Analysis of Characteristics and Causes of Anemia in 46 Severe and Critically Severe COVID-19 Patients

WU Yu Han1*, Meng Shan Shan1, Che Chun Li1#
The First Affiliated Hospital of Harbin Medical University
Che Chun Li (zhanglijuan18sui@163.com)
The First Affiliated Hospital of Harbin Medical University
Meng Shan Shan
The First Affiliated Hospital of Harbin Medical University

Research Article

Keywords: COVID-19, autoimmune hemolytic anemia (AIHA), Arbidol

Posted Date: September 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-71864/v1

Abstract

Background By analyzing characteristics and causes of anemia in 46 severe and critically severe COVID-19 patients that we treated, we aim to provide information on pathogenesis and the treatment method of COVID-19.

Methods: A retrospective analysis was performed on clinical data and laboratory findings of patients confirmed with severe and critically severe COVID-19. They were treated in Severe COVID-19 Treatment Center in Heilongjiang during the period of February 12, 2020 to March 19, 2020. We clarified the type of anemia and explored the relationship between novel coronavirus infection, therapeutic drugs and anemia.

Results: Among the 50 COVID-19 patients, 46 patients suffered from normocytic autoimmune hemolytic anemia. The occurrence and severity of anemia was irrelated to changes in titer of novel coronavirus IgM and IgG antibodies (r=0.071, *P*=0.897; r =0.41, *P*=0.361). The changes of the average Hb concentration and the changes of average drug concentration of patients with anemia showed that anemia had a significant negative correlation with Arbidol (r=- 0.758, *P*=0.029), but no significant correlation with Ribavirin, Interferon α-2b and magnesium isoglycyrrhizinate(*P* 0.05).

Conclusion: Severe and critically severe COVID-19 patients are more likely to have AIHA. Arbidol is significantly associated with AIHA in severe and critically severe COVID-19 patients.

Background

COVID-19 (Corona Virus Disease 2019, COVID-19) is an acute respiratory infectious disease caused by a novel coronavirus. It is characterized by rapid transmission, rapid disease progression and high mortality. At present, it has spread all over the world, with a total of more than 2 msevereion people infected, and it seriously endangers human life.

In the course of treating severe and critically severe COVID-19 patients in the Severe COVID-19 Treatment Center in Heilongjiang from February, 2020 to March, 2020, we found that 46 of the 50 patients showed a decrease in Hb.To determine the cause of anemia, we analyzed possible causes and pathogenesis, which are reported below.

1. **General Data**

After the approval of the ethics committee of our hospital (ethics no. 2020xs04-02), we collected the clinical data of COVID-19 patients treated in the Severe COVID-19 Treatment Center in Heilongjiang Province from February 12, 2020 to March 19, 2020. The diagnostic criteria and severity of COVID-19 were based on the diagnosis and treatment guidelines (Version 7) issued by the Chinese National Health Commission on April 8, 2020 [1]. Patients with a history of anemic disease or severe hemorrhagic disease were excluded from this study.

Methods

2.1 We obtained clinical data of those patients, which included basic diseases, the date of disease onset, hospital admission and discharge date, hospitalization time, the severity of COVID-19, therapeutic drugs and complications. Laboratory tests were also recorded, including routine blood, APTT, PT, C-reactive protein (CRP), PCT, serum iron, folic acid, vitamin B12, percentage of reticulocytes and Coombs tests. The onset date was defined as the day when any symptoms were noticed by the patients. The total hospitalization time(N) means that the hospitalization time spent in the previous hospital (N_1) plus the hospitalization time in our center (N_2).

2.2 Calculation of average Hb concentration, average IgM and IgG antibody concentration and average drug concentration: N_2 represents the hospitalization time in our center. We collected laboratory tests (including Hb, red blood cell count, MCV, MCH, MCHC, serum IgM and IgG antibodies) and duration and dosage of drugs (including RBV, IFN, Arbidol and magnesium isoglycyrrhizinate) of COVID-19 patients at different N_2 (N_2 = 0/1/3/5/7/9/11/13 day).

2.2.1 Average Hb concentration was defined as the sum of Hb of all patients tested on the same hospitalization date divided by 46 (46 patients).

2.2.2 Average IgM and IgG antibody concentrations were defined as the sum of IgM or IgG of all patients tested on the same hospitalization date divided by 46 (46 patients).

