Impact of hemoglobin levels and anemia on mortality in acute stroke: analysis of UK regional registry data, systematic review and meta-analysis

Barlas, Impact of hemoglobin on stroke mortality

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Total Word Count: 7039

Subject Codes: [8], [43], [44]

Abstract

Background: The impact of hemoglobin levels and anemia on stroke mortality remains controversial. We aimed to systematically assess this association and quantify the evidence.

Methods and Results: We analysed data from a cohort of 8,013 stroke patients (mean (sd) 77.81±11.83 years) consecutively admitted over 11 years (January 2003–May 2015) using a UK Regional Stroke Register. The impact of hemoglobin levels and anemia on mortality was assessed by sex-specific values at different time points (7-day, 14-day, 1-month, 3-month, 6-month, 1 year), using multiple regression models controlling for confounders. Anemia was present in 24.5% of the cohort on admission and was associated with increased odds of mortality at most of the time points examined up to 1 year following stroke. The association was less consistent for males with hemorrhagic stroke. Elevated haemoglobin was also associated with increased mortality, mainly within the first month. We then conducted a systematic review using the EMBASE and Medline databases. Twenty studies met the inclusion criteria. When combined with the cohort from the current study, this gave a pooled population of 29,943 patients with stroke. The evidence base was quantified in a meta-analysis. Anemia on admission was found to be associated with an increased risk of mortality in both ischemic stroke (8 studies); OR 1.97(1.56– 2.47) and hemorrhagic stroke (4 studies); OR 1.46(1.23–1.74).

Conclusions: There is strong evidence that patients with anemia have increased mortality in stroke. Targeted interventions in this patient population may improve outcomes and therefore require further evaluation.

Key Words

Hemoglobin, mortality, prognosis, stroke

Introduction

Anemia is common in patients presenting with acute stroke. Hospital based studies reported prevalence up to ~ 30% [1, 2]. While anemia has been independently associated with increased mortality in a variety of conditions including chronic kidney disease [3], heart failure[4] and acute coronary syndromes[5], observational studies investigating the association between anemia and mortality in stroke have shown conflicting results. Early studies found no association between anemia and stroke outcomes [6, 7], however, others have found both low and high hemoglobin levels to be associated with increased mortality [8 -10] suggesting a U-shaped relationship. Guidelines have so far been unable to specify the optimal treatment options in acute stroke patients with anemia[11].

Previous studies were limited by small sample sizes and a majority of them did not report outcomes by stroke subtype. In addition, no previous study stratified analysis by sex-specific hemoglobin levels. This is particularly important due to the natural variance in the normal hemoglobin ranges between sexes. The literature describes various plausible mechanisms which explain how anemia could directly contributes to poor outcomes [12]. However, there is a paucity of information investigating the impact of an important clinical factor; whether stroke patients with anemia receive less preventative medications pertinent to stroke such as antiplatelets and anticoagulants (antithrombotics). In addition, there is a lack of data with regard to the co-morbidity burden in anemic stroke patients and inadequate controlling for this in statistical analyses.

The current study aimed to clarify these important questions by assessing the impact of admission hemoglobin levels and anemia on stroke mortality at different time points, up to one-year follow-up. A systematic review and meta-analysis was also carried out in order to further quantify the impact of admission hemoglobin/anemia on stroke mortality outcomes.

Methods

Database study

The study population consisted of 8,013 patients with acute stroke, consecutively admitted between January 2003 – May 2015 to Norfolk and Norwich University Hospital, a regional tertiary center in East Anglia, UK, with a catchment population of approximately 750,000. Ethical approval was obtained from the Newcastle and Tyneside National Health Service (NHS) Research Ethics Committee (12/NE/0170) and the study protocol was approved by the Steering Committee of the Register.

The data collection methods for this prospective hospital-based register have been previously reported[13]. Briefly, the data were obtained from paper and electronic records, reviewed and then entered onto the register database. This was done by the hospital stroke data team and vetted by clinical team members for accuracy. For each patient admitted, the pre-stroke modified Rankin score (mRS) (see footnote Table 1), as modified by UK-TIA investigators [14], was ascertained from nursing and medical records by stroke specialist nurses. At discharge, the dead or alive status was recorded to capture in-hospital mortality. Follow-up for mortality was obtained by electronic record linkage with Office of National Statistics data through hospital episodes in May 2015. For the purposes of this study, the follow-up was truncated at 365 days for all patients.

The variables included were age, sex, stroke sub-type (ischemic/hemorrhagic), pre-stroke disability depicted by mRS (0 – 5), Oxfordshire Community Stroke Project (OCSP) classification (Total Anterior Circulation Stroke, Partial Anterior Circulation Stroke, Posterior Circulation Stroke, Lacunar Stroke), hemoglobin levels at admission, co-morbidities (Coronary Heart Disease, Congestive Heart Failure, Atrial Fibrillation, Hypertension, Hyperlipidemia, Previous Stroke, Diabetes Mellitus, Peripheral Vascular

Disease, Gastrointestinal Bleeding, Peptic Ulcers, Chronic Obstructive Pulmonary Disease, Chronic Kidney Disease, Falls, Malignancy, Dementia) and prior use of antithrombotics. Mortality was assessed at several different time points; in patient, 7 days, 14 days, 1 month, 3 months, 6 months and 1 year. However, results were displayed selectively in Tables 2 and 3 – data for the 7 and 14 day time points was not included due to the similarity in results and in order to ensure brevity. Only confirmed cases of stroke were included. Stroke was diagnosed using evidence from clinical features and neuroimaging (typically CT and in some cases MRI). Anemia was defined according to the WHO criteria of Hb <12.0 g/dL in females and <13.0 g/dL in males and elevated hemoglobin was defined as >15.5g/dL in females and >17.0g/dL in males[15].

The associations between hemoglobin levels and age, sex, pre-stroke mRS, stroke type, OCSP classification, co-morbidities, prior antithrombotic use and inpatient mortality were assessed using chi-squared test. Logistic regression models were constructed to assess the impact of hemoglobin levels (by quintiles) and anemia on odds of death. Univariate and multivariate models were used to calculate unadjusted and adjusted odds ratios. Sex and stroke type specific analyses were performed controlling for age, OCSP classification, pre-stroke mRS, co-morbidities and prior antithrombotic usage.

To better understand the potential mediating factors for the observed associations, we examined the distribution of selected chronic co-morbidities between patients with anemia and no anemia and also assessed the differences in proportions of patients receiving antithrombotic medications by a vascular indication (defined as presence of previous Stroke, Coronary Heart Disease, Diabetes Mellitus, Peripheral Vascular Disease, Hypertension and Atrial Fibrillation). The analysis was performed using the SPSS Version 23.0 (SPSS Inc., Chicago, Illinois, USA).

Systematic review and meta-analysis

We selected full journal articles reporting on studies that evaluated the association between baseline hemoglobin or anemia and subsequent mortality in patients diagnosed with stroke. PubMed and EMBASE were searched from inception until December 2014 using the terms shown in Figure 1, with no language restriction. In addition, we checked the bibliographies of relevant articles for any studies that met our selection criteria.

Two reviewers (RB and KH) independently screened abstracts and titles. Potentially relevant studies were reviewed in order to confirm their eligibility. The selection and data extraction of included studies was performed by RB and KH and checked by a senior reviewer, YKL. In order to assess study validity, included studies were assessed for the following; methods used for diagnosing stroke, determination of hemoglobin levels and anemia, ascertainment of mortality/outcome subsequent to the stroke and the analytic procedures aimed at minimizing the risk of bias from confounders. We pooled the reported associations (adjusted odds ratio where available) using the inverse variance method and random effects model in RevMan 5.3 software (Nordic Cochrane Center, Copenhagen, Denmark). The comparisons of interest were for categories of anemia versus no anemia, in patients with ischemic and hemorrhagic stroke, versus the referent normal category. We evaluated heterogeneity by calculating the I^2 statistic, whereby a value >50% was indicative of substantial heterogeneity. We also aimed to check for publication bias through a funnel plot if there were more than 10 eligible studies in our systematic review.

Results

Database study

Of the 11,886 episodes recorded in the registry, 3,873 were excluded due to various reasons, which are presented in Figure 2. 2,659 of these patients were excluded due to missing data and 991 were excluded because they were episodes which related to secondary entry into the register due to subsequent stroke. The sample included in the current study consisted of 8,013 patients with acute stroke, admitted consecutively between January 2003 – May 2015. The mean age in the cohort was 77.81 ± 11.83, with 52.4% females, and 86.7% had ischemic stroke. The most common OCSP stroke classification was PACS (33.1%) and the majority of patients (62.6%) had a pre-stroke mRS of 0. Inpatient mortality was 21.3% and 1 in 4 patients (24.5%) had anemia on admission.

