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Concurrent thiol-disulfide and glutathione redox imbalance in acute ischemic stroke and its relationship to functional outcome: A prospective case-control study

Faruk Danış[1]*, Murat Alışık[2], Kaan Çelik[1], Özgür Mehmet Yis[2]

[1]Department of Emergency Medicine, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye

[2]Department of Medical Biochemistry, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye

ABSTRACT

Aim: To concurrently assess serum thiol-disulfide and erythrocyte glutathione redox status in patients with acute ischemic stroke and determine their associations with stroke severity, infarct volume, and 90-day functional outcome.

Method: In this prospective case-control study, 85 stroke patients sampled within 24 hours of onset and 79 healthy controls were studied. Native and total thiol were measured by an automated assay and reduced (GSH) and total glutathione by a validated colorimetric method, with disulfide and oxidized glutathione (GSSG) derived by calculation. Markers were compared between groups with effect sizes and age- and sex-adjustment, correlated with NIHSS and infarct volume, and tested against poor 90-day outcome (modified Rankin Scale 3-6) in exploratory analyses.

Results: Both systems shifted toward oxidation. Native and total thiol were lower and the disulfide fraction higher; the glutathione system shifted most, with lower GSH, higher GSSG, and a markedly higher GSSG/GSH ratio (0.119 vs 0.046; rank-biserial r= 0.88; adjusted p<0.001). No marker correlated with NIHSS or infarct volume. A poor outcome occurred in 32 of 85 patients (37.6%). Among the 50 patients with glutathione measured, 16 had a poor outcome; the ratio was associated with it univariably (odds ratio 2.27 per SD, 95% CI 1.08-4.76) and after age adjustment, but not after adjustment for admission NIHSS (area under the curve 0.72).

Conclusions: Acute ischemic stroke was characterized by a coordinated redox imbalance, most pronounced in erythrocyte glutathione homeostasis. The GSSG/GSH ratio was associated with poor 90-day outcome and merits further validation as a prognostic marker.

Keywords: Ischemic stroke, oxidative stress, glutathione, sulfhydryl compounds, prognosis, biomarkers.

✉ *Faruk Danış* Department of Emergency Medicine, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Türkiye*

E-mail: farukdanis@gmail.com

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1. Introduction

Acute ischemic stroke remains a leading cause of death and long-term disability worldwide, and the extent of neuronal injury after arterial occlusion is shaped not only by the initial perfusion deficit but also by a cascade of secondary biochemical events [1]. Among these, oxidative stress has emerged as a central mechanism: the abrupt restoration of oxygen to ischemic tissue, together with mitochondrial dysfunction and excitotoxicity, generates reactive oxygen species faster than endogenous antioxidant systems can neutralize them [2,3].

The balance between oxidants and antioxidants can be captured by measuring the redox state of thiol-containing molecules, which act as the principal buffers of the

extracellular and intracellular redox environment [4]. In serum, the dynamic thiol-disulfide couple reflects the reversible oxidation of protein and low-molecular-weight thiols to disulfides; an automated assay now allows native thiol, total thiol, and their difference (disulfide) to be quantified reproducibly [5,6]. Inside erythrocytes, glutathione is the dominant thiol antioxidant, and the ratio of its oxidized (GSSG) to reduced (GSH) forms is a sensitive index of intracellular oxidative load [7,8].

Prior clinical studies have examined these two systems separately in cerebrovascular disease and have generally reported a shift toward oxidation in stroke patients, with lower native and total thiol and higher disulfide fractions [9,10,11]. Erythrocyte glutathione has been studied less often, and the few available reports differ in direction and magnitude [8,12]. Critically, the two redox couples have rarely been measured concurrently in the same patients, so whether they move together, and which is more closely tied to clinical severity or outcome, is unknown.

