



BMJ Open High haemoglobin levels and mortality in males with intracerebral haemorrhage: a retrospective cohort study

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ABSTRACT

Objectives To examine the association between high haemoglobin levels and outcomes in intracerebral haemorrhage (ICH) in a multicentre cohort study.

Design Prospective multicentre cohort study.

Settings 21 tertiary hospitals across mainland China.

Participants A total of 5318 consecutive in-hospital spontaneous ICH patients were recruited between January 2012 and June 2016.

Primary and secondary outcome

measures Haemoglobin levels were measured on admission. Binary or ordinary logistic regression was used to evaluate the independent relationship of haemoglobin level with clinical outcomes at 3 months, measured as death or disability. Restricted cubic spline regression was fitted to examine the potential non-linear shape of the dose–response curve between the whole haemoglobin levels and 3-month poor outcomes.

Results A total of 5031 patients with ICH were analysed (64.3% male; mean age (SD), 57.8 (15.2) years). We found that the highest haemoglobin quintile was associated with poor outcomes 3 months in males (adjusted OR (aOR) 1.65, 95% CI 1.21 to 2.25) but not in females, which was also observed in the pooled analysis of three subcohorts in male patients (average aOR 1.70, 95% CI 1.23 to 2.33). The spline regression suggested a non-linear association between haemoglobin levels and outcomes and a linear relationship was observed between an elevated haemoglobin level and 3-month disability/death in males (haemoglobin level per 10 g/L: aOR 1.24, 95% CI 1.10 to 1.40, $p < 0.001$), which was mediated by larger haematoma volume (effect size: 0.115, 95% CI 0.012 to 0.231).

Conclusions This study found a sex-specific association between an elevated haemoglobin level and poor 3-month outcomes, which might be mediated by larger haematoma volume.

INTRODUCTION

Intracerebral haemorrhage (ICH) has the most unfavourable outcome among all types of stroke, and therefore represents a significant health burden.^{1 2} However, there is no specific treatment for ICH and biomarkers are of limited value to predict the outcome of the disorder. Reduced haemoglobin or anaemia is frequently reported to be associated with poor outcomes in patients with

Strengths and limitations of this study

- A multicentre cohort study included the large sample size and heterogeneous intracerebral haemorrhage patient population.
- Multivariable linear logistic analysis and sensitivity subgroup analysis based on meta-analyses pooled estimates were performed to draw the conclusion.
- Mediation analysis was performed to explore the mediator of the association between high haemoglobin levels and mortality.
- The haemoglobin levels based one measurement on admission might be prone to regression attenuation bias.

ICH.^{3–5} However, elevated haemoglobin levels are double-edged. Elevated haemoglobin accompanied with higher heme production, which was a prominent breakdown product of haemoglobin. In subarachnoid haemorrhage (SAH), high levels of heme were found in the cerebrospinal fluid during SAH-induced vasospasm and to activate an inducible isoform of nitric oxide synthase in vascular smooth-muscle cells.^{6 7} Animal studies demonstrated that heme-induced oxidative stress and vascular expression of heme oxygenase-1, which impaired the platelet-dependent thrombosis process via stimulating production of Cyclic guanosine monophosphate (cGMP), an inhibitor of platelet aggregation.^{8–11} It was possible that the heme-induced platelet dysfunction might exacerbate poor outcomes in either in ischaemic stroke or in ICH.

In acute stroke, it was reported that an elevated haemoglobin concentration was associated with higher cardiovascular disease or stroke incidence.^{12–14} In ischaemic stroke, recent study also reported that the highest quartile of haemoglobin level or the elevated admission haemoglobin levels (153 g/L in male and 142 g/L in female) was correlated with poorer 3-month or 1-year outcomes,

respectively.^{14 15} However, there was a lack of evidence concerning the relationship between the high haemoglobin levels and outcomes in ICH. Our aims were, therefore, to investigate in a large multicentre cohort of patients with ICH, the association between the elevated haemoglobin levels and outcomes and explore the possible underlying mechanisms.

