# Can red cell distribution width and hemoglobin level variations be utilized to predict the prognosis of Stage III operative colorectal cancer patients with adjuvant chemotherapy treatment?

**Jie Li**
Sichuan Cancer Hospital and Research Institute https://orcid.org/0000-0003-4043-640X
**Yuan-Yi Rui**
Sichuan Cancer Hospital and Research Institute
**Bo Song**
Sichuan Cancer Hospital and Research Institute
**Ke Zhang**
Sichuan Cancer Hospital and Research Institute
**Bo Yi**
Sichuan Cancer Hospital and Research Institute
**Ying Zhou**
Sichuan Cancer Hospital and Research Institute
**Yang-Chun Zheng** ( dr_chunge@163.com )

**Research article**

**Keywords:** red cell distribution width, hemoglobin level variations, prognosis, Stage III operative colorectal cancer, adjuvant chemotherapy treatment

**Posted Date:** February 4th, 2020

**DOI:** https://doi.org/10.21203/rs.2.22551/v1

## Abstract

Background: The aim of this study was to find that if the red cell distribution width (RDW) or hemoglobin (Hb) level variations had prognostic value in stage III colorectal cancer patients treated with operation and adjuvant chemotherapy. Methods: One hundred and twenty-two patients were included in this retrospective study. All were diagnosed and re-staged as stage III colorectal cancer in Sichuan Cancer Hospital according to the AJCC Cancer Staging Manual, 8 th edition, 2018. The patients received R0 resection before adjuvant chemotherapy. The baseline information, routine blood examination data, pathological outcome and prognostic stature was retracted from the database. Receiver operating characteristic (ROC) curve analysis was utilized to determine the cut-off value, while the survival analyses were performed with Kaplan-Meier curve, the log-rank test and the Cox regression analysis. Results: The chemotherapy-associated hemoglobin change (change between the pre- and post-chemotherapy hemoglobin levels) was identified to be associated with the metastasis (P=0.030). The optimal cut-off point was calculated to be -9.5 by the ROC curve of the hemoglobin change, while the area under the curve was 0.648 (95% CI: 0.524-0.772). The results showed that patients with larger hemoglobin decrease had significantly worse disease free survival (DFS) than those with smaller decrease (P=0.020). Factors associated with DFS in uni-variate COX regression analysis were the number of harvested lymph nodes (P=0.040) and the perineural invasion (P=0.020). The peri-chemotherapy change of hemoglobin level was estimated to have significant effect on patient survival (P=0.010). Conclusions: We concluded that chemotherapy-associated Hb change (change between the pre- and post-chemotherapy) was a DFS prognostic factor for the stage III colorectal cancer patients who underwent operation and adjuvant chemotherapy.


## Background

Operation and adjuvant chemotherapy is the standard treatment for operative stage III patients[1, 2]. However, there are limited prognostic factors can be utilized to predict the survival of those patients, despite of traditional pathological sub-stage and indications of poorer prognosis. Bio-markers, such as ras or B-raf mutation,micro-satellite instability etc., have been recommended as effective evidence, while there are few publications confirmed their predictive capability when taking chemotherapy in consideration.

Red blood cell volume distribution width (RDW), which is the quantitative measurement of heterogeneity of the red blood cell size, can reflect impaired erythropoiesis and abnormal red blood cell survival. Although the early studies focused on the correlation between RDW and benign and chronic disease, recent publications have indicated its potential utilization in malignant tumors. Seretis [3] reported that RDW was related to the metastasis of breast cancer, and Lippi [4] concluded that RDW value was related to the stage of lung cancer. The information suggested the RDW might be utilized as prognostic factor, since the relationship between RDW and clinical characteristic of malignancies.

Several publications indicated certain values of hemoglobin or RDW in colorectal cancers. Yang [5] found that red blood cell distribution width was closely related to colorectal cancer metastasis. Wei [6] reported that chemotherapy-associated hemoglobin might be a prognostic bio-marker. Zhang [7] claimed that elevated red blood cell volume distribution width- the change of diversity (RDW-CV) might be an independent predictive factor in resectable rectal cancer patients. However, few articles focused on the change of red blood cell index and it's relationship with survival in those stage III patients who received surgery and adjuvant chemo-treatment.

