical bio-chemical parameters at the time of diagnosis provides a powerful prediction value for overall survival and better discrimination of the risk outcomes of iCCA patients. We also showed that iCCA patients in the lower score group (0-1, low risk) had longer expected survival than those in higher score groups (2-4 and 5-8). Our results using the Cox hazard model and multivariate analysis suggested that the score was an independent prognostic factor for the overall survival. Also, the score was able to predict the risk of advanced tumor stage of iCCA patients and the odds of this outcome were highest in the score group of 5-8. Besides, lymph node metastasis and advanced tumor stage were observed in high-score groups such as 2-4 and 5-8 ($p$=0.03 and $p$=0.008 respectively) (Table III). Additionally, this scoring system could discriminate the death rates per 100 person-years across score categories. These findings indicate that our AACC scoring system can effectively stratify iCCA patients including low risk patients having no apparent chronic diseases based on simple risk assessments. Our study provides important results because the indicators used in the scoring system have potential value for prediction risk of subclinical diseases and prognosis. The two components of the score (Cys C and eGFRcr) might be stronger predictors of survival than the other two, because Cys C and eGFRcr may integrate many processes that contribute to poor prognosis of iCCA patients. For example, Cys C may represent many organ dysfunctions, such as cognitive impairment, inflammation, and stroke; eGFRcr may reflect kidney dysfunction and treatment complications. Although AST and ALP levels of iCCA patients at the time of diagnosis were often extremely high, they seem to be weaker predictors because they are confounded by medical treatments for myocardial infarction, obstructive jaundice *etc*., whereas only few preoperative treatments affect Cys C and eGFRcr levels. Both AST and ALP may indicate early detection of comorbid conditions and can impact risk for poor survival; though it would be difficult to know if high values were due to the presence of asymptomatic comorbid diseases or due to cancer-treatment complications.

Very recently our team found that CCDC-25 protein is over-expressed in CCA tissues and elevated serum CCDC-25 level can be a unique bio-diagnostic marker for CCA (44, 45). Since higher serum CCDC-25 level is associated with longer survival of CCA patients (45), integration of CCDC-25 in our AACC prognostic scoring system reported here should be considered in future for further improvement of the system.

The limitation of this study is the lack of detailed information regarding the tumors, such as tumor size, tumor boundary type, residual tumor classification and history of clinically recognized diseases, which have an impact on the prognosis of iCCA patients. Also, since the parameters used for scoring are related to general health conditions or organ related subclinical diseases, the applicability of this AACC score to predict the prognosis of other malignancies should be examined.

In summary, the AACC score provides a useful tool to identify iCCA patients with low risk prognosis. In addition, the characteristics of easy-to-use cut-points and reasonable values for prognosis risk suggest that our new score may be useful in clinical research such as risk assessment, cancer care management and prediction of asymptomatic subclinical disease among cancer patients and in iCCA. We also hope that pathological information together with our prognostic score may help the physician in the decision for treatment regimens. To the best of our knowledge, this is the first report on a prognostic scoring system using organ related biochemical parameters at the time of diagnosis of iCCA to predict survival and stratify prognostic risk of iCCA patients independent of pathological features.

## Conclusion

In conclusion, the AACC score was identified as an independent prognostic score for prediction of overall survival and stratification of risk of outcomes in iCCA patients at the time of diagnosis. However, whether our proposed scoring system can perform well when evaluating the survival benefits of iCCA patients throughout treatment needs external validation with prospective studies or clinical evaluation studies.

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## Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

Conceptualization, M.W., P.M., T.P. and S.P.; methodology, H.H.M.-O. and S.P.; formal analysis, H.H.M.O. and S.P.; investigation, H.H.M.O.; T.P. and S.P.; data curation, H.H.M.O. and S.P.; writing-original draft preparation, H.H.M.O.; writing—review and editing, T.P., T.M.A. and S.P.; funding acquisition, S.P. All Authors have read and agreed to the published version of the manuscript.

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