Stroke severity is routinely quantified at admission with the National Institutes of Health Stroke Scale (NIHSS) [13], and functional outcome is summarized at 90 days with the modified Rankin Scale (mRS). Whether a bedside redox marker adds prognostic information beyond these established measures is a clinically relevant question, because a simple blood-based index could help identify patients at risk of poor recovery [14].

We therefore conducted a prospective case-control study with three prespecified aims: first, to compare serum thiol-disulfide and erythrocyte glutathione markers between acute ischemic stroke patients and healthy controls; second, to examine whether these markers are associated with stroke severity (NIHSS) and infarct volume; and third, as a secondary aim, to determine whether any marker is associated with poor 90-day functional outcome.

2. Materials and methods

2.1. Study design and setting: This was a prospective observational case-control study conducted in the emergency department of a tertiary-care hospital. The study followed the STROBE recommendations for observational research and adhered to the principles of the Declaration of Helsinki. The protocol was approved by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University (approval no. 2022/37; 22 February 2022), and written informed consent was obtained from all participants or their legal representatives.

2.2. Participants: Consecutive adults presenting with acute ischemic stroke confirmed by diffusion-weighted magnetic resonance imaging were screened for eligibility. Patients were enrolled if they were older than 18 years, presented acutely and were sampled within 24 hours of symptom onset or the time last known well, and had no condition known to alter systemic redox balance. Individuals with active infection, active malignancy, known chronic inflammatory or autoimmune disease, hepatic or renal dysfunction, or recent transfusion were not considered eligible and were not enrolled, as were patients with hemorrhagic stroke. A control group of volunteers without acute illness and free of the same conditions were recruited in parallel. Of 171 eligible participants enrolled, 7 were excluded after enrolment because of a hemolyzed or otherwise unsuitable sample with missing core biomarker data, leaving 85 stroke patients and 79 controls for analysis (Figure 1). Eight stroke patients died during the study; because the primary

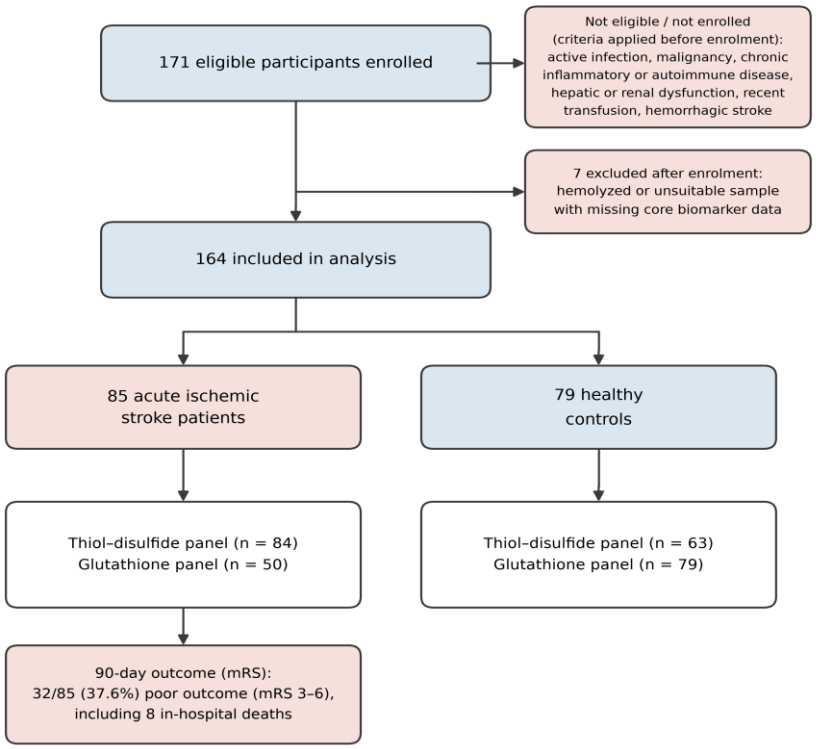

Figure 1. Study flow diagram. Of 171 eligible participants enrolled, 7 were excluded after enrolment because of a hemolyzed or unsuitable sample with missing core biomarker data, leaving 85 acute ischemic stroke patients and 79 healthy controls for analysis. A poor 90-day outcome (modified Rankin Scale 3–6) occurred in 32 of 85 stroke patients (37.6%), including 8 in-hospital deaths.

functional endpoint spans death (modified Rankin Scale 6), these patients were retained and counted as having a poor outcome.

