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Original Article

Clinicopathological characteristics and prognostic outcomes of resectable hepatoid adenocarcinoma of the stomach: insights from a multicenter case-control study

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Abstract: Hepatoid adenocarcinoma of the stomach (HAS) is a rare subtype of gastric cancer (GC). This multicenter case-control study aimed to elucidate the clinicopathological features and prognosis of patients with resectable HAS. This retrospective study included 1387 GC patients treated at Ningbo No. 2 Hospital between January 2016 and December 2023, among whom 23 were HAS cases and incorporated 61 HAS patients from three external centers. Prognostic factors were analyzed using the Cox proportional hazards model. Propensity score matching (PSM) at a ratio of 4:1 and Kaplan-Meier survival curves were employed for analysis. The prevalence of HAS in this cohort was 1.1%. Among the 84 HAS patients with a median follow-up of 28 months, 47.6% had serum alpha-fetoprotein (AFP) levels exceeding 20 ng/mL. During the follow-up period, 44.0% of patients experienced relapses, predominantly through hepatic metastasis (62.2%). Univariate and multivariate analyses identified preoperative serum AFP levels between 200-500 ng/mL and TNM stages III/IV as independent prognostic factors for overall survival (OS). Elevated preoperative levels of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and TNM stages III/IV were independently associated with poorer disease-free survival (DFS). Conversely, open surgery and a Ki-67 proliferation index exceeding 50% were found to act as protective factors for both OS and DFS, with postoperative chemotherapy improving OS outcomes. After PSM adjustment, the analysis included 248 non-HAS patients and 62 HAS patients, revealing significantly better OS (P=0.043) and DFS (P=0.009) among non-HAS patients. Open radical surgery followed by adjuvant chemotherapy is recommended for the treatment of resectable HAS. Overall, patients with HAS exhibit a less favorable prognosis compared to those with non-HAS.

Keywords: Hepatoid adenocarcinoma of the stomach, open surgery, alpha-fetoprotein, clinicopathological features, risk factors, prognosis

Introduction

Gastric cancer (GC) is the fifth most prevalent cancer worldwide and the fourth leading cause of cancer-related mortality, with an overall five-year survival rate of approximately 30% [1]. Among its rare subtypes, hepatoid adenocarcinoma of the stomach (HAS) was first described in 1985 by Ishikura et al., who defined HAS as a primary GC exhibiting hepatoid differentiation and often associated with elevated alpha-fetoprotein (AFP) levels [2]. A subsequent 1993 study by Nagai et al. refined this definition, emphasizing the importance of histopathological features for diagnosis and asserting that AFP production, while common, is not mandatory for the identification of HAS [3].

To date, the reported incidence of HAS ranges from 0.17% to 1% of all GC cases [4-7]. This low prevalence significantly hampers the timely identification and accurate diagnosis of HAS,

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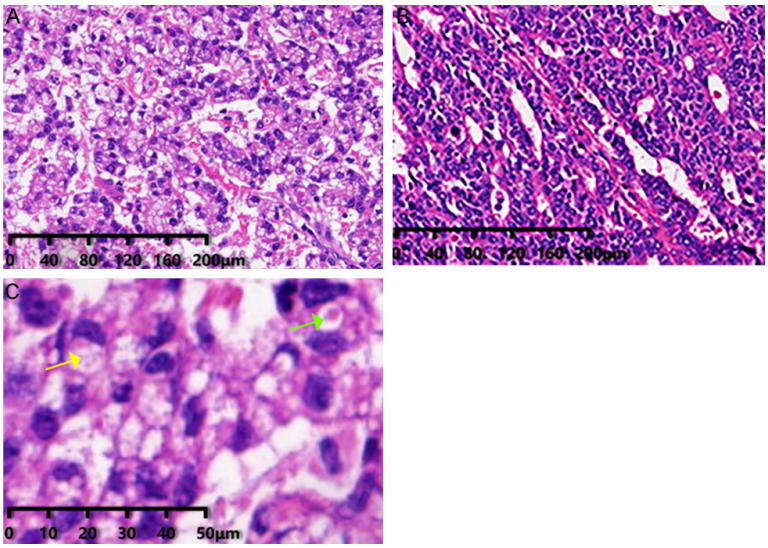

Figure 1. Pathological sections demonstrating differences between hepatoid adenocarcinoma of the stomach (HAS) and non-HAS patients (A: HAS group (100×), the tumor cells were arranged in trabecular shape with abundant interstitial blood sinuses; B: Non-HAS group (100×); C: HAS group (400×), the cytoplasm of the tumor cells is rich or transparent, and can be pseudo-granular (yellow arrow), and transparent globules (green arrow)).

presenting substantial challenges to clinical practice [8, 9]. HAS is characterized by its high malignancy, as well as a propensity for early metastasis and recurrence. Huang et al. demonstrated that the median time to recurrence after HAS was only 9 months, with a median follow-up time of 36.7 months. The 1-year and 3-year overall survival (OS) rates were reported as 83.3% and 61.2%, respectively [10]. Lin et al. also reported a 3-year OS rate of 58.1% in patients with HAS [11].

Despite several studies addressing the clinicopathological features, treatment modalities, and survival outcomes of HAS patients [4, 9, 11-13], these investigations are often constrained by small sample sizes or potential institutional biases. Furthermore, the low resectability rate of HAS further limits the scope of comprehensive analyses. This study aggregates data from multiple healthcare institutions to provide a detailed review and analysis of the clinicopathological characteristics and prognosis of patients with resectable HAS.

Methods

Patients

This study utilized a multicenter design. A total of 1387 patients diagnosed with GC, who underwent surgical treatment between January 2016 and December 2023 were retrospectively enrolled from Ningbo No. 2 Hospital. The inclusion criteria for these patients were: 1) histologically confirmed primary gastric adenocarcinoma; 2) absence of prior gastrectomy or other malignant tumors; 3) availability of complete clinical and pathological data. Additionally, to further expand the HAS cohort, 61 patients with histologically confirmed HAS from the First Affiliated Hospital of Ningbo University, Ningbo Medical Center Li Huili Hospital, and Sir Run Run Shaw Hospital affiliated to the Medical College of Zhejiang University were included during the same period. All participants underwent D2 lymph node dissection.

Clinical data were retrospectively collected analyzed. Specimen collection and use received ethical approval from Ningbo No. 2 Hospital (Ethical approval number: PJ-NBEY-KY-2019-153-01). The study adhered to ethical standards across all participating institutions, and all patients provided informed consent, documented through signed forms.

Histological examination

Pathology serves as the definitive diagnostic criterion for HAS, based on histological features reminiscent of hepatocellular carcinoma. Tumor specimens underwent hematoxylin and eosin staining and were examined under a light microscope. Interpretation was conducted through a double-blind process by three pathologists. GCs exhibiting hepatoid differentiation - characterized by trabecular, pseudo-granular patterns, and hyaline globules - were diagnosed as HAS, irrespective of the extent of hepatoid differentiation observed. Conversely, gastric adenocarcinomas without hepatoid differentiation in specimen sections were classified as non-HAS. **Figure 1A** and **1B** illustrate the pathological sections of the HAS and non-HAS groups, respectively. **Figure 1A** showed that the tumor cells of HAS were arranged trabecularis with abundant interstitial blood sinuses

(100×). **Figure 1C** showed that the cytoplasm of HAS cells was rich or clear and could be pseudo-granular patterns (yellow arrow) or transparent globules (green arrow) (400×).

