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

Raphae S. Barlas MA(Hons)¹, Katie Honney MRCP², Yoon K. Loke MD³, Stephen J. McCall BSc¹,², Joao H Bettencourt-Silva PhD³, Allan B. Clark PhD³, Kristian M. Bowles PhD³, Anthony K. Metcalf MBChB², Mamas A. Mamas DPhil⁵, John F. Potter DM³, Phyo K. Myint MD ¹,²,³

¹Epidemiology Group, Institute of Applied Health Sciences, Aberdeen, UK
²Stroke Research Group, Norfolk and Norwich University Hospital, Norwich, UK
³Norwich Medical School, University of East Anglia, Norwich, UK
⁴Nuffield Department of Population Health, University of Oxford, Oxford, UK
⁵Keele Cardiovascular Research Group, Institutes of Science and Technology in Medicine and Primary Care and Health Sciences, Keele University, Stoke-on-Trent, UK

Correspondence to:

Phyo Kyaw Myint
Room 4:013, Polwarth Building,
School of Medicine and Dentistry
University of Aberdeen
Foresterhill
AB25 2ZD
Aberdeen, Scotland, UK
Tel: +44 (0) 1224 437974
Fax: +44(0) 1224 437971
Mail to: phyo.myint@abdn.ac.uk

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.
References

[1] Sico JJ, Concato J, Wells CK, Lo AC, Nadeau SE, Williams LS, Peixoto AJ, Gorman M, Boice JL, Bravata DM. Anemia is associated with poor outcomes in patients with less severe ischemic stroke. *J Stroke Cerebrovasc Dis* 2013; 22:271–278.

[2] Hao Z, Wu B, Wang D, Lin S, Tao W, Liu M. A cohort study of patients with anemia on admission and fatality after acute ischemic stroke. *J Clin Neuroscience* 2013; 20:37–42.

[3] Levin A. The relationship of haemoglobin level and survival: direct or indirect effects? *Nephro Dial Transplant*. 2002; 17:8–13.

[4] Silverberg D. Outcomes of anaemia management in renal insufficiency and cardiac disease. *Nephrol Dial Transplant*. 2003; 19:ii7–12.

[5] Sabatine MS, Morrow DA, Giugliano RP, Burton PB, Murphy SA, McCabe CH, Gibson CM, Braunwald E. Association of hemoglobin levels with clinical outcomes in acute coronary syndromes. *Circulation*. 2005; 111:2042–2049.

[6] Bhatia RS, Garg RK, Gaur SP, Kar AM, Shukla R, Agarwal A, Verma R. Predictive value of routine hematological and biochemical parameters on 30-day fatality in acute stroke. *Neurology India*. 2004; 52:220–223.

[7] Czlonkowska A, Ryglewicz D, Lechowicz W. Basic analytical parameters as the predictive factors for 30-day case fatality rate in stroke. *Acta Neurol Scand*. 1997; 95:121–124.

[8] Kellert L, Martin E, Sykora M, Bauer H, Gussmann P, Diedler J, Herweh C, Ringleb PA, Hacke W, Steiner T, Bosel J. Cerebral oxygen transport failure?. *Stroke*. 2011; 42:2832–2837.

[9] Park YS, Kim BJ, Kim JS, Yang MH, Jang MS, Kim N, Han MK, Lee JS, Lee J, Kim S, Bae HJ. Impact of Both Ends of the Hemoglobin Range on Clinical Outcomes in Acute Ischemic Stroke. *Stroke*. 2013; 44:3220–3222.

[10] Tanne D, Molshatski N, Merzeliak O, Tsabari R, Toashi M, Schwammenthal Y. Anemia status hemoglobin concentration and outcome after acute stroke: a cohort study. *BMC Neurol*. 2010; 10:22.