METHODS

Study design and participants

We used a cohort study design based on a prospective, multicentre, hospital-based registry that collected data of patients with acute ICH admitted to 21 tertiary hospitals across a wide range of cities in China from January 2012 to June 2016. A total of 5318 patients who received a clinical diagnosis of spontaneous ICH confirmed by brain imaging were screened. The study was approved by the Biomedical Research Ethics Committee and the Committee on Human Research of West China Hospital, Sichuan University (2013 [124]).¹⁶

Patients with first-ever ICH were consecutively recruited. Patients were included if they (1) were at least 18 years old, (2) had been diagnosed with ICH based on non-contrast computed tomography (NCCT) performed within 72 hours from the presumed symptom onset and (3) had undergone CT or MRI to distinguish haemorrhagic stroke from ischaemic stroke. Patients underwent assessments at baseline (on admission) and at 3 months after stroke. Patients were excluded (1) if they were diagnosed with traumatic ICH, primary subdural/epidural haematoma, intracranial venous thrombosis or haemorrhage due to a tumour or recurrent ICH; (2) if they had stroke due to primary SAH with or without ICH and haemorrhagic transformation of a cerebral infarction or (3) haemoglobin measurements on admission were missing.

Procedures

Information about baseline demographic characteristics was obtained predominantly through in-person interviews. In-hospital details, including clinical features and diagnosis, were obtained through medical records and interviews with patients or their families. Follow-up details were obtained primarily through telephone interviews at 3 months after stroke. The state of consciousness was assessed by the Glasgow Coma Scale (GCS) Score with a score from 3 to 15, with a lower score indicating a worse level of consciousness, and the severity of neurological deficits was assessed using National Institutes of Health Stroke Scale (NIHSS) Score from 0 to 42, with higher scores indicating a more severe neurological deficit. CT scans of the head were performed in all patients on admission. The NCCT scans were acquired with a 5 mm-thick axial slice, 120–140 kVs (peak), 10–500 mA, and reviewed for determination of haematoma location and volume. haematoma volume was determined by the formula of ellipsoids ($A \times B \times C / 2$).¹⁷ Haemoglobin levels

were measured on admission using the hemiglobincyanide method. Drinking was confirmed if the patients were weekly drinkers, those who usually drank alcohol at least once per week during the past 12 months. Smoking was confirmed if the patients were regular smokers, who admitted to smoke at least one cigarette (or equivalent) per day. The primary outcome was disability/death at 3 months, defined by scores of 3–6 on the Modified Ranking Scale (mRS) and the follow-up period was 3 months. Subgroup was determined according to the geographic distribution of these subcentres. West China Hospital was allocated to cohort 1; the subcentres within Sichuan province were allocated to cohort 2; the subcentres of other provinces in China were allocated to cohort 3.

The follow-up period was 3 months with a follow-up rate of 88% (4418/5035). The baseline characteristics of the missing cases were not significantly different from those included (data not shown).

Statistical analysis

Categorical variables were presented as counts (%), and the continuous or discrete variables were presented as mean (SD) or median (IQR). Student's *t* test, the χ^2 test, Analysis of Variance (ANOVA), Mann-Whitney *U* test, Fisher's exact test and Kruskal-Wallis test were used for univariate analysis among groups with relevant variables as appropriate. Associations of clinical characteristics with death/disability were analysed using logistic regression models. Data are reported as ORs and 95% CIs. Where appropriate, adjusted ORs (aORs) were reported. Age, premorbid antithrombotics use, hyperlipidemia, diabetes mellitus, alcohol consumption, GCS, NIHSS, haematoma volume and locations, blood creatine levels, blood glucose, activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen, blood pressure, chronic kidney disease, lung disease, antihypertension treatment, cerebral oedema treatment (dehydration treatment) and surgical interventions were the selected potential confounders, which were adjusted in all multivariable models. To investigate the association between an elevated haemoglobin level and outcomes, the haemoglobin concentration was categorised into quintiles with similar sample numbers in each quintile (126, 137, 148 and 156 g/L for males and 115, 123, 130 and 139 g/L for females). The forest plot of the estimates of the three subcohorts was performed using the Mantel-Haenszel random-effect model of the meta-analysis (Revman V.5.0). Restricted cubic spline regression was fitted to examine the potential non-linear shape of the dose-response curve between the whole haemoglobin levels and 3-month poor outcomes and to check whether the breakpoints identified were in accordance with results of the haemoglobin quartiles. A threshold of $p < 0.10$ was used to declare statistical significance for heterogeneity. Two-sided *p* values are reported, with $p < 0.05$ considered statistically significant in all tests unless another threshold was given. Statistical analyses were performed in R Core

Team (2017) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (<https://www.R-project.org/>). The calculations and plottings were conducted using the algorithm packages in R including splines, MASS, dplyr and ggplot2 packages.