## Methods

# Patients

We retrospectively reviewed recorded database of patients with stage III colorectal cancer(CRC) who underwent surgery and adjuvant chemotherapy between April 2014 and January 2017 at Gastrointestinal Surgery Center, Sichuan Cancer Hospital. Pathological stage was determined and revised following the AJCC Cancer Staging Manual, 8th edition, 2018. The inclusion criteria were: colorectal adenocarcinoma confirmed by historical biopsy, undergoing radical resection and adjuvant chemotherapy. The exclusion criteria were: 1. Stage I, II or IV colorectal cancer, 2. Any systemic treatment utilized before surgery, 3. R1 or R2 resection, 4. Combined with severe infection, 5. Anemia induced by other diseases, such as chronic renal diseases or hematologic diseases. Baseline index, such as gender, age, operation time was collected, while blood transfusion, carcinoembryonie antigen(CEA), carbohydrate antigen 125(CA-125), carbohydrate antigen 199(CA199), TNM stage, AJCC stage, harvested lymph node number, differentiation, perineural invasion, vascular invasion and intestinal obstruction was retrieved, too. Hemoglobin, RDW-CV as well as RDW-SD (red blood cell volume distribution width- standard deviation), with four groups data included pre- and post-operation, and pre- and post-chemotherapy for each index, were also collected. The data of pre-operative timing was determined as the day before surgery while post-operative was 48–72 hours

after surgery. The data of the pre-chemotherapy was also determined as the day before the first chemotherapy while post-chemotherapy was the day after final treatment.

## Follow-up

Follow-up was performed every 3 months for the first 2 yeas, every 6 months in the next 3 yeas. The end-up time of follow-up was March 2019. The routine laboratory and radiological examinations included physical examination, blood test, serum tumor markers, Computed Tomography(CT) of chest and enhanced abdominal and pelvic CT or nuclear magnetic resonance (MRI, every 6 months within the first 2 years and every 12 months after 2 years ) and colonoscopy (every 2 years). Local recurrence was defined as the recurrent disease in the pelvis or at the incision, whereas the distant recurrence was defined as the recurrence beyond the above parts.

## Statistical analysis

Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value for the retrieved index changes. The disease-free survival (DFS) was analyzed by using of Kaplan-Meier survival curve and the log-rank test. Multivariate analysis was performed using COX proportional hazards regression. And statistical analyses were performed using the SPSS software version 17.0 (SPSS, Inc, Chicago, IL). $P < 0.05$ was considered statistically significant.

# Results

## Characteristics of the study population

In this study, one hundred and twenty two CRC stage III patients' information was gathered from a prospectively collected database. All patients received R0 operation from April 2014 to January 2017, followed by adjuvant chemotherapy. There were 69 (56.6%) males and 53 (43.4%) females with a median follow-up time of 42 months (12 to 59 months). The mean age ± standard deviation (SD) of patients at the time of operation was 54.75±11.28 years. The patients' demographic information is presented(Table 1).

## Changes in red cell distribution width and Hb values during therapy

The baseline Hb level (Hb before operation) was 125.84±23.11 g/L. At 48 hours after surgery, the patients' Hb levels was 113.6±21.19 g/L. The pre- and post-chemotherapy Hb levels were 116.49±16.49 g/L and 124.91±13.74 g/L, respectively. Using the same methods, we also analyzed the value of RDW-CV, RDW-SD and the Hb changes. Only 8 of 122 patients received intra-operative blood transfusion. Only twenty-one (17.2%) patients completed the chemotherapy, while the others could not tolerate the whole treatment or suffered tumor recurrence. Majority of patients (89.3%) received oxaliplatine in combine with fluorouracil and calcium folinate (mFOLFOX6). The RDW-CV, RDW-SD and Hb levels were presented (Table 2).

## Correlation between therapy-associated hemoglobin change and tumor progression

ROC curve analysis was performed for the purpose of finding an optimal cut-off for separation of patients into two groups regarding Hb changes values. According to the cut-off value, patients were divided into low group and high group. For the value of Hb changes (pre- and post-chemotherapy ), the cut-off was set at -9.50, area under the curve (AUC) was 0.648 (P=0.030) in Fig.1. For the value of Hb changes (pre-operation and post-chemotherapy), the cut-off was set at 6.50, and AUC was 0.794 (P=0.026) in Fig.2.

The median follow-up was 42 months (from 12 to 59 months). Patients with smaller Hb changes (pre-operation and post-chemotherapy) had superior DFS in comparison to those with larger Hb changes after the whole treatment (pre-operation and post-chemotherapy) (P=0.014) in Fig.3. Patients with larger Hb changes (pre-and post-chemotherapy) showed worse DFS in comparison to those with smaller Hb changes (pre- and post-chemotherapy) (P=0.020) in Fig.4.