[11] Retter A, Wyncoll D, Pearse R, Carson D, McKechnie S, Stanworth S, Shubha A, Thomas D, Walsh T. Guidelines on the management of anaemia and red cell transfusion in adult critically ill patients. *Br J Haematol.* 2013; 160:445–464.

[12] Hare GM, Tsui AK, McLaren AT, Ragoonana TE, Yu J, Mazer CD. Anemia and cerebral outcomes: many questions, fewer answers. *Anesth Analg*. 2008; 107:1356–1370.
[13] Bettencourt-Silva J, De La Iglesia B, Donnel S, Rayward-Smith V. On creating a patient-centric database from multiple Hospital Information Systems. *Methods Inf Med.* 2012; 51:210–220.

[14] Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *J Neurol, Neurosurg and Psychiatry.* 1991; 54:1044–1054.

[15] Blanc B, Finch CA, Hallberg L, Lawkowicz W, Layrisse M, Mollin DL, Rachmilewitz M, Ramalingaswami V, Sanchez-Medal L, Wintrobe MM. Nutritional Anaemias. Report of WHO Scientific Group. *World Health Organ Tech Rep Ser.* 1968; 405:1–40.

[16] Bussiere M, Gupta M, Sharma M, Dowlatshahi D, Fang J, Dhar R. Anaemia on admission is associated with more severe intracerebral haemorrhage and worse outcomes. *Int J Stroke.* 2015; 10:382–387.

[17] Chang TR, Boehme AK, Aysenne A, Albright KC, Burns C, Beasley TM, Martin-Schild S. Nadir hemoglobin is associated with poor outcome from intracerebral hemorrhage. *SpringerPlus.* 2013; 13:379.

[18] Diedler J, Sykora M, Hahn P, Heerlein K, Scholzke MN, Kellert L, Bosel J, Poli S, Steiner T. Low hemoglobin is associated with poor functional outcome after non-traumatic, supratentorial intracerebral hemorrhage. *Critical Care.* 2010; 14:R63.

[19] Huang WY, Chen IC, Meng L, Weng WC, Peng TI. The influence of anemia on clinical presentation and outcome of patients with first-ever atherosclerosis-related ischemic stroke. *J Clin Neurosci.* 2009; 16:645–649.

[20] Kumar MA, Rost NS, Snider RW, Chanderraj R, Greenberg SM, Smith EE, Rosand J. Anemia and hematoma volume in acute intracerebral hemorrhage. *Crit Care Med.* 2009; 37:1442–1447.

[21] Kuramatsu JB, Gerner ST, Lucking H, Kloska SP, Schellinger PD, Kohrmann M, Huttner HB. Anemia is an independent prognostic factor in intracerebral hemorrhage: an observational study. *Critical Care.* 2013; 17:R148.

[22] Millonis H, Papavasileiou V, Eskandari A, D’Ambrogio-Remillard S, Ntaios G, Michel P. Anemia on admission predicts short and long term outcomes in patients with acute ischemic stroke. *Int J Stroke.* 2015; 10:224–230.

[23] Nybo M, Kristensen SR, Mickley H, Jensen JK. The influence of anaemia on stroke prognosis and its relation to N-terminal pro-brain natriuretic peptide. *Eur J Neurol.* 2007; 14:477–482.

[24] Sharma M, Pearce LA, Benavente OR, Anderson DC, Connolly SJ, Palacio S, Coffey CS, Hart RG. Predictors of mortality in patients with lacunar stroke in the secondary prevention of small subcortical strokes trial. *Stroke.* 2014; 45:2989–2994.
[25] Wade JP, Taylor DW, Barnett HJ, Hachinski VC. Hemoglobin concentration and prognosis in symptomatic obstructive cerebrovascular disease. *Stroke*. 1987; 18:68–71.

[26] Zeng YJ, Liu GF, Liu LP, Wang CX, Zhao XQ, Wang YJ. Anemia on admission increases the risk of mortality at 6 months and 1 year in hemorrhagic stroke patients in China. *J Stroke Cerebrovasc Dis*. 2014; 23:1500–1505.

