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Combined detection of plasma HSP90α with conventional tumor markers improves postoperative recurrence detection and predicts survival in advanced gastric cancer: a retrospective cohort study

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Abstract

Background

Plasma heat shock protein 90α (HSP90α) is an emerging pan-cancer biomarker, but its prognostic value in advanced gastric cancer (GC) and its utility for postoperative recurrence surveillance remain incompletely defined. This study evaluated the prognostic significance of plasma HSP90α in advanced GC and its combined use with conventional tumor markers (CEA, CA19-9 and CA72-4) for postoperative recurrence detection.

Methods

A total of 293 GC patients with plasma HSP90α records at Jilin Cancer Hospital were retrospectively enrolled and classified into postoperative recurrence-free follow-up (n = 30), postoperative recurrence (n = 123) and initially advanced (n = 133) groups; seven unclassifiable cases were excluded. Pre-treatment marker levels were compared among groups with the Kruskal-Wallis test. Receiver operating characteristic analysis was used to assess discrimination of postoperative recurrence. In the advanced cohort (n = 256), Kaplan-Meier analysis, log-rank tests and Cox proportional hazards regression were performed to evaluate associations between baseline markers and overall survival (OS) and progression-free survival (PFS). Associations between marker decline after two treatment cycles and survival were also analyzed. The data cutoff date was July 30, 2026.

Results

Median OS was 30.0 months and median PFS was 10.0 months in the advanced cohort. Patients with baseline HSP90α > 82 ng/ml had significantly shorter OS than those with ≤ 82 ng/ml (23.0 vs 47.1 months, P = 0.011) and shorter PFS (10.0 vs 12.0 months, P = 0.042); the difference was most pronounced in the postoperative recurrence subgroup (26.0 vs 58.0 months, P = 0.006). HSP90α > 82 ng/ml was an independent adverse prognostic factor for OS (HR = 1.61, 95%CI 1.02–2.53, P = 0.042). At recurrence, the positivity rates of HSP90α, CEA, CA19-9 and CA72-4 were 36.2%, 23.5%, 19.0% and 39.0%, respectively, reaching 67.2% for any-positive combined testing. The areas under the curve for discriminating recurrence were 0.572, 0.547, 0.546 and 0.667, and 0.702 for the combined model. HSP90α decline after two treatment cycles was not associated with OS (P = 0.890) or PFS (P = 0.437).

Conclusions

Elevated plasma HSP90α (> 82 ng/ml) is an independent adverse prognostic factor in advanced gastric cancer, particularly in patients with postoperative recurrence. Combined testing with conventional

markers improves the sensitivity of recurrence detection. The prognostic relevance of early on-treatment HSP90α dynamics warrants prospective validation.

Background

Gastric cancer (GC) is one of the most common malignancies worldwide. According to GLOBOCAN 2020, more than one million new cases of GC are diagnosed annually, with approximately 769,000 deaths, ranking fifth in incidence and fourth in mortality among all malignancies; China bears a particularly heavy disease burden as a high-incidence country [1]. Radical surgery is the main curative approach for early-stage GC, but a considerable proportion of patients develop postoperative recurrence and metastasis, and many patients present with unresectable locally advanced or metastatic disease at initial diagnosis. Recurrence and metastasis are the leading causes of treatment failure and death; how to achieve early warning of postoperative recurrence and prognostic stratification of advanced disease remains a central issue in the whole-course management of GC [2, 3].

Serum tumor markers are widely used for auxiliary diagnosis and follow-up surveillance of GC because sampling is convenient and testing is reproducible. However, the sensitivity of any single conventional marker is limited. Deng et al. [2] retrospectively analyzed 564 GC patients and found that the areas under the curve (AUC) of carcinoembryonic antigen (CEA), carbohydrate antigen 19 – 9 (CA19-9) and carbohydrate antigen 72 – 4 (CA72-4) for diagnosing GC were only 0.633, 0.565 and 0.621, respectively, and that preoperative levels could not effectively predict postoperative recurrence. Shibata et al. [3] followed 149 patients after curative gastrectomy and showed that recurrence rates were higher in patients with positive postoperative CA19-9 than in negative patients (47% vs 16%), yet sensitivity remained unsatisfactory. A meta-analysis indicated that combined detection of CEA, CA19-9 and CA72-4 yielded a sensitivity of 0.67 and a specificity of 0.89 for diagnosing GC, outperforming any single marker [4]. Based on a multicenter cohort of 1,708 patients, Zhang et al. [5] further suggested that surveillance strategies should be stratified according to baseline CEA levels. Therefore, identifying novel blood-based biomarkers and optimizing combined testing strategies are of practical significance for improving recurrence surveillance.

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone involved in the folding and stabilization of numerous tumor-associated proteins [6]. The HSP90α isoform can be secreted by tumor cells into the extracellular space (eHsp90α), where it promotes tumor cell survival, migration, invasion and epithelial-mesenchymal transition through autocrine and paracrine mechanisms, and has become a research focus in liquid biopsy [7, 8]. In a multicenter cohort of 1,558 subjects, Liu et al. [9] demonstrated that plasma HSP90α is a pan-cancer diagnostic biomarker, with an optimal cutoff of 69.19 ng/ml for distinguishing cancer patients from controls (AUC 0.895; sensitivity 81.33%, specificity 81.65%). The diagnostic value of plasma HSP90α has subsequently been reported in hepatocellular carcinoma [10], lung cancer [11–13] and colorectal cancer liver metastasis [14], as well as its value in indicating chemotherapy efficacy and poor prognosis in small cell lung cancer [15]. In GC, Liang et al. [16] enrolled 976 patients and found that plasma HSP90α levels were significantly higher than in patients with gastric

inflammatory diseases and healthy controls, correlated with CEA and CA19-9 levels, and were higher in patients with distant metastasis (M1) than in those without (M0), yielding moderate diagnostic performance; however, no association with survival was observed in their full-stage cohort dominated by operable patients. Qu et al. [17] confirmed in 130 advanced GC patients treated with programmed cell death protein 1 (PD-1) inhibitors that plasma HSP90α was an independent prognostic factor for overall survival, decreasing in responders and increasing in patients with progression, and constructed a prognostic nomogram combining HSP90α with the neutrophil-to-lymphocyte ratio.

In summary, the diagnostic value of plasma HSP90α in GC is relatively well established, but its prognostic value in advanced disease—particularly in patients with recurrence after radical surgery—and real-world evidence for its combined use with conventional markers in postoperative recurrence surveillance remain limited. Based on the clinical data of 293 GC patients with plasma HSP90α testing records at Jilin Cancer Hospital, the present study systematically analyzed: (1) differences in marker levels among patients in three clinical states (postoperative recurrence-free follow-up, postoperative recurrence and initially advanced disease); (2) the performance of each marker, alone and in combination, for indicating postoperative recurrence; (3) the association between baseline HSP90α levels and overall survival (OS) and progression-free survival (PFS) in advanced GC; and (4) the association between early on-treatment marker dynamics and survival, aiming to provide real-world evidence for the clinical application of HSP90α in recurrence surveillance and prognostic assessment of GC.