In COX regression multivariate analysis, the Hb changes (pre- and post-chemotherapy) remained as a statistically significant risk factor for the tumor metastasis. The higher Hb changes (pre- and post-chemotherapy) was associated with worse DFS (P=0.010). However, there was no correlation between Hb changes (pre-operation and post-chemotherapy) and the tumor recurrence. The results of the

statistical calculations on COX regression multivariate analysis was presented (Table 3). In addition, the harvested lymph nodes number (P=0.040) and the perineural invasion (P=0.020) were significant prognostic factor for the DFS.

## Discussion

Anemia is a common comorbidity in CRC patients, while Hb level changes can be observed in those who received surgery and adjuvant chemotherapy. In our study, we find that Hb level changes, especially the changes after the whole treatment, may be related to the prognosis of stage III CRC patients. After Cox multivariate analysis, the change between pre- and post- adjuvant chemotherapy remains as an individual risk factor for locally advanced CRC patients. The analysis also confirms the harvested lymph nodes number and the perineural invasion are independent prognostic factors, which indicated the accuracy of the study.

Amount of studies reported the level of Hb is related to the survival of malignant tumor patients. However, most of the studies focused on the preoperative anemia and its relationship with prognosis [8-10]. Current studies indicate that pre-operative and post-operative Hb levels are related to the survival of malignant patients received radical resection [11]. The potential mechanism is estimated to be the relation between low Hb levels and hypoxia status of malignant tissues and the matrix [12]. Hypoxia sensitive gene, such as hypoxia-induced factors (HIFs) , Snail gene, FRFG3 and OCT4 may be involved in the mechanism [13-16]. However, tumor induced anemia, caused by chronic blood loss, should be regarded as a significant influence on the variation of Hb levels, which can be intervened by surgery and adjuvant chemotherapy.

Wei also reported that Hb changes after adjuvant chemotherapy is related to the prognosis of CRC patients [17]. The point is stage II and III patients are all confounded in that study and receive FOLFOX regimen treatment, while IDEAL study suggests only stage II CRC patients with high risks are recommended to be adjuvantly treated. The study focused on proving chemotherapy indicated anemia is a significant prognostic factor.

Above all, our current study indicates the prognostic significance of Hb change during the treatment in stage III CRC patients. Hb test (blood count) is a non-invasive and mature routine blood test, which is economical, effective, simple and convenient. It is also universally accepted as routine test for post-operative monitoring. Therefore, Hb test has the potential of being predictor of survival for locally advanced patients who receiving adjuvant treatment. However, further prospective studies, especially studies with interventions on Hb level, are needed to confirm the accuracy and effectiveness of the index.

During our research periodicity, no control study with intervention, such as ferralia supplement treatment, was reported. We expect related large-sized prospective studies will reveal whether this potential trend can be weakened or even reversed.

## Conclusions

Hemoglobin level changes between before initiation of adjuvant chemotherapy and after the whole treatment may be a potential predictor of survival in stage III CRC patients.

## Abbreviations

RDW: Red cell distribution width; Hb: Hemoglobin; AJCC: American Joint Committee on Cancer; ROC: Receiver operating characteristics; CI: Confidence interval; DFS: Disease free survival; RDW-CV: red blood cell volume distribution width- the change of diversity; RDW-SD: red blood cell volume distribution width- standard deviation; CRC: Colorectal cancer; CEA: carcinoembryonie antigen; CA-125: carbohydrate antigen 125; CA-199: carbohydrate antigen 199; CT: Computed Tomography; MRI: nuclear magnetic resonance; SD: Standard deviation; mFOLFOX: means chemotherapy for 6 months using capecitabine, fluorouracil and calcium folate; CAPEOX: means capecitabine and oxaliplatin; AUC: Area under the curve; HIFs: Hypoxia-induced factors; FRFG3: the name of one gene; OCT4: the name of one gene; IDEAL: the name of one study; SPSS: Statistical Product and Service Solutions.

## Declarations

**Ethics approval and consent to participate**

This study was conducted in accordance with the declaration of Helsinki. We've got verbal consent from all individuals before starting any study procedure. And we've got the ethics approval named Sichuan Cancer Hospital Ethics Committee Approval for Clinical Research Protocol of this study. The approval date was 6 March 2019 and the principal investigator is Jie Li. The name of the ethics committee that approved the study is "Ethics Committee of Sichuan Cancer Hospital". The completing approval comments is: The ethics committee reviewed and discussed this clinical research accordance with the ICH-GCP principle and related regulations/guidelines strictly. The committee agreed to carry out this study. And the author can provide the picture of the "Ethics Approval" at any time if necessary. All treatment methods and data are necessary for patients during the whole treatment process, and our research does not increase the additional burden or pressure of patients. Despite the retrospective study of patient data, there was no substantial injury or risk to the patient. So the verbal-only consent to participate was taken, and the ethics committee approved this.

