Role of initial anemia degree and treatment model on the prognosis of recurrent gastric cancer

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Abstract
Background: Anemia is highly prevalent in recurrent gastric cancer patients. The role of initial hemoglobin levels on prognosis prediction of in recurrent gastric cancer has not been well determined. Our present study aims to evaluate the relationship of the anemia degree and the Overall survival (OS) and Progression-free survival (PFS) of the patients with recurrent gastric cancer.
Methods: Our study enrolled 598 patients who were treated with chemotherapy when the recurrent gastric cancer were diagnosed after radical resection. Univariate and multivariate analyses were preformed to identify risk factors which has the potential to affect the patient prognosis. Besides, the relationship between clinicopathological characteristics including treatment styles and chemotherapy-related hemoglobin reduction degree were further analyzed. Results: Our results revealed that patients with HBini level ≤ 80 g/L had also a trend of shortened median OS and PFS (p=0.009 and p=0.049, respectively). Interesting, we also found that, HBdec ≥ 30 g/L was associated with significantly shortened median OS and PFS (p=0.039 and p=0.001, respectively). Multivariate analysis showed that HBini level ≤ 80 g/L could be used as independent prognostic factors for recurrent gastric cancer. More important, HBdec ≥ 30 g/L and treatment response also were significantly associated with OS and PFS. Besides, the degree of hemoglobin decrease was associated with chemotherapy include platinum, the number of chemotherapy cycles, treatment response rather than red blood cell (RBC) transfusion. Conclusion: Our study concludes that initially degree of anemia can serve as a biomarker of recurrent GC patients prognosis prediction, while chemotherapy treatment rather than RBC transfusion can improve those patient’s OS and PFS. Besides, paclitaxel should not be recommended to treat the recurrent GC patients.

Background
Gastric cancer (GC) is the fifth most common malignant tumour and the third leading cause of death worldwide (1). Recurrence and metastasis are the most important characteristics of cancers including GC (2, 3). The incidence of anaemia in advanced gastric cancer patients is high, with a large variability ranging from 10% to 30% (4, 5). Anaemia can weaken fragile patients and has been reported to be associated with a poor clinical outcome. However, the role of the degree of anaemia
and treatment mode in recurrent or metastatic GC patient prognosis is unclear. Therefore, managing and improving the condition of GC-related anaemia through medical approaches are urgently needed to improve the prognosis of patients with recurrent or metastatic GC.

Cancer-related anaemia (CRA) is considered to be associated with multiple pathological and clinical factors, such as bleeding, nutritional deficiency, and bone marrow suppression (6). Bone marrow suppression can be caused by both malignant cell infiltration and chemotherapy treatment (7, 8). Functional iron deficiency is usually associated with insufficient iron intake because of cancer-related appetite loss and bleeding (9, 10). At present, the treatments of anaemia and cancer are complementary. Under these circumstances, it is critical to identify the association of relevant elements, including clinicopathological characteristics and GC treatment model, with anaemia in recurrent or metastatic GC.

Our study aimed to determine the role of initial degree of anaemia and chemotherapy-related haemoglobin reduction in the prognosis of recurrent or metastatic GC patients. The relationships between clinicopathological characteristics, including treatment regimens, and chemotherapy-related haemoglobin reduction degree were further analysed. Our study will contribute to the determination of treatment approaches for recurrent or metastatic GC-related anaemia patients.

Methods

**Patients and enrolment**

All procedures followed were in accordance with the ethical standards of the ethical committee of Shandong Provincial Hospital regarding human experimentation and with the 1964 Helsinki Declaration and later versions. Informed consent for inclusion in the study was obtained from all patients.

Our retrospective study analysed the data collected from patients diagnosed with metastatic GC or recurrent GC at Shandong Provincial Hospital in China from January 1, 2010, to December 31, 2014. The entry criteria included the following: 1) metastatic GC or recurrent GC after radical surgical treatment was histologically confirmed as gastric adenocarcinoma. Radical gastric resection was defined as negative margins, en bloc resection of the greater and lesser omentum, and D2 lymph node
dissection, and standard lymphadenectomy was defined as when the number of retrieved lymph nodes was ≥15; 2) The Eastern Cooperative Oncology Group performance score (ECOG PS) was used to estimate a life expectancy of more than 3 months (11); and 3) patients had received at least one cycle of chemotherapy. The exclusion criteria included the following: 1) accompaniment by other types of malignancies, 2) use of neoadjuvant chemotherapy, and 3) loss to follow-up. All the pathologic specimens were reviewed by at least 2 pathologists to confirm the diagnosis of GC.