Mediation analysis was performed to estimate whether haematoma volume (as the mediator) was the mediator for any relationship between haemoglobin level (independent variable) and poor outcome (dependent variable) by analysing all three variables together with four steps: (1) path c: the total effect of the an elevated haemoglobin level (group factor) on the outcome; (2) path a: the group effect on haematoma volume; (3) path b: the correlation between haematoma volume and outcomes, after controlling for the group factor and (4) the $a \times b$ effect, which was referred to as the indirect effect and was indicative of whether the predictor–outcome relationship was significantly reduced after controlling for the mediator. If all four tests reached the level of significance, haematoma volume was considered to significantly mediate the group effect on the poor outcome. The $a \times b$ indirect effect was evaluated using the PROCESS macro implemented in SPSS (Hayes, 2018). A bootstrap method with 5000 repetitions was used to estimate the CIs for the indirect effects. An empirical 95% CI that did not include zero indicated significance at the 0.05 level.^{18 19} Analyses were performed using SPSS (IBM, Armonk, New York).

RESULTS

A total of 5318 patients with spontaneous ICH were screened and 5031 patients were included in the final analysis (figure 1). Of the 5031 patients, 3233 (64.3%) patients were male, the mean age was 57.8 (15.2) years (table 1). The 3-month mortality rate was 19.2% (19.5% for males and 18.8% for females) and 3-month disability/death rate was 48.8% (49.3% for males and 48.0% for females).

Association of an elevated haemoglobin with poor outcomes in male patients with ICH across three different subcohorts

Table 2 depicts the association of haemoglobin levels with 3-month disability/death by quintiles of sex-specific baseline haemoglobin levels. Compared with the male patients with haemoglobin levels of 138–146 g/L, after adjusting for potential confounders, the haemoglobin level within the highest quintile was associated with higher poor outcomes (aOR 1.65, 95% CI 1.21 to 2.25, $p=0.002$). Those significant associations between high haemoglobin levels and poor outcomes were not observed in the female patients.

To further confirm the association between the highest haemoglobin quintile (>156 g/L) and 3-month poor outcomes in males, patients were divided into three different subcohorts: cohort 1, cohort 2 and cohort 3 according to the geographical distribution of the included medical subcentres and stratified by the

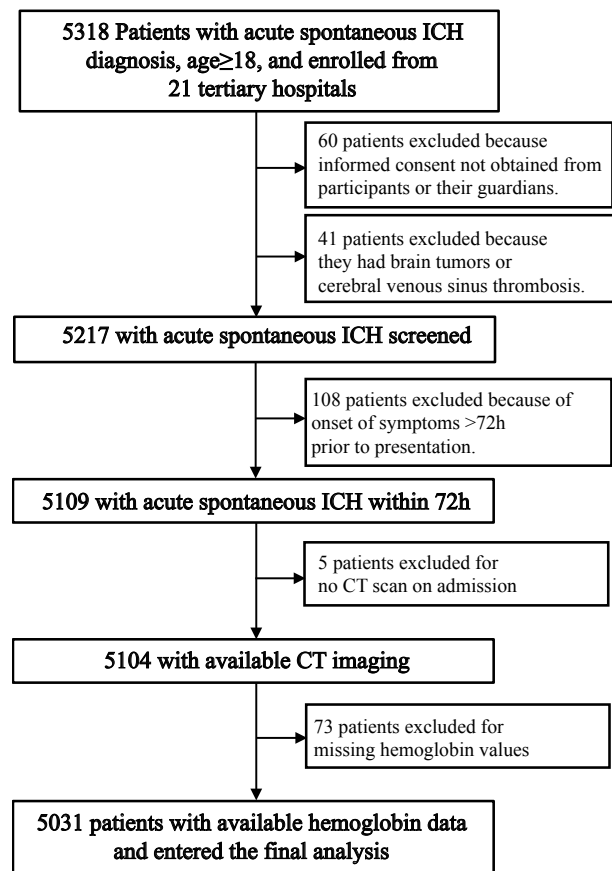


Figure 1 Flow chart of patients screened, describing both those included and excluded from the study. ICH, intracerebral haemorrhage.

haemoglobin quintiles with the third quintile as the reference (figure 2). A significant association between the highest haemoglobin quintile and poor outcomes was observed in cohort 1 (aOR 2.08, 95% CI 1.01 to 4.28) and cohort 3 (aOR 1.67, 95% CI 1.10 to 2.55). In cohort 2, there was a similar trend but not significant. Pooling and analysing the results from three subcohorts using the generic inverse variance method of meta-analysis, the highest quintile, compared with the reference, was associated with worse outcomes (average aOR 1.70, 95% CI 1.23 to 2.33). In females, the association was not observed in any subcohorts (online supplemental figure 1).