[27] Gray CS, French JM, James OF, Bates D, Cartlidge NE. The prognostic value of hematocrit in acute stroke. *Age and Ageing*. 1988; 17:406–409.

[28] Del Fabbro P, Luthi JC, Carrera E, Michel P, Burnier M, Burnand B. Anemia and chronic kidney disease are potential risk factors for mortality in stroke patients: a historic cohort study. *BMC Nephrol*. 2010; 11:10.

[29] Shahar A, Sadeh M. Severe anemia associated with transient neurological deficits. *Stroke*. 1991; 22:1201–1202.

[30] Hsiao KY, Hsiao CT, Lin LJ, Shiao CJ, Chen IC. Severe anemia associated with transient ischemic attacks involving vertebrobasilar circulation. *Am J Emerg Med*. 2008; 26:e3–4.

[31] Van Bommel J, Trouwborst A, Schwarte L, Siegemund M, Ince C, Henny ChP. Intestinal and cerebral oxygenation during severe isovolemic hemodilution and subsequent hyperoxic ventilation in a pig model. *Anesthesiology*. 2002; 97 660–670.

[32] Tomiyama Y, Jansen K, Brian JE, Todd MM. Hemodilution, cerebral O2 delivery, and cerebral blood flow: a study using hyperbaric oxygenation. *Am J Physiol*. 1999; 276:H1190-6.

[33] Kim JS, Kang SY. Bleeding and subsequent anemia: a precipitant for cerebral infarction. *Eur Neurol*. 2000; 43:201–208.

[34] Nagel T, Resnick N, Atkinson WJ, Dewey CF, Gimbrone MA. Sheer stress selectivity upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J Clin Invest*. 1994; 94:885–891.

[35] Morigi M, Zoja C, Figliuzzi M, Foppolo M, Micheletti G, Bontempelli M, Saronni M, Remuzzi G, Remuzzi A. Fluid shear stress modulates surface expression of adhesion molecules by endothelial cells. *Blood*. 1995; 85:1696–1703.

[36] McLaren AT, Marsden PA, Mazer CD, Baker AJ, Stewart DJ, Tsui AK, Li X, Yucel Y, Robb M, Boyd SR, Liu E, Yu J, Hare GM. Increased expression of HIF-1alpha, nNOS, and VEGF in the cerebral cortex of anemic rats. *Am J Physiol*. 2007; 292:R403–414.

[37] Felszeghy K, Banisadr G, Rostene W, Nyakas C, Haour F. Dexamethasone downregulates chemokine receptor CXCR4 and exerts neuroprotection against hypoxia/ischemia-induced brain injury in neonatal rats. *Neuroimmunomodulation*. 2004; 11:404–413.
[38] Moro MA, Cardenas A, Hurtado O, Leza JC, Lizasoain I. Role of nitric oxide after brain ischaemia. *Cell Calcium.* 2004; 36:265–275.

[39] Wu WC, Rathore SS, Wang Y, Radford MJ, Krumholz HM. Blood Transfusion in Elderly Patients with Acute Myocardial Infarction. *N Engl J Med.* 2001; 345:1230–1236.

[40] Kwok CS, Sherwood MW, Watson SM, Nasir SB, Sperrin M, Nolan J, Kinnaird T, Kiatchoosakun S, Ludman PF, de Belder MA, Rao SV, Mamas MA. Blood transfusion after percutaneous coronary intervention and risk of subsequent adverse outcomes: a systematic review and meta-analysis. *JACC Cardiovasc Interv.* 2015; 8:436–446.