2.2.3 Average drug concentration = (the sum dose of a drug that patients used on the same hospitalization date + the amount of drug remaining in the body after the elimination of half-life of the drug on the last day) / the number of patients who used the drug on the same hospitalization date. According to the half-life of these drugs ($t_{½}$), after 1 $t_{½}$, the total dose was eliminated by 50%; after 2 $t_{½}$, the total dose was eliminated by 75%; after 5 $t_{½}$, the drug was basically eliminated in vivo. The $t_{½}$ of Arbidol was 10.55±4.01 hours [2], the $t_{½}$ of IFN was 4 to 12 hours[3], and the $t_{½}$ of magnesium isoglycyrrhizinate was 23 ~ 24 hours [4]. Thus, the effect of drug accumulation should be considered for the above three drugs. As the $t_{½}$ of RBV was short(2~4 hours)[5], it is unnecessary to consider the effect of the drug accumulation. According to the above formula, the average drug concentration of RBV remained unchanged. To determine the effect of RBV on anemia, we divided anemia patients into an RBV group (19 cases used RBV) and non-RBV group (27 cases did not use RBV), and compared the average Hb concentration on the different hospitalization times between the two groups. Both groups were treated with IFN plus Arbidol for antiviral therapy.

2.3 Study end point: the patient was discharged or died.

3. **Statistical Analysis**

All statistical analysis was performed using the SPSS 21.0 software package, and the tables are all standard three-line statistical tables. The ranked data are described in terms of frequency and

percentage. The independent sample t test, analysis of variance and rank sum test were used to compare the data between groups. The variable correlation relation adopts linear correlation. $P<0.05$ was considered statistically significant.

Results

4.1 We collected a total of 50 COVID-19 patients, and 46 patients developed anemia. Among the anemia patients, there were 24 (52%) males and 22 (48%) females, with an age range of 23-87 years (mean 64.74±13.2). There were 32 severe cases and 14 critical severe cases. The average date of disease onset was 11 days. The average hospitalization time spent in the previous hospital (N_1) was 5.5 days and the average hospitalization time in our center was 22 days by the end of the study. The number of patients with different underlying diseases (N0, %): diabetes (7/46,15%), chronic cardiovascular diseases (10/46,22%), chronic pulmonary diseases (5/46,11%), malignant tumor (1/46,2%), mental diseases(2/46,4%), hypertension(12/46,26%), hyperthyroidism(1/46,2%), cerebral infarction(3/46,6%), intestinal diseases(2/46,4%), and pleural diseases(2/46,4%). The number of patients with different comorbidities related to COVID-19(N0, %): pulmonary hypertension (2/46,4%), pneumothorax (2/46,4%), pleural effusion (8/46,17%), emphysema (3/46,6%), ARDS (4/46,8%). The number of patients with different comorbidities developed during COVID-19 (N0, %): deep vein thrombosis (2/46,4%), liver injury (3/46,6%), arrhythmia (1/46,2%), myocardial injury (2/46,4%), and cardiac insufficiency (1/46,2%). Therapeutic drugs for COVID-19: (1) antiviral drugs: IFN α-2b (3 msevereion IU, Bid), RBV (500mg, Bid), Arbidol (0.2g, Tid).(2) antibiotics: Piperacseverein sodium tazobactam sodium, cefoperazone sodium sulbactam sodium, moxifloxacin, etc. (3) Other drugs: methylprednisolone, thymus methoxine, gamma globulin, ulinastatin, magnesium isoglycyrrhizinate, etc.

4.2 Analysis of anemia characteristics in 46 patients with COVID-19

We first analyzed the anemia characteristics of the 46 patients, and the results are shown in Table 1.

Table 1 Analysis of anemia characteristics of the 46 patients

Lab tests	Mean Value	Abnormal Index %(No.) (46 patients)
Hb	96.82	100
RBC (10^12/L)	3.18	100
MCV (fL)	92.2	8.7(4/46)
MCH (pg)	30.89	8.7(4/46)
MCHC (g/L)	330.57	26.1(12/46)
CRP (mg/L)	25.52	39 (18/46)
PCT (ng/ml)	0.81	6.5 (3/46)
APTT (sec)	31.63	8.7(4/46)
PT (sec)	13.15	10.9(5/46)
serum iron (μmol/L)	15.7	17.4 (8/46)
vitamin B12 (pg/ml)	533	10.9(5/46)
folic acid (ng/ml)	6.47	8.7(4/46)
RET%	3.33	69.6 (32/46)
DAT (+)	44	95.6(44/46)
LAT (+)	0	0

As Table 1 shows, among the 46 patients, 71.7% had normocytic anemia, 95.6% DAT (direct antiglobulin test) was positive. It was suggested that the anemia of severe and critically severe covid-19 patients in this intensive care area was autoimmune hemolytic anemia (AIHA).

4.3 We further analyzed the changes in the severity of anemia in 46 severe and critically severe COVID-19 patients during hospitalization, and the results showed that average Hb concentration and red blood cell count declined progressively with the extension of hospitalization time (P<0.05). MCH, MCV and MCHC showed no significant changes, and their mean values remained in the normal range, as shown in Table 2. It is suggested that the severity of anemia increased with longer total hospitalization time(N). The mean hospitalization time spent in the previous hospital (N_1) was 5.5 days.