Methods

Study design and participants

This single-center retrospective observational study was conducted at Jilin Cancer Hospital. Clinical data of patients with pathologically confirmed GC who had plasma HSP90α testing records between November 2020 and July 2026 were retrospectively collected, and 293 patients were included. Seven patients with insufficient grouping information to determine clinical status (neither radical surgery nor recurrence information recorded) were excluded. Patients were classified into three groups according to clinical status: (1) postoperative recurrence-free follow-up group (n = 30): patients who underwent radical surgery with no evidence of recurrence during follow-up; (2) postoperative recurrence group (n = 123): patients with recurrence or metastasis confirmed by imaging and/or pathology after radical surgery; (3) initially advanced group (n = 133): patients with unresectable locally advanced or metastatic GC at initial diagnosis. The postoperative recurrence and initially advanced groups were merged into an advanced cohort (n = 256) for survival analysis. Collected data included age, sex, primary tumor site, pathological differentiation, Lauren classification, Tumor-Node-Metastasis (TNM) stage, Eastern Cooperative Oncology Group performance status (ECOG PS), treatment regimens and follow-up information. The follow-up cutoff date was July 30, 2026.

Marker measurement

Plasma HSP90α was measured by enzyme-linked immunosorbent assay (ELISA) using a kit from Yantai Progen Biotechnology Development Co., Ltd. (Yantai, China), strictly according to the manufacturer's instructions; the upper limit of the kit reference range, 82 ng/ml, was used as the positivity cutoff. Serum CEA, CA19-9 and CA72-4 were routinely measured by electrochemiluminescence immunoassay in the hospital's clinical laboratory, with positivity cutoffs of 5 ng/ml, 37 U/ml and 6.9 U/ml, respectively. Blood sampling time points included before systemic treatment (at recurrence/before post-recurrence treatment for the postoperative recurrence group), after two treatment cycles and after four treatment cycles.

Outcome definitions

In the advanced cohort, OS was defined as the time from confirmation of recurrence or initiation of systemic treatment for advanced disease to death or last follow-up. PFS was defined as the time from treatment initiation to radiologically confirmed disease progression (with reference to the Response Evaluation Criteria in Solid Tumours version 1.1 (RECIST 1.1) [18]), death or last follow-up. Follow-up records documented as "alive", "no progression", "not reached" or "≥" were treated as censored; records with OS blank but PFS completed were treated as alive-censored at the data cutoff date (July 30, 2026).

Statistical analysis

Continuous variables with skewed distributions are presented as median (lower quartile, upper quartile) [M (Q1, Q3)] and were compared between two groups using the Mann-Whitney U test and among three groups using the Kruskal-Wallis test; categorical variables are presented as counts (percentages). Survival curves were generated by the Kaplan-Meier method and compared by the log-rank test. Univariate and multivariate Cox proportional hazards models were used to estimate hazard ratios (HRs) with 95% confidence intervals (95%CIs). Systemic treatments in the advanced cohort were categorized according to regimen composition by review of treatment records, and a sensitivity analysis additionally adjusting for immunotherapy-containing and targeted therapy-containing regimens was performed for the multivariate Cox model of OS. Receiver operating characteristic (ROC) curves and the AUC were used to evaluate the discrimination of postoperative recurrence by each marker, and a logistic regression model was constructed for the combined four-marker panel (AUC is the apparent value without cross-validation). All tests were two-sided with $\alpha = 0.05$. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [19] and, where applicable, the REporting recommendations for tumor MARKer prognostic studies (REMARK) [20]. Statistical analyses were performed using SPSS (version 19.0; IBM Corp., USA). All statistical results were independently performed by the authors with the assistance of an independent statistician, and verified by Python 3.12 (scipy, statsmodels and scikit-learn libraries).

Results

Baseline characteristics by clinical group

Of the 286 classifiable patients, 30 were under surveillance after radical surgery without recurrence (postoperative recurrence-free follow-up group), 123 had confirmed recurrence or metastasis after radical surgery (postoperative recurrence group) and 133 had unresectable locally advanced or metastatic disease at initial diagnosis (initially advanced group) (Fig. 1). Median ages were 64.5, 63.0 and 64.0 years, respectively; all three groups were predominantly male with poorly differentiated adenocarcinoma, and ECOG PS was mainly 0–1 (Table 1). Stage IV disease predominated in the initially advanced group (83/133), whereas preoperative stage III was most common in the postoperative recurrence group (46/123). The three groups were thus broadly comparable in demographic composition and performance status, differing mainly in tumor stage in a manner consistent with their clinical definitions.

Table 1
Baseline characteristics of the three patient groups

Characteristic	Recurrence-free follow-up (n = 30)	Postoperative recurrence (n = 123)	Initially advanced (n = 133)
Age, years, M (Q1, Q3)	64.5 (55.8, 71.0)	63.0 (56.0, 69.0)	64.0 (54.0, 69.0)
Sex, male/female	18/12	87/36	93/39
Primary site, n			
Antrum	10	50	58
Body	9	27	56
Angle	3	17	17
Cardia/EGJ	6	10	12
Whole stomach/multiple	1	5	4
Differentiation, n			
Poor (incl. signet-ring)	19	85	83
Moderate	6	23	23
Well	0	2	1
Lauren classification, n			
Intestinal	2	38	21
Diffuse	7	31	19
Mixed	10	20	12
Stage, n			
I-II	4	24	2
III	10	46	28
IV	2	30	83
ECOG PS, 0–1/≥2	28/2	112/10	112/20

Note: individual items were missing for some patients, so item counts may not sum to group totals; stage refers to postoperative pathological stage for the two surgical groups and clinical stage for the initially advanced group. EGJ: esophagogastric junction; ECOG PS: Eastern Cooperative Oncology Group performance status.

Pre-treatment marker levels by clinical group

Pre-treatment levels of HSP90α, CEA, CA19-9 and CA72-4 across the three groups are shown in Table 2 and Fig. 2. Median levels of all four markers increased stepwise with advancing clinical state (recurrence-free follow-up < postoperative recurrence < initially advanced), broadly paralleling tumor burden. This trend reached statistical significance for CEA, CA19-9 and CA72-4 (all $P < 0.05$) but not for HSP90α ($P = 0.057$), suggesting that plasma HSP90α was already substantially elevated at recurrence and distinguished the three clinical states less sharply than the conventional markers. More importantly for the question of recurrence detection, direct comparison between the postoperative recurrence and recurrence-free follow-up groups showed a significant difference only for CA72-4 ($P = 0.025$), but not for HSP90α ($P = 0.264$), CEA ($P = 0.477$) or CA19-9 ($P = 0.488$), indicating that no single marker separated recurrence from recurrence-free follow-up well enough to be used alone.

Table 2
Pre-treatment plasma/serum marker levels by clinical group [M (Q1, Q3)]

Marker	Recurrence-free follow-up	Postoperative recurrence	Initially advanced	P (three groups)
HSP90α, ng/ml	62.9 (46.7, 104.8) (n = 25)	71.4 (49.6, 116.3) (n = 105)	86.6 (56.6, 138.3) (n = 119)	0.057
CEA, ng/ml	2.1 (1.3, 4.0) (n = 24)	2.4 (1.4, 4.6) (n = 102)	3.9 (1.8, 11.6) (n = 113)	0.010
CA19-9, U/ml	6.5 (3.6, 18.9) (n = 24)	10.1 (4.3, 20.8) (n = 105)	25.1 (10.3, 99.3) (n = 116)	< 0.001
CA72-4, U/ml	1.6 (1.2, 9.3) (n = 19)	4.4 (1.9, 15.6) (n = 77)	8.1 (2.5, 38.3) (n = 88)	0.002

Note: Kruskal-Wallis test for three-group comparison; pairwise comparison of postoperative recurrence vs recurrence-free follow-up (Mann-Whitney U test): HSP90α $P = 0.264$, CEA $P = 0.477$, CA19-9 $P = 0.488$, CA72-4 $P = 0.025$.