[41] Sheth KN, Gilson AJ, Chang Y, Kumar MA, Rahman RM, Rost NS, Schwab K, Cortellini L, Goldstein JN, Smith EE, Greenberg SM, Rosand J. Packed red blood cell transfusion and decreased mortality in intracerebral hemorrhage. *Neurosurgery.* 201; 68:1286–1292.
Figure Legend

Figure [1]. Search Strategy
Figure [2]. Patient Inclusion Chart
Figure [3]. Prevalence of Co-Morbidities by Anemia Status in Males
Figure [4]. Prevalence of Co-Morbidities by Anemia Status in Females
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
Table [2]. The Impact of Hemoglobin Levels on Mortality at Different Time Points (Logistic Regression)
Table [3]. Effect of Anemia and Elevated Hemoglobin on Stroke Outcomes at Different Time Points (Logistic Regression)
Table [4]. Use of Prior Antithrombotic by Anemia Status and Vascular Indication (Chi-Square Test)
Table [5]. Characteristics of Studies Examining the Relationship Between Anemia/Hemoglobin Levels and Stroke Outcomes
Table [6]. Characteristics Determining Study Validity
Table [7]. Odds Ratios From Studies Evaluating Association Between Elevated Hemoglobin and Stroke Mortality
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

11,886 strokes on the register

Exclusions:
991 - subsequent strokes in the same patient
767 - missing data for prior anti-thrombotic
689 - missing data for pre-morbid mRs
674 - missing data for OSCP classification
529 - missing hemoglobin values
207 - subarachnoid haemorrhage
10 - missing previous stroke data
6 - below 18 years of age

8,013 patients used as final sample population
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.
1424 Citations from database searches and checking of reference lists.
Screened on title and abstract for potentially relevant studies

Excluded (n=1389) as clearly did not cover association between baseline anemia or hemoglobin with mortality after stroke

Records for further detailed checking (n=35)

Did not fulfill selection criteria (n=15) on basis of:
- Abstracts only (n=7)
- Critical care patients only (n=1)
- Did not report on relationship between baseline anemia or hemoglobin and subsequent mortality after stroke (n=7)

Studies included in systematic review (n=20) + current study

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

| Study or Subgroup         | Weight | Odds Ratio | Odds Ratio |
|---------------------------|--------|------------|------------|
|                           |        | IV, Random, 95% CI | IV, Random, 95% CI |
|                           |        | 0.1 | 0.2 | 0.5 | 1 | 2 | 5 | 10 |
| 1.1.1 Categorical Anaemia |        |     |     |     |     |     |     |     |     |
| Current Study Female Ischaemic | 15.4% | 1.48 | 1.23, 1.79 |   |
| Current Study Male Ischaemic | 15.2% | 2.25 | 1.84, 2.74 |   |
| Hao 2013                   | 11.3%  | 1.56 | 1.05, 2.32 |   |
| Huang 2009                 | 6.8%   | 2.22 | 1.12, 4.39 |   |
| Milionis 2014             | 13.4%  | 1.35 | 1.01, 1.80 |   |
| Nybo 2007                  | 8.5%   | 4.70 | 2.69, 8.20 |   |
| Sharma 2014               | 13.8%  | 1.60 | 1.22, 2.10 |   |
| Tanne 2010                 | 7.9%   | 1.90 | 1.05, 3.44 |   |
| Subtotal (95% CI)          | 92.3%  | 1.85 | 1.49, 2.32 |   |

Heterogeneity: \( \text{Tau}^2 = 0.07; \text{Chi}^2 = 25.66, \text{df} = 7 (P = 0.0006); I^2 = 73\% 
Test for overall effect: \( Z = 5.45 (P < 0.00001) \)

1.1.2 Lowest vs. referent

| Study or Subgroup         | Weight | OddsRatio | OddsRatio |
|---------------------------|--------|-----------|-----------|
|                           |        | IV, Random, 95% CI | IV, Random, 95% CI |
|                           |        | 0.1 | 0.2 | 0.5 | 1 | 2 | 5 | 10 |
| Park 2013                 | 7.7%   | 3.74 | 2.03, 6.89 |   |
| Subtotal (95% CI)         | 7.7%   | 3.74 | 2.03, 6.89 |   |