Table 2 Changes routine in blood parameters with the length of hospital stay

Items	The total hospitalization time(N)（d）								P value
	N=5.5	N=6.5	N=8.5	N=10.5	N=12.5	N=14.5	N=16.5	N=18.5	
Hb （g/L）	118.24	115.28	112.78	106.04	103.42	102.08	98.16	96.82	0
RBC（10^{12}/L）	3.88	3.75	3.64	3.45	3.35	3.32	3.22	3.18	0
MCV （fL）	89.87	90.64	91.43	92.55	91.82	92.21	91.72	92.2	0.134
MCH （pg）	30.56	30.56	30.56	30.56	30.56	30.56	30.56	30.89	0.832
MCHC（g/L）	340.24	338.94	338.62	341.92	333.52	332.34	333.76	330.57	0.139

* *The above data are the mean values of each indicator

4.4 Correlation analysis of anemia with novel coronavirus infection

We analyzed the correlation between the average Hb concentration and the average IgM and IgG antibody concentrations in 46 COVID-19 patients. The average onset date before patients were treated in our hospital was 11 days. We started to examine novel coronavirus antibodies on the first day of hospitalization, so the statistical starting point was the 12th day of disease onset.

Table 3 Correlation analysis between average concentration of Hb and novel coronavirus IgM and IgG

Items	The Onset Date (M)							R value	P value
	12d（N_2=1）	14d（N_2=3）	16d（N_2=5）	18d（N_2=7）	20d（N_2=9）	22d（N_2=11）	24d（N_2=13）		
Hb (g/L)	115.28	112.78	106.04	103.42	102.08	98.16	98.98		
IgM (AU/ml)	66.39	13.11	168.48	452.23	73.1	46.37	44.94	r=0.071	P=0.897
IgG (AU/ml)	100.32	125.98	85.49	68.57	114.08	96.59	86.25	r=0.41	P=0.361

* *The above data are the mean values of each indicator

As shown in table 3 and figure 1, with the extension of hospitalization time, the daily average concentration of Hb in COVID-19 patients gradually decreased. The average serum IgM concentration gradually increased on the 14th day of disease onset, reached its peak on the 18th day, then began to decline, and reached its lowest point on the 25th day of disease onset. The fluctuation of average serum IgG concentration was not obvious, and it had a significant increase on the 25th day of onset. Novel coronavirus antibodies (IgM and IgG) had no significant correlation with a decline in Hb (r=0.071, P=0.897; R =0.41, P=0.361), so it was considered that new coronavirus infection was not the cause of anemia.

4.5 Correlation between anemia and therapeutic drugs: We first analyzed the relationship between anemia and Arbidol. As shown in Figure 2. We can see the changes in the average daily drug

concentration of Arbidol and the mean value of Hb of the 46 COVID-19 patients. When the total hospitalization time(N) was less than 11.5 days, the average daily drug concentration of Arbidol rose and the mean value of Hb decreased. However, when the total hospitalization time(N) was more than 11.5 days, the average daily drug concentration of Arbidol decreased and the mean value of Hb rebounded. Through Pearson linear correlation analysis, anemia had a significant inverse correlation with Arbidol (r=-0.758, P=0.029). It can be seen that there was a significant correlation between the occurrence of anemia in severe and critically severe patients and the use of Arbidol.

We used the same method to analyze the correlation between the changes in the average daily drug concentration of IFN α-2b and magnesium isoglycyrhizinate and anemia. As shown in figure 3-4, with the increase in the average daily drug concentration of IFN α-2b, the mean value of Hb decreased initially, while the average daily drug concentration of IFN α-2b gradually decreased in the later stage, while the mean value of Hb continued to decrease. Through Pearson linear correlation analysis, there was no significant correlation between IFN α-2b and Hb (r=-0.223, P=0.595), and anemia was independent of IFN α-2b.With the increase in the average dose of magnesium iso-glycyrrhizinate, the mean value of Hb decreased, and both showed a downward trend at the later stage. Through Pearson linear correlation analysis, there was no significant correlation between magnesium iso-glycyrrhizinate and Hb (r=-0.637, P=0.089).

There was no significant difference in the general characteristics (sex, age, number of severe and critically severe patients) and Hb, red blood cell count, MCV, MCH and MCHC at admission between the RBV group (19 patients) and the non-RBV group (27 patients) (P >0.05).As can be seen in Figure 5, the Hb in both the RBV group and the non-RBV group decreased gradually. After statistical analysis, there was no significant difference in the change in Hb between the two groups (P=0.504). Therefore, the application of RBV had no significant effect on the decrease of Hb and there was no significant relationship between RBV and anemia.

In addition to the abovementioned drugs, some of the 46 patients were also treated with antibiotics, thymalfasin, ulinastatin, glucocorticoids and other drugs. However, due to the small number of cases and short application time of these drugs, we did not make statistics about these drugs. In addition, some patients were treated with invasive or noninvasive ventilation, or ECMO. As the above operations fail to cause blood loss, no analysis was performed in this study. We also analyzed other possible causes of anemia and found that anemia was not associated with gastrointestinal bleeding. The increased percentage of reticulocytes suggested no myelosuppression. The patients' serum iron, folic acid and B12 were all in the normal range, and the patients' anemia was normocytic anemia, not dystrophic anemia. CRP was elevated slightly, PCT was normal, and sepsis was excluded. The patients' APTT and PT were normal, and there was no serious coagulation dysfunction.