**Haemoglobin level measurement**

The initial haemoglobin level (HB\text{ini}) was collected at the initial diagnosis of recurrent or metastatic GC. The lowest haemoglobin level was determined as the lowest level obtained from the day of diagnosis to the date of death or the final follow-up visit. The decrease in haemoglobin (HB\text{dec}) was defined by subtracting the lowest haemoglobin level from the initial haemoglobin level. Evaluation and grading of anaemia were performed according to National Comprehensive Cancer Network (NCCN) guidelines for cancer- and chemotherapy-induced anaemia (12). The volume of transfused units in anaemia patients whose haemoglobin levels were less than 80 g/L was adopted.

**Chemotherapy regimens**

The regimens used to treat the patients included the combination chemotherapy of docetaxel, cisplatin, and 5-fluorouracil (DCF) and related modifications (docetaxel 75 mg/m\textsuperscript{2} on day 1, cisplatin 60 mg/m\textsuperscript{2} or oxaliplatin 130 mg/m\textsuperscript{2} on day 1, fluorouracil 2500 mg/m\textsuperscript{2} continuous infusion 120 hours, cycled every 21 days); XP or modifications (capecitabine 1000 mg/m\textsuperscript{2} twice daily (BID) on days 1-14, cisplatin 75 mg/m\textsuperscript{2} or oxaliplatin 130 mg/m\textsuperscript{2} on day 1); FOLFIRI (irinotecan 180 mg/m\textsuperscript{2} on day 1, leucovorin 400 mg/m\textsuperscript{2} on day 1, fluorouracil 400 mg/m\textsuperscript{2} IV push on day 1, fluorouracil 2400 mg/m\textsuperscript{2} continuous infusion 46 hours, cycled every 14 days); and single agents such as docetaxel 75-100 mg/m\textsuperscript{2} on day 1, capecitabine 1000-1250 mg/m\textsuperscript{2} BID on days 1-14, or S-1 80-120 mg on days 1-14, cycled every 21 days.

**Follow-up**

Tumour responses to the chemotherapy regimens were evaluated after every 2-3 cycles of
chemotherapy and categorized based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guidelines (13). Malignant ascites and peritoneal cytology were also considered when assessing the antitumour effects.

Overall survival (OS) was calculated as the time from the date of initial diagnosis of metastatic GC or the date of recurrence after GC resection to the date of either death or the final follow-up. Progression-free survival (PFS) was calculated as the date of either disease progression, confirmed by magnetic resonance imaging or computed tomography using a contrast medium if possible, or death from any cause.

Clinical variables for risk assessment consisted of patient demographics, surgical and pathological factors, chemotherapy regimens, and packed red cell transfusion. Data regarding recurrence, defined as disease recurrence at any site, and survival outcomes were also collected.

**Statistical analysis**

Survival analyses were performed by Kaplan-Meier curves with log-rank tests for significance. Univariable Cox regression analyses were performed using PFS, OS and HB\textsubscript{dec} as the outcomes, with a significance level of $p<0.05$. Multivariate analysis was carried out with a Cox proportional hazards model to evaluate prognostic factors with respect to PFS, OS and HB\textsubscript{dec}. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. A value of $p<0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS statistical software (Version 24.0; IBM Corporation, Armonk, NY, USA).

**Results**

**Patients**

Based on the inclusion and exclusion criteria, 598 patients were included in our study. Our study included 170 recurrent GC patients and 428 metastatic GC patients. The general characteristics of all enrolled patients are listed in Table 1. The age and gender proportions and surgical and pathological factors of the patient population were similar to those observed in other studies [4].

**Follow-up and survival**

Of the 598 GC patients, the median follow-up time was 11.60 months (range 0-76), and the median
OS after chemotherapy was 12 months (95% CI 11.221–12.779), with 1-, 3-, and 5-year OS rates of 45.40%, 3.80%, and 0.90%, respectively.