Performance of individual markers and their combination for indicating postoperative recurrence

This limitation of individual markers was also evident in their positivity rates. At recurrence, the positivity rates of HSP90α, CEA, CA19-9 and CA72-4 in the postoperative recurrence group were 36.2% (38/105), 23.5% (24/102), 19.0% (20/105) and 39.0% (30/77), respectively; in other words, testing with any single marker would have missed more than 60% of recurrences. Among the 67 patients with all four markers measured, 45 were positive for at least one marker, corresponding to a combined positivity rate of 67.2% (Fig. 3A), which indicates that combined testing captures substantially more recurrences than any individual marker.

ROC analysis pointed to the same conclusion. With the postoperative recurrence group as positives and the recurrence-free follow-up group as controls, the AUCs of HSP90α, CEA, CA19-9 and CA72-4 were 0.572 (n = 130), 0.547 (n = 126), 0.546 (n = 129) and 0.667 (n = 96), respectively, none exceeding 0.70; the

combined four-marker logistic regression model achieved an AUC of 0.702 (n = 85) (Fig. 3B). Combined testing thus offered the best discrimination, although discrimination remained moderate.

Survival of the advanced cohort

Among the 256 patients in the advanced cohort, 165 were analyzable for OS (92 deaths, 73 alive-censored), with a median OS of 30.0 months; 179 were analyzable for PFS (142 progression or death events, 37 censored), with a median PFS of 10.0 months. The median follow-up time of censored patients was 24.0 months (interquartile range [IQR] 10.0-56.9 months), providing a reasonably informative basis for survival estimation in this real-world cohort.

Systemic treatment of the advanced cohort is summarized in Table 3. Chemotherapy-containing regimens predominated (194 of 256 patients, 75.8%); 83 patients (32.4%) received immunotherapy-containing regimens and 70 (27.3%) received targeted therapy-containing regimens during the disease course, whereas 50 (19.5%) received no conventional systemic therapy. Importantly, the distribution of treatment categories did not differ significantly between the HSP90α > 82 ng/ml and ≤ 82 ng/ml groups (chi-square, P = 0.066), making a major confounding effect of treatment composition on the survival analyses that follow unlikely.

Table 3
Systemic treatment of the advanced cohort

Treatment category	Postoperative recurrence (n = 123)	Initially advanced (n = 133)	Total (n = 256)
Chemotherapy alone	59 (48.0)	41 (30.8)	100 (39.1)
Chemotherapy + immunotherapy	14 (11.4)	22 (16.5)	36 (14.1)
Chemotherapy + targeted therapy	4 (3.3)	19 (14.3)	23 (9.0)
Chemotherapy + immunotherapy + targeted therapy	14 (11.4)	21 (15.8)	35 (13.7)
Immunotherapy or targeted therapy without chemotherapy	4 (3.3)	8 (6.0)	12 (4.7)
No conventional systemic therapy	28 (22.8)	22 (16.5)	50 (19.5)

Note: values are counts (percentages of column totals); categories were assigned by review of treatment records according to regimen composition during the disease course.

Association between baseline HSP90α levels and survival in advanced GC

Patients were stratified by whether baseline HSP90α exceeded 82 ng/ml. Median OS was 23.0 months in the HSP90α > 82 ng/ml group, significantly shorter than 47.1 months in the ≤ 82 ng/ml group (log-rank P = 0.011, Fig. 4A); median PFS was also shorter (10.0 vs 12.0 months, P = 0.042, Fig. 4B). Subgroup

analysis showed that in the postoperative recurrence subgroup, median OS of patients with HSP90α > 82 ng/ml was 26.0 months, significantly shorter than 58.0 months in those with ≤ 82 ng/ml (P = 0.006, Fig. 4C); in the initially advanced subgroup, the difference was not significant (23.0 vs 20.0 months, P = 0.641, Fig. 4D). The prognostic separation by HSP90α was therefore driven mainly by patients who recurred after radical surgery — the clinical setting in which a prognostic marker is arguably most needed.

Regarding other markers, median OS was 23.0 months in patients with CEA > 5 ng/ml versus 41.0 months in those with ≤ 5 ng/ml, a numerical difference that did not reach statistical significance (P = 0.107); median OS did not differ significantly by CA19-9 > 37 U/ml or CA72-4 > 6.9 U/ml status (P = 0.286 and P = 0.298, respectively). The association with OS thus appeared specific to HSP90α rather than a generic feature of tumor marker elevation.

Cox proportional hazards regression analysis of OS in the advanced cohort

Univariate analysis showed that HSP90α > 82 ng/ml was associated with shorter OS (HR = 1.75, 95%CI 1.13–2.72, P = 0.012); analyzed as a continuous variable, each 10 ng/ml increase in HSP90α was associated with a 1.1% increase in the risk of death (HR = 1.011, 95%CI 1.004–1.017, P < 0.001). CEA > 5 ng/ml showed a numerical but non-significant association with shorter OS (HR = 1.46, 95%CI 0.92–2.33, P = 0.110); elevated CA19-9 and CA72-4, initially advanced status (vs postoperative recurrence), age and ECOG PS were not significantly associated with OS. When HSP90α group, disease status, age and ECOG PS were entered into a multivariate Cox model (n = 148, 85 death events), HSP90α > 82 ng/ml remained an independent adverse prognostic factor for OS (HR = 1.61, 95%CI 1.02–2.53, P = 0.042) (Table 4, Fig. 5). In a sensitivity analysis additionally adjusting for immunotherapy-containing and targeted therapy-containing regimens (n = 148, 85 death events), the association between HSP90α > 82 ng/ml and shorter OS persisted in direction and magnitude (HR = 1.55, 95%CI 0.97–2.48, P = 0.067), indicating that the association was not materially explained by differences in the types of systemic treatment received.

Table 4
Cox proportional hazards regression analysis of overall survival in the advanced cohort

Factor	Univariate HR (95%CI)	P	Multivariate HR (95%CI)	P
HSP90α > 82 ng/ml	1.75 (1.13–2.72)	0.012	1.61 (1.02–2.53)	0.042
HSP90α per 10 ng/ml increase	1.011 (1.004–1.017)	< 0.001	—	—
CEA > 5 ng/ml	1.46 (0.92–2.33)	0.110	—	—
CA19-9 > 37 U/ml	1.30 (0.80–2.09)	0.290	—	—
CA72-4 > 6.9 U/ml	1.30 (0.79–2.14)	0.301	—	—
Initially advanced (vs postoperative recurrence)	1.19 (0.79–1.79)	0.415	1.40 (0.89–2.18)	0.144
Age (per 1-year increase)	0.99 (0.97–1.01)	0.199	0.99 (0.97–1.01)	0.372
ECOG PS (per 1-score increase)	1.02 (0.73–1.43)	0.898	1.08 (0.76–1.54)	0.659

Note: the multivariate model included HSP90α group, disease status (initially advanced), age and ECOG PS (n = 148, 85 death events); "—" indicates factors not included in the multivariate model.