Haemoglobin level linearly and negatively correlates with poor outcomes in male patients with an elevated haemoglobin level (>156 g/L) via larger haematoma volume

The spline regression was employed to investigate any non-linear correlation between haemoglobin levels and outcomes. In the spline plot, the nadir of the curve was observed at a haemoglobin level of approximately 150 g/L ($P_{\text{non-linearity}}=0.004$), as shown in figure 3A; however, the non-linear correlation was not found in female patients ($P_{\text{non-linearity}}=0.818$). The turning points of the U-shaped curve of male patients, beyond which there was a sharp increase in the prevalence of poor outcomes, were

Table 1 Baseline characteristics of study population according to gender

Characteristics	Total	Female	Male
	n=5031	n=1798	n=3233
Age, years	57.8±15.2	58.0±15.5	57.6±15.1
Comorbidities and risk factors, n (%)			
HD history	359 (7.1%)	142 (7.9%)	217 (6.7%)
Hypertension	2965 (58.9%)	1081 (60.1%)	1884 (58.3%)
Hyperlipidemia	1413 (33.0%)	521 (34.2%)	892 (32.3%)
Diabetes mellitus	430 (8.5%)	151 (8.4%)	279 (8.6%)
Chronic kidney disease	256 (5.1%)	58 (3.2%)	198 (6.1%)
Antithrombotics	432 (8.6%)	130 (7.2%)	302 (9.3%)
Alcohol	1068 (21.2%)	56 (3.1%)	1012 (31.3%)
Smoking	1290 (25.6%)	70 (3.9%)	1220 (37.7%)
Clinical status			
GCS	13.0 (8.0, 15.0)	13.0 (8.0, 15.0)	13.0 (8.2, 15.0)
NIHSS	8.0 (3.0, 16.0)	9.0 (3.0, 17.0)	8.0 (3.0, 16.0)
Haematoma volume, mL	13.0 (5.2, 27.8)	11.0 (5.0, 23.6)	14.4 (5.4, 30.0)
SBP, mm Hg	162.3±31.3	160.2±32.3	163.4±30.7
DBP, mm Hg	95.0±18.1	92.7±17.8	96.3±18.2
Haemoglobin, g/L	136±20	127±16	141±20
HCT	0.41±0.06	0.38±0.05	0.42±0.06
Albumin, g/L	41.7 (38.5, 44.9)	41.9 (38.7, 44.9)	41.7 (38.3, 44.8)
Platelet count, 10 ⁹ /L	161 (121, 207)	168 (123, 216)	158 (119, 202)
PT, s	11.8 (11.0, 12.9)	11.8 (10.9, 12.8)	11.8 (11.0, 12.9)
APTT, s	26.5 (23.6, 30.0)	26.1 (23.2, 29.5)	26.7 (23.8, 30.3)
Fibrinogen, g/L	2.8 (2.3, 3.5)	2.9 (2.4, 3.5)	2.8 (2.3, 3.5)
INR	1.02 (0.95, 1.10)	1.01 (0.94, 1.09)	1.02 (0.95, 1.10)
Blood glucose, mmol/L	7.25 (6.03, 9.12)	7.34 (6.14, 9.40)	7.20 (6.00, 9.01)
Creatine, µmol/L	70.0 (57.0, 85.8)	58.0 (48.9, 71.0)	76.0 (64.9, 91.0)
Haematoma location, n (%)			
BG or thalamus	2969 (64.1%)	1053 (63.9%)	1916 (64.2%)
Lobar	1626 (33.7%)	575 (33.3%)	1051 (33.8%)
Brainstem	405 (8.4%)	135 (7.8%)	270 (8.7%)
Cerebellar	292 (6.0%)	101 (5.8%)	191 (6.2%)
IVH	1632 (33.8%)	601 (34.8%)	1031 (33.2%)
Therapies and complications, n (%)			
Antihypertension	2826 (58.9%)	978 (57.8%)	1848 (59.5%)
Cerebral oedema treatment	4285 (89.3%)	1516 (89.7%)	2769 (89.2%)
Surgical intervention	1354 (26.9%)	500 (27.8%)	854 (26.4%)
Respiratory infection	1097 (21.8%)	344 (19.1%)	753 (23.3%)

Descriptive statistics were calculated using mean±SD or median (IQR) for continuous variables and frequencies for categorical variables. APTT, activated partial thromboplastin time; BG, basal ganglia; DBP, diastolic blood pressure; GCS, Glasgow Coma Scale; HCT, haematocrit; HD, heart disease; INR, international normalised ratio; IVH, intraventricular haemorrhage; NIHSS, National Institutes of Health Stroke Scale; PT, prothrombin time; SBP, systolic blood pressure.

approximately between 150 and 160 g/L, which was in accordance with the results of the haemoglobin quartiles.