Discussion

Anemia may increase the risk of mortality in patients suffering from coronavirus pneumonia [6], and it is important to discover and correct anemia in a timely manner. According to the results of our study, anemia in COVID-19 patients was associated with Arbidol. Arbidol hydrochloride is an indole derivative molecule with broad-spectrum antiviral activity. It has been reported that antiviral effect of ARD can be partially achieved by blocking and downregulating the expression of nuclear factor-κB (NF-κB) protein [7]. NF-κB proteins selectively bind to enhancers of B cell κ-light chain and regulate the expression of many genes. NF-κB protein plays a key role in cellular inflammation and the immune response. Incorrect regulation of NF-κB can lead to autoimmune diseases, chronic inflammation and many cancers [8]. Therefore, ARD may cause immune disorders through incorrect regulation of NF-κB protein, and immune disorders further cause AIHA.

Multiple studies have confirmed that when RBV is used to treat viral infection, patients may develop hemolytic anemia [6,9-11]. Red blood cells are nucleated, and they lack phosphatase. Then RBV accumulates in red blood cells abnormally, which can inhibit ATP development in red blood cells, change the nature of the red blood cells [12] and weaken the antioxidant capacity of red blood cells. These changes promote the removal of red blood cells by the reticular system, accelerate the destruction of red blood cells, and eventually lead to hemolytic anemia. Hemolytic anemia caused by RBV can be improved by adjusting the therapeutic dose of RBV or by discontinuing RBV [6]. RBV was irrelevant to anemia in this research, which may be due to the short time use of RBV.

Conclusions

The data of COVID-19 patients in this study are all first hand, which are of great value for research on therapy and pathogenesis. At present, ARD has not been reported to have side effects of anemia. This is the first study to find the relationship between anemia and ARD, and we further speculate that ARD and AIHA have a certain correlation, which is innovative and of great value.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from each participant, and the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University: Reference number: 2020XS04-02.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Bethune Medical Research Fund (Grant No. SG059EN). The funding agency had a role in writing the manuscript, the study design and the collection of data.

Authors' contributions

WYH collected, analyzed and interpreted the patient data. CCL designed the research and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements:

Not applicable.

References

[1]国家卫生健康委办公厅.新型冠状病毒肺炎诊疗方案(试行第七版)[J].传染病信息,2020,33(1):1-6,26.

[2] Deng P, Zhong D, Yu K, et al. Pharmacokinetics, metabolism, and excretion of the antiviral drug Arbidol in humans[J]. Antimicrobial agents and chemotherapy,2013,57(4): 1743-1755.DOI:10.1128/AAC.02282-12

[3] Shaaban R, El-Sayed WM, Samir S. Molecular and Biological Characterization of a Prepared Recombinant Human Interferon Alpha 2b Isoform[J]. Applied biochemistry and biotechnology,2019,188(1): 72-86.DOI:10.1007/s12010-018-2908-y

[4] Sun L, Shen J, Pang X, et al. Phase I safety and pharmacokinetic study of magnesium isoglycyrrhizinate after single and multiple intravenous doses in Chinese healthy volunteers[J]. Journal of clinical pharmacology,2007,47(6): 767-773.DOI:10.1177/0091270007299757

[5] 杨宝峰、陈建国.药理学[M] (第九版) 人民卫生出版社, 2018.

[6] Chiou Heli CL, Buttrey M J, et al. Adverse effects of ribavirin and outcome in severe acute respiratory syndrome: experience in two medical centers[J]. Chest ,2005,128(1): 263-272.DOI:10.1378/chest.128.1.263

[7] Ma N, Shen M, Chen T, et al. Assessment of a new Arbidol derivative against herpes simplex virus II in human cervical epithelial cells and in BALB/c mice[J]. Biomedicine & pharmacotherapy = Biomédecine &

pharmacothérapie,2019,118():109359.DOI:10.1016/j.biopha.2019.109359

[8] Napetschnig J, Wu H. Molecular Basis of NF-κB Signaling[J]. Annual Review of Biophysics, 2013, 42(1):443-468.

[9] Sung JJ, Wu A, Joynt GM, et al. Severe acute respiratory syndrome: report of treatment and outcome after a major outbreak[J]. Thorax ,2004,59(5): 414-420.DOI:10.1136/thx.2003.014076

[10] Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: Role of membrane oxidative damage[J]. Hepatology, 2000, 31(4).

[11]Gentile, Ivan, Viola, Chiara, Reynaud, Laura,. Case Report: Hemolytic Anemia During Pegylated IFN-α2b Plus Ribavirin Treatment for Chronic Hepatitis C: Ribavirin Is Not Always the Culprit[J]. Journal of Interferon & Cytokine Research, 2005, 25(5):283-285.