Early on-treatment marker dynamics and survival

Median levels of each marker before treatment and after two and four treatment cycles in the advanced cohort are shown in Fig. 6A-D. Patients were grouped according to whether marker levels declined after two treatment cycles compared with baseline. Median OS (29.0 vs 40.0 months, P = 0.890, Fig. 6E) and median PFS (10.0 vs 8.0 months, P = 0.437, Fig. 6I) did not differ significantly between the HSP90α-decline and non-decline groups. Median OS was numerically longer in the CA19-9-decline group than in the non-decline group, without statistical significance (48.0 vs 24.0 months, P = 0.207, Fig. 6G), whereas CA19-9 decline was not significantly associated with PFS (12.0 vs 8.0 months, P = 0.233, Fig. 6K). CEA and CA72-4 declines were not significantly associated with OS (P = 0.496 and P = 0.513, respectively; Fig. 6F, H), and CA72-4 decline was not associated with PFS (P = 0.782, Fig. 6L). Unexpectedly, median PFS was shorter in the CEA-decline group than in the non-decline group (10.0 vs 12.0 months, P = 0.023, Fig. 6J); this is likely a chance finding attributable to multiple comparisons and should be interpreted with caution. Overall, early changes in marker levels showed no consistent association with survival in this cohort, suggesting that a single early on-treatment measurement has limited value for judging treatment benefit and should be interpreted together with radiological assessment.

Discussion

Based on the real-world data of 293 GC patients with plasma HSP90α testing records, this study has three main findings. First, in the advanced GC cohort (initially advanced plus postoperative recurrence, n

= 256), patients with baseline plasma HSP90α > 82 ng/ml had significantly shorter median OS (23.0 vs 47.1 months), and HSP90α > 82 ng/ml was a prognostic factor independent of age, ECOG PS and disease status (multivariate HR = 1.61, P = 0.042), with the association most pronounced in the postoperative recurrence subgroup (26.0 vs 58.0 months, P = 0.006). Second, individual markers had limited ability to indicate postoperative recurrence (positivity rates at recurrence 19.0%-39.0%; discrimination AUC 0.546–0.667), whereas combined testing of all four markers increased the positivity rate to 67.2% and the combined model achieved an AUC of 0.702. Third, in this retrospective cohort, HSP90α decline after two treatment cycles was not significantly associated with survival.

The present findings regarding the prognostic value of HSP90α are generally consistent with recent literature. Qu et al. [17] demonstrated that plasma HSP90α was an independent prognostic factor for OS in 130 advanced GC patients treated with PD-1 inhibitors, and Huang et al. [15] reported shorter OS in small cell lung cancer patients with high plasma eHsp90α (prognostic cutoff 61.2 ng/ml, AUC 0.791). Notably, Liang et al. [16] found no association between plasma HSP90α and survival in a full-stage cohort of 976 GC patients, which appears to contradict the present results but may in fact stem from differences in cohort composition: that cohort consisted mainly of operable patients with few survival events and no stratification by disease status, whereas the present study focused on initially advanced and postoperative recurrence populations—precisely the advanced population with the greatest prognostic heterogeneity, in which the stratification value of HSP90α became apparent. In addition, the AUC of HSP90α for discriminating recurrence from recurrence-free follow-up in the present study was only 0.572, which does not conflict with the "moderate diagnostic performance" reported by Liang et al. [16]: diagnosis (distinguishing cancer from non-cancer) and recurrence discrimination (distinguishing recurrence from postoperative remission) are two different application scenarios, and the value of HSP90α in the recurrence setting lies more in prognostic stratification than in recurrence screening. Mechanistically, tumor cell-secreted eHsp90α can promote tumor invasion and migration through autocrine loops, and its plasma level correlates with tumor burden and invasiveness [7–9], providing biological plausibility for the association between high HSP90α levels and poor prognosis. The 82 ng/ml cutoff used in this study is the upper limit of the kit reference range, requiring no additional optimization and facilitating direct clinical translation.

Regarding postoperative recurrence surveillance, the present results corroborate existing evidence. Deng et al. [2] reported AUCs of only 0.565–0.633 for CEA, CA19-9 and CA72-4 individually in diagnosing GC; in the present study, the positivity rates of individual markers at recurrence were likewise only 19.0%-39.0%, indicating that no single marker is sufficient for independent recurrence warning. A meta-analysis showed that combined detection of CEA, CA19-9 and CA72-4 improved the sensitivity of GC diagnosis (0.67) over individual markers [4]; consistently, the any-positive rate of the four-marker panel (including HSP90α) at recurrence reached 67.2%, and the AUC of the combined model (0.702) exceeded that of any single marker. The studies of Shibata et al. [3] and Zhang et al. [5] both highlighted the surveillance value of tumor markers during follow-up after gastrectomy and the importance of stratified application. Accordingly, these results suggest that for GC patients after radical surgery, periodic combined testing of HSP90α with CEA, CA19-9 and CA72-4 may improve the sensitivity of early

recurrence detection, and that in patients with detected recurrence, the HSP90α level can be further used for prognostic stratification to guide follow-up intensity and treatment decisions.

No significant association was observed in this study between early on-treatment HSP90α dynamics and survival, which is inconsistent with the report by Qu et al. [17] of "decrease in responders and increase in progressors" in patients treated with PD-1 inhibitors. Possible explanations include: (1) this was a retrospective real-world cohort with highly heterogeneous treatment regimens (chemotherapy alone, chemotherapy combined with immunotherapy or targeted therapy), which may have diluted marker-change patterns across treatment modalities; (2) blood sampling time points were not strictly standardized, and the actual time window of the after-two-cycles measurement was wide; (3) as treatment progressed, fewer patients had available test data, introducing survivor bias—the rise rather than decline of median levels of some markers at later time points in Fig. 6A-D reflects this compositional change; and (4) Zhang et al. [21] reported that approximately 37.1% of patients receiving first-line systemic therapy for advanced GC experienced transient marker elevation (the "flare" phenomenon), suggesting that a single early increase or decrease has inherently limited interpretive value. Studies by Yu et al. [22], who constructed a slope model based on serial CEA measurements, and Zhang et al. [23], who developed a tumor marker index from the geometric mean of CEA and CA19-9, suggest that the value of dynamic monitoring may depend on more refined modeling and more standardized sampling time points, warranting validation in prospective cohorts. In addition, an anomalous result of shorter PFS was observed in the CEA-decline group ($P = 0.023$); in the absence of a biological explanation and without multiplicity correction, this finding is more likely a chance result.

This study has several limitations: (1) the single-center retrospective design, with non-uniform treatment regimens and testing time points—although treatment categories were balanced between the HSP90α groups and the association between HSP90α and OS persisted in direction and magnitude after adjustment for treatment regimens, residual confounding by treatment era, number of treatment lines and dosing cannot be excluded; (2) missing data for some patients—CA72-4 was recorded in only 186 of 293 patients and OS was analyzable in 165 of 256 patients—which may introduce selection bias; (3) the AUC of the combined model is an apparent value without cross-validation or independent-cohort validation; (4) the small sample size of the recurrence-free follow-up group ($n = 30$) limited the statistical power of the recurrence discrimination analysis.