We compared the potential risk factors of male patients with different haemoglobin quintiles. Compared with

reference quintile, the patients with the highest haemoglobin quintile were characterised with younger age (51.0 years), higher frequency of diabetes (62.2%), higher alcohol consumption (36.7%), higher platelet count

Table 2 Quintiles of haemoglobin on admission and 3-month disability/death in male and female patients with ICH

3-month death/ disability	Quartiles of haemoglobin (g/L)			Crude		Multivariable	
	Category	n	Events, n (%)	OR (95% CI)	P	OR (95% CI)	P
Male	<126	605	289 (54.1)	1.46 (1.16 to 1.85)	0.002	0.95 (0.69 to 1.31)	0.773
	126–137	658	317 (52.8)	1.39 (1.11 to 1.75)	0.005	1.14 (0.84 to 1.54)	0.391
	138–146	675	261 (44.6)	ref	ref	ref	ref
	147–156	662	252 (42.8)	0.93 (0.74 to 1.17)	0.527	0.97 (0.73 to 1.31)	0.863
	>156	633	290 (52.8)	1.39 (1.10 to 1.76)	0.006	1.65 (1.21 to 2.25)	0.002
Female	<115	359	166 (51.6)	1.28 (0.93 to 1.77)	0.125	1.04 (0.67 to 1.60)	0.856
	115–123	364	152 (46.1)	1.07 (0.78 to 1.47)	0.678	1.12 (0.75 to 1.69)	0.575
	124–130	343	131 (44.4)	ref	ref	ref	ref
	131–139	380	146 (45.8)	1.06 (0.77 to 1.45)	0.735	1.04 (0.68 to 1.57)	0.869
	>139	352	154 (52.2)	1.37 (0.99 to 1.89)	0.058	1.21 (0.80 to 1.83)	0.375

All models were adjusted for age, premorbid antithrombotics use, hyperlipidemia, diabetes mellitus, alcohol consumption, GCS, NIHSS, haematoma volume and locations, blood creatine levels, blood glucose, activated partial thromboplastin time, prothrombin time, fibrinogen, blood pressure, chronic kidney disease, lung disease, antihypertension treatment, cerebral oedema treatment and surgical interventions. GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage; NIHSS, National Institutes of Health Stroke Scale.

($171 \times 10^9/L$), higher haematoma volume (15.4 mL), higher frequency of antihypertensive (66.4%) and cerebral oedema treatment (92.4%; online supplemental table 1), which were all adjusted in the following analysis as potential confounders. When we analysed the linear correlation between haemoglobin levels and outcomes in the patients with haemoglobin levels of the highest quartile ($>156 g/dL$), a linear relationship was observed between an elevated haemoglobin level and 3-month disability/death in males (haemoglobin level per 10 g/dL: aOR 1.24, 95% CI 1.10 to 1.40, $p < 0.001$), but not in females ($p = 0.700$; figure 3B).

To investigate the potential mediators between an elevated haemoglobin level and poor outcome, we performed a mediation analysis in male ICH patients with haemoglobin level $>156 g/L$ (figure 4). We define path a, path b and path c as group difference in haematoma volume, group-independent haematoma-outcomes relations and group difference in 3-month disability/death, respectively. Given the requirement for a significant path c, path a and path b, there was only one candidate mediation path, in which the group factor, haematoma volume and 3-month disability/death are the predictor, mediator and outcome, respectively (path c', $\beta = 0.398$, $p = 0.005$; path a, $\beta = 3.20$, $p = 0.031$; path b, $\beta = 0.036$, $p < 0.001$). The bootstrap simulation ($n = 5000$) confirmed a significant indirect effect (effect size: $a \times b = 0.115$, 95% CI 0.012 to 0.231, $p < 0.05$), implying that 3-month death was expected to increase by 0.115 for every 10 g/L increase in haemoglobin when haemoglobin levels were more than 156 g/L, if one considers only the indirect influence via haematoma volume. Therefore, an elevated haemoglobin level might impair 3-month disability/death through larger haematoma volume.

DISCUSSION

In a prospective cohort of 5031 consecutive intracerebral haemorrhage patients, we found that the association of the highest haemoglobin level with an increased 3-month poor outcome risk was observed in males but not in females. There was a linear association among men such that every 10 g/L increment of haemoglobin level was associated with 1.24-fold of increased 3-month poor outcomes risk when haemoglobin was more than 156 g/L.