Immunohistochemistry (IHC) staining and evaluation

Given that postoperative IHC staining frequently indicates AFP positivity in patients with HAS [14, 15], AFP detection via IHC was employed to enhance diagnostic precision in this study. IHC staining followed a standardized protocol, with 4-μm-thick sections cut from freshly embedded paraffin blocks. Non-specific binding was blocked using a dedicated blocking reagent (Dako, Glostrup, Denmark). The sections were then incubated with a polyclonal antibody against AFP (ab169552, Abcam) at a 100-fold dilution (150 μl per section) for 120 minutes.

Three pathologists independently evaluated the AFP staining, quantifying both the percentage of positive cells [0 - absent, 1 - (1%-50%), 2 - (51%-100%)] and staining intensity [0 - absent, 1 - mild, 2 - moderate, 3 - strong]. This dual-parameter assessment facilitated a comprehensive evaluation of AFP expression.

Collection of clinical variables

Clinical data regarding patient characteristics were collected, including age, gender, body mass index (BMI), comorbidities, and preoperative serum levels of AFP, carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9). Additionally, clinical TNM (cTNM) stage, type of surgery, and preoperative and postoperative treatments were recorded. Postoperative pathological evaluations encompassed tumor location, size, perineural invasion, lymphovascular invasion, pathological TNM (pTNM) stage, number of lymph nodes dissected, and expression levels of human epidermal growth factor receptor 2 (HER-2), mismatch repair proteins (MMR), and the marker of proliferation Ki-67 (MKI-67). Clinical and pathological staging were performed according to the American Joint Committee on Cancer (AJCC) TNM Staging Classification for Carcinoma of the Stomach (Eighth Edition, 2016).

Follow-up

Patients were systematically monitored for recurrence and metastasis through regular outpatient visits, telephone follow-ups, physical examinations, laboratory tests, imaging assessments, and gastroscopy. Follow-up intervals were set every 3-6 months for the first two years post-surgery and annually thereafter, up to a minimum of five years or until the patient's death. Patients lost to follow-up were censored, and the date of last known contact was recorded. OS was defined as the duration from the date of surgery to death from any cause. Disease-free survival (DFS) was defined as the interval from surgery to the occurrence of local recurrence, distant recurrence, or death from any cause. All patients included in this study were followed until April 2024.

Statistical analysis

Data were analyzed using SPSS software (version 25.0, IBM Corp.). Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data and as median with interquartile range (IQR) for non-normally distributed data. Categorical variables were expressed as frequencies and percentages. Continuous variables were compared using the independent samples t-test or the Wilcoxon rank-sum test, as appropriate, while categorical data were analyzed using the Pearson chi-square test or Fisher's exact test, depending on expected frequencies.

The Cox proportional-hazards model facilitated both univariate and multivariate analyses of OS and DFS, presenting results as hazard ratios (HRs) with 95% confidence intervals (CIs). To mitigate selection bias, propensity score matching (PSM) was implemented at a 4:1 ratio between non-HAS and HAS groups using a caliper width of 0.02. The variables included in the PSM were age, gender, BMI, comorbidities, type of gastrectomy, tumor location, tumor size, perineural and lymphovascular invasion, pathological T (pT) and N (pN) categories, preoperative serum levels of CEA, number of lymph nodes dissected, and postoperative chemotherapy. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. All statistical tests were two-tailed, with a significance level set at $P<0.05$.

Results

Study population

Between January 2016 and December 2023, a total of 2,031 patients underwent gastrectomy

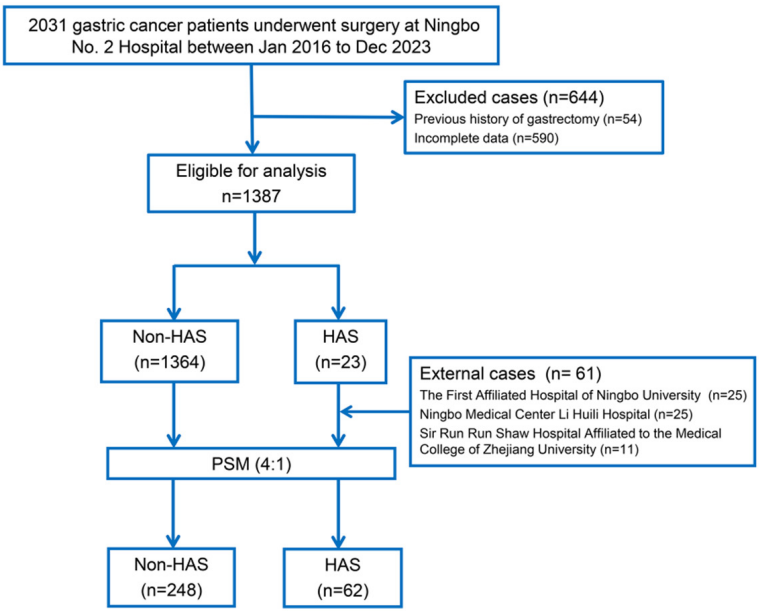

Figure 2. Flowchart detailing the selection process for the study participants.

at Ningbo No. 2 Hospital, as depicted in **Figure 2**. Of these, 644 patients were excluded based on the inclusion criteria: 54 had a previous gastrectomy and 590 had incomplete data. Ultimately, 1,387 patients were deemed eligible for analysis. Within this cohort, 23 patients were identified with HAS through histological examination and IHC. Additionally, 61 patients with HAS confirmed by histological examination and IHC were included from three other institutions: the First Affiliated Hospital of Ningbo University (n=25), Ningbo Medical Center Li Huili Hospital (n=25), and Sir Run Run Shaw Hospital affiliated with the Medical College of Zhejiang University (n=11), bringing the total HAS cohort to 84 patients. After excluding 14 patients who did not undergo radical surgery, 310 patients were selected through 4:1 nearest-neighbor matching, comprising 248 non-HAS and 62 HAS patients.

Clinicopathological characteristics of patients with HAS

The baseline clinical characteristics of the 84 patients with HAS are summarized in **Table 1**. The average age was 69.3 years, with a predominance of males (85.7%) over females (14.3%). A majority (65.5%) had comorbidities such as hypertension, diabetes mellitus type 2, and chronic obstructive pulmonary disease. Regarding preoperative serum AFP levels, 50.0% of patients had levels below 20 ng/mL, 20.2% ranged from 20 to 200 ng/mL, 6.0% from 200 to 500 ng/mL, and 21.4% exceeded 500 ng/mL. Elevated preoperative serum CEA (>5 ng/mL) was observed in 36.9% of patients, and 15.5% had elevated preoperative serum CA19-9 (>37 U/mL). Clinically, 6.0% were in stage I, 13.1% in stage II, 51.2% in stage III, and 10.7% in stage IV, with clinical staging unknown for 19.0%. Surgical approaches were nearly evenly split between laparoscopic (56.0%) and open surgeries (44.0%). Distal gastrectomy was the most frequent surgical type (59.5%), followed by total gastrectomy (38.1%), and proximal gastrectomy (2.4%). Preoperative chemotherapy, predominated by SOX or FOLFOX regimens, was administered in 7.1% of patients, with programmed cell death protein 1 (PD-1) immunotherapy included for three patients. Postoperative chemotherapy was administered to 75.0% of patients, primarily using the SOX regimen (42.9%) and Tegafur monotherapy (11.9%), and, in some advanced cases, combined with targeted therapy against HER-2 or PD-1 immunotherapy.