Conclusions

Elevated plasma HSP90α (> 82 ng/ml) is an independent adverse prognostic factor in patients with advanced gastric cancer, with particularly clear implications in the postoperative recurrence population. Individual tumor markers have limited ability to indicate postoperative recurrence, whereas combined testing of HSP90α with CEA, CA19-9 and CA72-4 improves the sensitivity of recurrence detection. It is therefore recommended that HSP90α be incorporated into the combined marker panel for postoperative follow-up of gastric cancer, and that its baseline level be used to assist prognostic stratification of

patients with advanced disease. The prognostic significance of early on-treatment HSP90α dynamics requires validation in prospective studies with standardized sampling time points.

Abbreviations

AUC
area under the curve
CA19-9
carbohydrate antigen 19 – 9
CA72-4
carbohydrate antigen 72 – 4
CEA
carcinoembryonic antigen
CI
confidence interval
ECOG PS
Eastern Cooperative Oncology Group performance status
EGJ
esophagogastric junction
eHsp90α
extracellular heat shock protein 90α
ELISA
enzyme-linked immunosorbent assay
GC
gastric cancer
HR
hazard ratio
HSP90
heat shock protein 90
HSP90α
heat shock protein 90α
IQR
interquartile range
OS
overall survival
PD-1
programmed cell death protein 1
PFS
progression-free survival
RECIST

Response Evaluation Criteria in Solid Tumours
REMARK
REporting recommendations for tumor MARKer prognostic studies
ROC
receiver operating characteristic
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
TNM
Tumor-Node-Metastasis

Declarations

Ethics approval and consent to participate

This retrospective observational study was approved by the Institutional Review for Science and Technology Ethics of Jilin Cancer Hospital (approval number: 202306-014-01). The requirement for individual informed consent was waived by the committee owing to the retrospective nature of the study and the use of anonymized data. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J.D. conceived and designed the study; Ying W. performed the statistical analysis and drafted the manuscript; Yaopeng W. collected the clinical data; Y.H. critically revised the manuscript. All authors read and approved the final manuscript.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Deng L, Yin T, Li H, Wang X, Li J, Liu K, Long T, Wang Y, Cheng L. CEA, CA19-9, and CA72-4 in Gastric Cancer Diagnosis and Progression: a Chinese Retrospective Case-Control Study. Clin Lab 2025, 71(4).
3. Shibata C, Nakano T, Yasumoto A, Mitamura A, Sawada K, Ogawa H, Miura T, Ise I, Takami K, Yamamoto K, et al. Comparison of CEA and CA19-9 as a predictive factor for recurrence after curative gastrectomy in gastric cancer. BMC Surg. 2022;22(1):213.
4. Wang H, Jin W, Wan C, Zhu C. Diagnostic value of combined detection of CA72-4, CA19-9, and carcinoembryonic antigen comparing to CA72-4 alone in gastric cancer: a systematic review and meta-analysis. Translational cancer Res. 2022;11(4):848–56.
5. Zhang R, Chen X, Chen G, Zhao Z, Zhang F, Chen Z, Wei C, Lin J, Liu Z, Liang Y, et al. Novel surveillance protocol for gastric cancer based on CEA: a high-volume multi-center study. BMC Cancer. 2025;25(1):1396.
6. Hoter A, El-Sabban ME, Naim HY. The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. Int J Mol Sci 2018, 19(9).
7. Jay D, Luo Y, Li W. Extracellular Heat Shock Protein-90 (eHsp90): Everything You Need to Know. Biomolecules 2022, 12(7).
8. Reynolds TS, Blagg BSJ. Extracellular heat shock protein 90 alpha (eHsp90α)'s role in cancer progression and the development of therapeutic strategies. Eur J Med Chem. 2024;277:116736.
9. Liu W, Li J, Zhang P, Hou Q, Feng S, Liu L, Cui D, Shi H, Fu Y, Luo Y. A novel pan-cancer biomarker plasma heat shock protein 90alpha and its diagnosis determinants in clinic. Cancer Sci. 2019;110(9):2941–59.

10. Wei W, Liu M, Ning S, Wei J, Zhong J, Li J, Cai Z, Zhang L. Diagnostic value of plasma HSP90α levels for detection of hepatocellular carcinoma. BMC Cancer. 2020;20(1):6.
11. Yuan Z, Wang L, Hong S, Shi C, Yuan B. Diagnostic value of HSP90α and related markers in lung cancer. J Clin Lab Anal. 2022;36(6):e24462.
12. Wang Y, Seyed Barghi SM, Yang Y, Akhavan-Sigari R. Value of HSP90α in Lung Cancer Diagnosis and Recurrence Prediction: A Cohort Study. Oncol Res Treat. 2021;44(11):583–9.
13. Wang M, Jıang Z, Lıu R. The diagnostic value of Hsp90α in monitoring treatment responses in lung cancer. Turk J Med Sci. 2022;52(3):747–53.
14. Ding Q, Sun Y, Zhang J, Yao Y, Huang D, Jiang Y. Utility and specificity of plasma heat shock protein 90 alpha, CEA, and CA199 as the diagnostic test in colorectal cancer liver metastasis. J Gastrointest Oncol. 2022;13(5):2497–504.
15. Huang B, Pan J, Liu H, Tang Y, Li S, Bian Y, Ning S, Li J, Zhang L. High Expression of Plasma Extracellular HSP90α is Associated With the Poor Efficacy of Chemotherapy and Prognosis in Small Cell Lung Cancer. Front Mol Biosci. 2022;9:913043.
16. Liang XQ, Li KZ, Li Z, Xie MZ, Tang YP, Du JB, Huang Y, Li JL, Hu BL. Diagnostic and prognostic value of plasma heat shock protein 90alpha in gastric cancer. Int Immunopharmacol. 2021;90:107145.
17. Qu Z, Zhang Y, Lu L, Qi X, Zhang T, Zhao Z, Zhang Y, Gu K. A nomogram based on plasma HSP90α and NLR for predicting prognosis in advanced gastric cancer patients treated with PD-1 inhibitors. J Cancer. 2025;16(11):3473–84.
18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47.
19. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet (London England). 2007;370(9596):1453–7.
20. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst. 2005;97(16):1180–4.
21. Zhang F, Zhai M, Yang J, Zhao L, Lin Z, Wang J, Zhang T, Yu D. FLARE' of tumor marker in advanced gastric cancer treated with first-line systemic therapy. Therapeutic Adv Gastroenterol. 2022;15:17562848221124029.
22. Yu S, Sun Y, Zhou J, Sun C, Wu Z. Dynamic CEA kinetics as a prognostic biomarker for HER2-negative advanced gastric cancer treated with chemoimmunotherapy. BMC Cancer. 2025;25(1):1857.
23. Zhang J, Yin X, Wang H, Fang T, Gao J, Zhu Z, Li C, Wang Y, Wang X, Lu Z, et al. Development and Validation of Tumor Marker Indices in Advanced Gastric Cancer Patients. Cancer control: J Moffitt Cancer Cent. 2023;30:10732748231202466.

Figures

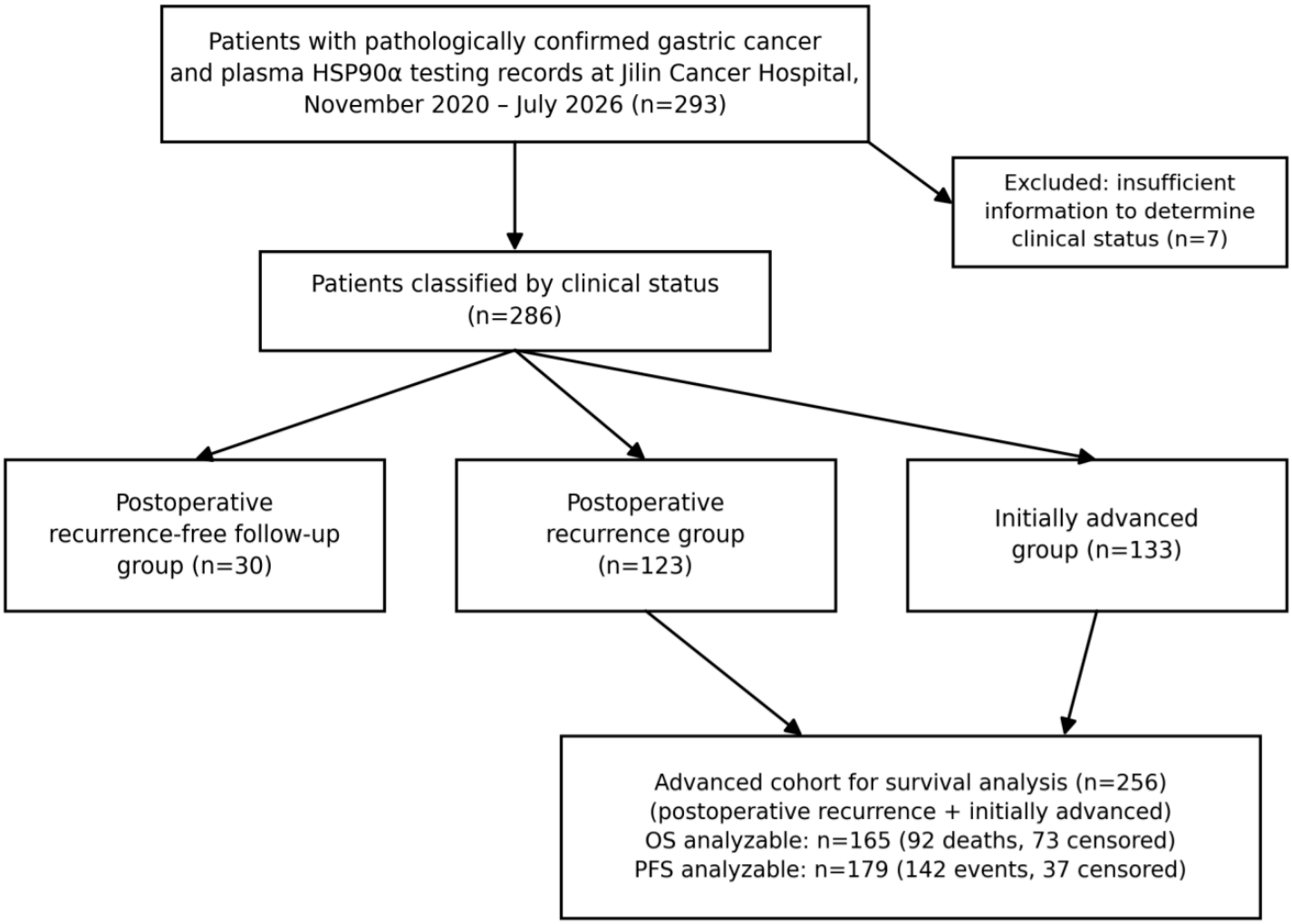


Figure 1

Flow diagram of patient selection and grouping. GC: gastric cancer; OS: overall survival; PFS: progression-free survival.

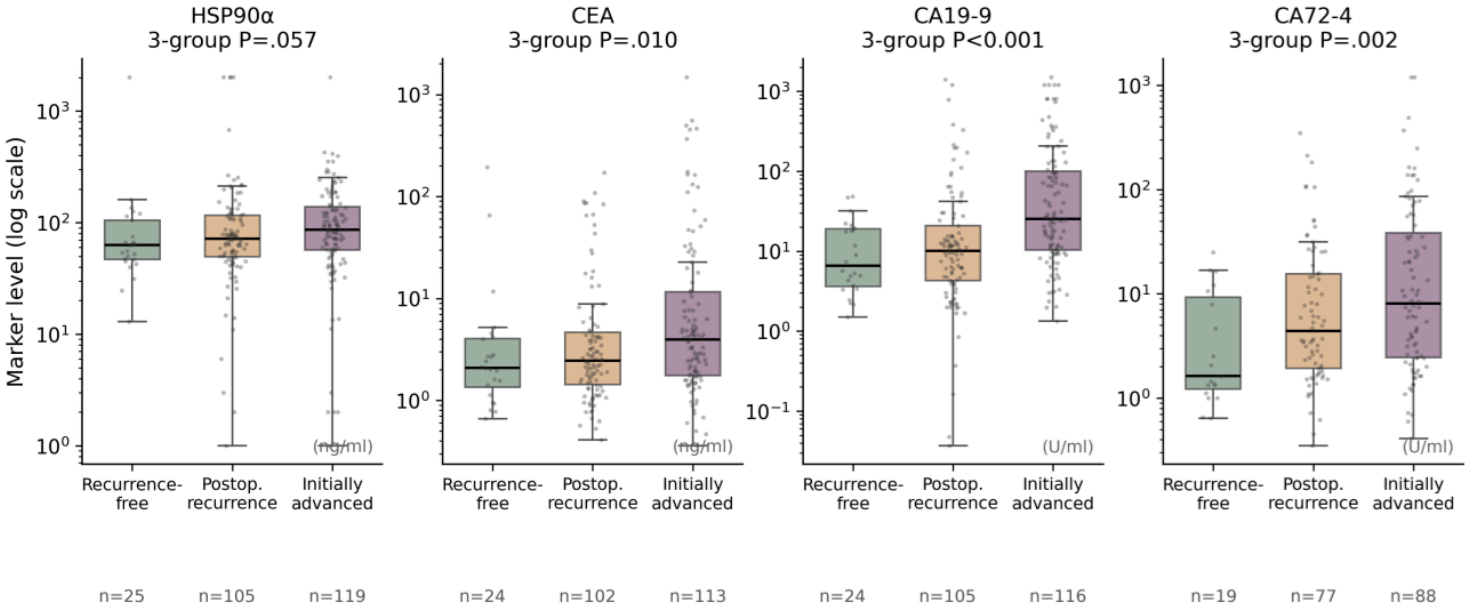


Figure 2

Pre-treatment plasma/serum marker levels in the three patient groups (log scale). Boxes indicate medians and interquartile ranges; dots represent individual values.