### Consent for publication

Not Applicable.

### Availability of data and materials

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

### Competing interests

None of the authors have any competing interests.


### Funding

This study was supported by two projects. One is research programme of Sichuan Health and Family Planning Commission (18PJ499 to Yuan-Yi Rui) and another one is Special plan project of Sichuan Youth Scientific and technological innovation research team(2017TD0029 to Yang-Chun Zheng).


### Authors' contributions

Conceptualization: YCZ.

Data curation: JL,YYR.

Funding acquisition: JL,YYR, BS, KZ, BY.

Methodology: JL, YYR,YCZ, YZ.

Supervision:YCZ.

Writing-original draft: JL,YYR.

Writing-review& editing: YCZ, YYR.

YCZ, JL,YYR, BS, KZ, BY,YZ agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. And all authors have read and approved the manuscript.


### Acknowledgements

Not Applicable.



### Authors' information

**1** Clinical Medical College, Chengdu Medical College, Chengdu, Sichuan Province, People's Republic of China, **2** Department of Gastrointestinal Surgery, Sichuan Cancer Hospital, Chengdu, Sichuan Province, China


## References

1. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. NCCN Clinical Practice Guidelines in Oncology:Colon Cancer,Version 2.2019.
2. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. NCCN Clinical Practice Guidelines in Oncology:Rectal Cancer,Version 2.2019.
3. Seretis C, Seretis F, Lagoudianakis E, Gemenetzis G, Salemis NS.
Is red cell distribution width a novel biomarker of breast cancer activity? Datafrom a pilot study. J Clin Med Res.2013 Apr;5(2):121-6. doi: 10.4021/jocmr1214w. Epub 2013 Feb 25.
4. Lippi G, Plebani M. Red blood cell distribution width (RDW) and human pathology. One size fits all. Clin Chem Lab Med.2014 Sep;52(9):1247-9. doi: 10.1515/cclm-2014-0585.
5. Yang DY, Quan WQ, Wu JL, Ji XY, Dai Y, Xiao WD, et al. The value of red blood cell distribution width in diagnosis of patients with colorectal cancer. Clin Chim Acta.2018 Apr;479:98-102. doi: 10.1016/j.cca.2018.01.022. Epub 2018 Feb 2
6. Wei YS, Zhou YG, Wang GY, Liang ZH, Luo MR, Yang TA, et al. The impact of chemotherapy-associated hemoglobin on prognosis of colorectal cancer patients receiving adjuvant chemotherapy. Cancer 2017 Dec 6;20(4):627-635. doi: 10.3233/CBM-170601
7. Zhang XB, Wu QB, Hu T, Gu CY, Bi L, Wang ZQ. Elevated red blood cell distribution width contributes to poor prognosis in patients undergoing resection for nonmetastatic rectal cancer. Medicine (Baltimore).2018 Jan;97(3):e9641. doi: 10.1097/MD.0000000000009641
8. Njoistad TS, Engerud H, Werner HM, Salvesen HB, Trovik J. Preoperative anemia,leukocytosis and thrombocytosis indentify aggressive endometrial carcinomas. Gynecol Oncol.2013 Nov;131(2):410-5. doi: 10.1016/j.ygyno.2013.08.032. Epub 2013 Sep 1
9. Rink M, Sharifi N, Fritsche HM, Aziz A, Miller F, Kluth LA, et al.
Impact of preoperative anemia on oncologic outcomes of upper tract urothelialcarcinoma treated with radical nephroureterectomy. J Urol.2014 Feb;191(2):316-22. doi: 10.1016/j.juro.2013.09.010. Epub 2013 Sep 12
10. Hamai Y, Hihara J, Emi M, Taomoto J, Aoki Y, Kishimoto I, et al.
Treatment outcomes and prognostic factors for thoracic esophageal cancerwith clinical evidence of adjacent organ invasion. Anticancer Res.2013 Aug;33(8):3495-502
11. Reichel O, Panzer M, Wimmer C, Dühmke E, Kastenbauer E, Suckfüll M. Prognostic implications of hemoglobin levels before and after surgery as wellas before and after radiochemotherapy for head and neck tumors. Eur Arch Otorhinolaryngol.2003 May;260(5):248-53.
12. Becker A, Stadler P, Lavey RS, Hänsgen G, Kuhnt T, Lautenschläger C, et al.
Severe anemia is associated with poor tumor oxygenation in head and necksquamous cell carcinomas. Int J Radiat Oncol Biol Phys.2000 Jan 15;46(2):459-66.
13. De Francesco EM, Lappano R, Santolla MF, Marsico S, Caruso A, Maggiolini M. HIF-1α/GPER signaling mediates the expression of VEGF induced by hypoxia in breast cancer associated fibroblasts (CAFs). Breast Cancer Res.2013;15(4):R64.
14. Liu S1, Kumar SM, Martin JS, Yang R, Xu X. Snail1 mediates hypoxia-induced melanoma progression. Am J Pathol. 2011 Dec;179(6):3020-31. doi: 10.1016/j.ajpath.2011.08.038. Epub 2011 Oct 1.
15. Blick C1, Ramachandran A, Wigfield S, McCormick R, Jubb A, Buffa FM, et al. Hypoxia regulates FGFR3 expression via HIF-1α and miR-100 and contributesto cell survival in non-muscle invasive bladder cancer. Br J 2013 Jul 9;109(1):50-9. doi: 10.1038/bjc.2013.240. Epub 2013 Jun 18.
16. Wu YC1, Ling TY, Lu SH, Kuo HC, Ho HN, Yeh SD, et al.
Chemotherapeutic sensitivity of testicular germ cell tumors under hypoxicconditions is negatively regulated by SENP1-controlled sumoylation of OCT4. Cancer Res. 2012 Oct 1;72(19):4963-73. doi: 10.1158/0008-5472.CAN-12-0673. Epub 2012 Sep 20.
17. Wei YS, Zhou YG, Wang GY, Liang ZH, Luo MR, Yang TA, et al. The impact of chemotherapy-associated hemoglobin on prognosis of colorectalcancer patients receiving adjuvant chemotherapy.Cancer 2017 Dec 6;20(4):627-635. doi: 10.3233/CBM-170601.

## Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

# Figures

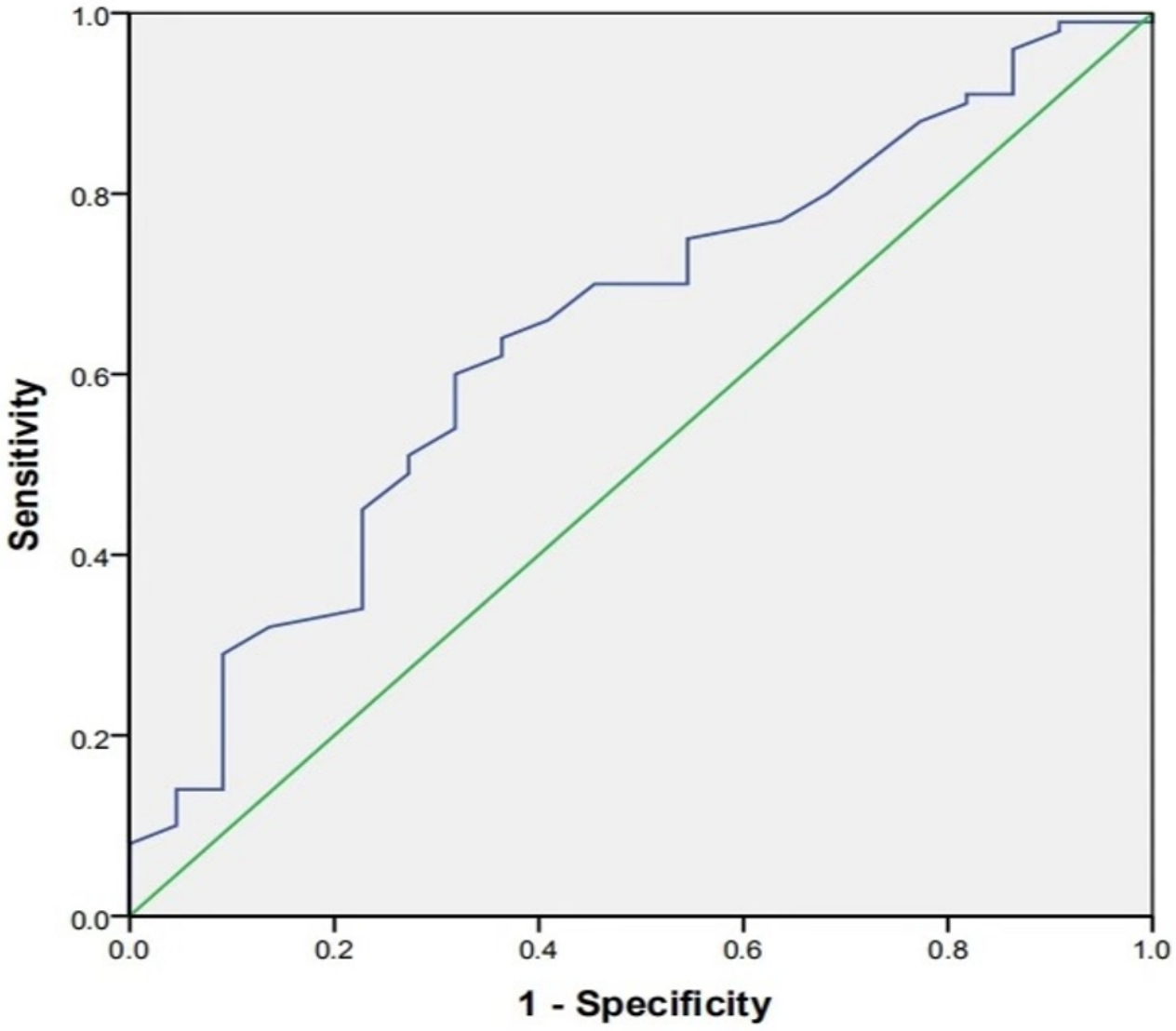


**Figure 1**

ROC curve for Hb changes (pre- and post-chemotherapy ).