[12] ,, ,, ,. α2b56C1[J]. = The Japanese journal of gastro-enterology, 2011, 108:1571-1578.

Figures

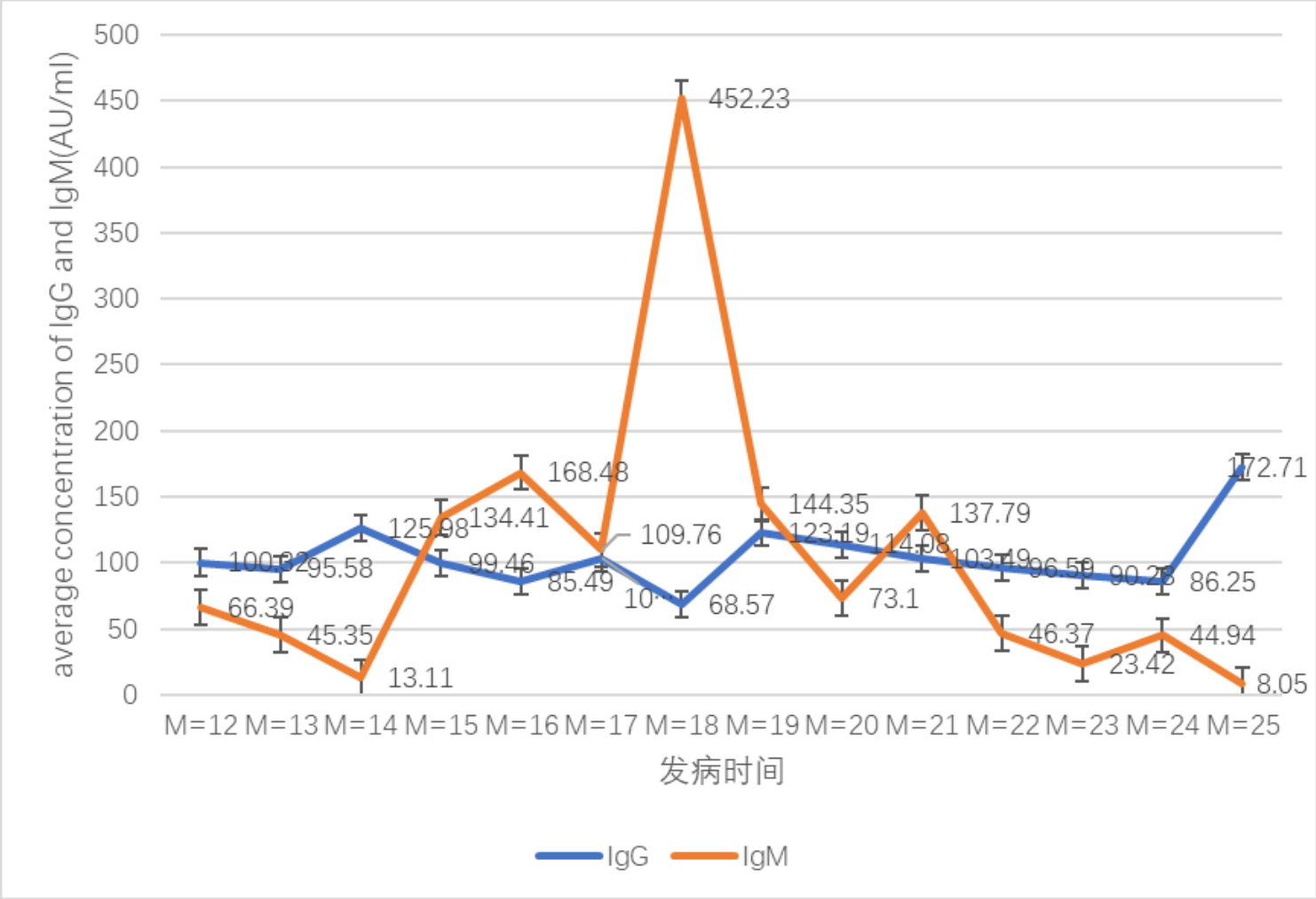

Figure 1

Changes in novel coronavirus IgM