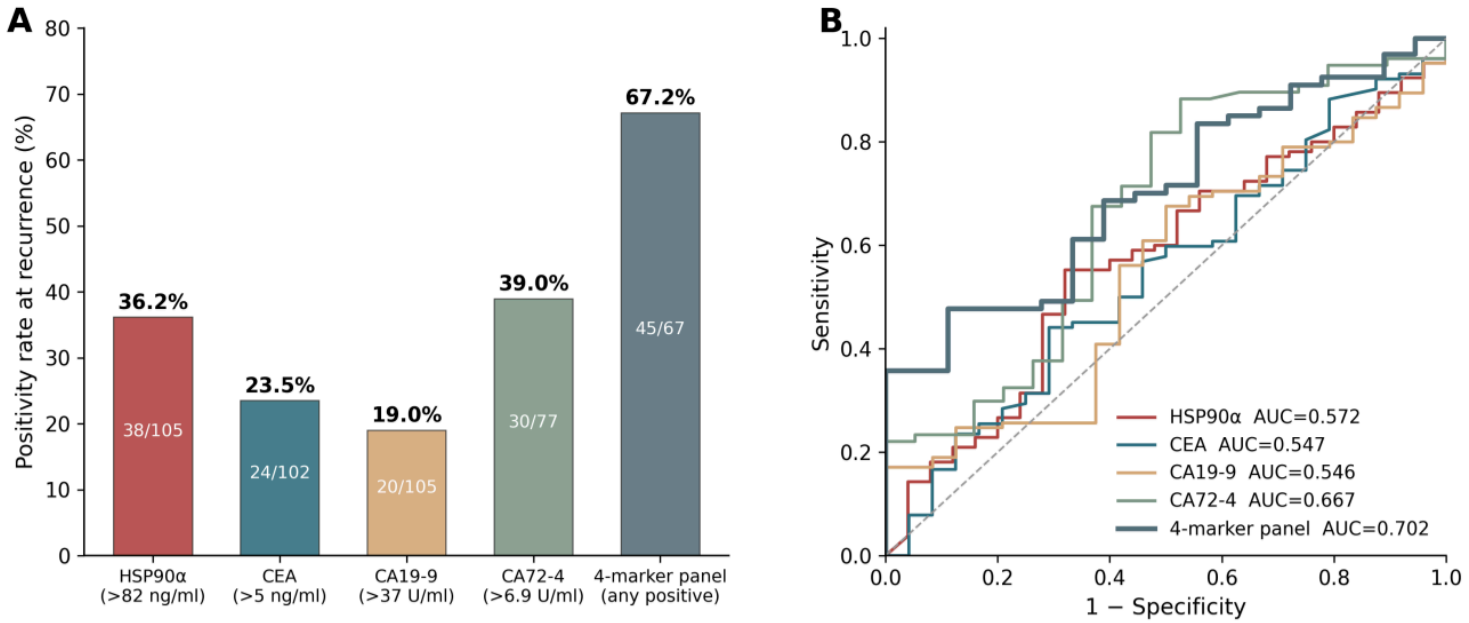


Figure 3

Performance of the markers for indicating postoperative recurrence. A Positivity rates of the markers at recurrence in the postoperative recurrence group (numbers inside the bars are positive/tested); B receiver operating characteristic curves of the markers for discriminating postoperative recurrence from recurrence-free follow-up.

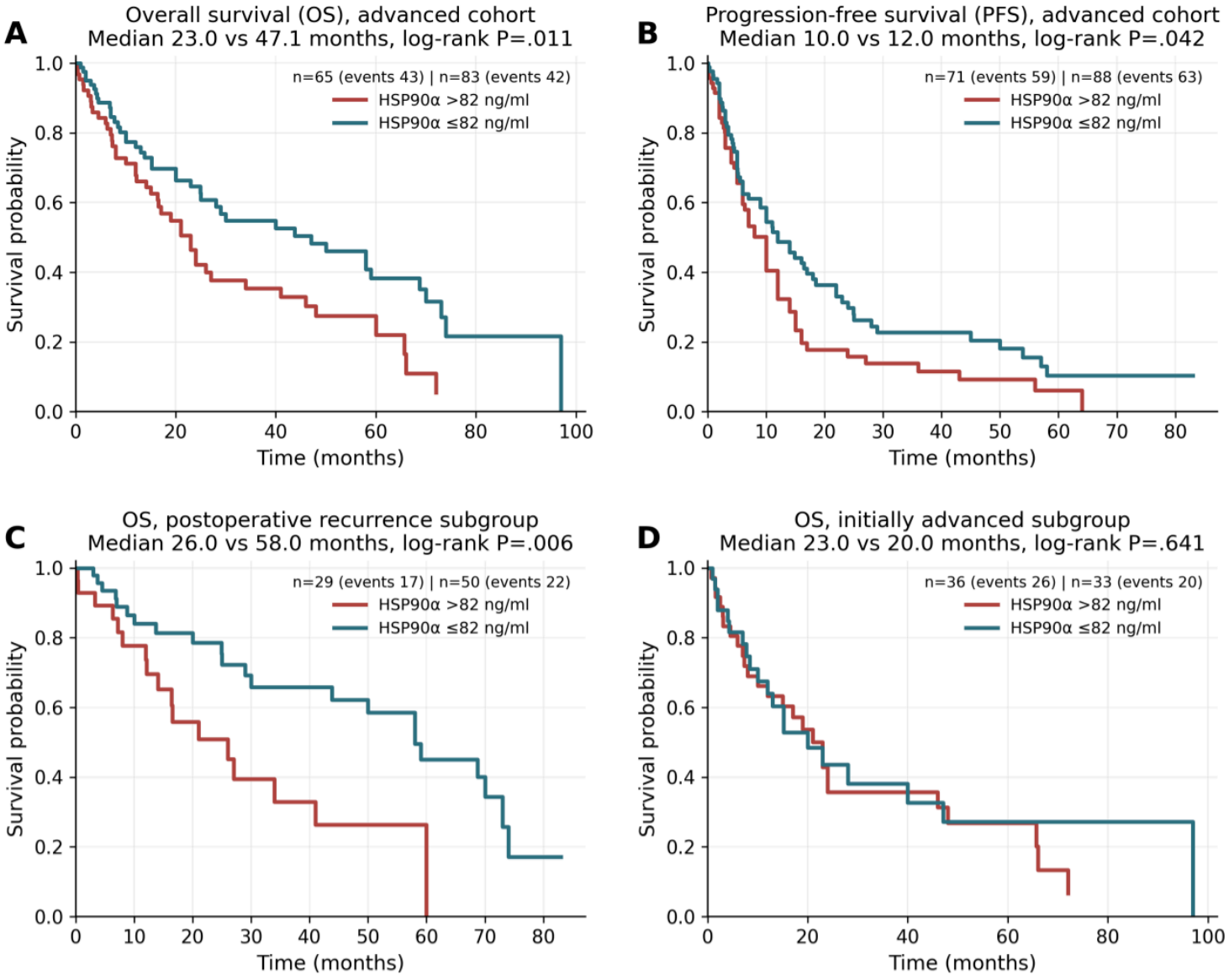


Figure 4

Kaplan-Meier survival curves stratified by baseline HSP90α level. A Overall survival (OS) in the advanced cohort; B progression-free survival (PFS) in the advanced cohort; C OS in the postoperative recurrence subgroup; D OS in the initially advanced subgroup.