Table 2 presents detailed pathological characteristics of the 84 patients with HAS. The tumors predominantly affected the lower third of the stomach (56.0%). Peripheral tissue invasion was significant, with perineural invasion in 42.9% of cases and lymphovascular invasion in 73.8%. The median number of lymph nodes dissected was 25. Postoperative pathological staging indicated 11.9% of patients in stage I, 19.0% in stage II, 52.4% in stage III, and 16.7% in stage IV.

Prognosis of patients with HAS

Table 3 outlines the prognosis for the 84 patients with HAS. The median follow-up period for the cohort was 28 months. During this time, 44.0% of the patients experienced a recurrence post-treatment. The liver was the most common site for initial metastasis, accounting

Table 1. Baseline clinical characteristics of patients with HAS

	N=84
Age (years) (mean ± SD)	69.3±7.8
Gender	
Male	72 (85.7%)
Female	12 (14.3%)
BMI (kg/m^2) (mean ± SD)	23.1±6.0
Comorbidity	
Absence	29 (34.5%)
Presence	55 (65.5%)
AFP (ng/mL)	
0-20	42 (50.0%)
20-200	17 (20.2%)
200-500	5 (6.0%)
>500	18 (21.4%)
NA	2 (2.4%)
CEA (ng/mL)	
≤5	51 (60.7%)
>5	31 (36.9%)
NA	2 (2.4%)
CA19-9 (U/mL)	
≤37	56 (66.6%)
>37	13 (15.5%)
NA	15 (17.9%)
cTNM	
I	5 (6.0%)
II	11 (13.1%)
III	43 (51.2%)
IV	9 (10.7%)
NA	16 (19.0%)
Surgery	
Laparoscope	47 (56.0%)
Open surgery	37 (44.0%)
Eradication	
No	14 (16.7%)
Yes	70 (83.3%)
Surgical combined organ resection	
Absence	67 (79.8%)
Presence	17 (20.2%)
Type of gastrectomy	
Distal subtotal	50 (59.5%)
Total	32 (38.1%)
Proximal subtotal	2 (2.4%)
Preoperative chemotherapy	
Absence	78 (92.9%)
Presence	6 (7.1%)
Postoperative chemotherapy	
Absence	21 (25.0%)
Presence	63 (75.0%)

HAS, hepatoid adenocarcinoma of the stomach; SD, standard deviation; BMI, body mass index; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; NA, not available.

for 62.2% of all first relapse cases. By the end of the observation period, 66.7% of patients were still alive; among these, 10.7% lived with persistent tumors. All deceased patients succumbed to GC.

Factors influencing the prognosis of HAS patients

Supplementary Table 1 presents the findings from a Cox univariate analysis of OS in the 84 patients with HAS, identifying seven potential prognostic factors: preoperative serum AFP, preoperative serum CA19-9, cTNM stage, surgical approach, pTNM stage, MKI-67, and postoperative chemotherapy. Subsequent multivariate analysis indicated that preoperative serum AFP levels ranging from 200-500 ng/mL and cTNM/pTNM stages III/IV were independent risk factors for OS. Conversely, open surgery, MKI-67 >50%, and postoperative chemotherapy emerged as independent protective factors for OS, as detailed in **Table 4**.

Furthermore, Supplementary Table 2 provides results from a Cox univariate analysis for DFS among the same cohort, highlighting eight factors potentially associated with DFS: preoperative serum AFP, preoperative serum CEA, preoperative serum CA19-9, cTNM stage, mode of surgery, perineural invasion, pTNM stage, and MKI-67. Multivariate analysis revealed that elevated preoperative serum CEA and CA19-9, along with cTNM stage III/IV, were independent risk factors for DFS. Open surgery and MKI-67 >50% were independently associated with improved DFS outcomes, as illustrated in **Table 5**.

Survival analysis of preoperative serum AFP and postoperative chemotherapy

The 84 patients with HAS were stratified based on their preoperative serum AFP levels into four groups: 0-20 ng/mL, 20-200 ng/mL, 200-500 ng/mL, and >500 ng/mL. Kaplan-Meier curves for OS and DFS were constructed for these subgroups, as depicted in **Figure 3**. The analysis indicated that while variations in serum AFP levels did not significantly impact OS (P=0.096), they were significantly associated with DFS (P=0.037).

Additionally, based on whether patients received postoperative chemotherapy, Kaplan-

Table 2. Pathological characteristics of HAS patients

	N=84
Tumor location	
Upper third	21 (25.0%)
Middle third	16 (19.0%)
Lower third	47 (56.0%)
Tumor size (cm)	
≤5	52 (61.9%)
>5	32 (38.1%)
Perineural invasion	
Absence	48 (57.1%)
Presence	36 (42.9%)
Lymphovascular invasion	
Absence	22 (26.2%)
Presence	62 (73.8%)
pT category	
T1	8 (9.5%)
T2	14 (16.7%)
T3	21 (25.0%)
T4a	32 (38.1%)
T4b	9 (10.7%)
pN category	
N0	18 (21.4%)
N1	20 (23.8%)
N2	23 (27.4%)
N3a	21 (25.0%)
N3b	2 (2.4%)
Number of lymph node dissection (median, IQR)	25 (20-35)
HER-2	
Negative	52 (61.9%)
Positive	5 (5.9%)
2+	12 (14.3%)
NA	15 (17.9%)
MMR	
pMMR	59 (70.2%)
dMMR	2 (2.4%)
NA	23 (27.4%)
MKI-67	
<50	9 (10.7%)
51-70	24 (28.6%)
71-90	37 (44.0%)
NA	14 (16.7%)

HAS, hepatoid adenocarcinoma of the stomach; IQR, interquartile range; HER-2, human epidermalgrowth factor receptor 2; NA, not available; MMR, mismatch repair; pMMR, mismatch repair-proficient; dMMR, mismatch repair-deficient; MKI-67, marker of proliferation ki-67.

Meier survival curves for OS and DFS were plotted for the cohort, as shown in **Figure 4**. Patients who underwent postoperative chemotherapy demonstrated a median OS of 64 months and a median DFS of 58 months. At the 1-year mark, OS rates were 50.8% for patients not receiving postoperative chemotherapy and 89.9% for those who did, indicating a statistically significant improvement in OS for patients receiving postoperative chemotherapy (P=0.019). Conversely, the 1-year DFS rates were 51.9% for patients without postoperative chemotherapy and 85.0% for those with, although this difference did not reach statistical significance (P=0.234).