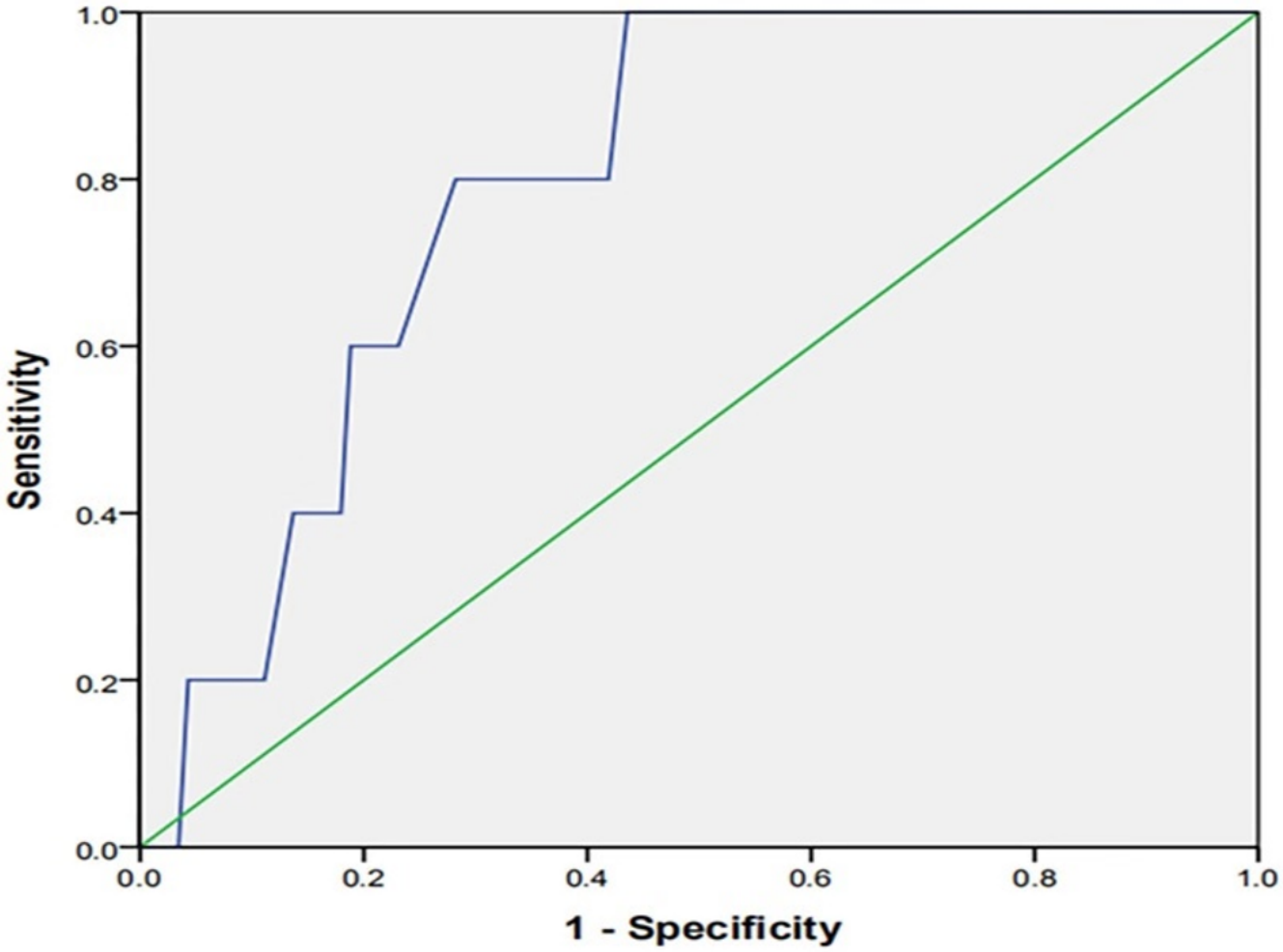


**Figure 2**

ROC curve for Hb changes (pre-operation and post-chemotherapy).

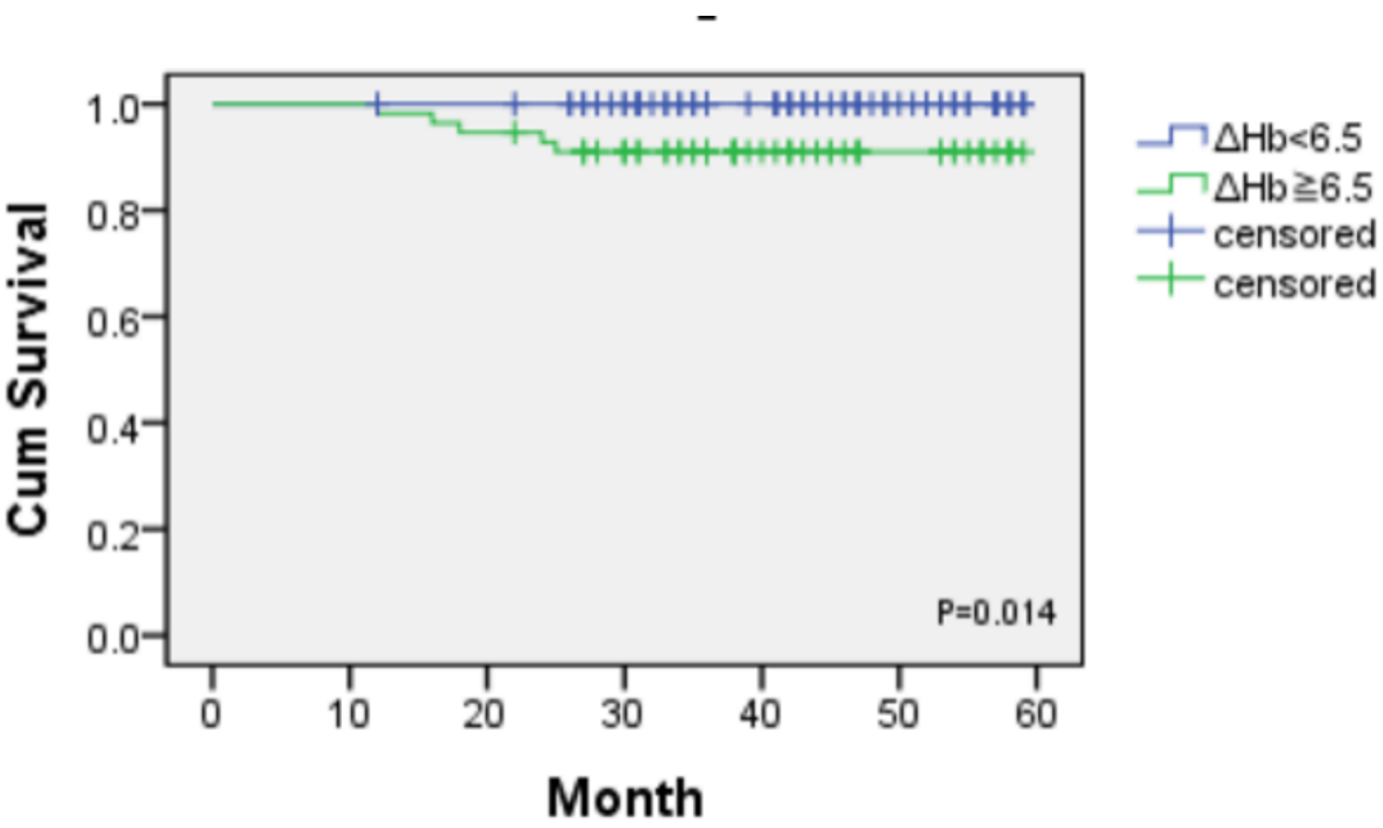


**Figure 3**

Smaller Hb changes (pre-operation and post-chemotherapy) are associated with superior DFS in patients with colorectal cancer stage III (P=0.014).