and IgG with the length of hospital stay

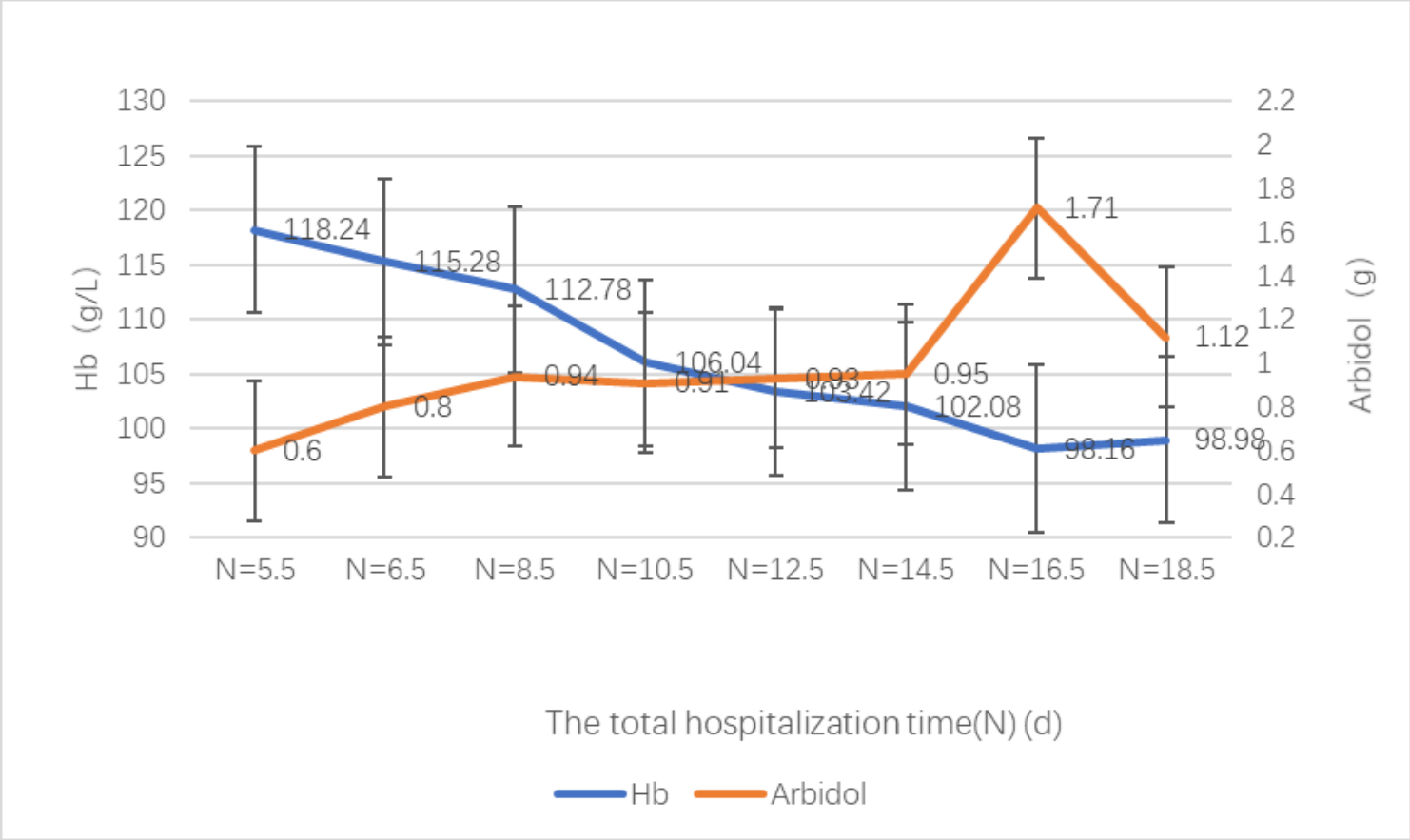

Figure 2

Curve of the relationship between average Arbidol concentration and average Hb concentration