The 598 patients were divided into the HB_{ini} \leq 80 \text{ g/L} cohort and the HB_{ini} level > 80 \text{ g/L} cohort. Our study included 40 patients in the HB_{ini} \leq 80 \text{ g/L} cohort and 558 patients in the HB_{ini} level > 80 \text{ g/L} cohort.

For the HB_{ini} \leq 80 \text{ g/L} cohort, the median OS was 10 months with 1-, 3-, and 5-year survival rates of 35.40%, 0%, and 0%, respectively, while in the HB_{ini} level > 80 \text{ g/L} cohort, the median OS was 12 months with 1-, 3-, and 5-year survival rates of 46.10%, 4.10%, and 3.00%, respectively. The OS of the HB_{ini} \leq 80 \text{ g/L} cohort was significantly worse than that of the HB_{ini} level > 80 \text{ g/L} cohort (p=0.009, Table 2).

Kaplan-Meier analysis was also used to analyse the correlation between HB_{ini} level and PFS. Our results revealed that patients with HB_{ini} levels \leq 80 \text{ g/L} also had a trend toward a shortened median PFS (p=0.049, Table 2). Interestingly, we found that HB_{dec} \geq 30 \text{ g/L} was associated with a significantly shortened median PFS (p=0.001), and a similar relationship was found with decreased median OS (p=0.039, Table 2).

Red blood cell (RBC) transfusion is an important treatment modality, while chemotherapy is beneficial for improving the prognosis of recurrent and metastatic GC patients. Thus, we analysed the different treatment modalities and clinicopathological parameters for the OS and PFS in our patients.

Using univariate analysis, we found that RBC transfusion was associated with neither median OS nor median PFS (Table 2 and Table 3). The factors that significantly influenced OS were HB_{ini} level, HB_{ini} \leq 80 \text{ g/L}, metastatic sites \geq 3, liver metastases, bone metastases, paclitaxel-based combination of three regimens, the number of chemotherapy cycles, treatment response, and HB_{dec} \geq 30 \text{ g/L} (p<0.05). Additionally, HB_{ini} level, the lowest haemoglobin level, metastatic sites \geq 3, liver metastases, bone metastases, number of chemotherapy cycles, chemotherapy including paclitaxel, treatment response and HB_{dec} \geq 30 \text{ g/L} were significantly associated with PFS (p<0.05) (Table 3).
Multivariate analysis showed that HB_{ini} level ≤80 g/L (HR=1.879, 95% CI=1.301-2.767, p=0.001), liver metastases (HR=1.234, 95% CI=1.022-1.490, p=0.029), chemotherapy including paclitaxel (HR=1.225, 95% CI=1.013-1.481, p=0.036), treatment response (HR=1.457, 95% CI=1.173-1.808, p=0.001), and HB_{dec} ≥30 g/L (HR=1.536, 95% CI=1.206-1.957, p=0.001) were significant adverse prognosis factors of OS (Table 4). More importantly, the number of chemotherapy cycles was also significantly correlated with improved OS (HR=0.879, 95% CI=0.855-0.904, p <0.001) (Table 4).

For PFS, HB_{ini} level ≤80 g/L (HR=1.516, 95% CI=1.082-2.126, p=0.016), chemotherapy including paclitaxel (HR=1.273, 95% CI=1.068-1.517, p=0.007), treatment response (HR=2.235, 95% CI=1.818-2.747, p<0.001), the number of chemotherapy cycles (HR=0.922, 95% CI=0.899-0.945, p<0.001), and HB_{dec} ≥30 g/L (HR=1.543, 95% CI=1.233-1.932, p<0.001) were independent prognostic factors.

**Relationship between the degree of decrease in haemoglobin levels and the clinicopathological parameters of our patients**

We then investigated whether we could identify correlations between the degree of decrease in haemoglobin levels and the clinicopathological parameters of our GC patients. Our results suggested that bone metastases, chemotherapy including platinum, the number of chemotherapy cycles, and treatment response were associated with the degree of haemoglobin decrease (p<0.05) (Table 5). Multivariate analyses revealed that the number of chemotherapy cycles and chemotherapy including platinum were significantly correlated with improved HB_{dec} (p<0.001 and p=0.019, respectively) (Table 6).