Elevated haemoglobin levels have been reported to be positively associated with a higher risk of various cardiovascular diseases. A study retrospectively analysed a cohort including 170 078 men and 122 116 women without cardiovascular diseases, which showed that, in men, an increased haemoglobin concentration outside $\geq 160 g/L$ was associated with an increase in all-cause mortality risk (adjusted HR: 1.10, 95% CI 1.01 to 1.20), the trend was similar in women but less significant.¹³ In ischaemic stroke, Guo *et al* reported that in a cohort of 3881 patients, the highest quartile of haemoglobin level was associated with an increased 3-month major disability and death rate (adjusted ORs 1.49, 95% CI 1.11 to 1.99).¹⁴ Tanne *et al* investigated haemoglobin concentration and 1-year outcome among 859 consecutive patients with acute stroke and found that elevated admission haemoglobin level was correlated with a higher risk of death in stroke (153 g/L in male and 142 g/L in female);¹⁵ however, only 15.4% patients of the cohort had an ICH. In contrast, Park *et al* reported that in a cohort of 2681 consecutive patients with acute ischaemic stroke, poor outcome was not related to the higher end of the haemoglobin range.²⁰ In summary, reports regarding the relationship between higher haemoglobin levels and outcomes of cerebrovascular disease are inconsistent and most ICH studies were limited by small sample size. Our results support that, in

Subgroup analysis	Events, No. (%)		Weight	Favors		aOR (95%CI)
	High or low Hgb	Normal Hgb		High or low Hgb	Normal Hgb	
Hemoglobin quintile 1:						
Cohort 1	45 (55.6)	29 (31.5)	2.9%			1.79 (0.71-4.49)
Cohort 2	109 (47.2)	79 (48.2)	7.6%			0.60 (0.34-1.06)
Cohort 3	135 (60.8)	153 (46.5)	11.9%			1.24 (0.79-1.95)
Subtotal (95%)	289 (54.1)	261 (44.6)	22.4%			1.02 (0.73-1.41)
Heterogeneity: Chi ² : 5.49, df=2 (P=0.06); I ² =64%						
Test for overall effect: Z=0.09 (P=0.93)						
Hemoglobin quintile 2:						
Cohort 1	41 (52.6)	29 (31.5)	3.7%			1.96 (0.87-4.42)
Cohort 2	115 (50.2)	79 (48.2)	7.5%			0.91 (0.51-1.61)
Cohort 3	161 (54.9)	153 (46.5)	14.9%			1.16 (0.77-1.74)
Subtotal (95%)	317 (52.8)	261 (44.6)	26.1%			1.16 (0.85-1.58)
Heterogeneity: Chi ² : 2.30, df=2 (P=0.32); I ² =13%						
Test for overall effect: Z=0.96 (P=0.34)						
Hemoglobin quintile 4:						
Cohort 1	39 (37.5)	29 (31.5)	4.2%			1.23 (0.57-2.64)
Cohort 2	67 (40.6)	79 (48.2)	7.1%			0.85 (0.47-1.53)
Cohort 3	146 (45.6)	153 (46.5)	15.9%			0.97 (0.66-1.44)
Subtotal (95%)	252 (42.8)	261 (44.6)	27.3%			0.97 (0.72-1.32)
Heterogeneity: Chi ² : 0.57, df=2 (P=0.75); I ² =0%						
Test for overall effect: Z=0.17 (P=0.86)						
Hemoglobin quintile 5:						
Cohort 1	61 (50.8)	29 (31.5)	4.7%			2.08 (1.01-4.28)
Cohort 2	65 (52.8)	79 (48.2)	5.6%			1.48 (0.76-2.87)
Cohort 3	164 (53.6)	153 (46.5)	14.0%			1.67 (1.10-2.55)
Subtotal (95%)	290 (52.8)	261 (44.6)	24.3%			1.70 (1.23-2.33)
Heterogeneity: Chi ² : 0.48, df=2 (P=0.79); I ² =0%						
Test for overall effect: Z=3.25 (P=0.001)						
Total (95%)	1000.0%					1.18 (1.01-1.38)
Heterogeneity: Chi ² : 16.20, df=11 (P=0.13); I ² =32%						
Test for overall effect: Z=2.04 (P=0.04)						
Test for subgroup difference: Chi ² : 7.36; df=3; (P=0.06); I ² =59.2%						

Figure 2 Pooled analysis of subcohorts in male patients with intracerebral haemorrhage. The haemoglobin concentration was categorised into quintiles by 126, 137, 148 and 156 g/L in males. Forest plots of adjusted ORs for male ICH patients with other haemoglobin quintiles, compared with the third haemoglobin quintile, by subcohorts for 3-month disability/death. All models were adjusted for age, premorbid antithrombotics use, hyperlipidemia, diabetes mellitus, alcohol consumption, GCS, NIHSS, haematoma volume and locations, blood creatine levels, blood glucose, activated partial thromboplastin time, prothrombin time, fibrinogen, blood pressure, chronic kidney disease, lung disease, antihypertension treatment, cerebral oedema treatment and surgical interventions. aOR, adjusted OR; GCS, Glasgow Coma Scale; Hgb, haemoglobin level; ICH, intracerebral haemorrhage; NIHSS, National Institutes of Health Stroke Scale.

male patients with ICH, the highest quintile of haemoglobin level was associated with poor outcomes. Further prospective studies from other samples of patients with ICH are needed to validate our findings.