Heterogeneity: Not applicable
Test for overall effect: \( Z = 4.23 (P < 0.0001) \)

Total (95% CI) 100.0% 1.97 [1.57, 2.47]

Heterogeneity: \( \text{Tau}^2 = 0.08; \text{Chi}^2 = 31.34, \text{df} = 8 (P = 0.0001); I^2 = 74\% 
Test for overall effect: \( Z = 5.82 (P < 0.00001) \)
Test for subgroup differences: \( \text{Chi}^2 = 4.47, \text{df} = 1 (P = 0.03), I^2 = 77.6\% 

Anaemia not harmful Anaemia harmful
Figure [7]. Meta-Analysis of Studies Analysing the Impact of Anemia on Admission on Mortality in Hemorrhagic Stroke

| Study or Subgroup       | Weight | Odds Ratio IV, Random, 95% CI | Odds Ratio IV, Random, 95% CI |
|-------------------------|--------|-------------------------------|-------------------------------|
| Bussiere 2013           | 29.9%  | 1.39 [1.01, 1.91]             |                               |
| Current Study Female Haemorrhagic | 9.2%  | 2.11 [1.19, 3.74]             |                               |
| Current Study Male Haemorrhagic | 10.0% | 1.76 [1.01, 3.04]             |                               |
| Kumar 2009              | 11.6%  | 1.50 [0.90, 2.50]             |                               |
| Zeng 2014               | 39.2%  | 1.33 [1.00, 1.75]             |                               |
| Total (95% CI)          | 100.0% | 1.46 [1.23, 1.74]             |                               |

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 2.58$, df = 4 ($P = 0.63$); $I^2 = 0$
Test for overall effect: $Z = 4.30$ ($P < 0.0001$)
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                  | P-Value | | Male                   | Female                  | P-Value   |
|--------------------|------------------------|-------------------------|---------|------------------------|-------------------------|---------|
| **Number**         | 1,017 (26.7)           | 2,794 (73.3)            |         |                       | 947 (22.5)              | 3,255 (77.5) |         |
| **Age**            |                        |                         |         |                        |                         |         |         |
| ≤ 60               | 46 (4.5)               | 424 (15.2)              | <0.001  | 29 (3.1)               | 208 (6.4)               | <0.001  |
| 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** |                        |                         |         |                        |                         | 0.286   |
|                | No          | Yes         | Pre-stroke Rankin Score† | <0.001 | <0.001 |
|----------------|-------------|-------------|--------------------------|--------|--------|
|                | 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 |
|----------|---------------|---------------|---------------|---------------|---------------|--------|
| 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    |

| 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    |

| 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   |

| 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, 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) |              |              | 1.85 (1.03 – 3.32)  | 511    |
| 1 Month        | 1.86 (1.46 – 2.38) |              | 1.79 (1.00 – 3.20)  | 488    |
| 3 Months       | 2.18 (1.75 – 2.72) |              | 1.86 (1.08 – 3.18)  | 674    |
| 6 Months       | 2.25 (1.83 – 2.78) |              | 1.46 (0.86 – 2.48)  | 796    |
| 1 Year         | 2.25 (1.85 – 2.75) |              | 1.50 (0.91 – 2.47)  | 971    |
| **Male - Hemorrhagic** |              |              |                     |        |
| Number         | 121          | 367          | 25                  |        |
| Inpatient      |              |              |                     |        |
| 1.33 (0.77 – 2.31) |              |              | 3.30 (1.19 – 9.17)  | 176    |
| 1 Month        | 1.42 (0.83 – 2.42) |              | 2.90 (1.08 – 7.75)  | 173    |
| 3 Months       | 1.39 (0.81 – 2.39) |              | 2.08 (0.75 – 5.78)  | 200    |
| 6 Months       | 1.64 (0.94 – 2.85) |              | 1.56 (0.56 – 4.40)  | 221    |
| 1 Year         | 1.76 (1.01 – 3.04) |              | 1.56 (0.56 – 4.35)  | 229    |
| **Female - Ischemic** |              |              |                     |        |
| Number         | 852          | 2,585        | 216                 |        |
| Inpatient      |              |              |                     |        |
| 1.20 (0.97 – 1.49) |              |              | 1.30 (0.87 – 1.94)  | 792    |
| 1 Month        | 1.29 (1.04 – 1.60) |              | 1.49 (1.00 – 2.21)  | 733    |
| 3 Months       | 1.39 (1.14 – 1.70) |              | 1.19 (0.81 – 1.75)  | 1007   |
| 6 Months       | 1.44 (1.18 – 1.75) |              | 1.12 (0.78 – 1.62)  | 1159   |
| 1 Year         | 1.47 (1.22 – 1.77) |              | 1.04 (0.73 – 1.48)  | 1328   |
| **Female - Hemorrhagic** |              |              |                     |        |
| Number         | 95           | 418          | 36                  |        |
| Inpatient      | 1.90 (1.09 – 3.33) |              | 2.76 (1.16 – 6.56)  | 229    |
| 1 Month        | 1.82 (1.06 – 3.11) |              | 2.11 (0.92 – 4.82)  | 226    |
| 3 Months       | 1.80 (1.04 – 3.13) |              | 2.08 (0.91 – 4.77)  | 256    |
| 6 Months       | 2.05 (1.17 – 3.59) |              | 2.99 (1.29 – 6.90)  | 278    |
| 1 Year         | 2.11 (1.19 – 3.74) |              | 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         |
| Male                     | No Antithrombotic | 237 (34.2)           | 456 (65.8)      | 0.971          |
|                          | Antithrombotic   | 447 (34.3)           | 857 (65.7)      |               |
| Female                   | No Antithrombotic | 270 (28.9)           | 665 (71.1)      | 0.032          |
|                          | Antithrombotic   | 350 (24.9)           | 1,057 (75.1)    |               |
|                          |                 |                       |                 | <0.001         |
|                          | Anemia          | 210 (16.3)           | 1082 (83.7)     |               |
|                          | No Anemia       | 123 (23.6)           | 399 (76.4)      |               |
|                          |                 |                       |                 | 0.334          |
|                          |                 |                       |                 |               |

* 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) |
| Study                                | Period       | Study Design | Sample Size | Variables Studied                                                                 | Findings                                                                                           |
|--------------------------------------|--------------|--------------|-------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| 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) |
| Authors         | Year Range   | Study Design | Sample Size | Hemoglobin Condition | Outcome Measure                          | Result                                                                 |
|-----------------|--------------|--------------|-------------|----------------------|------------------------------------------|----------------------------------------------------------------------|
| 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) |
| 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                |
| Study                  | Method    | Follow-up | Data Collection                          | Key Variables                                                                 | Anemia Definition | Stroke Type |
|-----------------------|-----------|-----------|------------------------------------------|-------------------------------------------------------------------------------|-------------------|-------------|
| 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    |
|                     | Imaging | Mortality at 1 and 1-year | Population registry | Age, sex, stroke type, NIHSS, prior disability, co-morbidities | Anemia (WHO definition) | Both considered separately |
|---------------------|---------|--------------------------|---------------------|----------------------------------------------------------------|------------------------|---------------------------|
| Tanne et al [10]    |         |                          |                     |                                                                 |                        | Both considered separately |
| Wade et al [25]     |         |                          |                     |                                                                 | Cut-off at 15 g/dL     | AIS Only                  |
| Zeng et al [26]     |         | Discharge, 30 days, 3, 6, 12 months | Telephone interview | Age, sex, pre-stroke mRS, NIHSS, BMI, GCS, ICH volume, glucose, comorbidities, 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

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