**Discussion**

CRA occurs as a result of multiple aetiologies, including blood loss, functional iron deficiency, erythropoietin deficiency due to renal disease, chemotherapy-induced myelosuppression, marrow involvement with tumours and other factors. The relationship between anaemia and the prognosis of GC patients remains unclear. Several studies have focused on the role of anaemia in predicting the prognosis of GC patients treated with chemotherapy or surgical treatment. Zhang et al. reported that
patients with less than $≤65$ g/L haemoglobin had a significantly shorter median OS than patients with
65 g/L to normal haemoglobin or patients with normal haemoglobin and demonstrated that a lower
haemoglobin level might predict poorer OS in advanced GC patients (15). There is little information to
evaluate the effect of anaemia status and RBC transfusion treatment on the OS and PFS of recurrent
or metastatic GC patients.

According to the NCCN guidelines for cancer- and chemotherapy-induced anaemia, a haemoglobin
level $≤80$ g/L is used to define severe-grade anaemia. Our present study also chose a haemoglobin
level of 80 g/L as the cut-off value for severe anaemia. Our results revealed that pretreatment of
severe anaemia could serve as a prognostic factor in metastatic GC or recurrent GC patients who
underwent radical resection and were then treated with chemotherapy. Multivariate analysis also
showed that an initial haemoglobin level $≤80$ g/L was an independent adverse prognostic factor for
our patients. In addition, the degree of haemoglobin decrease during chemotherapy or the follow-up
period was also an important risk factor for the prognosis of recurrent or metastatic GC.

The cause of anaemia in patients with cancer is often multifactorial. The malignancy itself can lead to
or exacerbate anaemia, and underlying comorbidities may also contribute to anaemia. Cancer cells
can directly suppress haematopoiesis through bone marrow infiltration and produce cytokines,
leading to iron sequestration. Chronic blood loss, nutritional deficiencies, myelosuppressive effects of
chemotherapy, and radiation therapy to the skeleton can further exacerbate anaemia in patients with
cancer (6-10). Due to the potentially multifactorial complexity of anaemia, defining the causes of
anaemia in cancer patients is essential; this knowledge will contribute to determining the appropriate
treatment method to apply. Our present study revealed that chemotherapy including paclitaxel and
$\text{HB}_{\text{dec}} \geq 30$ g/L were independent adverse prognostic factors. Chemotherapy including platinum was
associated with a decrease in haemoglobin. Our results were similar to those of previous studies.

Groopman et al. reported that platinum-based regimens were well known to induce anaemia due to
the combined bone marrow and kidney toxicity, and the use of chemotherapy regimens including
paclitaxel was an adverse prognostic factor for the decrease in haemoglobin, although this effect was
not significant (16). Another article also showed a similar result, in that treatment with docetaxel as a
single agent could cause a progression in anaemia from grade III to IV in 9% of patients (17). Therefore, we consider that the improvement in anaemia may be one of the most important reasons for the improved prognosis of GC patients observed after chemotherapy treatment, and paclitaxel should not be recommended to treat recurrent or metastatic GC patients.

The most common treatment options for CRA include erythropoietic-stimulating agents, RBC transfusion and nutritional therapy, such as iron intake. Previous studies have reported that the lowest postoperative haemoglobin level and postoperative transfusion were the most significant risk factors for postoperative complications in GC surgery (18). Squires et al. reported that perioperative allogeneic blood transfusion was associated with decreased PFS and OS after resection of GC, independent of adverse clinicopathologic factors (19). In addition, RBC transfusion could not improve the chemotherapy outcomes by increasing the haemoglobin level (20). However, the role of RBC transfusion in improving the prognosis of recurrent or metastatic GC patients remains unclear. Our present data support the notion that transfusion neither significantly improved the OS and PFS nor served as a risk factor for PFS and OS in recurrent or metastatic GC. These results may be attributed to the fact that transfusion was used only when haemoglobin was not more than 80 g/L in our hospital. Insufficient blood transfusion may be another possible reason for this result.