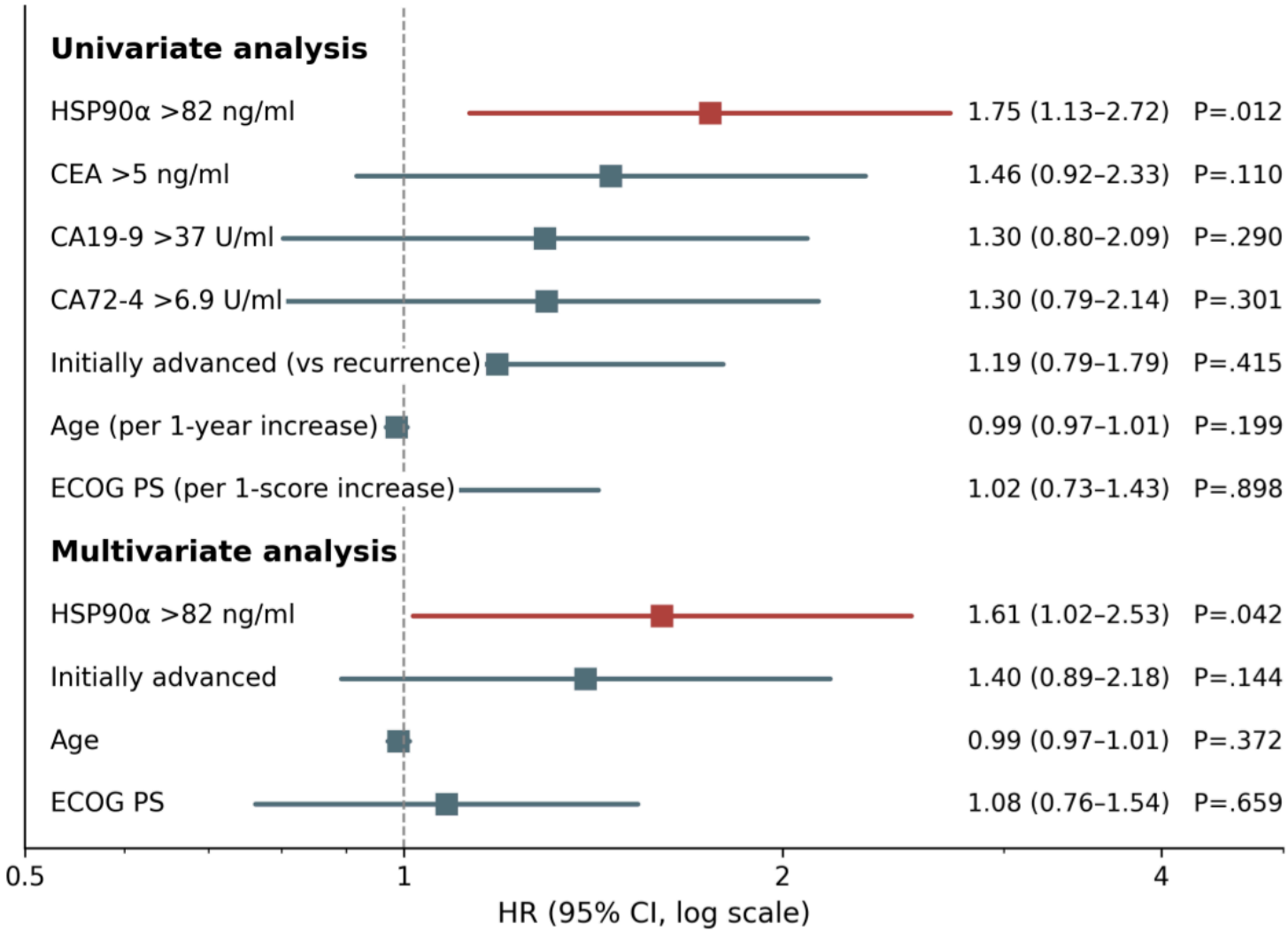


Figure 5

Forest plot of Cox proportional hazards regression analysis of overall survival in the advanced cohort.

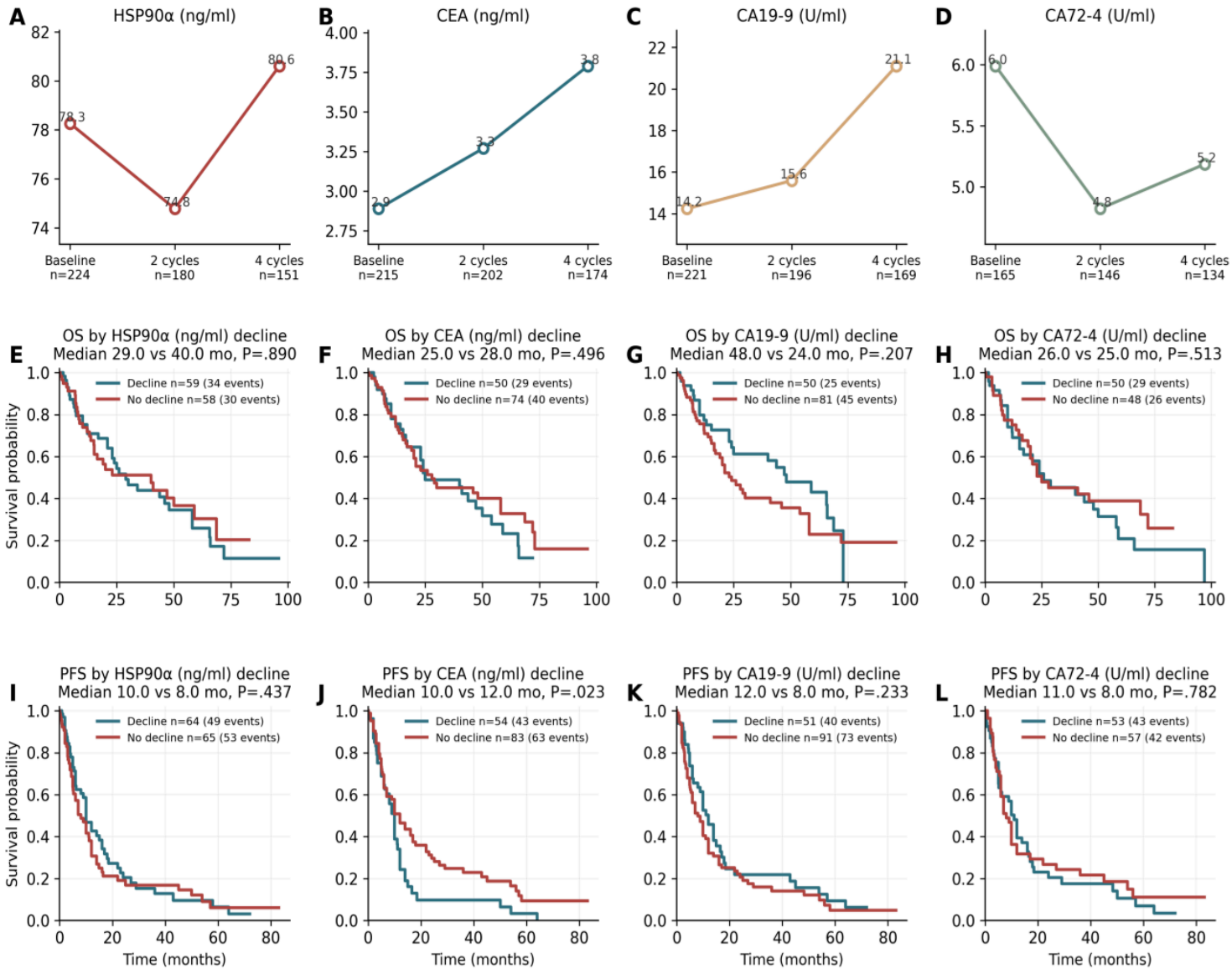


Figure 6

Early on-treatment marker dynamics and survival in the advanced cohort. A-D Median levels of each marker during treatment (baseline, after two cycles and after four cycles); E-H Kaplan-Meier curves of overall survival stratified by whether the level of each marker declined after two treatment cycles (E HSP90α; F CEA; G CA19-9; H CA72-4); I-L corresponding Kaplan-Meier curves of progression-free survival.

Supplementary Files

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- STROBEChecklist.docx