2.3. Data collection and clinical variables: Demographic data, vascular risk factors, and routine admission laboratory values were recorded for all participants. In stroke patients, admission stroke severity was scored with the NIHSS, and the time from symptom onset (or the time the patient was last known to be well) to blood sampling at emergency-department arrival was recorded. Functional outcome was assessed at 90 days with the mRS by a study physician, either from the institutional medical records or by telephone interview; a poor outcome was defined as a priori as mRS 3 to 6. Follow-up at 90 days was complete for all 85 stroke patients, and because all biochemical assays were performed in a single batch after enrolment was complete (see below), the outcome assessor was unaware of the redox-marker results.

2.4. Infarct volume measurement: Acute infarct volume was quantified retrospectively from the admission diffusion-weighted MRI using the region-of-interest planimetric method prespecified in the study protocol. De-identified studies were retrieved from the institutional picture archiving and communication system (PACS) and assessed by a single board-certified emergency physician with 10 years of experience in stroke imaging, without reference to the biochemical redox results.

On each axial slice the hyperintense lesion was manually traced, and its area was computed automatically in mm² by the PACS; restricted diffusion was confirmed as a corresponding low signal on the apparent-diffusion-coefficient (ADC) map to exclude T2 shine-through. Areas from all involved slices were summed and multiplied by the slice thickness to yield the total infarct volume, and coexisting lesions were summed [15].

2.5. Biochemical measurements:

Venous blood was drawn from an antecubital vein at admission, before any intervention that could affect redox status, and collected into a plain serum tube and an EDTA-containing tube. Serum was separated by centrifugation at 1500 × g for 15 min at room temperature, aliquoted within 1 h of collection, and stored at −80 °C until analysis. Blood collected in the EDTA-containing tube was washed three times with 0.9% NaCl, the erythrocytes were lysed, and intracellular proteins were precipitated with trichloroacetic acid. The resulting supernatant was separated and stored at −80 °C within 30 min of blood collection. Control samples were collected and processed using the same protocol.

All samples were thawed and assayed together in a single batch after enrolment was complete, so each sample underwent a single freeze–thaw cycle and the laboratory staff performed the assays without access to any clinical information.

Serum thiol-disulfide parameters were determined with the automated Erel and Neselioglu method, which measures native thiol and total thiol; the disulfide concentration was derived as (total thiol minus native thiol) divided by two, and the disulfide/native thiol, disulfide/total thiol, and native/total thiol percentages were computed from these values [5]. Erythrocyte glutathione was measured with a validated colorimetric method.[8] Whole blood was washed three times with 0.9% NaCl, after which the erythrocytes were lysed, and intracellular proteins were precipitated with trichloroacetic acid. The resulting supernatant was stored at −80 °C until analysis. After thawing, reduced glutathione (GSH) and total glutathione concentrations were measured spectrophotometrically. Oxidized glutathione (GSSG) was calculated as (total glutathione − GSH)/2, and the GSSG/GSH ratio was subsequently calculated.

2.6. Statistical analysis:

All analyses were performed in R version 4.5.3 (R Foundation for Statistical Computing, Vienna, Austria) using RStudio. The study design, the primary and secondary outcomes, and the statistical analysis plan were defined before the start of the study. Continuous variables are summarized as mean (standard deviation) or median [interquartile range] according to distribution, and categorical variables as counts (percentages). Between-group comparisons used the Mann-Whitney U test for continuous variables and the chi-square or Fisher exact test for categorical variables; the Welch t test was used for age. For each redox marker a between-group effect size with a bootstrap 95% confidence interval was calculated-Cohen d for the normally distributed markers (native thiol, total thiol, and GSSG) and the rank-biserial correlation for the remaining markers compared with the Mann-Whitney U test; marker comparisons were additionally adjusted for age and sex in linear models. To control the family-wise error rate across the ten redox markers, the Holm correction was applied. The associations of the markers with stroke severity (NIHSS) and infarct volume were prespecified aims and were assessed with the Spearman rank correlation. For the secondary outcome aim, the association of each marker with poor 90-day outcome was examined with logistic regression, expressed as the odds ratio per standard deviation; the erythrocyte GSSG/GSH ratio was carried into an adjusted model. The decision to carry the GSSG/GSH ratio, rather than another marker, into the adjusted models was based on the univariable findings; these outcome analyses were prespecified only as a secondary aim, and this marker selection is therefore reported as

exploratory and hypothesis-generating. Adjusted models were fitted with Firth's penalized-likelihood method because of the small number of events. In line with recommendations for prognostic-marker studies, the model adjusted for admission NIHSS-the established prognostic factor in acute ischemic stroke—is reported as the adjusted estimate of primary interest; the age-adjusted and age-plus-NIHSS-adjusted models are reported as secondary analyses. Discrimination was summarized with the area under the receiver operating characteristic curve. Sensitivity analyses repeated the principal comparisons using multiple imputations for missing values and after trimming values beyond three standard deviations. A two-sided p value below 0.05 was considered statistically significant. The sample size was based on a priori power analysis using effect sizes from a previous acute ischemic stroke cohort. The governing calculation was the marker–infarct-volume correlation: detecting r = 0.378 with 90% power at a two-sided alpha of 0.05 required approximately 69 patients, while the primary between-group difference in total thiol (Cohen d = 1.09) required fewer than 20 participants per group. Consecutive eligible patients presenting during the study period were enrolled, and the resulting sample of 85 stroke patients and 79 controls exceeded this requirement and provided adequate power for the primary between-group comparisons. Because of the limited number of patients and outcome events, the outcome analyses are reported as exploratory.

3. Results

3.1. Baseline characteristics: The analysis included 85 acute ischemic stroke patients and 79 healthy controls. Stroke patients were older than controls (mean age 70.4 ± 10.8 vs 54.7 ± 8.9 years; $p<0.001$) and less often female (41 [48.2%] vs 56 [70.9%]; $p=0.005$). As expected for an acute stroke population, vascular risk factors were common among patients: hypertension in 67 (78.8%), diabetes mellitus in 42 (49.4%), atrial fibrillation in 32 (37.6%), hyperlipidemia in 29 (34.1%), and coronary artery disease in 15 (17.6%). Red-cell distribution width was higher in stroke patients (14.0 ± 1.6 vs 13.1 ± 1.2%; $p<0.001$), whereas hemoglobin, white-cell count, and platelet count did not differ between groups. Among stroke patients, the median admission NIHSS was 5 [4–12] and the median infarct volume was 13.3 [6.9–23.6] mL; the median 90-day mRS was 2 [1–3], and 32 (37.6%) had a poor 90-day outcome (mRS 3 to 6), including 8 in-hospital deaths. The median time from symptom onset (or last known well) to blood sampling was 4.2 hours (interquartile range 2.8–7.8; range 0.6–16.4), and all patients were sampled within 24 hours. Baseline characteristics are shown in Table 1.