We also found that haematoma volume mediated the association between an elevated haemoglobin level and poor outcomes in ICH. The pathophysiologic mechanism for this might be the result of multiple factors, such as the hemin-induced platelet-dependent thrombosis dysfunction or the iron toxicity and its related inflammation and coagulopathy.^{8 21-23} Although iron overload is not directly associated with high haemoglobin levels, however, iron dysregulation could be found in haematological disease such as polycythemia vera. A possible explanation of the larger haematoma volume could be similar to that found in the less frequent haemorrhagic presentations of patients with polycythemia vera or other iron-induced abnormal blood coagulation.²⁴ We noted that, although the platelet was higher in these patients, the coagulation function measured by PT, APTT and fibrinogen was not

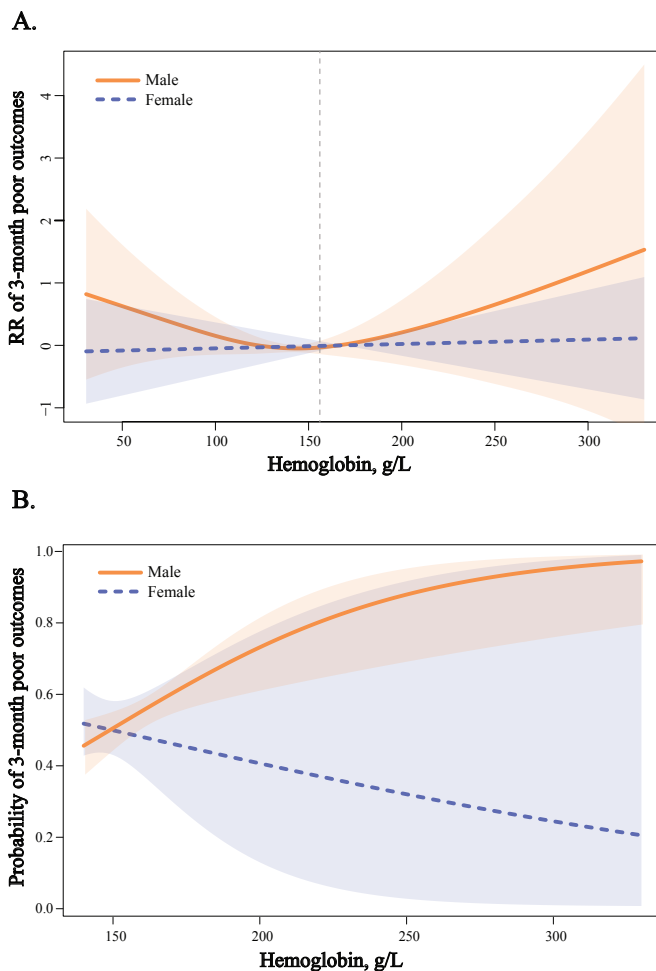


Figure 3 A. The nonlinear correlation was found in male patients with a nadir value between 150 and 160 g/L; B. Predicted poor 3-month outcome probabilities by baseline haemoglobin levels in male and female ICH patients with an elevated haemoglobin level (>156 g/L). All models were adjusted for age, premorbid antithrombotics use, hyperlipidemia, diabetes mellitus, alcohol consumption, GCS, NIHSS, haematoma volume and locations, blood creatine levels, blood glucose, activated partial thromboplastin time, prothrombin time, fibrinogen, blood pressure, chronic kidney disease, lung disease, antihypertension treatment, cerebral oedema treatment and surgical interventions. The grey dashed line indicated a haemoglobin level at 156/dL. GCS, Glasgow Coma Scale; ICH, intracerebral haemorrhage; NIHSS, National Institutes of Health Stroke Scale.

significantly different from the reference group, indicating that other factors or coagulation pathways might be underlying the observation. In the future, it would be interesting to study if there would be some haematological aspects associated with high haemoglobin levels.

Our findings also suggested a gender difference in the association between elevated haemoglobin levels and poor outcomes. The gender differences we observed could be partly explained by the different haemoglobin levels between men and women. We observed a wider haemoglobin distribution in males with a median value of 140 g/L, compared with a more focused distribution

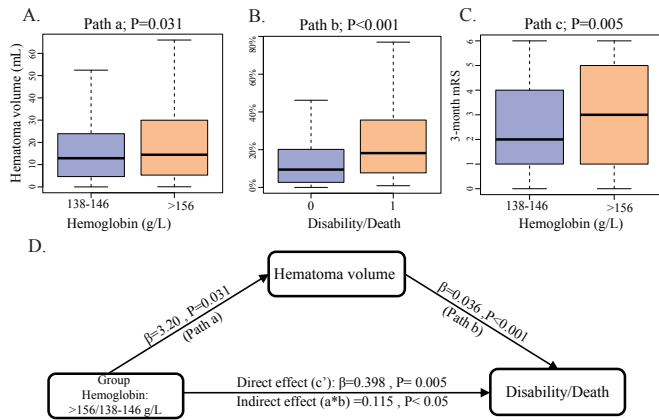


Figure 4 Mediation model of an elevated haemoglobin–haematoma volume–3-month disability/death pathway in male ICH patients. (A) Path a: Significant elevated haemoglobin (>156 g/L) group effect on haematoma volume. (B) Path b: Significant haematoma volume–disability/death correlation after controlling for group factor, age and urea nitrogen. (C) Path c: Significant elevated haemoglobin (>156 g/L) group effect on 3-month mRS (disability/death). (D) Schematic diagram of the mediation model of the pathway from an elevated haemoglobin (>156 g/L) to larger haematoma volume to disability/death in the male patients with ICH. ICH, intracerebral haemorrhage; mRS, Modified Ranking Scale.

in females with a median value of 128 g/L (online supplemental figure 2). It is plausible that the dispersed distribution of haemoglobin in males might partly explain why male ICH patients were more vulnerable to elevated haemoglobin than females. In females, repeatedly altered haemoglobin status caused by menstruation might make the women’s cardiovascular system resistant to the change of haemoglobin levels.²⁵ Similar to our results, the Apolipoprotein Mortality Risk Study reported that elevated haemoglobin levels were associated with acute myocardial infarction only in men.²⁶ Another study analysing 18 413 participants over a mean 7±2 years of follow-up demonstrated that the highest quartiles of haemoglobin in both gender (154 g/L in male and 140 g/L in female) were associated with higher risk of incident stroke (HR, 1.59, 95% CI 1.08 to 2.35).²⁷ So far, the sex differences on the effect of elevated haemoglobin levels on various cardiovascular diseases has been still inconsistent. Further studies with large sample or individual patient data meta-analysis concerning the issue are needed.

Based on our multivariable analysis, an elevated haemoglobin level in male (>156 g/L) could be an independent risk factor in male ICH patients. Therefore, haemodilution therapy aiming to decrease the haemoglobin levels might be beneficial to improve the prognosis in those patients. Moreover, mannitol-induced osmotic diuresis may cause or worsen dehydration, hypovolaemia and haemoconcentration. A rodent model study suggested that using mannitol during active ICH may interfere with collagen-stimulated platelet aggregation.²⁸ Considering the routine use of mannitol in ICH treatment, caution

should be exercised when using mannitol in men with an elevated haemoglobin concentration. Further studies on whether mannitol should be cautiously applied in ICH patients with elevated haemoglobin levels are needed.

Recently, Acosta *et al* did an exploratory analysis of two randomised clinical trials and one multiethnic observational study and found that higher haemoglobin levels were associated with better outcome in ICH.²⁹ The study did not perform the analysis based on male and female subgroup. Moreover, the different conclusion might be the result of the different ethnic groups, different distribution of outcomes as well as the strict exclusion criteria of both randomised clinical trials. Future individual patient data meta-analysis is needed to evaluate the high haemoglobin levels as the predictor of outcome.

Strengths of our study include the large and heterogeneous patient population which had rigorous prospective and systemic evaluations early after the onset of acute ICH. Nevertheless, some limitations need to be mentioned. First, we only included tertiary care hospitals in our study, which might lead to missing patients with less severe ICH. Second, the haemoglobin level was evaluated based on one single measurement on admission with various intervals from symptom onset to hospitalisation and thus prone to regression attenuation bias because this was based only on one measurement at presentation.

CONCLUSION

In summary, in this multicentre cohort of ICH, we revealed a sex-specific association between elevated haemoglobin levels and a poor 3-month outcome. Our data also suggested that haematoma volume might mediate the linear association between the haemoglobin levels and worse outcomes in male ICH patients with a haemoglobin level>156 g/L.

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