Despite its several limitations, including being retrospective and having a small specific patient population size, our study has some advantages. First, a large range of clinical and pathological factors was comprehensively collected and compared. Second, this is the first study to analyse the effect of initial severe anaemia on the prognosis of recurrent or metastatic GC patients.

Conclusions
Our study demonstrated that the initial degree of anaemia can serve as a biomarker for predicting the prognosis of recurrent or metastatic GC patients, while chemotherapy treatment rather than RBC transfusion can improve OS and PFS. In addition, paclitaxel should not be recommended to treat severely anaemic GC patients.

Abbreviations
GC, Gastric cancer; CRA, Cancer-related anaemia; ECOG PS; The Eastern Cooperative Oncology Group
performance score; \( \text{HB}_{\text{ini}} \), the initial haemoglobin level; \( \text{HB}_{\text{dec}} \), the decrease in haemoglobin; NCCN, National Comprehensive Cancer Network; OS, Overall survival; PFS, Progression-free survival; HRs, Hazard ratios; Cis, confidence intervals.

Declarations

**Ethics approval and consent to participate**

All procedures followed were in accordance with the ethical standards of the ethical committee of Shandong Provincial Hospital regarding human experimentation and with the 1964 Helsinki Declaration and later versions. Informed consent for inclusion in the study was obtained from all patients.

**Consent for Publication**

Not applicable.

**Availability of data and material**

Yes.

**Competing interests**

The authors declare no conflict of interest. There are no financial and non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript.

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**Authors' contributions**

WH L and XX C conceived the study. WH L and XX C made substantial contributions to data acquisition, WH L, JY Z, WH L and XX C performed measurements, analysed the data and drafted the manuscript. All authors have read and approved the final manuscript.

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Tables

Table 1 Patients characteristics

|                      | Total N=598          |
|----------------------|----------------------|
| Age                  |                      |
| <65 years, N (%)     | 230(38.5)            |
| ≥65 years, N (%)     | 368(61.5)            |
| Gender               |                      |
| Male, N (%)          | 469(78.4)            |
| Female, N (%)        | 129(21.6)            |
| Palliative setting   |                      |
| Initially metastatic | 428(71.6)            |
| Recurrent            | 170(28.4)            |
| Operation method     |                      |
| Proximal gastrectomy | 60(35.3)             |
| Distal gastrectomy   | 83(48.8)             |
| Total gastrectomy    | 27(15.9)             |
| Pathological type    |                      |
| Well differentiated  | 4(0.7)               |
| Moderately differentiated | 59(9.8)       |
| Poorly differentiated | 250(41.8)            |
| Signet ring cell     | 61(10.2)             |
| Unassorted           | 224(37.5)            |
| Fecal occult blood#  |                      |
| Positive             | 129(33.9)            |
| Negative             | 361(74.7)            |
| Combination of three regimens | 217(36.3) |
| Treatment response   |                      |
| Partial response     | 14(2.3)              |
| Stable disease       | 246(41.1)            |
| Progressive disease  | 188(31.4)            |
| Not evaluable        | 150(25.1)            |
| Tumor location       |                      |
| Upper part (U)       | 253(42.3)            |
| Middle part (M)      | 91(15.2)             |
| Lower part (L)       | 206(34.4)            |
| ML                   | 29(4.8)              |
| MU                   | 19(3.2)              |
| T/N stage            |                      |
| Ia+Ib                | 4+7(6.5)             |
| Iia+Iib              | 8+15(13.5)           |
| Iii+Iiib+IIIc        | 34+46+56(80.0)       |
| Hemoglobin level (g/L) |        |
| Initial              |                      |
| >110                 | 399                   |
| 100-110              | 78                    |
| 80-100               | 82                    |
| 65-80                | 26                    |
| <65                  | 13                    |
| Post-treatment       |                      |
| Etiology of anemia   |                      |
| Fecal occult blood + | 33                    |
| Erosion and bleeding by endoscopy | 92                |
| Hematemesis          | 21                    |
| Iron deficiency anemia | 3                  |
| Cancer- and chemotherapy-induced anemia | 0    |
| Unknown              | 50                    |
| DIC                  | 0                     |
| Bone marrow infiltration | 0          |
| During treatment     |                      |
| Initial              | 33                    |
| Erosion and bleeding by endoscopy | 92     |
| Hematemesis          | 21                    |
| Iron deficiency anemia | 3          |
| Cancer- and chemotherapy-induced anemia | 0     |
| Unknown              | 50                    |
| DIC                  | 0                     |
| Bone marrow infiltration | 0      |
Table 2 Median OS and PFS