PSM analysis

To ensure the validity of comparisons between resectable HAS and non-HAS patients, stage IV cases were explicitly excluded from both cohorts during PSM. By 4:1 PSM, there were no significant differences between the non-HAS group (248 patients) and the HAS group (62 patients) in terms of age, gender, BMI, type of gastrectomy, tumor location, tumor size, perineural invasion, lymphovascular invasion, pT category, pN category, preoperative serum CEA, number of lymph node dissection and postoperative chemotherapy, as detailed in **Table 6**.

The median follow-up time for the overall population after PSM was 41 months. For the non-HAS group, the median follow-up time was 44 months, and the median OS and DFS data for the non-HAS group were immature. The median follow-up time for the HAS group was 29 months, with median OS and DFS values of 64 months and 58 months, respectively. Regarding postoperative chemotherapy, 77.8% of patients in the non-HAS group and 75.8% in the HAS group received treatment, with the SOX regimen as the primary choice. In terms of postoperative pathological stage, 12.5% of patients in the non-HAS group were in stage I, and 87.5% were in stage II/III. In the HAS group, 12.9% were in stage I, and 87.1% were in stage II/III.

Table 3. Prognosis of patients with HAS

	N=84
Postoperative recurrence	
Absence	47 (56.0%)
Presence	37 (44.0%)
First recurrence location	
Liver	23 (62.2%)
Others	14 (37.8%)
Number of patients surviving	56 (66.7%)

HAS, hepatoid adenocarcinoma of the stomach.

Table 4. Multivariate analysis to determine the risk of OS in patients with HAS

	HR	95% CI	P
AFP (ng/mL)			
0-20	1		
20-200	4.31	0.99-18.87	0.052
200-500	7.70	1.30-45.62	0.024
>500	0.51	0.08-3.21	0.477
cTNM			
I/II	1		
III/IV	48.86	4.58-520.80	0.001
Surgery			
Laparoscope	1		
Open surgery	0.23	0.07-0.77	0.017
MKI-67			
<50	1		
51-70	0.24	0.05-1.15	0.075
71-90	0.11	0.03-0.49	0.004
pTNM			
I	1		
II	3.90	0.19-82.24	0.382
III	17.48	1.49-205.86	0.023
IV	23.15	1.41-378.81	0.028
Postoperative chemotherapy			
Absence	1		
Presence	0.14	0.03-0.54	0.005

OS, overall survival; HAS, hepatoid adenocarcinoma of the stomach; HR, hazard ratios; CI, confidence interval; AFP, alpha-fetoprotein; MKI-67, marker of proliferation ki-67.

Figure 5 demonstrates the Kaplan-Meier curves of OS and DFS between the non-HAS group and the HAS group after PSM treatment. In our study, OS was better in the non-HAS group than in the HAS group, and the difference was statistically significant (P=0.043). The 1-year, 3-year, and 5-year OS in the non-HAS group was 93.9%, 76.2%, and 67.9%, respectively. Whereas in the HAS group, the 1-year, 3-year, and 5-year OS was 79.2%, 67.7%, and 67.7%, respectively. In addition, the DFS of the non-HAS group was also better than that of the HAS group, and the difference was statistically significant (P=0.009). The 1-year, 3-year, and 5-year DFS of the non-HAS group was 85.7%, 68.4%, and 65.2%, respectively. Whereas in the HAS group, the 1-year, 3-year, and 5-year DFS was 67.6%, 55.7%, and 37.1%, respectively.

Discussion

This study examined the clinicopathologic features and prognosis of patients with resectable HAS. We analyzed the prevalence of HAS among patients undergoing surgical treatment for GC, identifying a prevalence of 1.1%. This rate aligns with previously reported figures in the literature, which range from 0.17% to 1% [1]. Unlike common GC, where the incidence is twice as high in males compared to females, our findings show that male patients with HAS are six times more prevalent than female patients. HAS is recognized as an aggressive tumor type. Zeng et al. reported a 78.4% incidence of preoperative lymphovascular invasion in HAS patients, significantly higher than the 68.4% observed in patients with common GC [9]. Our results are comparable, with lymphovascular invasion present in 73.8% of HAS patients and perineural invasion in 42.6%. These findings corroborate those of Huang et al., who documented perineural invasion in 40.2% of HAS cases [16].

Regarding tumor markers, half of the HAS patients in our cohort exhibited preoperative serum AFP levels below 20 ng/mL, challenging the notion of an absolute association between elevated AFP and HAS. In contrast to findings by Huang et al., where 60.4% of HAS patients had serum AFP levels ≥20 ng/mL, a stark contrast to only 2.9% in common GC patients [16], our study observed a similar proportion of 47.6%. While AFP levels are not solely diagnostic for HAS, both our research and that of Huang et al. indicate a

Table 5. Multivariate analysis to determine the risk of DFS in patients with HAS

	HR	95% CI	P
CEA (ng/mL)			
≤5	1		
>5	4.11	1.33-12.71	0.014
CA19-9 (U/mL)			
≤37	1		
>37	4.67	1.56-14.02	0.006
cTNM			
I/II	1		
III/IV	6.30	1.18-33.78	0.032
Surgery			
Laparoscope	1		
Open surgery	0.33	0.13-0.86	0.023
MKI-67			
<50	1		
51-70	0.26	0.06-1.14	0.073
71-90	0.24	0.06-0.95	0.043

DFS, disease-free survival; HAS, hepatoid adenocarcinoma of the stomach; HR, hazard ratios; CI, confidence interval; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; MKI-67, marker of proliferation ki-67.

prevalent trend of elevated AFP among HAS patients. The higher proportion of patients with AFP levels >20 ng/mL observed in the study by Huang et al. compared with our cohort may be due to the relatively high proportion of patients with pathologic stage III in their study population (66.1% versus 50.4%) and the possible inter-regional differences in laboratory testing methods. Prior studies have shown that common GC patients exhibit preoperative CEA levels >5 ng/L and CA19-9 levels >37 U/mL in 4.3% to 17% and about 7% of cases, respectively [17, 18]. In contrast, our HAS cohort demonstrated substantially higher rates, with 36.9% showing elevated CEA and 15.5% elevated CA19-9. The insidious onset and high rate of HAS diagnosis at stage III (52.4%) underscore the necessity to enhance diagnostic accuracy and implement timely and effective treatment strategies.

It is well established that HAS is associated with a poor prognosis. In our study, the recurrence rate was 44.0%, with the liver being the predominant site for metastasis, accounting for 62.2% of cases. The hematogenous route is widely recognized as the principal pathway for liver metastases in GC, primarily due to cancer cell dissemination via the portal venous system. This anatomical arrangement positions the liver as the initial filter for circulating neoplastic cells [19]. Additionally, lymphatic-venous anastomoses, resulting from lymphatic vessel obstruction and subsequent lymphatic reflux, also play a significant role in the metastatic spread to the liver and peritoneum [20].

Jiang et al. reported a notably higher incidence of liver metastases in HAS patients compared to non-HAS individuals (41.1% vs. 17.8%), suggesting that this discrepancy might be linked to specific alterations in cell cycle pathway genes and signaling pathways related to stem cell pluripotency. However, these associations were not significant in patients with non-HAS [21]. Furthermore, they proposed that the development of polyclonal structures in HAS could be intricately linked to its propensity for liver metastasis.