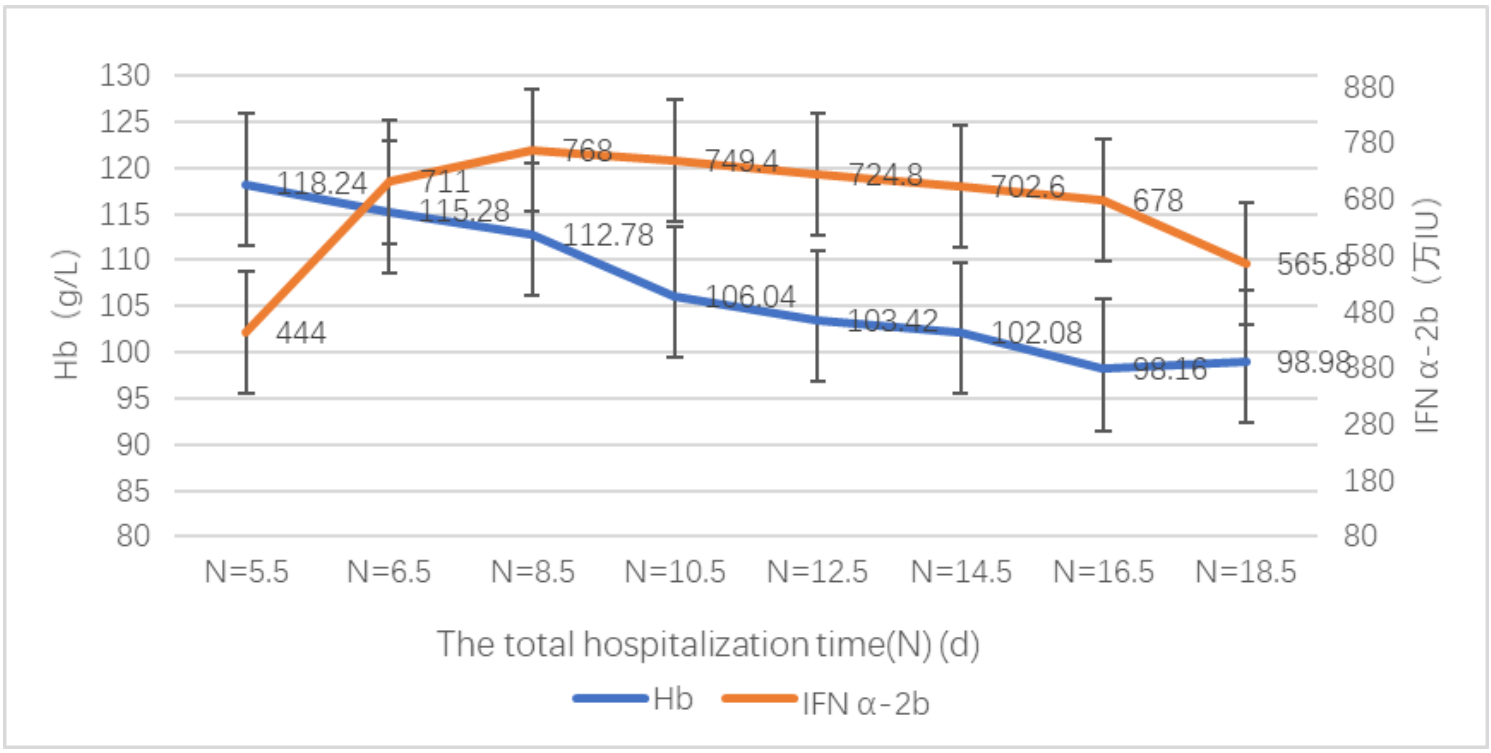

Figure 3

Curve of the relationship between average IFN α-2b concentration and average Hb concentration