| Variable        | Median OS (m) | 95% CI       | p value | Median PFS (m) | 95% CI     | p value |
|-----------------|---------------|--------------|---------|---------------|------------|---------|
| HB<sub>ini</sub> ≤80 g/L | 10.0          | 6.147-13.853 | 0.009   | 5.0           | 3.038-6.962 | 0.049   |
| HB<sub>ini</sub> >80 g/L | 12.0          | 11.191-12.809| 7.0     | 3.038-6.962   | 0.049      |
| HB<sub>dec</sub> ≥30 g/L | 11.0          | 8.899-13.101 | 0.039   | 5.0           | 4.097-5.903 | 0.001   |
| HB<sub>dec</sub> <30 g/L | 12.0          | 11.151-12.849| 7.0     | 4.097-5.903   | 0.001      |
| Transfusion yes | 12.0          | 9.436-14.564 | 0.769   | 6.0           | 4.495-7.505 | 0.468   |
| Transfusion no  | 12.0          | 11.185-12.815| 7.0     | 6.266-7.734   | 0.468      |

Table 3 Univariate analyses of risk factors for OS and PFS,
|                  | N=598 | OS |                  |                  | PFS |                  |                  |
|------------------|-------|----|------------------|------------------|-----|------------------|------------------|
|                  |       |    | p value   | HR    | 95%CI  | p value   | HR    | 95%CI  |
| HB<sub>ini</sub> |       |    | 0.010     | 0.995  | 0.991-0.999 | 0.013     | 0.995  | 0.995-0.999 |
| HB<sub>lowest</sub> |       |    | 0.575     | 0.999  | 0.995-1.003 | 0.010     | 0.995  | 0.995-0.999 |
| HB<sub>ini</sub> ≤80 g/L | 40(6.7) |    | 0.012     | 1.608  | 1.109-2.332 | 0.065     | 1.371  | 1.9- |
| HB<sub>ini</sub> >80 g/L | 558(93.3) |    |           |        |        |           |        |        |
| Metastases       |       |    |           |        |        |           |        |        |
| Metastatic sites ≥3 |       |    | 0.033     | 1.268  | 1.020-1.577 | 0.015     | 1.289  | 1.0- |
| Metastatic sites <3 |       |    | 0.849     | 0.980  | 0.794-1.209 | 0.276     | 0.896  | 1.0- |
| Lymph node       |       |    | 0.010     | 1.271  | 1.059-1.525 | 0.001     | 1.354  | 1.1- |
| Lung             |       |    | 0.399     | 0.899  | 0.703-1.151 | 0.221     | 1.150  | 1.0- |
| Bone             |       |    | 0.072     | 1.495  | 0.964-2.318 | 0.017     | 1.651  | 1.0- |
| Chemotherapy regimen |       |    |           |        |        |           |        |        |
| Included paclitaxel |       |    | 0.116     | 1.160  | 0.964-1.397 | 0.018     | 1.232  | 1.0- |
| Included platinum |       |    | 0.290     | 0.849  | 0.626-1.150 | 0.734     | 0.985  | 0.7- |
| Number of cycles | <0.001 |    | 0.916     | 0.894-0.940 | 0.006 | 0.97 | 0.9- |
| Number of PTX3* | 0.023  |    | 0.937     | 0.885-0.991 | 0.940 | 1.002 | 0.9- |
| Treatment response |       |    |           |        |        |           |        |        |
| Progressive disease |       |    | 0.041     | 1.223  | 1.008-1.484 | <0.001   | 1.959  | 1.6- |
| Non-progressive disease |       |    |           |        |        |           |        |        |
| HB<sub>dec</sub> ≥30 |       |    | 0.048     | 1.244  | 1.002-1.546 | <0.001   | 1.594  | 1.3- |
| <30 Transfusion |       |    | 0.778     | 1.038  | 0.802-1.342 | 0.492     | 1.085  | 0.8- |
| No transfusion |       |    | 0.735     | 1.010  | 0.954-1.070 | 0.470     | 0.981  | 0.9- |
| Toxicity of chemotherapy |       |    |           |        |        |           |        |        |
| Degree I | 103 |    | 0.001     | 3.117  | 1.809-4.371 | 0.148     | 1.47  | 0.8- |
| Degree II | 137 |    | 0.075     | 1.371  | 0.911-2.943 | 0.598     | 1.157  | 0.3- |
| Degree III | 63  |    | 0.102     | 1.591  | 0.911-2.776 | 0.680     | 1.119  | 0.6- |
| Degree IV | 15  |    | 0.998     | 1.001  | 0.552-1.814 | 0.600     | 0.860  | 0.4- |