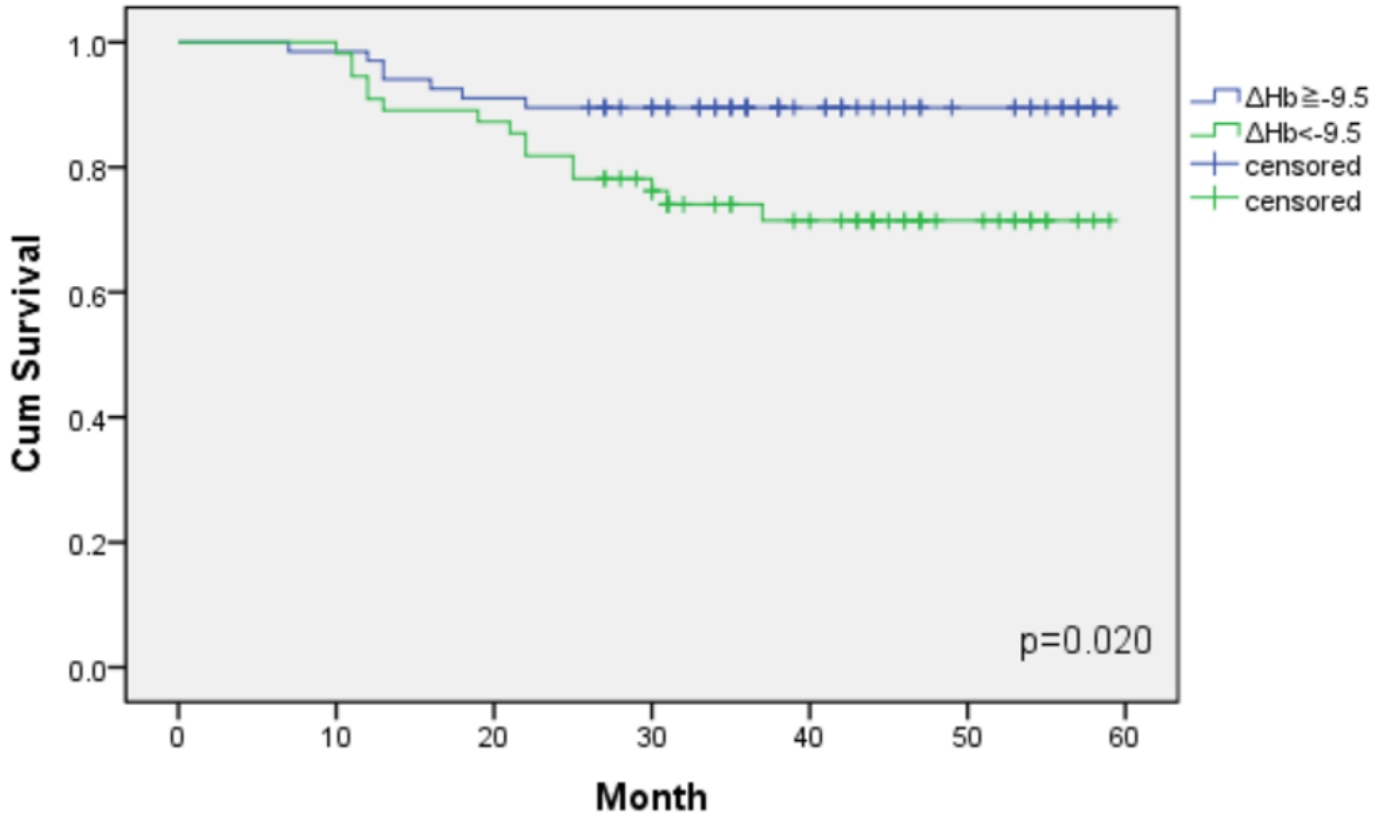

**Figure 4**

High Hb changes (pre-and post-chemotherapy) are associated with superior DFS in patients with colorectal cancer stage III (P=0.020).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf