

Original Paper

Loss of Penumbra by Impaired Oxygen Supply? Decreasing Hemoglobin Levels Predict Infarct Growth after Acute Ischemic Stroke

Stroke: Relevant Impact of Hemoglobin, Hematocrit and Transfusion (STRAIGHT) –
An Observational Study

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Key Words

Stroke · Acute stroke · Thrombolysis · Hemoglobin · Anemia · Magnetic resonance imaging ·
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Abstract

Background: The association of mortality and poor outcome with reduced levels of hemoglobin (Hb) and hematocrit (Hct) in patients admitted for ischemic stroke was recently demonstrated. The mechanisms behind this have remained unclear. **Aims:** Here, we aimed to investigate a putative association between low Hb and Hct levels and infarct growth. **Methods:** All consecutive patients who received intravenous thrombolysis based on multimodal magnetic resonance imaging during the years 1998–2009 were screened. Laboratory data as well as admission magnetic resonance images and follow-up computed tomography scans of 257 patients were assessed. Overall, data of 100 patients were of sufficient quality and further analyzed. **Results:** Decrease in Hb and Hct as well as perfusion-weighted imaging volume, mismatch volume, and final infarct size on follow-up computed tomography were associated with infarct growth. A linear regression model revealed Hb decrease ($\beta = 0.23$, $p = 0.02$) to be a predictor of infarct growth, independent of mismatch volume ($\beta = 0.27$, $p = 0.004$) and minimum sodium ($\beta = -0.21$, $p = 0.03$), and adjusted to the non-predicting variables age, National Institute of Health Stroke Scale score, maximum leucocytes and C-reactive protein, blood glucose, and Hct decrease. **Conclusion:** Hb levels that decrease after admission independently predict infarct growth in thrombolized stroke patients. The clinical implications of this relationship remain to be investigated.

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Introduction

Tissue fate after ischemic stroke depends upon timely reperfusion and thus re-establishment of oxygen supply to the penumbra. This is the rationale for thrombolysis in acute stroke, and the significant impact of recanalization on infarct growth and functional outcome has been demonstrated [1–4]. Additionally, other factors might influence infarct growth and functional outcome, such as age [5], admission National Institute of Health Stroke Scale (NIHSS) [5], treatment with r-tPA [6], time to treatment [7], elevated hematocrit (Hct) [8], and hyperglycemia [9].

Anemia is a common condition in elderly patients and nearly every fifth patient with acute ischemic stroke presents with anemia on admission [10]. However, an optimal oxygen-carrying capacity in the blood, i.e. level of hemoglobin (Hb), would appear to be decisive for the penumbral salvage. There are only few studies on the role of Hb in ischemic stroke and their results are heterogeneous. Hct, in contrast, has been addressed in some studies with regard to the effect of viscosity and cerebral blood flow in acute ischemic stroke, but again with controversial results. Hemodilution, once considered a potential treatment principle in stroke, has not proved successful. Thus, optimal Hb and Hct levels in acute ischemic stroke are still unknown and guideline recommendations are vague or lacking [11].

We have recently demonstrated that low and further decreasing levels of Hb and Hct after admission in patients thrombolized for ischemic stroke were associated with poor outcome and mortality [12]. Although the results of our analysis hinted at anemia being an independent factor rather than an epiphenomenon merely reflecting illness severity, the question whether this is an association or a pathophysiological causative relation remained unanswered. In particular, it is unclear whether anemia is indeed involved in loss of the penumbra, i.e. infarct growth.

Here, we have analyzed infarct development between admission and follow-up imaging in patients who received intravenous thrombolysis (IVT) based on magnetic resonance imaging (MRI) and the associations with clinical and laboratory parameters.

Methods

Consecutive patients with acute ischemic stroke treated with IVT based on MRI in the years 1998–2009 were retrospectively analyzed. Baseline and demographic characteristics, cardiovascular risk factors, treatment time intervals, and stroke severity upon admission as reflected by the NIHSS score were prospectively collected. All Hb and Hct concentrations during hospital stay were extracted from the laboratory registry of the hospital. All patients had their first blood sample including a blood count taken in our emergency room. IVT was started in our emergency room and continued on our certified stroke unit, where patients stayed for at least 3 days. We assessed the levels of Hb and Hct on admission (baseline) and minimum levels until the follow-up computed tomography (CT) scan. Anemia was defined by WHO criteria as Hb <12 g/dl in women and Hb <13 g/dl in men. Other relevant laboratory parameters such as blood glucose, leukocytes, platelets, C-reactive protein (CRP), creatinine, blood urea nitrogen (BUN), sodium and potassium were analyzed as well.

MR and CT images were available either as hard copies or in digital format. The imaging investigator (P.G.) was blinded to the laboratory and outcome data of the patients. Images from a dedicated multimodal stroke MR examination on admission were used to outline and quantify the initial volume of diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI). The volume of the latter was calculated as area under the curve of a time/bolus graph. The DWI volume was regarded as the initial infarct size. CT images from

the latest follow-up examination were used to determine the final infarct size. Infarct size was determined using the ABC/2 method as follows: the slice with the largest lesion extension (hyperintense on DW and PW MRI, hypodense on CT images) diameter was chosen. This measurement is referred to as A. The second distance (B) was the largest lesion diameter perpendicular to A on the same slice. Multiplying the number of slices showing the lesion by the slice thickness yielded the third distance (C). After multiplication of the 3 distances, the product was divided by 2, resulting in the final volume in cubic millimeters (mm³). Infarct growth was defined as the difference between the ABC/2 result of the initial DWI lesion and the final follow-up CT lesion. The in-plane resolution of both the DW and PW MR images was 96 × 96 with a slice thickness of 5 mm; CT images had a resolution of 512 × 512 with a slice thickness of either 6 or 8 mm. Mismatch was defined as a visually detected PW MRI lesion that was at least 20% larger than the DWI lesion [13]. Most patients were routinely examined with extra- and intracranial ultrasound during the first day of hospital stay to detect recanalization.

The study was approved by our local ethics committee (application No. S324/2009).

Statistical Analysis

Normally distributed data are presented as means ± standard deviation (SD) and non-normally distributed data as medians and interquartile range or counts and percentages in parentheses. Correlation of variables with infarct growth was analyzed using the Pearson correlation coefficient for normally distributed and the Spearman correlation coefficient for non-normally distributed data. Multivariable analysis was performed using a linear regression model for calculating predictors of infarct growth to present regression coefficients B (non-standardized) and β (standardized), with the level of significance set at p < 0.05. Because of possible co-linearity of the included variables, a forward stepwise approach was chosen. All statistical analyses were performed using the SPSS software package (SPSS 19.0) for Windows.

Results

Of 257 patients treated with IVT selected by MRI criteria in the years 1998–2008, we excluded the following from further analysis: 59 patients due to incomplete imaging data, 35 patients because there was no infarction displayed in the follow-up imaging scan, 50 patients due to lack of DWI-PWI mismatch, 6 patients who received intra-arterial recanalization, and 7 patients with incomplete laboratory data.

Table 1 shows baseline characteristics, radiological and clinical outcome data of the remaining 100 patients, as well as their correlation with infarct growth. Mean age was 70 years, stroke severity was reflected by a median NIHSS of 14, and mean time to IVT treatment was 195 min. Mean initial DWI volume was 13.7 ml and mismatch volume was 121.7 ml. Median modified Rankin Scale (mRS) after 3 months was 4; 33% of the patients reached functional independence and 16% deceased. Correlations were found between infarct growth and PWI volume, mismatch volume, and infarct size on follow-up CT.

With regard to laboratory parameters, we found that decrease of Hb and Hct until follow-up CT was associated with infarct growth. In addition, there were correlations between infarct growth and maximum values of leucocyte count and CRP, as well as minimum values of sodium and blood glucose (table 2).

Figure 1 shows two examples with different courses of tissue at risk in relation to Hb level development after stroke onset. Figure 2 presents the correlation between infarct growth and Hb decrease.

Table 1. Correlation of baseline characteristics with infarct growth

Parameters		p (infarct growth, ml)
Baseline characteristics		
Infarct growth, ml	2.9 (15)	1
Age, years ^a	70 ± 12.9	0.60
Female sex ^b	40 (40%)	0.53
NIHSS on admission ^b	14 (5)	0.09
Hypertension ^b	74 (74%)	0.58
Diabetes mellitus ^b	23 (23%)	0.15
Hyperlipidemia ^b	23 (23%)	0.44
Smoking ^b	20 (20%)	0.84
Previous stroke ^b	9 (9%)	0.30
Coronary heart disease ^b	11 (11%)	0.74
Peripheral artery disease ^b	1 (1%)	0.12
Atrial fibrillation ^b	39 (39%)	0.52
Off-label treatment ^b	61 (61%)	0.64
Time to treatment, min ^{b, d}	195 (207)	0.80
Recanalization ^{b, c, e}	64 (88%)	0.39
Time to follow-up imaging scan, h ^b	24 (22)	0.23
Radiological data		
DWI volume, ml ^b	13.7 (25)	0.09
PWI volume, ml ^{b, f}	155 (111)	0.001
Mismatch, ml ^b	121.7 (126)	0.001
Infarct size on CT, ml ^b	17 (42)	0.001
Outcome data		
mRS at 3 months ^b	4 (3)	0.09
mRS 0–2 at 3 months ^b	33 (33%)	0.07
Death at 3 months ^b	16 (16%)	0.72

Values are means ± SD, median (IQR) or numbers with percentages in parentheses.

^a Pearson. ^b Spearman. ^c As assessed by transcranial doppler sonography at various time points. ^d n = 93, 7 unknown (3 vs. 4). ^e 28 unknown. ^f n = 91, missing 4 vs. 5.

A linear regression model showed Hb decrease ($\beta = 0.23$, $p = 0.02$) to predict infarct growth independently of mismatch volume ($\beta = 0.27$, $p = 0.004$) and minimum sodium ($\beta = -0.21$, $p = 0.03$), and adjusted to the non-predicting variables age, NIHSS score, maximum leucocyte count and CRP, blood glucose, and Hct decrease (table 3).

Discussion

Anemia on admission and thereafter is associated with worse outcome and mortality in stroke patients [12]. The pathophysiological mechanisms behind this phenomenon, however, are unknown. Here, we demonstrate that decreasing Hb levels after admission are independently associated with infarct growth in stroke patients who received MRI-based thrombolysis.

Loss of the ischemic penumbra, i.e. extension of irreversibly damaged infarct tissue, depends on mechanisms so diverse as excitotoxicity, inflammation, and apoptosis, but the most critical initial factor certainly is failure of supply of the core component of aerobic cellular metabolism, i.e. oxygen [14]. Infarct growth by penumbral transition was thought to be a rapid process during the acute stroke phase in the past, but imaging studies have revealed

Table 2. Correlation of laboratory data with infarct growth

Parameters		p* (infarct growth, ml)
Hb at baseline, g/dl	13.6 (2.2)	0.81
Hb _{min} until CT scan, g/dl	13.0 (2)	0.20
Hb decrease until CT scan, g/dl	0.8 (1.4)	0.02
Anemia at baseline	19 (19%)	0.20
Anemia until CT scan	40 (40%)	0.51
Hct at baseline, %	40 (6)	0.59
Hct _{min} until CT scan, %	38 (5)	0.18
Hct decrease until CT scan, %	2 (4)	0.001
Leukocytes at baseline, /nl	8.7 (4)	0.63
Leukocytes _{max} until CT scan, /nl	10.4 (4)	0.02
Platelets at baseline, /nl	230 (90)	0.86
Platelets _{max} until CT scan, /nl	240 (90)	0.39
Platelets _{min} until CT scan, /nl	205 (85)	0.82
CRP at baseline, mg/l	6 (15)	0.72
CRP _{max} until CT scan, mg/l	13 (22)	0.05
Creatinine at baseline, mg/dl	0.94 (0.25)	0.12
Creatinine _{max} until CT scan, mg/dl	0.98 (0.18)	0.13
BUN at baseline, mg/dl	38 (21)	0.21
BUN _{max} until CT scan, mg/dl	42 (21)	0.48
Sodium at baseline, mmol/l	139 (4)	0.11
Sodium _{max} until CT scan, mmol/l	141 (3)	0.32
Sodium _{min} until CT scan, mmol/l	138 (4)	0.05
Potassium at baseline, mmol/l	4.0 (0.6)	0.96
Potassium _{max} until CT scan, mmol/l	4.2 (0.6)	0.85
Potassium _{min} until CT scan, mmol/l	3.7 (0.4)	0.96
Blood glucose, mg/dl	125 (40)	0.04

Values are means ± SD, median (IQR) or numbers with percentages in parentheses. * Spearman correlation.

that this process can continue for more than 36–48 h in stroke patients [15, 16] and for more than 2 weeks in animal experiments [17]. It should therefore be assumed that optimal oxygen supply to the penumbra and adjacent oligemic tissue is not only warranted in the acute phase of stroke but also during the following days. Healthy brain tissue is able to compensate falling Hb levels by increasing regional cerebral blood flow and oxygen extraction up to extremely low levels of 5–6 g/dl [18, 19]. In ischemic brain tissue, these compensatory mechanisms might fail at considerably higher critical Hb levels already [20, 21]. Thus, we hypothesized that infarct growth in this selected group of patients with confirmed presence of an ischemic penumbra on admission and a homogeneous recanalization regime might be associated with differences in Hb levels, as our data showed.

The main result of this study is that falling Hb levels after admission seem to be more relevant to infarct growth than levels on admission. This observation is in good agreement with our recently published data, showing outcome parameters to have a stronger association with decreasing and minimum Hb and Hct levels than with admission levels [12]. Surprisingly, this significant effect on infarct growth was observed with a relative reduction of Hb within a range of as little as 0.8 g/dl. Apparently, even little variations in oxygen-carrying capacity might contribute to loss of cerebral tissue. The negative association of good functional outcome with infarct growth was only a non-significant trend in this study, although this is an obvious clinical observation. Probably, the study population was too small for this demonstration due to our strict selection criteria.

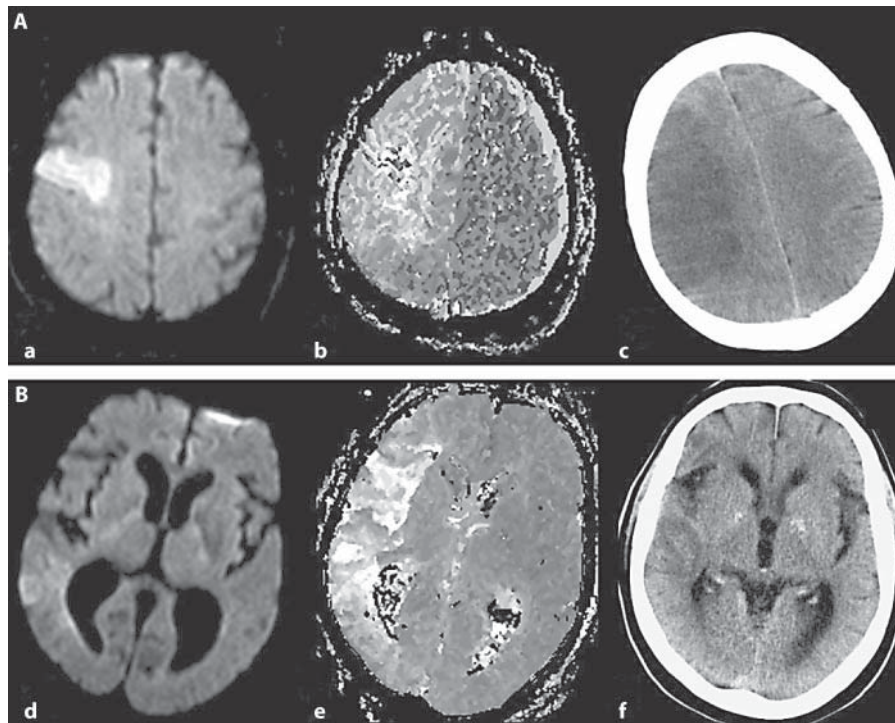


Fig. 1. Different courses of tissue at risk. Examples of tissue fate in a patient developing anemia between stroke onset and follow-up CT scan (patient 1; **A**) and in a patient without anemia (patient 2; **B**). Displayed are DW (**a, d**) and PW (**b, e**) MR images on admission demonstrating a substantial mismatch in both patients. In patient 1, the follow-up CT image demonstrates infarct growth in comparison to the DWI lesion (**c**). In patient 2, no relevant change of the initial DWI lesion size was observed (**f**).

Table 3. Linear regression model to predict infarct growth

Variable	B	β	p
Age			0.98
NIHSS on admission			0.19
Mismatch volume	0.19	0.27	0.004
CRP _{max} until CT scan			0.84
Leukocytes _{max} until CT scan			0.13
Sodium _{min} until CT scan	-4.49	-0.21	0.03
Blood glucose			0.63
Hb decrease until CT scan	16.33	0.23	0.02
Hct decrease until CT scan			0.87

With regard to Hct, the linear regression model failed to indicate an independent association with infarct growth, although in the univariate analysis, there was a highly significant correlation. The most probable explanation is the strong interaction of Hct with the statistically similarly effective minimum sodium levels, as both reflect fluid overload. Additionally, this discrepancy might point to a relatively higher relevance of oxygen-carrying capacity, i.e. Hb level, in comparison to the amount of blood cells, i.e. Hct, which on its own

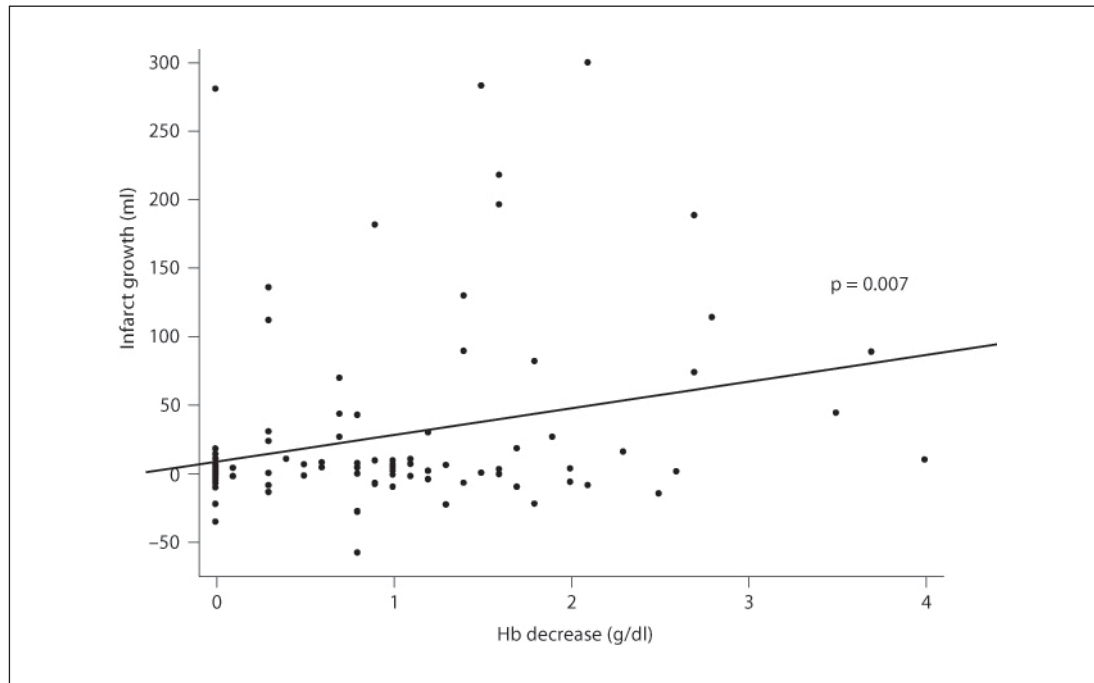


Fig. 2. Correlation between Hb decrease and infarct growth.

has to be balanced against flow-impairing increase in viscosity. However, our study was not designed to allow for further differentiation of these effects, and clearly, Hb and Hct can hardly be looked at separately from a clinical point of view.

Not only low, but also extremely high Hb levels have shown to be deleterious in stroke patients by other authors, who have described a U-shaped relationship between Hb and outcome or Hct and cerebral reperfusion deficits [8, 10]. This constellation might be equally fatal for the penumbral tissue, probably through viscosity-related impairment of microcirculation. We do not consider our findings in disagreement with this observation. However, we cannot confirm such a relationship by our analysis, as almost none of our patients had elevated Hb or Hct levels.

Our study has several limitations. As a retrospective analysis, it is naturally prone to diverse biases and can only detect an association but not demonstrate a causal relationship between decreasing Hb and infarct growth. Our findings should therefore certainly not be interpreted as support of a more active transfusion practice. Data on recanalization based on the initial MRI angiography and follow-up transcranial ultrasound suggested a rate of >80% of recanalization, which is considerably higher than usually reported after IVT and even after intra-arterial thrombolysis [22, 23]. However, in 28% of our patients, the retrospective analysis of the available recanalization data did not allow for confident determination of the recanalization status, and there was no systematic timing of transcranial doppler sonography either. This might have contributed to the appearance of a falsely high recanalization rate. Of note, however, when we dichotomized the population according to high and low infarct growth in relation to the median infarct growth and compared recanalization rates, these were evenly distributed between the groups, so we do not assume recanalization to have relevantly affected our findings (data not shown). The application of quite strict selection criteria aiming at homogeneity and sufficient quality of imaging data had led to a study pop-

ulation of only 100 patients, in which some effects might have been statistically undetected. However, one reason why these criteria were chosen was to overcome another limitation, i.e. the difficulty to perform an intermodal comparison of infarct sizes between MRI and CT. This latter problem, together with the application of the ABC/2 method to assess infarct growth (the method is more established for assessing ellipsoid hemorrhages) and the fact that we did not use an up-to-date software to analyze images are also methodological shortcomings of our study. Finally, we have made our observations in IVT stroke patients only and they might thus not apply to other stroke patients, or at least not to the same extent. Possibly, patients are more prone to anemia after thrombolysis, e.g. by a higher incidence of undetected gastrointestinal bleeding.

Despite these limitations, this is the first study showing the *independent* association of decreasing levels of Hb with infarct growth in MRI-based thrombolysed stroke patients, independent of mismatch volume and recanalization rate.

Conclusion

Decreasing Hb levels are associated with further increase in infarct size after acute ischemic stroke. This might point to insufficient oxygen supply to the ischemic penumbra. The more detailed pathophysiology behind this observation and, more importantly, the clinical implications for hematologic management of stroke patients remain to be investigated prospectively.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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