Table 1 shows sex-specific sample characteristics by anemia status. Increasing age, higher pre-stroke disability, increased stroke severity, inpatient mortality and all co-morbidities (with the exceptions of Hyperlipidemia in females) were associated with anemia (see Figures 3 & 4). Prior antithrombotic use in males and ischemic stroke in females were also associated with anemia.

Table 2 depicts the impact of hemoglobin levels on stroke mortality by quintiles of sex-specific admission hemoglobin levels, presented separately for ischemic and hemorrhagic stroke. Quintile 1 contains those with the lowest values and Quintile 5 with the highest. The cut-off points were 12.40, 13.80, 14.64 and 15.60 (g/dL) for males and 11.70, 12.80, 13.60, 14.50 (g/dL) for females. In males with ischemic stroke, low hemoglobin (Quintile 1) was significantly associated with increased mortality at all of the time points measured, compared to those with normal hemoglobin levels (Quintile 3). High hemoglobin (Quintile 5) was also associated with increased odds of mortality at four time points; inpatient, 7 days, 14 days and

1 month. This suggested a U-shaped relationship between hemoglobin levels and short-term mortality in males with ischemic stroke. In females with ischemic stroke, low hemoglobin levels were significantly associated with mortality at five time points; inpatient, 1 month, 3 months, 6 months and 1 year. In females with hemorrhagic stroke, low hemoglobin levels were associated with increased mortality at all time points.

Table 3 shows the impact of anemia and elevated hemoglobin levels on mortality. In males with ischemic stroke, anemia was associated with higher odds of death at all time points assessed and elevated hemoglobin was associated with increased odds of death at 3 time points; inpatient, 1 month and 3 months. In males with hemorrhagic stroke, anemia was associated with increased mortality at 1 year and elevated hemoglobin was associated with increased mortality at four time points; inpatient, 7 days, 14 days and 1 month. In females with ischemic stroke, anemia was associated with increased mortality at 1 month, 3 months, 6 months and 1 year, while elevated hemoglobin was associated with increased mortality at 7 days, 14 days and 1 month. In females with hemorrhagic stroke, anemia was associated with increased mortality at all time points assessed, while elevated hemoglobin was associated with increased mortality at three time points; inpatient, 6 months and 1 year.

Table 4 depicts prior antithrombotic use by anemia status and vascular indication. In females with a positive vascular indication, those with anemia were less likely to be on prior antithrombotics compared to those without anemia (p-value 0.032). Conversely, in males with a negative vascular indication, those with anemia were more likely to be on prior antithrombotics than those without anemia (p-value <0.001). In addition, anemia was associated with increased co-morbidity burden in both sexes (Figures 3 & 4)

Systematic review and meta-analysis

Our search identified 1,424 citations. After detailed screening, 20 studies were included in our systematic review; the flow chart of study selection is shown in Figure 5. Ten studies assessed the impact of anemia on stroke [1, 2, 10, 19 – 23, 26, 28] and ten evaluated the association between stroke and hemoglobin levels [6 - 9, 16 - 18, 24, 25, 27]. In terms of study design, three were retrospective cohort studies[1, 22, 28], thirteen were prospective cohort studies[2, 6 - 10, 16 - 21, 26] and two were secondary analyses of a randomized control trials[24, 25]. There were also two studies which did not state their design [23, 27]. There was a high degree of geographical location, with cohorts from Germany[8, 18, 21], Switzerland[22, 28], United States[17, 20], China[2], Canada[16], India[6], Israel[10], South Korea[9], Denmark[23], Taiwan[19], United Kingdom[27] and Poland[7]. There were also three studies conducted across multiple states[1, 24, 25]. Regarding stroke type, nine studies assessed patients with ischemic stroke[1, 2, 8, 9, 19, 22 - 25], six assessed patients with hemorrhagic stroke[16 - 18, 20, 21, 26] and five evaluated both types of stroke[6, 7, 10, 27, 28]. The number of participants in the studies ranged from 106 to 3,020. When combined with the participants from the current study, this resulted in a total pooled study population of 29,943 participants of whom 24,816 were meta-analysed. Odds ratios included in the meta-analysis were from the mortality time-point of 12 months, or the closest available to this. Tables 5 and 6 shows the key features of the selected studies.

Validity Assessment

Different methods were used for ascertainment of stroke diagnosis. Imaging (CT, MRI or both) was used in seventeen studies[1, 2, 6, 7, 10, 16 – 26], one study relied on clinical evaluation alone [27] and two did not state the method used[9, 28]. The methods used to ascertain mortality also varied. Attending doctors were used to confirm in-hospital mortality

in two studies[17, 18], while death registry data was used in three[10, 16, 23]. Telephone interviews were used by nine studies, typically in conjunction with other methods such as outpatient visit, home visit, mailed questionnaires, analysis of death registries or review of medical records[6, 8, 9, 20 - 22, 26, 28]. One study used outpatient visits only[19] and the method used to establish mortality status was unclear in six studies[1, 2, 7, 24, 25, 27]. Despite the variety of approaches taken to ascertain mortality, none were notably unreliable.

Eleven studies used the WHO definition of anemia as hemoglobin cut-offs[2, 8, 10, 17, 19, 20 - 23, 26, 28], seven used pre-specified values [1, 9, 16, 18, 24, 25, 27] and two did not specify the values used[6,7]. By using pre-specified thresholds in constructing categorical comparisons for anemia, it is possible that cut-points have been drawn up which favour statistically significant findings. Eighteen studies adjusted for potential confounders[1, 2, 6, 7, 8, 10, 16 - 27], however, there was great variation in terms of the variables adjusted for. These ranged from age and NIHSS[1] to age, sex, insurance status, smoking, time to treatment, type of intervention, pre-stroke medication, body mass index, blood pressure, heart rate, TOAST classification, metabolic parameters and co-morbidities[22]. Many studies were therefore liable to residual confounding (Table 6).

Meta-analysis of pooled results show anemia is associated with an increased risk of mortality in ischemic stroke; pooled OR of 1.97 (1.56 – 2.47) (Figure 6).We also found a significant association for the evaluation of anemia and mortality in hemorrhagic stroke, albeit at a lower magnitude of association; OR of 1.47 (1.23 – 1.74) (Figure 7).The number of studies providing ORs on the relationship between elevated hemoglobin and stroke mortality were insufficient for a meta-analysis to be carried out. While available data suggests elevated hemoglobin predicts short-term mortality in ischemic stroke, it is less consistent for hemorrhagic stroke (Table 7). The funnel plot, depicting odds ratios for mortality in anemic ischemic stroke patients, shows asymmetry (Figure 8), with an under-representation of

studies on the right side which we would typically expect to consist of those reporting no significant harm in the relationship between anemia and stroke mortality. We encountered five such studies that reported no significant association in our systematic review, which we could not incorporate into the meta-analysis because the odds ratios were not given, thus causing asymmetry in the funnel plot.

Discussion

Our study examined the association between anemia/hemoglobin levels and mortality in acute stroke in a large unselected stroke patient population and also sought to quantify this association using systematic review and meta-analysis. At 24.5%, anemia had a high prevalence in the cohort analysed in the current study. Low hemoglobin levels were associated with older age, increased stroke severity, higher pre-stroke disability and the increased co-morbidity burden. This suggested that outcomes were mediated by the impact of confounders. However, we found anemia to be independently associated with mortality, subsequent to making the appropriate adjustments. A systematic review and meta-analysis of the literature confirmed our findings. In addition, we found elevated hemoglobin to be associated with poorer outcomes in acute stroke suggesting a U-shaped relationship between hemoglobin levels and stroke mortality.

The literature has described several pathological mechanisms which can plausibly explain the independent association between anemia and increased mortality risk in stroke. Firstly, by lowering the oxygen carrying capacity of blood, anemia may intensify ischemia and therefore hypoxia within the penumbral lesions in patients with ischemic stroke[29,30]. Secondly, anemia can compromise cerebrovascular autoregulation leading to fluctuations in cerebral perfusion, which in turn alters the delivery of oxygen to the brain[31, 32], thereby

exacerbating damage caused by ischemia or hemorrhage. Thirdly, augmentation of cerebral blood flow can create turbulence, which can in turn trigger the migration of an existing thrombus leading to a thromboembolism[33]. Fourthly, anemia may lead to hyperdynamic circulation, which has been shown to modulate the expression of adhesion molecules on vascular endothelial cells by upregulating their production. This may trigger an inflammatory response which leads to thrombus formation in a process similar to atherosclerosis[34, 35]. Fifthly, anemia may worsen outcomes in stroke due to its relationship with inflammatory mediators; it can upregulate the production of iNOS and CXCR4[36], both of which have been associated with brain damage during ischemia[37, 38].

In addition to the pathophysiological mechanisms described above, there is also a plausible clinical explanation for the excess mortality risk in stroke patients with anemia. It may be the case that anemic patients were less likely to be prescribed antithrombotics due to the increased risk of bleeding. This was suggested by the finding in Table 4, where fewer anemic females, who had a positive vascular indication, were on prior antithrombotics compared to those without anemia. This finding potentially supports the well documented differential management of cardiovascular risk factors between sexes. The reverse trends are observed for those without vascular indications thus supporting previous observations that inappropriate prescribing may be more prevalent in females.

The association between anemia and mortality suggests that interventions may improve outcomes. While previous studies have shown packed red blood cell (pRBC) transfusions to reduce mortality at 30-days in anemic patients with myocardial infarction[39], a recent systematic review and meta-analysis found blood transfusion after percutaneous coronary intervention to be associated with adverse outcomes [40] thus casting doubt on the potential benefits of pRBC transfusions in anemic stroke patients. Observational studies reporting the association between mortality and transfusion in anemic patients with

hemorrhagic stroke have had varied results, with one finding a reduction in mortality [41] and another finding no change[18]. To the knowledge of the authors, no studies assessing the impact of pRBC transfusion on anemic ischemic stroke patients have been conducted. Due to the paucity of evidence, guidelines have been unable to specify hemoglobin targets or optimal management options [11]. A randomized controlled trial is required to gauge the impact of transfusions and establish optimum hemoglobin ranges in patients with acute stroke.

Our study has a number of strengths. The stroke cases were prospectively identified and the cohort had an almost complete follow-up using validated methods. As a large sample population was used, it was possible to conduct a rigorous analysis by sex and stroke type, enabling us to provide new insights. We were also able to control for a diverse array of confounders thereby mitigating the effects of residual confounding. The meta-analysis included individuals from a wide array of countries increasing the generalizability of our findings. The inclusion of a large number of participants in the meta-analysis provided sufficient statistical power to obtain results for both stroke sub-types. Finally, all studies included in the meta-analysis were of high methodological quality.

This study has some limitations. The small sample number of patients with hemorrhagic stroke may have contributed to the non-significant p-values. Some of the models used did not fit the data well. Hosmer-Lemeshow tests were significant for ischemic stroke in males at 3, 6 and 12 months (see Table 2). Although this does not alter the associations found it indicates that for this subgroup there may be other factors or interactions which might help better predict mortality outcome at these time points. It is therefore possible that we were not able to control for unknown factors. As a registry based study we were not able to fully adjust for treatment effect (e.g. blood transfusion, use of iron supplements and erythropoietin stimulating agents). Nonetheless, transfusion for mild to moderate anemia in

stroke is not a routine practice and the likelihood of such confounding is thus minimal. We were unable to take into account the duration of anemia or assess the impact of abnormal hemoglobin levels subsequent to a stroke. The independent association between anemia and excess mortality in stroke cannot, therefore, be described as a causal relationship. The studies in the meta-analysis had high heterogeneity for ischemic stroke (I^2>50%). Finally, the possibility of under-representation of studies that reported no significant harm in the relationship between anemia and stroke mortality raises the possibility of selective reporting. As a consequence of this, our meta-analysis may over-inflate estimates of the association between anemia and excess mortality risk.

To conclude, we have shown that a significant proportion of stroke patients have anemia at the time of stroke onset and that this is associated with increased mortality up to one year. The optimal treatment option in this patient group is unclear. Studies are required to examine the clinical and cost effectiveness of interventions in this patient population in an acute stroke setting.

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Figure Legend

Contributions of Authors

PKM is the PI of NNUSTR. PKM conceived the study. JBHS performed data linkages. RSB and SJM analysed the data for cohort study. JFP, KMB and AKM are co-I of NNUSTR. RSB and KH performed systematic review & meta-analysis under supervision of YKL. RSB, YKL and PKM drafted the manuscript. All authors contributed in writing the paper. PKM is the guarantor.

Acknowledgments

We thank the stroke data team for their contribution to maintain the NNUH stroke & TIA registers.

Funding Sources

The NNUH Stroke and TIA Register is maintained by the NNUH NHS Foundation Trust Stroke Services and data management for this study is supported by the NNUH Research and Development Department through Research Capability Funds.

Conflicts of Interest Disclosures

PKM received small honorarium <£1000 from ViForPharma as an advisory panel member on one occasion.

Figure [1]. Search Strategy

Disease term: (stroke OR intracranial-hemorrhage OR intracerebral-hemorrhage)

AND

Anemia term: (haemoglobin OR hemoglobin OR anaemia OR anemia)

AND

Outcome term: (mortality OR fatal* OR survival OR death)

NOT

(rivaroxaban OR dabigatran OR apixaban OR sickle OR surgery OR glycated OR glycosylated OR HbA1C OR erythropoie*)