3.2. Redox markers in stroke patients versus controls: Acute ischemic stroke was characterized by a coordinated shift toward oxidation across both redox systems (Figure 2, Table 2). In the serum thiol-disulfide couple, native thiol was lower in stroke patients (median 304.6 [261.2–353.6] vs 371.8 [325.9–403.7] µmol/L; Cohen d -0.97) and total thiol was likewise lower (343.1 [309.3–397.3] vs 403.1 [361.1–442.0] µmol/L; d -0.81), while the disulfide concentration was higher (22.4 [17.9–27.4] vs 17.9 [13.0–22.4] µmol/L; rank-biserial r 0.37). The proportional indices moved consistently: the disulfide/native thiol and disulfide/total thiol ratios were higher and the native/total thiol ratio lower in stroke patients (all absolute r approximately 0.55). Every serum index differed significantly after Holm correction and after adjustment for age and sex; for native thiol the age- and sex-adjusted mean

difference was −70.6 µmol/L (95% CI −95.5 to −45.7).

The erythrocyte glutathione system showed the largest differences. Reduced glutathione (GSH) was markedly lower in stroke patients (median 597.7 [429.7–709.5] vs 930.6 [750.0–1054.7] µmol/L; rank-biserial r -0.80), oxidized glutathione (GSSG) was higher (68.0 [53.2–86.3] vs 43.8 [30.2–57.2] µmol/L; Cohen d 1.16), and total glutathione was lower (707.4 [549.5–886.2] vs 999.7 [835.5–1136.8] µmol/L; r -0.70).

Table 1. Baseline characteristics of stroke patients and controls.

Characteristic	Stroke (n=85)	Control (n=79)	*p* value
Age, years (mean ± SD)	70.4 ± 10.8	54.7 ± 8.9	<0.001
Female, n (%)	41 (48.2)	56 (70.9)	0.005
Hypertension, n (%)	67 (78.8)	0 (0.0)	
Diabetes mellitus, n (%)	42 (49.4)	0 (0.0)	
Hyperlipidemia, n (%)	29 (34.1)	0 (0.0)	
Atrial fibrillation, n (%)	32 (37.6)	0 (0.0)	
Coronary artery disease, n (%)	15 (17.6)	0 (0.0)	
Current smoking, n (%)	17 (20.0)	11 (13.9)	0.41
Hemoglobin, g/dL	14.0 ± 2.0	13.9 ± 1.5	0.92
RDW, %	14.0 ± 1.6	13.1 ± 1.2	<0.001
White-cell count, ×10⁹/L	8.3 ± 2.9	7.5 ± 1.9	0.19
Platelet count, ×10⁹/L	258.6 ± 73.3	272.6 ± 59.6	0.23

Data: means± standard deviation or n (%). Comorbidities were absent by design in the healthy control group, so no comparative p value is reported. RDW, red-cell distribution width. Stroke-cohort clinical measures: median admission NIHSS 5 [4–12]; infarct volume 13.3 [6.9–23.6] mL; 90-day mRS 2 [1–3]; poor outcome (mRS 3–6) 32 (37.6%), including 8 in-hospital deaths.

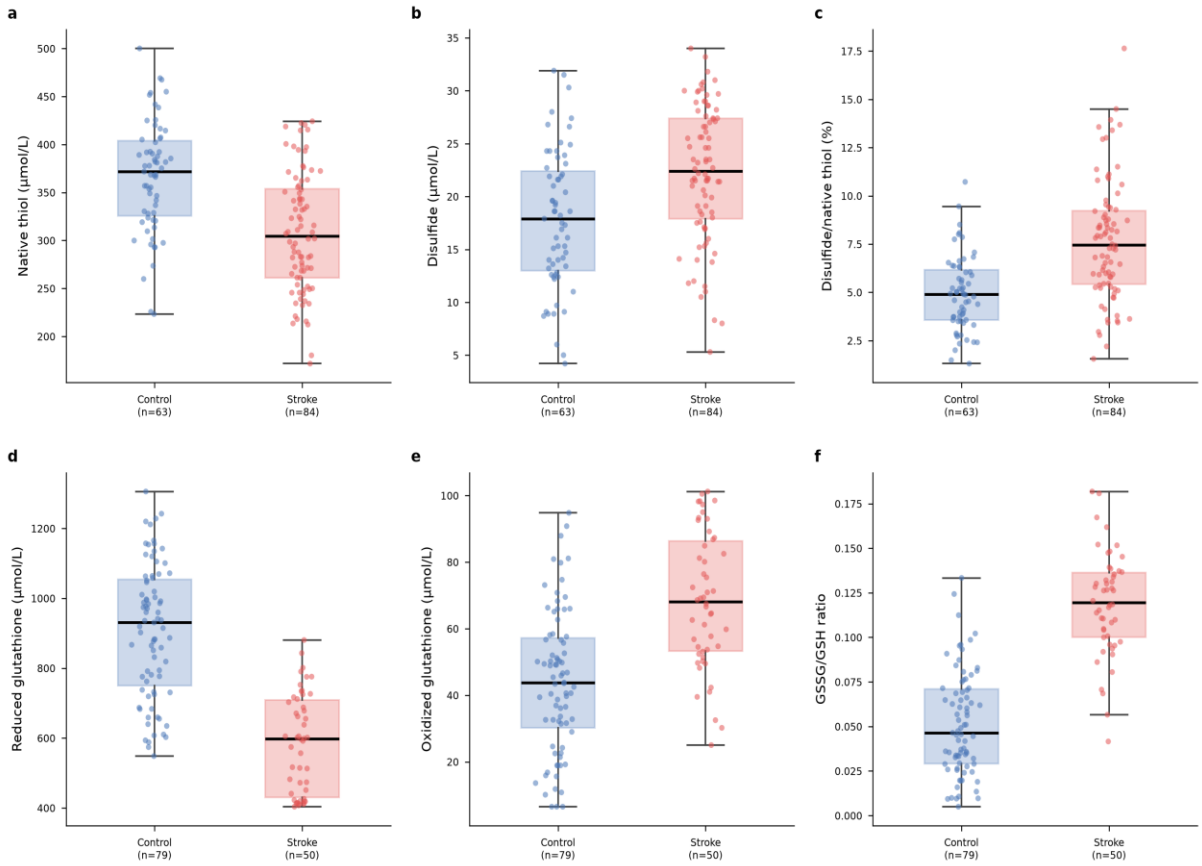

Figure 2. Serum thiol-disulfide and erythrocyte glutathione redox markers in stroke patients versus controls.
Boxes show the median and interquartile range with whiskers to 1.5× the interquartile range; each point is an individual participant. All markers differed between groups after Holm correction (all adjusted p<0.001).

Table 2. Thiol-disulfide and glutathione markers in stroke patients versus controls.

Marker	Stroke	Control	Effect size [95% CI]	Holm-adjusted *p*
Native thiol, µmol/L	304.6 [261.2–353.6] (n=84)	371.8 [325.9–403.7] (n=63)	d -0.97 [-1.33, -0.65]	<0.001
Total thiol, µmol/L	343.1 [309.3–397.3] (n=84)	403.1 [361.1–442.0] (n=63)	d -0.81 [-1.21, -0.48]	<0.001
Disulfide, µmol/L	22.4 [17.9–27.4] (n=84)	17.9 [13.0–22.4] (n=63)	r 0.37 [0.19, 0.54]	<0.001
Disulfide/native thiol, %	7.5 [5.4–9.2] (n=84)	4.9 [3.6–6.2] (n=63)	r 0.55 [0.40, 0.69]	<0.001
Disulfide/total thiol, %	6.5 [4.9–7.8] (n=84)	4.5 [3.3–5.5] (n=63)	r 0.55 [0.39, 0.69]	<0.001
Native/total thiol, %	87.0 [84.4–90.2] (n=84)	91.1 [89.0–93.4] (n=63)	r -0.55 [-0.69, -0.39]	<0.001
GSH, µmol/L	597.7 [429.7–709.5] (n=50)	930.6 [750.0–1054.7] (n=79)	r -0.80 [-0.89, -0.69]	<0.001
GSSG, µmol/L	68.0 [53.2–86.3] (n=50)	43.8 [30.2–57.2] (n=79)	d 1.16 [0.78, 1.55]	<0.001
Total glutathione, µmol/L	707.4 [549.5–886.2] (n=50)	999.7 [835.5–1136.8] (n=79)	r -0.70 [-0.82, -0.57]	<0.001
GSSG/GSH ratio	0.119 [0.100–0.136] (n=50)	0.046 [0.029–0.071] (n=79)	r 0.88 [0.80, 0.95]	<0.001

Values are median [interquartile range] (n). The effect size is Cohen d for the normally distributed markers (native thiol, total thiol, and GSSG) and the rank-biserial correlation (r) for the remaining markers compared with the Mann-Whitney U test, each with a bootstrap 95% confidence interval; the metric is indicated in each cell (d or r). The p values are Holm-corrected across the ten markers for multiple comparisons; all comparisons also remained significant in linear models additionally adjusted for age and sex (reported in the text). GSH, reduced glutathione; GSSG, oxidized glutathione.

The GSSG/GSH ratio, which integrates both directions of change, was the single most discriminating marker: median 0.119 [0.100–0.136] in stroke patients versus 0.046 [0.029–0.071] in controls (rank-biserial r 0.88, 95% CI 0.80 to 0.95; Holm-adjusted $p<0.001$; adjusted for age and sex $p<0.001$). After adjustment for age and sex the differences persisted for every glutathione marker; the adjusted mean difference was −364 µmol/L (95% CI −441 to −288) for GSH and 0.069 (95% CI 0.057 to 0.083) for the GSSG/GSH ratio. The group differences were unchanged in sensitivity analyses using multiple imputations and after trimming values beyond three standard deviations.

3.3. Association with stroke severity and infarct volume: Within the stroke group, none of the redox markers correlated significantly with admission NIHSS or with diffusion-weighted infarct volume. All Spearman correlations were weak (absolute $\rho \leq 0.28$; all $p \geq 0.05$), including the GSSG/GSH ratio (NIHSS ρ 0.28, p=0.05; infarct volume ρ 0.14, $p=0.32$). The oxidized-glutathione concentration showed the strongest, but still non-significant, association with infarct volume (ρ 0.28, $p=0.050$). Thus, in this cohort, the redox markers were not significantly associated with either the clinical (NIHSS) or the radiological (infarct volume) severity of the stroke.

3.4. Redox markers and 90-day functional outcome: A poor 90-day outcome (modified Rankin Scale 3 to 6) occurred in 32 of 85 stroke patients (37.6%) overall, including 8 in-hospital deaths. The prognostic analyses were restricted to the 50 patients in whom erythrocyte glutathione was measured, among whom 16 (32.0%) had a poor outcome; given the small number of events, these analyses were considered exploratory. In univariable logistic regression, the erythrocyte GSSG/GSH ratio was the only marker associated with poor outcome (odds ratio 2.27 [1.08, 4.76] per standard deviation; p=0.03); the serum thiol

indices and the individual glutathione concentrations were not. Adjusted models were fitted with Firth's penalized likelihood because of the small subsample. The association persisted after adjustment for age (odds ratio 2.05 [1.06, 4.59] per standard deviation; p=0.03). However, after adjustment for admission NIHSS-the established prognostic anchor in stroke—the association was attenuated and no longer significant (odds ratio 1.71 [0.86, 3.90] per standard deviation; p=0.13), and the same held after adjustment for both age and NIHSS (odds ratio 1.73 [0.85, 4.05]; p=0.13); with only 16 events (events-per-variable approximately 8) the study is underpowered to establish independence from NIHSS. The ratio discriminated poorly from good outcome with an area under the receiver operating characteristic curve of 0.72 (95% CI 0.56 to 0.88; Figure 3), and the proportion of patients with a poor outcome rose across ascending tertiles of the ratio (11.8%, 37.5%, and 47.1%; trend p=0.03). A Youden-derived cutoff of 0.116 (sensitivity 0.88, specificity 0.59) is reported as exploratory and requires external validation.