We hypothesize that tumor-homing effects play a crucial role in directing liver metastasis in HAS. Tumor homing refers to the ability of circulating tumor cells to return and thrive in the organ of their origin after vascular dissemination, often without needing further adaptation to the local microenvironment [22]. Although HAS originates in the stomach, its solid growth pattern - indicative of high invasive potential - and pathological similarities to hepatocellular carcinoma may predispose HAS cells to thrive particularly well in the liver's nutrient-rich environment [23]. This "fertile ground" offers an optimal setting for the survival and proliferation of HAS cells, enhancing their colonization efficiency and impacting the clinical outcomes of HAS patients.

In our study, AFP, CEA, and CA19-9 emerged as significant prognostic markers for patients with HAS. Multivariate analysis identified elevated serum AFP as an independent risk factor for OS. Although Kaplan-Meier survival analysis indicated a significant correlation between varying levels of serum AFP and DFS, its impact on OS was not statistically significant, potentially due to limited sample size, follow-up duration, or other confounding factors. This finding is consistent with research by Li et al. and Yang et al., who also recognized higher serum AFP

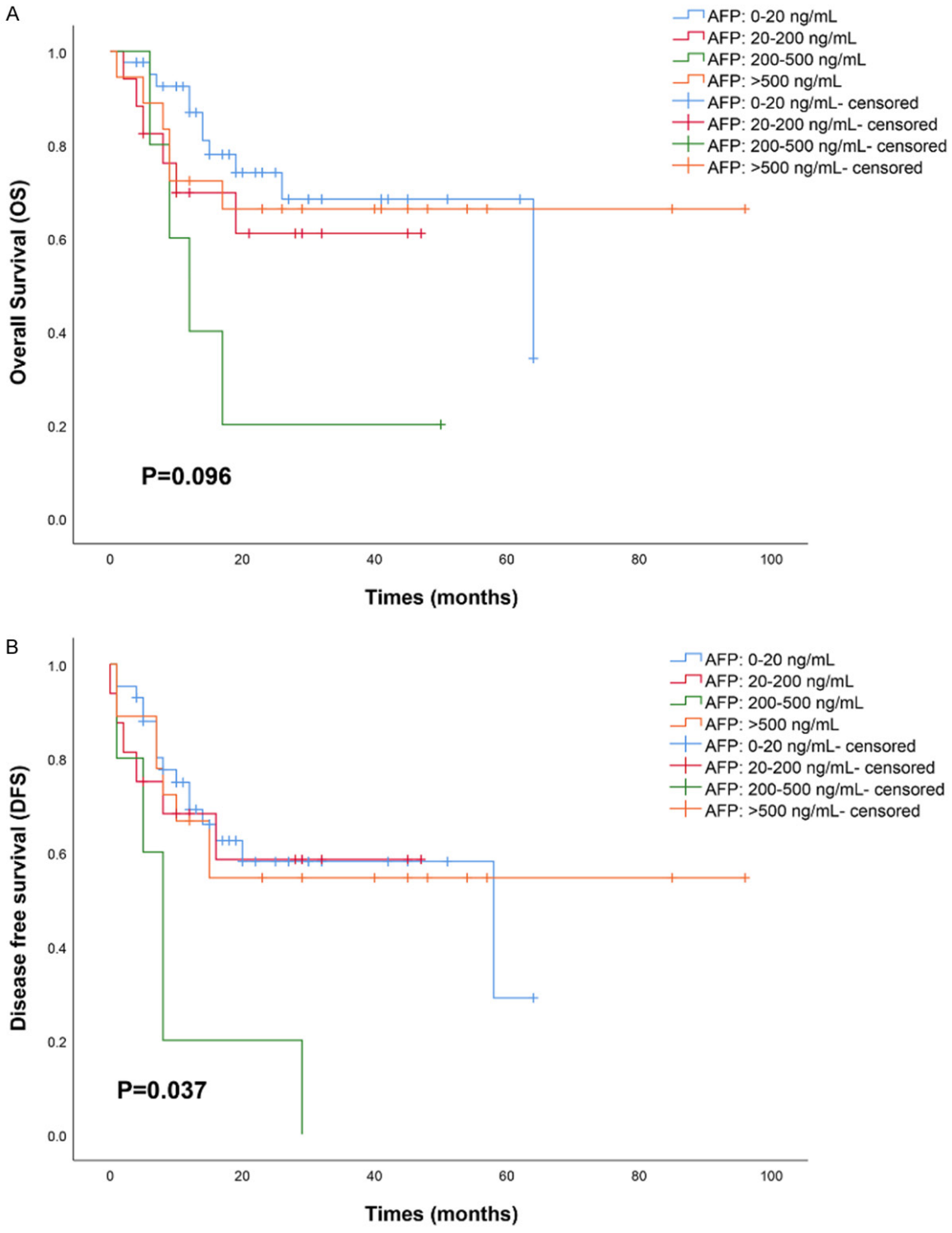

Figure 3. Kaplan-Meier survival curves illustrating the impact of different preoperative serum alpha-fetoprotein (AFP) levels on 84 HAS patients (A: Overall survival (OS), B: Disease-free survival (DFS)).

levels as an independent risk factor for OS in HAS patients [15, 24].

The precise mechanisms by which AFP influences HAS prognosis remain partially unde-

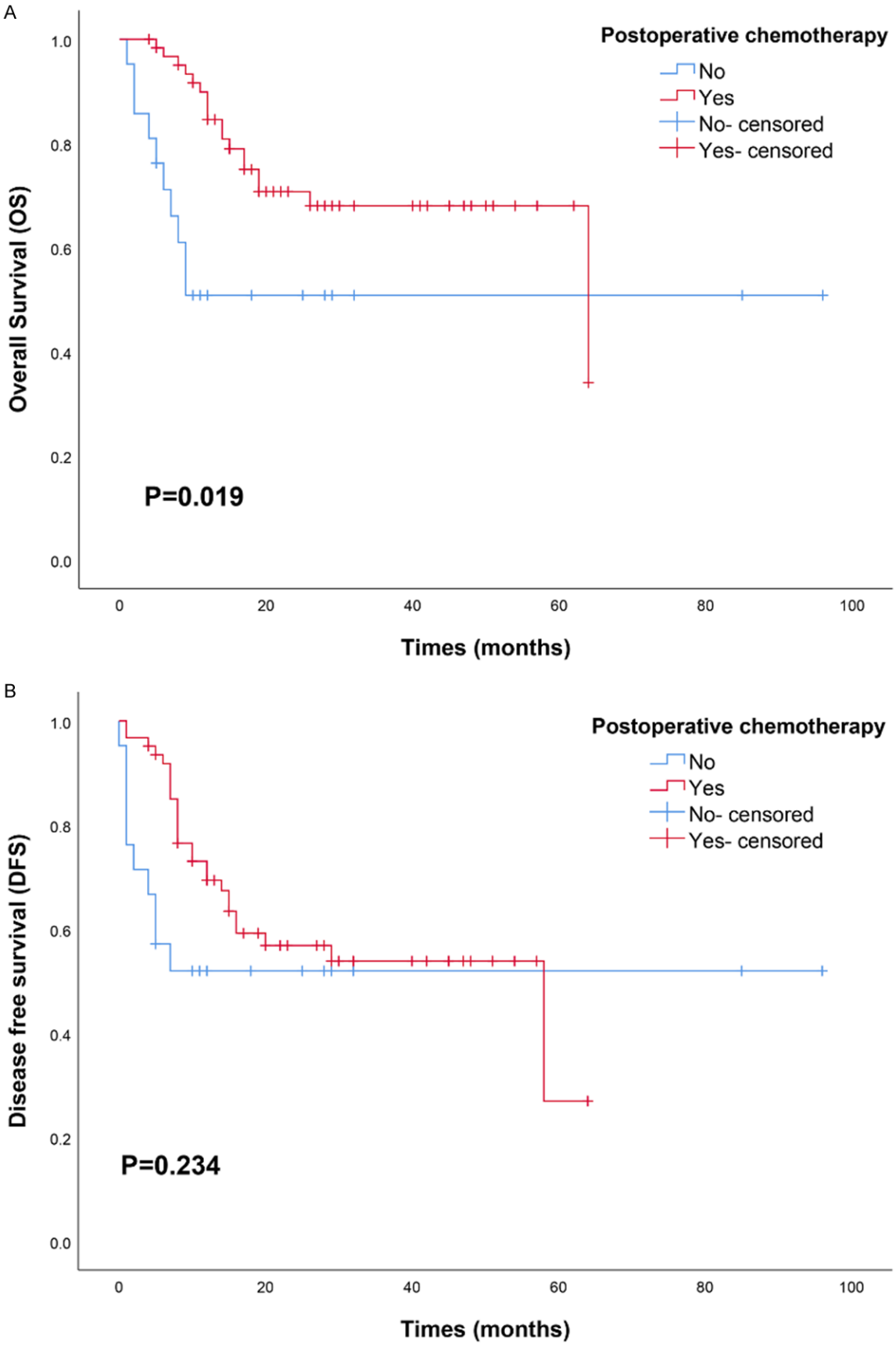

Figure 4. Kaplan-Meier survival curves showing the effect of postoperative chemotherapy on 84 HAS patients (A: OS, B: DFS).

Table 6. Baseline characteristics of HAS patients and non-HAS patients after propensity score matching (4:1)

Clinicopathological feature	Non-HAS patients N=248	HAS patients N=62	P
Age (years)			
≤60	11 (4.4%)	4 (6.5%)	
>60	237 (95.6%)	58 (93.5%)	0.741
Gender			
Male	221 (89.1%)	55 (88.7%)	
Female	27 (10.9%)	7 (11.3%)	0.928
BMI (kg/m^2)			
<18.5	26 (10.5%)	6 (9.7%)	
18.5-23.9	179 (72.2%)	45 (72.6%)	
≥24	43 (17.3%)	11 (17.7%)	0.873
Comorbidity			
Absence	34 (13.7%)	16 (25.8%)	
Presence	214 (86.3%)	46 (74.2%)	0.021
Type of gastrectomy			
Distal subtotal	154 (62.1%)	37 (59.7%)	
Total	93 (37.5%)	24 (38.7%)	
Proximal subtotal	1 (0.4%)	1 (1.6%)	0.477
Tumor location			
Upper third	51 (20.6%)	16 (25.8%)	
Middle third	40 (16.1%)	12 (19.4%)	
Lower third	151 (60.9%)	34 (54.8%)	
Two-thirds or more	6 (2.4%)	0 (0)	0.499
Tumor size (cm)			
≤5	162 (65.3%)	39 (62.9%)	
>5	86 (34.7%)	23 (37.1%)	0.721
Perineural invasion			
Absence	151 (60.9%)	38 (61.3%)	
Presence	97 (39.1%)	24 (38.7%)	0.954
Lymphovascular invasion			
Absence	74 (29.8%)	17 (27.4%)	
Presence	174 (70.2%)	45 (72.6%)	0.708
pT category			
T1	31 (12.5%)	6 (9.7%)	
T2	44 (17.7%)	10 (16.1%)	
T3	39 (15.7%)	16 (25.8%)	
T4a	118 (47.6%)	25 (40.3%)	
T4b	16 (6.5%)	5 (8.1%)	0.962
pN category			
N0	92 (37.1%)	14 (22.6%)	
N1	43 (17.3%)	16 (25.8%)	
N2	46 (18.5%)	17 (27.4%)	
N3a	53 (21.4%)	14 (22.6%)	
N3b	14 (5.7%)	1 (1.6%)	0.322
CEA (ng/mL)			
≤5	159 (64.1%)	36 (58.1%)	
>5	89 (35.9%)	26 (41.9%)	0.378
Number of lymph node dissection (median, IQR)	26 (20-32)	25 (21-35)	0.499
Postoperative chemotherapy			
Absence	65 (26.2%)	15 (24.2%)	
Presence	183 (73.8%)	47 (75.8%)	0.746

HAS, hepatoid adenocarcinoma of the stomach; BMI, body mass index; CEA, carcinoembryonic antigen; IQR, interquartile range.

fined, yet several studies have offered insightful hypotheses. Munson et al. proposed that tumor-derived AFP exerts a broad immunosuppressive effect on various immune cells, including natural killer (NK) cells, natural killer T (NKT) cells, and dendritic cells (DCs). They suggested that AFP not only indirectly suppresses NK cell function through the induction of regulatory T cells but may also directly inhibit or destroy NK cells. For DCs, AFP can restrict interleukin-12 production and disrupt mitochondrial homeostasis and metabolic balance, consequently impairing their antigen-presenting capabilities [25]. Additionally, Li et al. postulated that AFP may activate the cAMP-PKA signaling pathway by binding to its receptor, thereby altering the expression of the K-Ras p21 signaling pathway and promoting tumor cell proliferation [22].

Furthermore, our findings indicated that elevated serum levels of CEA and CA19-9 are independent risk factors for DFS in HAS patients. CEA, an acidic glycoprotein with embryonic antigenic properties, enhances tumor invasiveness through its adhesion, immunosuppressive, and protease-inhibitory functions [26]. CA19-9, an oligosaccharide tumor-associated antigen, reflects the proliferative, metastatic, and invasive capabilities of GC. Therefore, the elevated levels of CEA and CA19-9 not only aid in diagnosing HAS but also serve as crucial indicators for assessing recurrence risk, emphasizing their importance in clinical practice.

The heightened incidence of lymphovascular and perineural invasion, alongside elevated serum levels of tumor markers such as AFP, CEA, and CA19-9, underscore the aggressive nature of HAS. It is widely recognized that HAS patients generally have a poorer prognosis than those with non-HAS [9, 27]. In contrast, a study by Zhou et al. suggested no significant difference in prognosis between HAS and non-HAS patients following radical surgery complemented by adjuvant chemotherapy [13]. To investigate this further, we employed a 4:1 PSM between non-HAS and HAS patients, all of whom underwent radical surgery. Subsequent survival analysis revealed that the 1-, 3-, and 5-year OS rates in the HAS group demonstrated marked improvements over previous findings by Liu et al. Nevertheless, our Kaplan-Meier analysis still indicated that, despite radical surgery and adjuvant therapy, both OS and DFS rates for HAS patients remained lower compared to those of non-HAS patients.