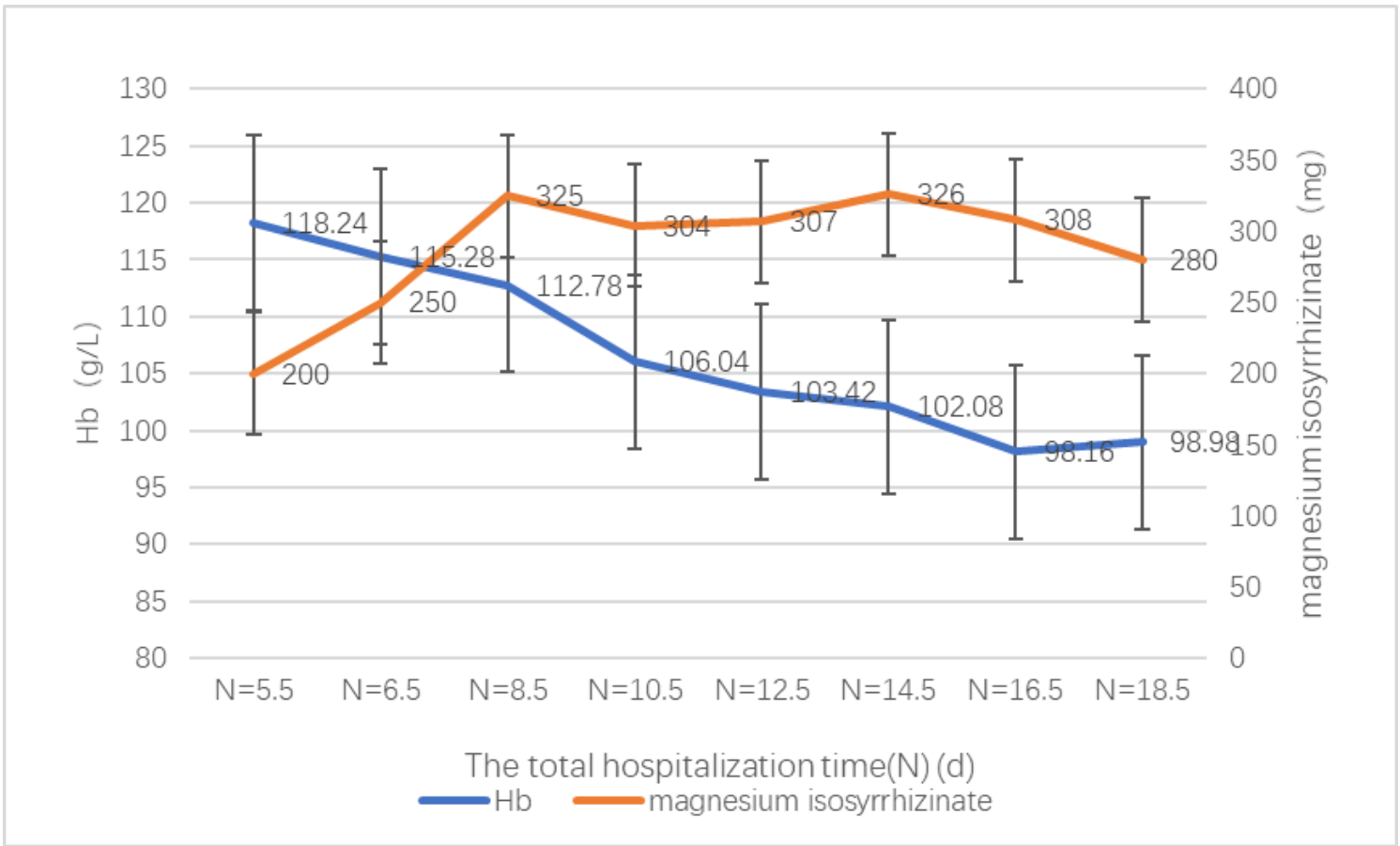

Figure 4

Curve of the relationship between average magnesium isosyrrhizinate concentration and average Hb concentration

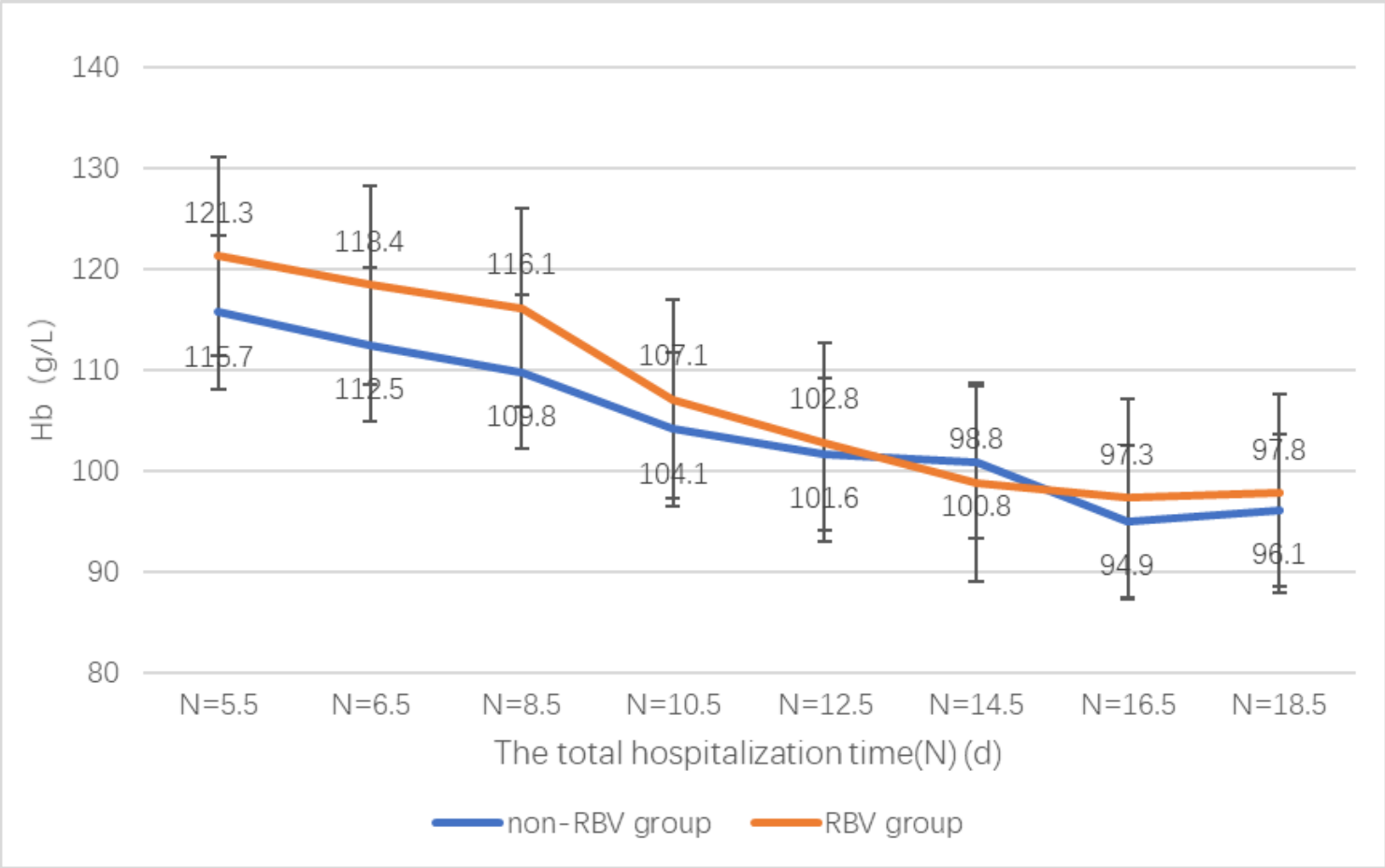

Figure 5

Changes of Hb with length of hospital stay in RBV group and non-RBV group