*PTX3 paclitaxel-based combination of three regimens

Table 4 Multivariate analyses of risk factors for OS and PFS
|                      | OS       | PFS      |
|----------------------|----------|----------|
|                      | p value  | HR       | 95%CI   | p value  | HR       | 95%CI   |
| HB_{ini} ≤80 g/L     | 0.001    | 1.879    | 1.301-2.767 | 0.016    | 1.516    | 1.082-2.126 |
| metastatic sites ≥3  | 0.063    | 1.246    | 0.989-1.572 | 0.823    | 1.026    | 0.821-1.281 |
| Liver metastases     | 0.029    | 1.234    | 1.022-1.490 | 0.057    | 1.188    | 0.885-1.420 |
| Bone metastases      | 0.269    | 1.293    | 0.820-2.040 | 0.685    | 1.094    | 0.709-1.689 |
| Chemotherapy included paclitaxel | 0.036    | 1.225    | 1.013-1.481 | 0.007    | 1.273    | 1.068-1.517 |
| Number of cycles     | <0.001   | 0.879    | 0.855-0.945 | <0.001   | 0.922    | 0.899-1.151 |
| Treatment response   | 0.001    | 1.457    | 1.173-1.808 | <0.001   | 2.235    | 1.818-2.747 |
| HB_{dec} ≥30 g/L     | 0.001    | 1.536    | 1.206-1.957 | <0.001   | 1.543    | 1.233-1.932 |

Table 5 Univariate analyses of risk factors for HB_{dec}

| Variable            | p value | HR       | 95%CI   |
|---------------------|---------|----------|---------|
| Metastases          |         |          |         |
| metastatic sites ≥3 | 0.141   | 0.838    | 0.663-1.060 |
| metastatic sites <3 |         |          |         |
| Metastatic site     |         |          |         |
| Lymph node          | 0.325   | 1.131    | 0.885-1.444 |
| Liver               | 0.328   | 1.107    | 0.903-1.358 |
| Lung                | 0.936   | 0.989    | 0.763-1.283 |
| Bone                | 0.017   | 0.574    | 0.365-0.905 |
| Chemotherapy regimen|         |          |         |
| Included paclitaxel | 0.876   | 0.984    | 0.802-1.207 |
| Included platinum   | 0.010   | 0.645    | 0.463-0.899 |
| Number of cycles    | <0.001  | 0.933    | 0.907-0.961 |
| Number of PTX3*     | 0.161   | 0.96     | 0.908-1.016 |
| Treatment response  |         |          |         |
| Progressive disease | 0.037   | 0.798    | 0.646-0.986 |
| Non-progressive disease |       |         |         |

Table 6 Multivariate analyses of risk factors for HB_{dec}

| Variable                                | p value | HR       | 95% CI   |
|-----------------------------------------|---------|----------|----------|
| Chemotherapy included platinum          | 0.019   | 0.661    | 0.468-0.934 |
| Metastatic sites ≥3                     | 0.371   | 0.895    | 0.702-1.141 |
| Bone metastases                         | 0.055   | 0.633    | 0.396-1.010 |
| Chemotherapy included paclitaxel        | 0.061   | 1.226    | 0.991-1.517 |
| Number of chemotherapy cycles           | <0.001  | 0.938    | 0.911-0.966 |
| Liver metastases                        | 0.060   | 1.227    | 0.991-1.520 |
| Treatment response                      | 0.111   | 0.833    | 0.665-1.043 |