Figure [2]. Patient Inclusion Chart

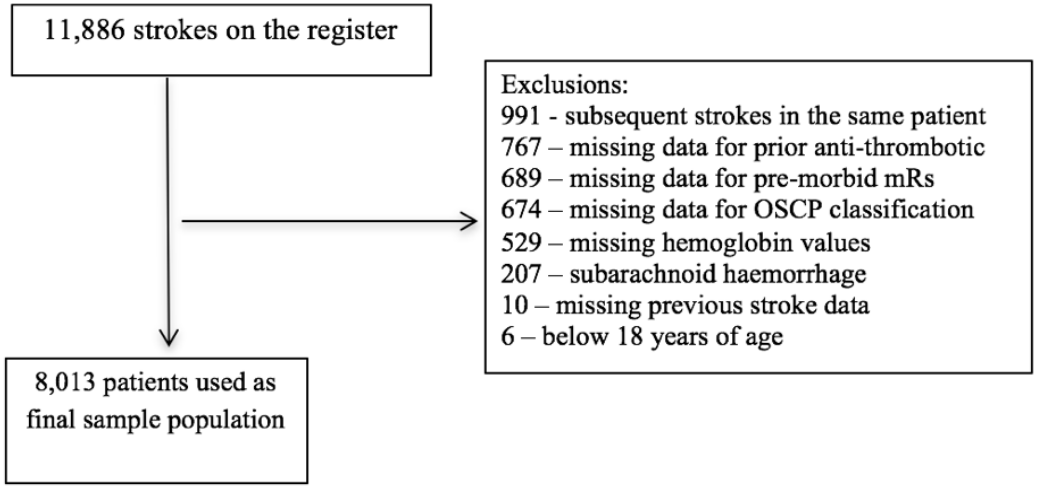
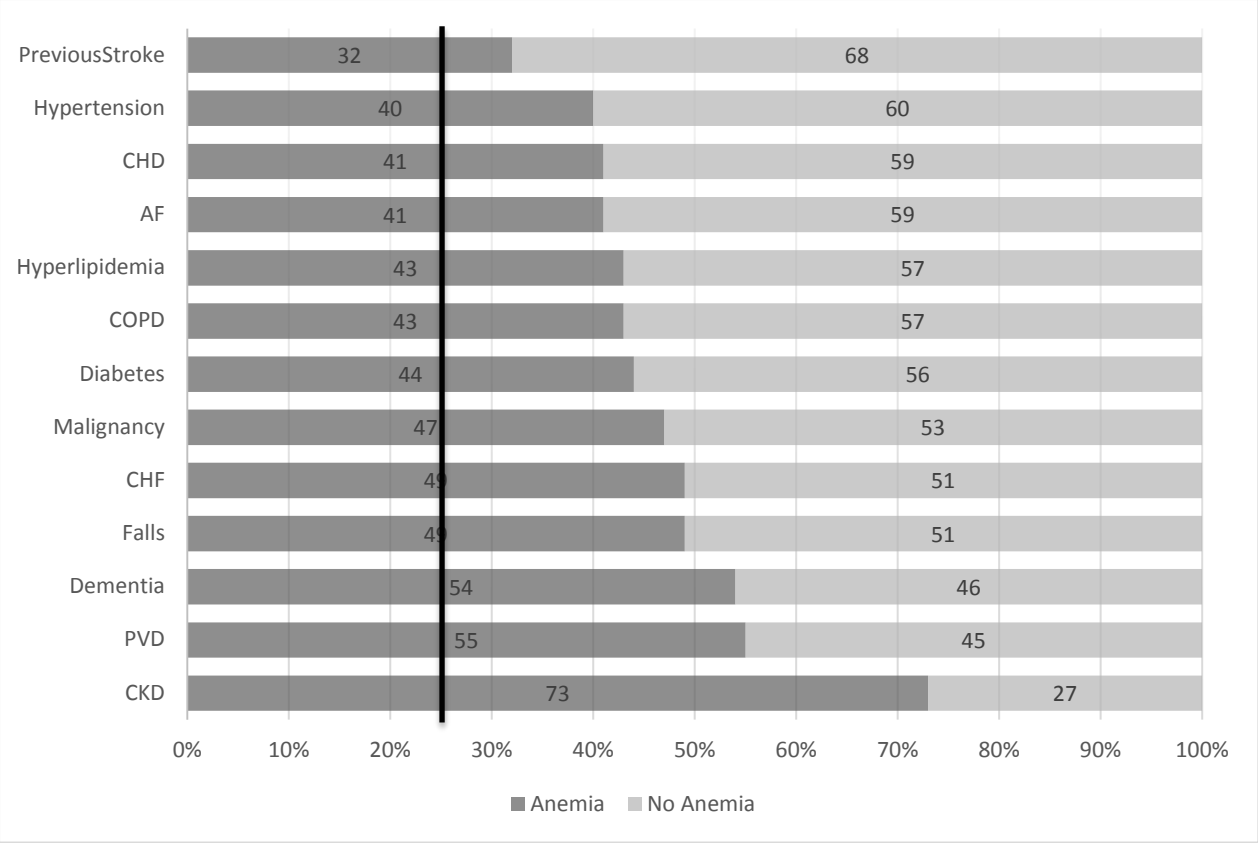
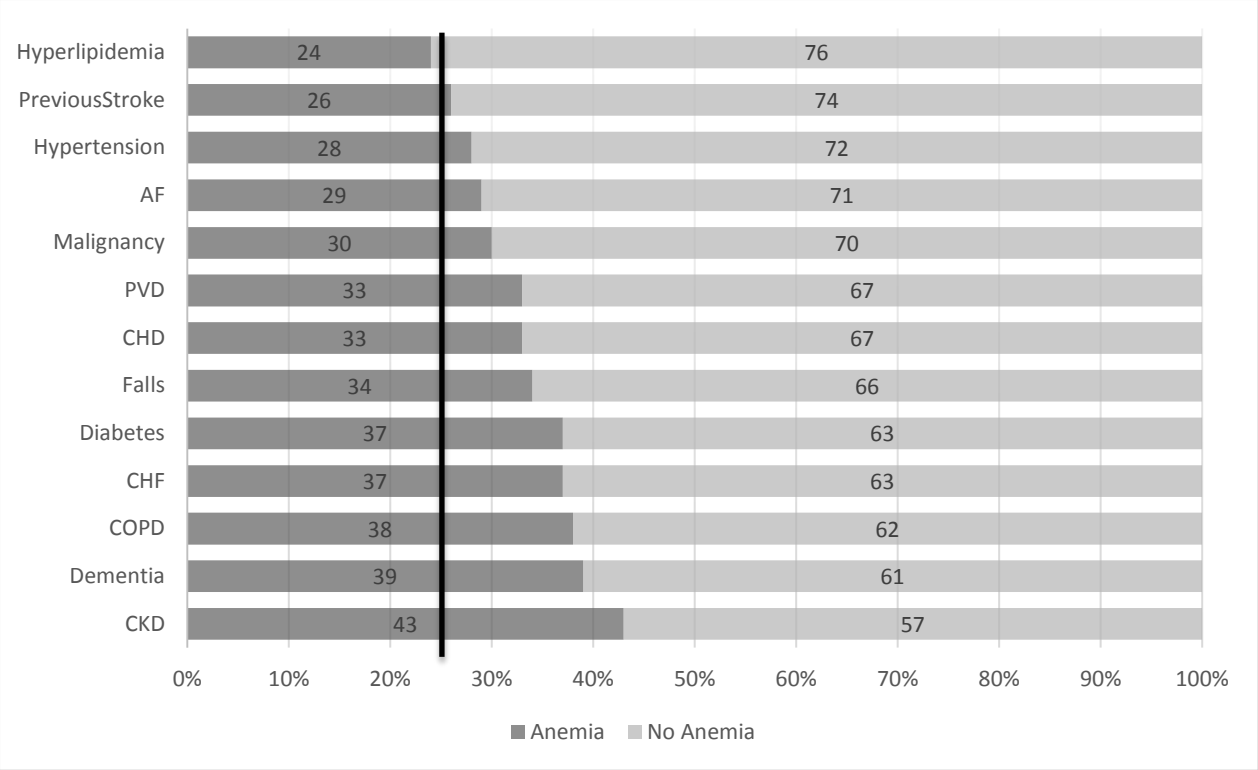


Figure [3]. Prevalence of Co-Morbidities by Anemia Status in Males



The vertical line in the figure above represents the expected proportion of co-morbidity based on the proportion of stroke patients with anemia. Therefore any dark bars to the right of the vertical line represent higher co-morbidity burden in anemic patients compared with patients who were not anemic.

Figure [4]. Prevalence of Co-Morbidities by Anemia Status in Females



The vertical line in the figure above represents the expected proportion of co-morbidity based on the proportion of stroke patients with anemia. Therefore any dark bars to the right of the vertical line represent higher co-morbidity burden in anemic patients compared with patients who were not anemic.

Figure [5] Flow Diagram of Study Selection

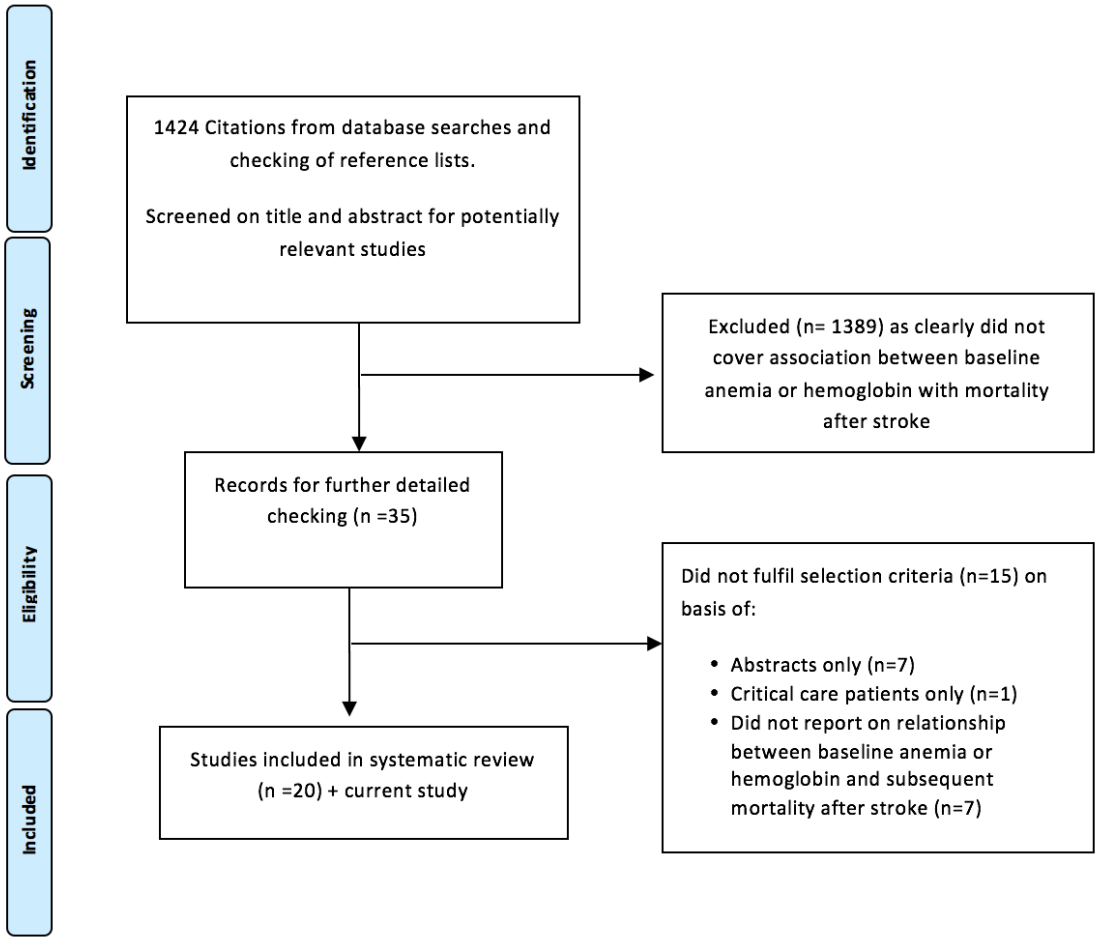


Figure [6]. Meta-Analysis of Studies Analysing the Impact of Anemia on Admission on Mortality in Ischemic Stroke

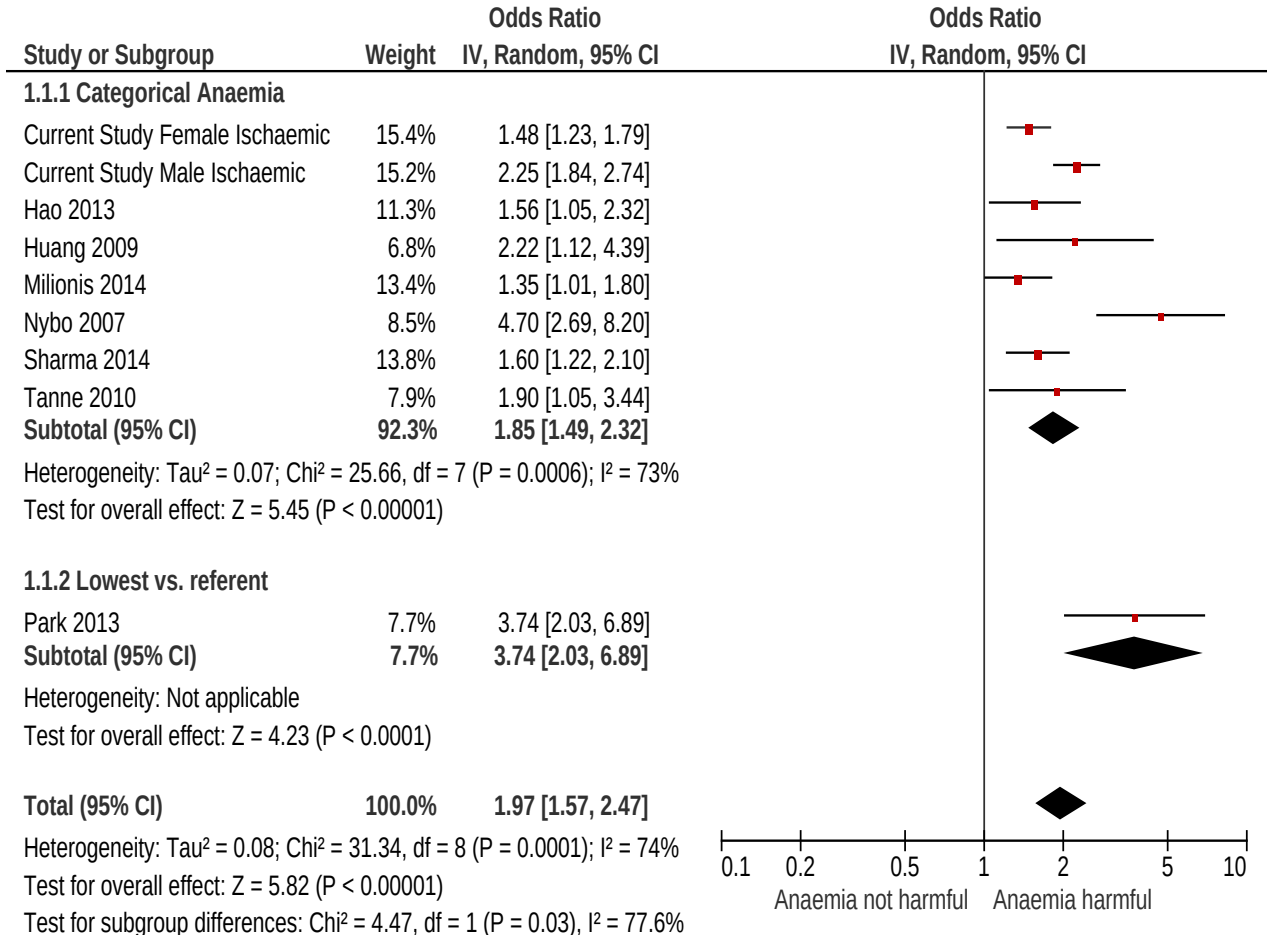


Figure [7]. Meta-Analysis of Studies Analysing the Impact of Anemia on Admission on Mortality in Hemorrhagic Stroke

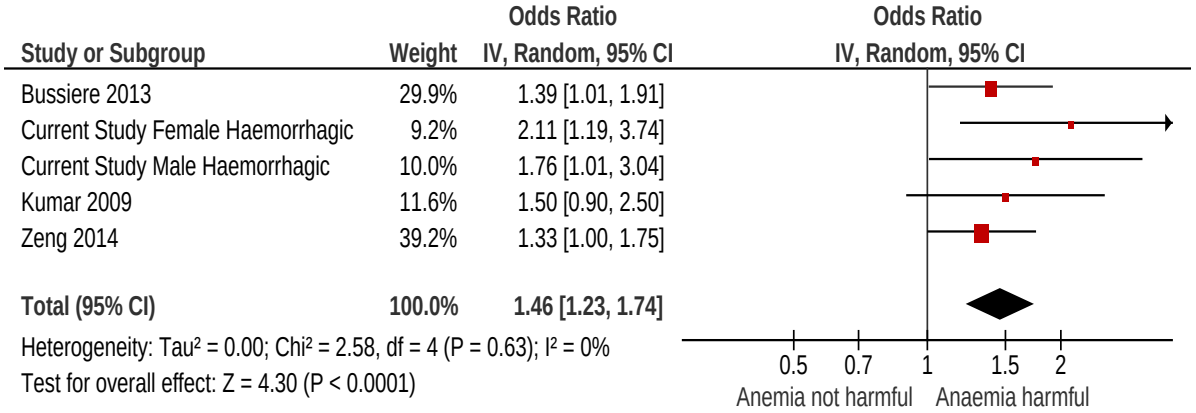


Figure [8]. Funnel Plot of Odds Ratios from Studies Analyzing the Impact of Anemia on Admission on Mortality in Ischemic Stroke

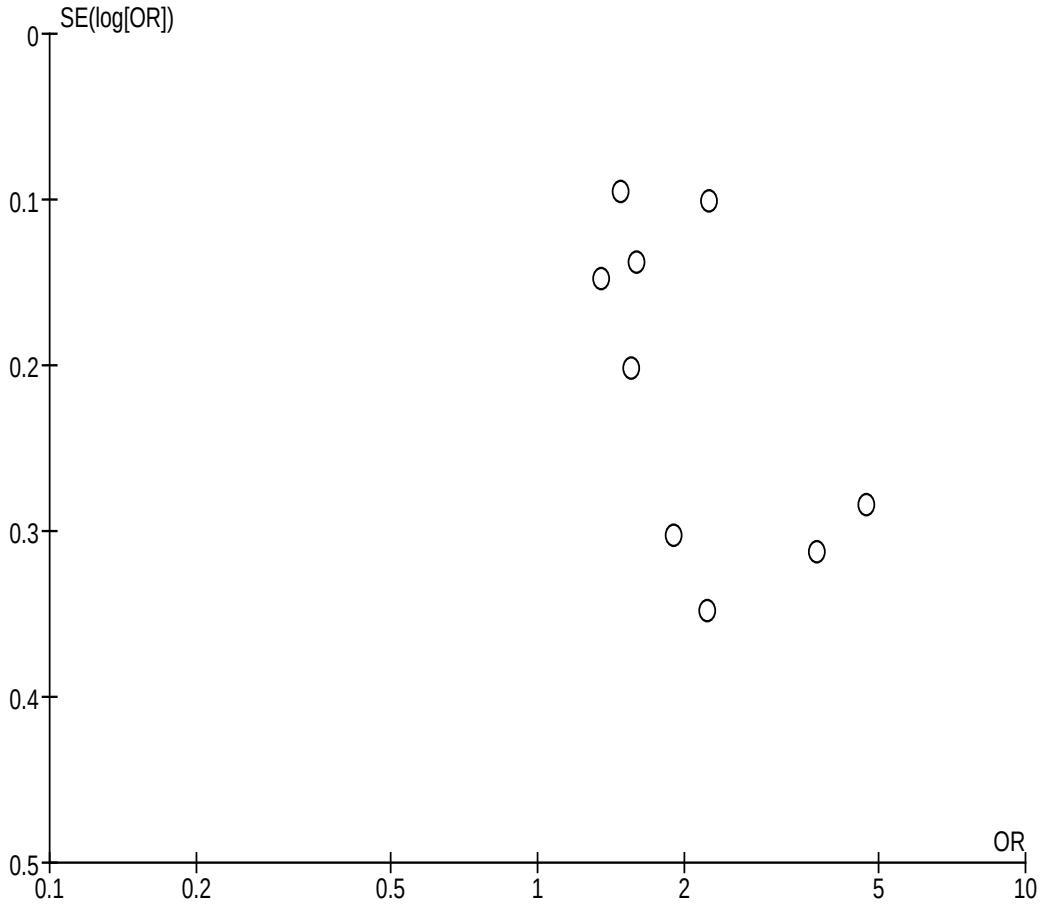


Table [1]. Sex-specific Sample Characteristics by Anemia Status

	Male			Female		
	Anemia	No Anemia	P-Value	Anemia	No Anemia	P-Value
Number	1,017 (26.7)	2,794 (73.3)		947 (22.5)	3,255 (77.5)	
Age			<0.001			<0.001
≤ 60	46 (4.5)	424 (15.2)		29 (3.1)	208 (6.4)	
61 – 65	32 (3.1)	282 (10.1)		21 (2.2)	143 (4.4)	
66 – 70	50 (4.9)	340 (12.2)		33 (3.5)	231 (7.1)	
71 – 75	127 (12.5)	411 (14.7)		65 (6.9)	344 (10.6)	
76 – 80	187 (18.4)	470 (16.8)		137 (14.5)	522 (16.0)	
81 – 85	247 (24.3)	475 (17.0)		232 (24.5)	747 (22.9)	
86 – 90	234 (23.0)	279 (10.0)		253 (26.7)	635 (19.2)	
≥ 91	94 (9.2)	113 (4.0)		177 (18.7)	435 (13.4)	
Pre-stroke Comorbidity						
Coronary Heart Disease	304 (29.9)	446 (16.0)	<0.001	230 (24.3)	475 (14.6)	<0.001
Previous Stroke	287 (28.2)	607 (21.7)	<0.001	261 (27.6)	763 (23.4)	0.009
Congestive Heart Failure	143 (14.1)	154 (5.5)	<0.001	147 (15.5)	255 (7.8)	<0.001
Atrial Fibrillation	217 (21.3)	307 (11.0)	<0.001	206 (21.8)	508 (15.6)	<0.001
Hypertension	422 (41.5)	650 (23.3)	<0.001	393 (41.5)	1,044 (32.1)	<0.001
Hyperlipidaemia	76 (7.5)	99 (3.5)	<0.001	42 (4.4)	135 (4.1)	0.698
Diabetes Mellitus	183 (18.0)	231 (8.3)	<0.001	137 (14.5)	242 (7.4)	<0.001
Peripheral Vascular Disease	63 (6.2)	49 (1.8)	<0.001	28 (3.0)	61 (1.9)	0.042
GI Bleeding and Peptic Ulcer	81 (8.0)	118 (4.2)	<0.001	62 (6.5)	142 (4.4)	0.006
COPD	90 (8.8)	113 (4.0)	<0.001	65 (6.9)	107 (3.3)	<0.001
Chronic Kidney Disease	93 (9.1)	37 (1.3)	<0.001	50 (5.3)	69 (2.1)	<0.001
Falls	161 (15.8)	160 (5.7)	<0.001	275 (29.0)	557 (17.1)	<0.001
Malignancy	240 (23.6)	274 (9.8)	<0.001	112 (11.8)	278 (8.5)	0.002
Dementia	47 (4.6)	43 (1.5)	<0.001			
Prior Antithrombotic Use			<0.001			0.286

No	447 (44.0)	1,538 (55.0)		499 (52.7)	1,779 (54.7)	
Yes	570 (56.0)	1,256 (45.0)		448 (47.3)	1,476 (45.3)	
Pre-stroke Rankin Score†			<0.001			<0.001
0	556 (54.7)	2,119 (75.8)		388 (41.0)	1,955 (60.1)	
1	155 (15.2)	286 (10.2)		135 (14.3)	399 (12.3)	
2	96 (9.4)	147 (5.3)		121 (12.8)	303 (9.3)	
3	131 (12.9)	142 (5.1)		160 (16.9)	354 (10.9)	
4	54 (5.3)	73 (2.6)		90 (9.5)	176 (5.4)	
5	25 (2.5)	27 (1.0)		53 (5.6)	68 (2.1)	
Stroke Type			0.088			0.002
Hemorrhagic	121 (11.9)	392 (14.0)		95 (10.0)	454 (13.9)	
Ischemic	896 (88.1)	2,402 (86.0)		852 (90.0)	2,801 (86.1)	
OCSP Classification			0.002			<0.001
TACS	215 (21.1)	500 (17.9)		248 (26.2)	699 (21.5)	
PACS	359 (35.3)	900 (32.2)		312 (32.9)	1,084 (33.3)	
POCS	169 (16.6)	548 (19.6)		118 (12.5)	521 (16.0)	
LACS	209 (20.6)	696 (24.9)		186 (19.6)	786 (24.1)	
Undefined	65 (6.4)	150 (5.4)		83 (8.8)	165 (5.1)	
Inpatient Mortality			<0.001			<0.001
Alive	731 (71.9)	2,393 (85.6)		635 (67.1)	2,546 (78.2)	
Dead	286 (28.1)	401 (14.4)		709 (21.8)	312 (22.5)	

*Hb = hemoglobin, OCSP = Oxfordshire Community Stroke Project, TACS = Total Anterior Circulation Stroke, PACS = Partial Anterior Circulation Stroke, POCS = Posterior Circulation Stroke, LACS = Lacunar Stroke, GI = Gastrointestinal

† 0 = no symptoms.

1 = no significant disability despite symptoms; able to carry out all usual duties and activities.

2 = slight disability; unable to perform all previous activities but able to look after own affairs without assistance.

3 = moderate disability; requires some help but able to walk without assistance.

4 = moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance.

5 = severe disability; bedridden, incontinent, and requiring constant nursing care and attention.

‡The data presented are number (%) for categorical variables.

Table [2]. The Impact of Hemoglobin Levels on Mortality at Different Time Points (Logistic Regression)

Variable	Hb Quintile 1	Hb Quintile 2	Hb Quintile 3	Hb Quintile 4	Hb Quintile 5*	Events
Male Ischemic						
Number	635	675	667	644	677	
Inpatient	**2.64 (1.83 – 3.81)**	**1.56 (1.08 – 2.25)**	1.00	**1.59 (1.06 – 2.38)**	**1.62 (1.08 – 2.42)**	511
1 Month	**2.99 (2.06 – 4.34)**	**1.74 (1.19 – 2.53)**	1.00	1.55 (1.03 – 2.34)	**1.79 (1.19 – 2.68)**	488
3 Months	**3.09 (2.24 – 4.25)**	1.34 (0.96 – 1.85)	1.00	1.18 (0.82 – 1.69)	1.37 (0.96 – 1.95)	674
6 Months	**2.92 (2.16 – 3.94)**	**1.37 (1.01 – 1.85)**	1.00	1.05 (0.75 – 1.46)	1.16 (0.83 – 1.63)	796
1 Year	**2.90 (2.18 – 3.86)**	**1.43 (1.08 – 1.90)**	1.00	1.17 (0.86 – 1.59)	1.17 (0.86 – 1.60)	971
Male Hemorrhagic						
Number	83	109	103	87	131	
Inpatient	1.23 (0.58 – 2.60)	0.83 (0.42 – 1.65)	1.00	0.86 (0.39 – 1.86)	1.05 (0.53 – 2.09)	176
1 Month	1.22 (0.59 – 2.51)	0.92 (0.48 – 1.78)	1.00	0.81 (0.39 – 1.70)	0.93 (0.48 – 1.80)	173
3 Months	1.16 (0.55 – 2.42)	0.67 (0.34 – 1.30)	1.00	0.76 (0.38 – 1.57)	0.68 (0.35 – 1.57)	200
6 Months	1.65 (0.77 – 3.55)	0.81 (0.41 – 1.60)	1.00	0.78 (0.38 – 1.62)	0.73 (0.38 – 1.41)	221
1 Year	1.97 (0.92 – 4.22)	0.81 (0.42 – 1.58)	1.00	0.73 (0.35 – 1.51)	0.79 (0.41 – 1.51)	229
Female Ischemic						
Number	698	748	700	752	755	
Inpatient	**1.47 (1.08 – 1.98)**	1.05 (0.77 – 1.43)	1.00	**1.39 (1.01 – 1.90)**	1.20 (0.88 – 1.63)	792
1 Month	**1.48 (1.09 – 2.01)**	1.16 (0.85 – 1.58)	1.00	1.23 (0.89 – 1.69)	1.26 (0.92 – 1.73)	733
3 Months	**1.70 (1.28 – 2.25)**	1.16 (0.87 – 1.54)	1.00	**1.34 (1.00 – 1.79)**	1.19(0.89 – 1.58)	1007
6 Months	**1.86 (1.42 – 2.44)**	1.22 (0.93 – 1.60)	1.00	**1.44 (1.09 – 1.89)**	1.26 (0.96 – 1.67)	1159
1 Year	**1.86 (1.44 – 2.41)**	1.23 (0.96 – 1.59)	1.00	1.29 (0.99 – 1.68)	1.13 (0.87 – 1.46)	1328
Female Hemorrhagic						
Number	72	100	132	127	118	
Inpatient	**2.56 (1.23 – 5.32)**	0.80 (0.41 – 1.57)	1.00	0.80 (0.54 – 1.81)	1.35 (0.74 – 2.47)	229
1 Month	**2.61 (1.31 – 5.36)**	0.80 (0.42 – 1.55)	1.00	1.12 (0.62 – 2.01)	1.46 (0.81 – 2.63)	226
3 Months	**2.26 (1.10 – 4.64)**	0.88 (0.47 – 1.67)	1.00	0.94 (0.53 – 1.68)	1.22 (0.68 – 2.19)	256
6 Months	**2.02 (0.98 – 4.16)**	0.97 (0.52 – 1.82)	1.00	0.80 (0.45 – 1.42)	1.22 (0.68 – 2.18)	278
1 Year	**2.59 (1.23 – 5.44)**	1.20 (0.64 – 2.25)	1.00	0.95 (0.54 – 1.68)	1.35 (0.75 – 2.41)	292

*Hb = hemoglobin

†The cut off points for the quintiles are as follows: Male: 12.4, 13.8, 14.6, 15.6 g/dL. Female: 11.7, 12.8, 13.6, 14.5 g/dL. The variables adjusted for were Age, Oxford Community Stroke Project Classification, Prestroke-Ranking Score, Prior Antithrombotic Use, Coronary Heart Disease, Previous Stroke, Congestive Heart Failure, Atrial Fibrillation, Hypertension, Hypertension, Hyperlipidemia, Diabetes Mellitus, Peripheral Vascular Disease, Chronic Obstructive Pulmonary Disease, Chronic Kidney Disease, Falls, Malignancy, Dementia, Gastrointestinal Bleeding and Peptic Ulcer. We also adjusted for International Normalised Ratio (INR) in patients with Hemorrhagic Stroke. INR was included as a dichotomous categorical variable; < 1.40 vs >= 1.40. Data for the time points of 7 and 14 days have been removed for brevity. Mean ages for the male quintiles were 82.80, 80.48, 78.49, 78.09 and 78.38 for Quintiles 1 – 5 respectively. Mean ages for female quintiles were 83.04, 81.81, 80.45, 78.96 and 78.06 for Quintiles 1 – 5 respectively.

Table [3]. Effect of Anemia and Elevated Hemoglobin on Stroke Outcomes at Different Time Points (Logistic Regression)

	Anemia	Normal	Elevated Hemoglobin	Events
Male - Ischemic				
Number	896	2,277	125	
Inpatient	**1.75 (1.37 – 2.25)**	1.00	**1.85 (1.03 – 3.32)**	511
1 Month	**1.86 (1.46 – 2.38)**	1.00	**1.79 (1.00 – 3.20)**	488
3 Months	**2.18 (1.75 – 2.72)**	1.00	**1.86 (1.08 – 3.18)**	674
6 Months	**2.25 (1.83 – 2.78)**	1.00	1.46 (0.86 – 2.48)	796
1 Year	**2.25 (1.85 – 2.75)**	1.00	1.50 (0.91 – 2.47)	971
Male - Hemorrhagic				
Number	121	367	25	
Inpatient	1.33 (0.77 – 2.31)	1.00	**3.30 (1.19 – 9.17)**	176
1 Month	1.42 (0.83 – 2.42)	1.00	**2.90 (1.08 – 7.75)**	173
3 Months	1.39 (0.81 – 2.39)	1.00	2.08 (0.75 – 5.78)	200
6 Months	1.64 (0.94 – 2.85)	1.00	1.56 (0.56 – 4.40)	221
1 Year	**1.76 (1.01 – 3.04)**	1.00	1.56 (0.56 – 4.35)	229
Female - Ischemic				
Number	852	2,585	216	
Inpatient	1.20 (0.97 – 1.49)	1.00	1.30 (0.87 – 1.94)	792
1 Month	**1.29 (1.04 – 1.60)**	1.00	**1.49 (1.00 – 2.21)**	733
3 Months	**1.39 (1.14 – 1.70)**	1.00	1.19 (0.81 – 1.75)	1007
6 Months	**1.44 (1.18 – 1.75)**	1.00	1.12 (0.78 – 1.62)	1159
1 Year	**1.47 (1.22 – 1.77)**	1.00	1.04 (0.73 – 1.48)	1328
Female - Hemorrhagic				
Number	95	418	36	
Inpatient	**1.90 (1.09 – 3.33)**	1.00	**2.76 (1.16 – 6.56)**	229
1 Month	**1.82 (1.06 – 3.11)**	1.00	2.11 (0.92 – 4.82)	226
3 Months	**1.80 (1.04 – 3.13)**	1.00	2.08 (0.91 – 4.77)	256
6 Months	**2.05 (1.17 – 3.59)**	1.00	**2.99 (1.29 – 6.90)**	278
1 Year	**2.11 (1.19 – 3.74)**	1.00	**2.63 (1.14 – 6.05)**	292

*The cut off points were as follows: Male: 13.00, 17.00 g/dL, Female: 12.00, 15.50 g/dL. The variables adjusted for were Age, Oxford

Community Stroke Project Classification, Prestroke-Ranking Score, Prior Antithrombotic Use, Coronary Heart Disease, Previous Stroke, Congestive Heart Failure, Atrial Fibrillation, Hypertension, Hypertension, Hyperlipidemia, Diabetes Mellitus, Peripheral Vascular Disease, Chronic Obstructive Pulmonary Disease, Chronic Kidney Disease, Falls, Malignancy, Dementia, Gastrointestinal Bleeding and Peptic Ulcer. We also adjusted for International Normalised Ratio (INR) in patients with Hemorrhagic Stroke. INR was included as a dichotomous categorical variable; < 1.40 vs >= 1.40. Data for the time points of 7 and 14 days have been removed for brevity.

Table [4]. Use of Prior Antithrombotic by Anemia Status and Vascular Indication (Chi-Square Test)

	Vascular Indication Yes*			Vascular Indication No		
	Anemia	No Anemia	P-Value	Anemia	No Anemia	P-Value
Male			0.971			**<0.001**
No Antithrombotic	237 (34.2)	456 (65.8)		210 (16.3)	1082 (83.7)	
Antithrombotic	447 (34.3)	857 (65.7)		123 (23.6)	399 (76.4)	
Female			**0.032**			0.334
No Antithrombotic	270 (28.9)	665 (71.1)		229 (17.1)	1114 (82.9)	
Antithrombotic	350 (24.9)	1,057 (75.1)		98 (19.0)	419 (81.0)	

* Indications considered were Previous Stroke, Coronary Heart Disease, Diabetes, Hypertension, Peripheral Vascular Disease, Atrial Fibrillation

Table [5]. Characteristics of Studies Examining the Relationship Between Anemia/Hemoglobin Levels and Stroke Outcomes

Author	Years Sampled	Study Design	n	Exposure(s)	Outcome(s)	Main Result
Bhatia et al [6]	2000 – 2001	Prospective	116	Admission hemoglobin	Mortality at 30 days	Hemoglobin not associated with outcome
Bussiere et al [16]	2003 – 2008	Prospective	2,406	Admission hemoglobin divided into quintiles. Cut-offs: 100, 120, 140, 160 g/l	Mortality at 1 year, mRs at discharge	Hemoglobin predicted mortality at 1 year: aOR 1.39 (1.01-1.91) in hemoglobin <100 vs 141-160 g/l
Chang et al [17]	2008 – 2010	Prospective	106	Admission hemoglobin, nadir hemoglobin and transfusion	In-hospital mortality, length of stay and disability at discharge	Admission anemia did not predict outcomes
Czlonkowska et al [7]	1991 – 1992	Prospective	345	Admission hemoglobin	Mortality at 30 days	Hemoglobin not associated with outcome
Diedler et al [18]	2004 – 2006	Prospective	196	Admission, mean and nadir hemoglobin	mRs at discharge and 6 months	Admission hemoglobin did not predict outcomes
Del Fabbro et al [28]	2001 – 2003	Retrospective	890	Anemia on admission	In-hospital mortality, survival at 1 year	Higher hemoglobin predicted decreased mortality at 1 year: HR 0.98 (0.97 – 1.00)
Gray et al [27]	1985 – 1986	-	122	Admission hemoglobin	Mortality at 4 and 12 weeks	Hemoglobin not associated with outcome
Hao et al [2]	2002 – 2008	Prospective	1,176	Anemia on admission	In-patient mortality, mortality and disability (mRs > 2) at 12 months	Anemia associated with in-patient mortality, aOR 1.66 (1.08 – 2.56) and mortality at 12 months, aOR 1.56 (1.05 – 2.31)
Huang et al [19]	2001 – 2003	Prospective	774	Anemia on admission	In-patient mortality, mRs at discharge, Stroke recurrence at 3 years	Anemia was associated at increased mortality at 3 years: aOR 2.22 (1.13–4.39)

Kellert et al [8]	1998 – 2009	Prospective	217	Admission, mean and nadir hemoglobin	Mortality and mRs at 3 months	Hb decrease was associated with increased mortality at 3 months (OR 1.34: 1.01 – 1.76) but admission hb was not (OR not given)
Kumar et al [20]	1999 – 2005	Prospective	685	Anemia on admission	Mortality at 30 days, ICH Volume	Anemia is not a predictor of mortality on multivariable analysis OR 1.5 (0.9–2.4)
Kuramatsu et al [21]	2006 – 2010	Prospective	435	Anemia on admission	mRs at 90 days and 1 year	Anemia was associated with poor long-term-outcome (mRS 4-6 at 1 year): OR 7.5
Milionis et al [22]	2003 – 2011	Retrospective	2,439	Anemia on admission	Mortality and disability at 12 months	Anemia associated with mortality at 12 months: OR 2.70 (2.12 – 3.43)
Nybo et al [23]	2003 – 2004	-	250	Anemia on admission	Mortality at 6 months	Anemia associated with greater risk of death at 6 months: OR 4.7 (1.1–8.2)
Park et al [9]	2004 – 2009	Prospective	2,681	Admission, nadir, time-averaged and discharge hemoglobin	Mortality and mRs at 3 months	Admission hemoglobin predicted mortality 3 months. aOR's: Q1 vs. Q3 was 3.74 (2.03–6.89) and Q5 vs. Q3 was 1.99 (1.02–3.91)
Sharma et al [24]	2003 – 2011	Post hoc analysis of RCT	3,020	Admission hemoglobin and hemoglobin < 13	All-cause mortality	Hb < 13g/dL was a significant predictor of mortality: HR 1.60 (1.22–2.10)
Sico et al [1]	1998 – 2003	Retrospective	1,306	Anemia on admission	In-patient mortality or discharge to hospice (combined end-point)	Anemia was associated with outcome in patients with less severe stroke on sub-group analysis, aOR: 4.17 (1.47 – 11.90)
Tanne et al [10]	2001 – 2002	Prospective	859	Anemia on admission	Mortality at 1 month and 1 year, functional outcome using Barthel Index	aOR for mortality at 1 month, 1.90 (1.05 – 3.43), 1 year, 1.72 (1.00 – 2.93)

Wade et al [25]	1977 – 1982	Post hoc analysis of RCT	1,377	Hemoglobin >15 vs ≥15 g/dl on study entry	Fatal and non-fatal strokes	No significant difference in hemoglobin levels amongst those who died compared to survivors.
Zeng et al [26]	2007 – 2008	Prospective	2,513	Anemia on admission	Mortality and dependency (mRs > 2) at 1, 3, 6 and 12 months	aOR for mortality in anemic patients compared to non-anemic were, 6-month 1.34 (1.01 – 1.78), 1 year 1.33 (1.00 – 1.75)

Table [6]. Characteristics Determining Study Validity

Study	Method of Stroke Diagnosis	Time of Mortality Measurement	Method of Mortality Measurement	Confounders Adjusted For in Multivariate Logistic Regression	Hemoglobin Cut-offs Used	Stroke Types Considered
Bhatia et al [6]	Imaging	30 days	Telephone, outpatient or home interview	-	-	Both considered together
Bussiere et al [16]	Imaging	30 days, 6 months, 1 year	Population registries	Age, sex, warfarin, INR, glucose, creatinine, blood pressure, IVH	Quintiles. Cut-offs;100, 120, 140, 160, anemia defined <120 g/l	ICH Only
Chang et al [17]	Imaging	In-hospital mortality	Attending physician	Age, nadir hemoglobin, ICH score, intubation	Anemia (WHO definition)	ICH Only
Czlonkowska et al [7]	Imaging or autopsy	30 days	-	Age, decreased consciousness, severity of weakness	-	Both considered together
Diedler et al [18]	Imaging	In-hospital mortality	Attending physician	Age, ICH volume, NIHSS, IVH, ICU stay, mechanical ventilation, RBC transfusion, mean hemoglobin	Anemia definition; <12.1 g/l for women <13.1 g/l for men	ICH Only
Del Fabbro et al [28]	-	In-hospital, 1 year	Telephone interview, population registries	Age, GFR, comorbidities, functional status	Anemia (WHO definition)	Both considered together
Gray et al [27]	Clinical Evaluation	4 weeks and 12 weeks	-	Age, white cell count, haematocrit, hemoglobin, urea	>16g/dL defined as elevated	Both considered together
Hao et al [2]	Imaging	1 year	-	Age, sex, co-morbidities, smoking, alcohol, NIHSS, eGFR	Anemia (WHO definition)	AIS Only

Huang et al [19]	Imaging	3 years	Outpatient interview	Age, co-morbidities	Anemia (WHO definition)	AIS Only
Kellert et al [8]	Imaging	3 months	Telephone and outpatient interview	Age, NIHSS, blood glucose, microcytic and hypochromic RBCs, leucocytosis, creatinine, CRP	Anemia (WHO definition)	AIS Only
Kumar et al [20]	Imaging	30 days	Telephone interview, medical records, population registry	Age, sex, warfarin, ICH volume, IVH, glucose, WBC	Anemia (WHO definition)	ICH Only
Kuramatsu et al [21]	Imaging	90 days and 1 year	Telephone interview, mailed questionnaire	NIHSS, GCS, ICH-Score, ICH Volume, IVH, Graeb score, midline shift, hemoglobin, hematocrit, mechanical ventilation, pneumonia	Anemia (WHO definition)	ICH Only
Milionis et al [22]	Imaging	7 days, 3 months, 12 months	Medical records, death certificate, population registry	Age, sex, smoking, insurance, time to treatment, type of intervention, co-morbidities, prior medication, BMI, blood pressure, heart rate, TOAST Classification, metabolic parameters	Anemia (WHO definition)	AIS Only
Nybo et al [23]	Imaging	6 months	Population registry	Age, sex, co-morbidities, Scandinavian stroke scale	Anemia (WHO definition)	AIS Only
Park et al [9]	-	3 months	Telephone interview, chart review	Age, sex, blood pressure, pre-stroke mRs, NIHSS, co-morbidities, blood glucose, thrombolysis	Pre-specified quintiles	AIS Only
Sharma et al [24]	Imaging	-	-	Age, BMI, co-morbidities, hemoglobin, eGFR	Hemoglobin <13g/dL	AIS Only
Sico et al	Imaging	In-hospital	-	Age, NIHSS	Hematocrit < 31%	AIS Only

[1]						
Tanne et al [10]	Imaging	Mortality at 1 and 1-year	Population registry	Age, sex, stroke type, NIHSS, prior disability, co-morbidities	Anemia (WHO definition)	Both considered separately
Wade et al [25]	Imaging	-	-	-	Cut-off at 15 g/dL	AIS Only
Zeng et al [26]	Imaging	Discharge, 30 days, 3, 6, 12 months	Telephone interview	Age, sex, pre-stroke mRS, NIHSS, BMI, GCS, ICH volume, glucose, co-morbidities, antithrombotic use, antihypoglycemic use, antihyperlipidemic use, family history, smoking, alcohol	Anemia (WHO definition)	ICH Only

Table [7]. Odds Ratios From Studies Evaluating Association Between Elevated Hemoglobin and Stroke Mortality

Ischemic Stroke				
Study	**Definition of Elevated Hemoglobin**	**Mortality time-point**	**Number of patients**	**Odds Ratio**
Park et al [9]	Pre-specified Quintile	3 months	2,681	**1.99 (1.02 – 3.91)**
Current Study Male	Over 17 g/dL	In-patient 1 month 3 months 6 months 1 year	3,298	**1.85 (1.03 – 3.32)** **1.79 (1.00 – 3.20)** **1.86 (1.08 – 3.18)** 1.46 (0.86 – 2.48) 1.50 (0.91 – 2.47)
Current Study Female	Over 15.5 g/dL	In-patient 1 month 3 months 6 months 1 year	3,653	1.30 (0.87 – 1.94) **1.49 (1.01 – 2.21)** 1.19 (0.81 – 1.75) 1.12 (0.78 – 1.62) 1.04 (0.73 – 1.48)
Hemorrhagic Stroke				
Study	**Definition of Elevated Hemoglobin**	**Mortality time-point**	**Number of patients**	**Odds Ratio**
Bussiere et al [16]	Over 16 g/dL	1 year	2,406	1.00 (0.74 – 1.33)
Current Study Male	Over 17 g/dL	In-patient 1 month 3 months 6 months 1 year	513	**3.20 (1.19 – 9.17)** **2.90 (1.08 – 7.75)** 2.08 (0.75 – 5.78) 1.56 (0.56 – 4.40) 1.56 (0.56 – 4.35)
Current Study Female	Over 15.5 g/dL	In-patient 1 month 3 months 6 months 1 year	549	**2.76 (1.16 – 6.56)** 2.11 (0.92 – 4.82) 2.08 (0.91 – 4.77) **2.99 (1.29 – 6.90)** **2.63 (1.14 – 6.05)**