Regarding prognostic staging, cTNM staging proved valuable for predicting both OS and DFS, aligning with findings from Zhou et al. [28]. Conversely, pTNM staging displayed significance only for OS, a divergence from Yang et al., who reported no prognostic relevance of pTNM staging for OS. This inconsistency may be attributed to the limited sample size and follow-up duration, or more fundamentally, to potential inadequacies of the current TNM staging system for HAS. In response, Huang et al. advocated for a modified pTNM staging system tailored for HAS based on multicenter data, which they found to offer enhanced predictive accuracy [16].

Our study highlights a significant finding: compared to laparoscopic surgery, open surgery positively impacts both OS and DFS in patients with HAS. While laparoscopic approaches have been validated for safety and efficacy in early-stage GC patients undergoing gastrectomy, the suitability of laparoscopy for advanced GC remains contentious and is a subject of ongoing debate. Recent research by Morino et al. supports this view, revealing that open surgery significantly enhances 5-year recurrence-free survival in patients with advanced GC compared to those undergoing laparoscopic procedures. They advocate for a cautious approach to expanding laparoscopic indications in such cases [29].

The detection of HAS often occurs at advanced stages, frequently accompanied by distant metastases, which complicates the surgical approach. This is especially true in laparoscopic procedures, where the intricacy of D2 lymph node dissection might compromise the thoroughness required for optimal outcomes. In aggressive cancers like HAS, comprehensive lymph node dissection is paramount for prognosis. Open surgery, offering a better surgical field and more adaptable operational space, potentially facilitates a more extensive and effective lymph node dissection compared to laparoscopic techniques. Therefore, for patients with HAS, particularly those with suspected or confirmed advanced disease, open surgery should be strongly considered to enhance surgical outcomes and prognosis.

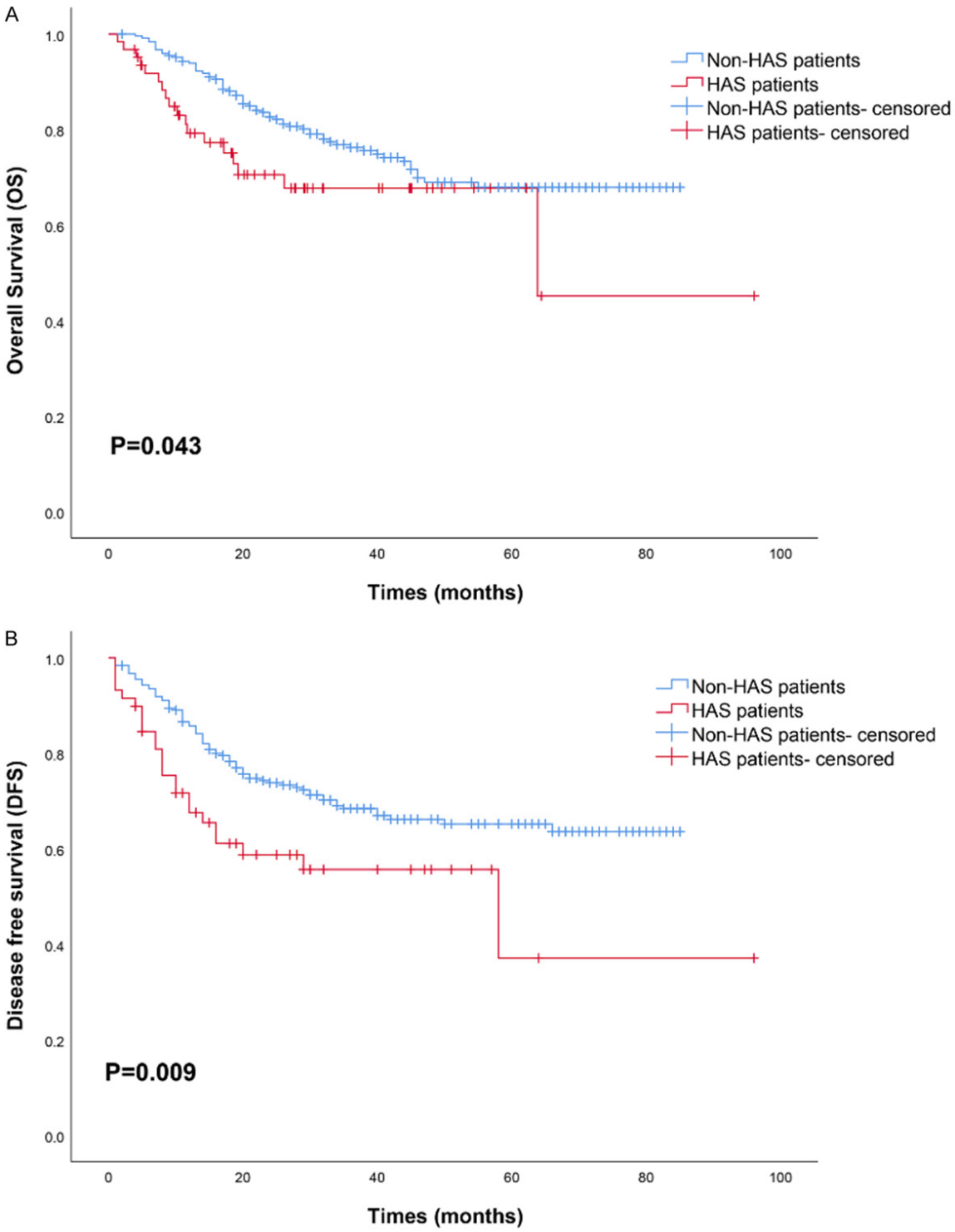

Figure 5. Kaplan-Meier survival curves comparing OS and DFS between HAS and non-HAS patients after propensity score matching (A: OS, B: DFS).

Our findings indicate that high levels of MKI-67, a cellular marker of proliferation detected from the G1 to the M phase of the cell cycle, serve as an independent protective factor for both OS and DFS in patients with HAS. Although MKI-67 is a well-established prognostic biomarker in

cancers such as breast cancer, lymphoma, and neuroendocrine tumors [30-32], its prognostic value in GC remains contentious. Some studies report no association between MKI-67 levels and GC prognosis [33, 34], while others suggest that high MKI-67 levels correlate with poor outcomes [35, 36]. Contrary to these findings, our study associates high MKI-67 expression with favorable prognoses in HAS, suggesting that MKI-67 may effectively predict chemotherapy response.

Considering the role of postoperative chemotherapy as a protective factor for OS in HAS, we propose that MKI-67's predictive capacity could be particularly relevant. Chemotherapy typically shows enhanced efficacy in rapidly proliferating and poorly differentiated tumors, such as small-cell lung cancer and acute lymphoblastic leukemia. The majority of our study participants underwent postoperative chemotherapy using the SOX regimen (Tegafur and Oxaliplatin), which inhibits DNA synthesis and forms disruptive DNA adducts. Thus, we hypothesize that cell cycle-blocking chemotherapeutic agents are especially beneficial in HAS cases characterized by high MKI-67 expression, leading to more effective tumor control.

Our study also explored the potential of combining chemotherapy with targeted therapies. In our study, the primary regimen employed was the SOX protocol, with additional inclusion of XELOX (Capecitabine and Oxaliplatin), monotherapy using Tegafur, and for patients with HER-2 positivity, a combined therapeutic approach of SOX along with targeted therapy was adopted. Among the HER-2 positive HAS patients, those treated with targeted therapies alongside chemotherapy showed mixed outcomes. While one patient relapsed within a year, another achieved an OS of five years. This observation underscores the potential benefits of integrating anti-HER-2 agents with chemotherapy in managing HER-2-positive HAS, potentially improving survival rates.

Despite its insights, this study faces several limitations. The rarity of HAS limits the sample size, despite incorporating data from multiple centers, which may affect the robustness of the results. Additionally, the relatively short follow-up period restricts our ability to fully assess long-term survival and disease progression in HAS patients.

Conclusion

HAS is characterized by its high aggressiveness and comparatively poor prognosis relative to the non-HAS. Our study identifies serum AFP levels ranging from 200-500 ng/mL and TNM stages III/IV (either clinical or pathological) as independent risk factors for OS in HAS patients. Similarly, elevated preoperative levels of CEA and CA19-9, along with clinical TNM stage III/IV, were found to be independent risk factors for DFS. Conversely, open surgery and a marker of MKI-67 >50% emerged as protective factors for both OS and DFS. Additionally, postoperative chemotherapy significantly enhanced OS, affirming its role as a critical component of HAS management.

Given these findings, open radical surgery followed by adjuvant chemotherapy is recommended for managing resectable HAS. However, the effectiveness of this approach warrants further investigation through larger, more comprehensive clinical studies to validate and refine the treatment protocols.

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Disclosure of conflict of interest

None.

Abbreviations

HAS, Hepatoid Adenocarcinoma of the Stomach; PSM, Propensity-Matched Analysis; AFP, Alpha-Fetoprotein; OS, Overall Survival; CEA, Carcinoembryonic Antigen; CA19-9, Carbohydrate Antigen 19-9; DFS, Disease-Free Survival; GC, Gastric Cancer; IHC, Immunohistochemistry; BMI, Body Mass Index; cTNM, Clinical TNM; pTNM, Pathological TNM; HER-2, Human Epidermal Growth Factor Receptor 2; MMR, Mismatch Repair; MKI-67, Marker of Proliferation Ki-67; AJCC, American Joint Committee on Cancer; SD, Standard Deviation; IQR,

Interquartile Range; HRs, Hazard Ratios; CIs, Confidence Intervals; PD-1, Programmed Cell Death Protein 1; NK cells, Natural Killer Cells; NKT cells, Natural Killer T Cells; DCs, Dendritic Cells.

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Supplementary Table 1. Univariate analysis to determine the risk of OS in patients with HAS

	HR	95% CI	P
Age (years)			
≤60	1		
>60	1.04	0.36-3.00	0.942
Gender			
Male	1		
Female	1.99	0.85-4.71	0.114
AFP (ng/mL)			
0-20	1		
20-200	1.64	0.60-4.46	0.331
200-500	3.85	1.22-12.16	0.022
>500	1.16	0.43-3.16	0.769
CEA (ng/mL)			
≤5	1		
>5	1.75	0.82-3.73	0.148
CA19-9 (U/mL)			
≤37	1		
>37	2.37	0.94-5.98	0.068
cTNM			
I/II	1		
III/IV	7.52	1.00-56.37	0.050
Surgery			
Laparoscope	1		
Open surgery	0.44	0.20-0.96	0.039
Tumor location			
Upper third	1		
Middle third	0.52	0.13-1.99	0.337
Lower third	1.07	0.45-2.58	0.872
Tumor size (cm)			
≤5	1		
>5	1.38	0.66-2.91	0.396
Perineural invasion			
Absence	1		
Presence	1.45	0.68-3.09	0.339
Lymphovascular invasion			
Absence	1		
Presence	0.90	0.39-2.06	0.804
pTNM			
I	1		
II	2.12	0.22-20.73	0.519
III	5.27	0.68-40.86	0.112
IV	6.76	0.77-58.88	0.083
HER-2			
Negative	1		
Positive	0.38	0.05-2.90	0.352
2+	0.48	0.11-2.09	0.328

MKI-67			
<50	1		
51-70	0.19	0.05-0.73	0.016
71-90	0.39	0.14-1.14	0.085
Preoperative chemotherapy			
Absence	1		
Presence	1.09	0.26-4.59	0.910
Postoperative chemotherapy			
Absence	1		
Presence	0.40	0.18-0.89	0.024

OS, overall survival; HAS, hepatoid adenocarcinoma of the stomach; HR, hazard ratios; CI, confidence interval; AFP, alpha-feto-protein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; HER-2, human epidermalgrowth factor receptor 2; MKI-67, marker of proliferation ki-67.

Supplementary Table 2. Univariate analysis to determine the risk of DFS in patients with HAS

	HR	95% CI	P
Age (years)			
≤60	1		
>60	0.98	0.38-2.54	0.974
Gender			
Male	1		
Female	1.66	0.72-3.79	0.234
AFP (ng/mL)			
0-20	1		
20-200	1.12	0.44-2.87	0.813
200-500	3.77	1.37-10.37	0.010
>500	1.04	0.44-2.43	0.936
CEA (ng/mL)			
≤5	1		
>5	2.01	1.04-3.92	0.039
CA19-9 (U/mL)			
≤37	1		
>37	3.11	1.37-7.04	0.007
cTNM			
I/II	1		
III/IV	5.26	1.24-22.29	0.024
Surgery			
Laparoscope	1		
Open surgery	0.44	0.22-0.87	0.019
Tumor location			
Upper third	1		
Middle third	0.73	0.24-2.24	0.582
Lower third	1.23	0.55-2.77	0.610
Tumor size (cm)			
≤5	1		
>5	1.67	0.87-3.23	0.124
Perineural invasion			
Absence	1		
Presence	1.91	0.98-3.71	0.057

Lymphovascular invasion			
Absence	1		
Presence	1.12	0.53-2.40	0.767
pTNM			
I	1		
II	3.43	0.40-29.52	0.262
III	7.86	1.00-61.86	0.050
IV	10.71	1.26-91.11	0.030
HER-2			
Negative	1		
Positive	1.20	0.36-4.04	0.765
2+	0.41	0.10-1.75	0.230
MKI-67			
<50	1		
51-70	0.32	0.10-1.05	0.060
71-90	0.66	0.24-1.80	0.411
Preoperative chemotherapy			
Absence	1		
Presence	0.77	0.19-3.22	0.722
Postoperative chemotherapy			
Absence	1		
Presence	0.65	0.31-1.35	0.246

DFS, disease-free survival; HAS, hepatoid adenocarcinoma of the stomach; HR, hazard ratios; CI, confidence interval; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; HER-2, human epidermal growth factor receptor 2; MKI-67, marker of proliferation ki-67.