4. Discussion

In this prospective case-control study we measured the serum thiol-disulfide couple and the erythrocyte glutathione couple concurrently in the same acute ischemic stroke patients. Both redox systems shifted toward oxidation relative to controls, with the erythrocyte glutathione system showing the most pronounced disturbance. This oxidative shift was not significantly associated with stroke severity or infarct volume in this cohort. Of the markers examined, the erythrocyte GSSG/GSH ratio showed the strongest association with poor 90-

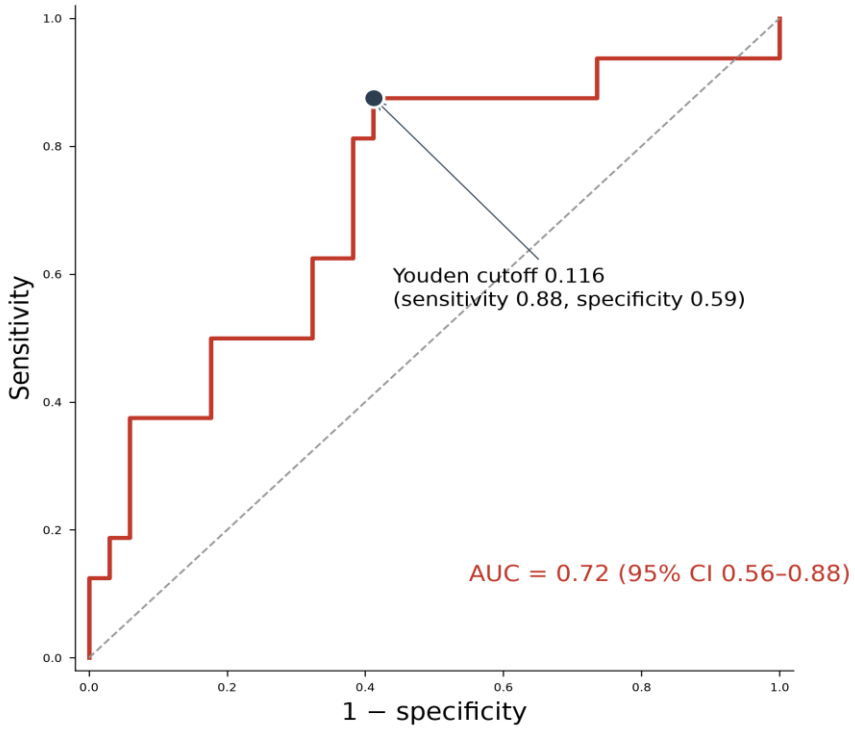

Figure 3. Receiver operating characteristic curve for the erythrocyte GSSG/GSH ratio in predicting poor 90-day functional outcome (modified Rankin Scale 3–6). *Area under the curve 0.72 (95% CI 0.56–0.88). The marked point is the Youden-derived cutoff (0.116; sensitivity 0.88, specificity 0.59), reported as exploratory.*

day functional outcome, although this association did not remain significant after adjustment for admission stroke severity.

The coordinated oxidative shift we observed is consistent with the accepted pathophysiology of ischemic stroke, in which reperfusion and mitochondrial dysfunction overwhelm endogenous antioxidant buffering [1,2,3]. The direction of the serum thiol-disulfide changes—lower native and total thiol with a higher disulfide fraction—matches earlier reports in cerebrovascular disease [9,10,11]. Our data extend these observations by showing that, when the two systems were measured together, the erythrocyte glutathione couple was disturbed more profoundly than the serum thiol pool. Among the redox markers evaluated in our study, the GSSG/GSH ratio showed one of the largest between-group differences (rank-biserial r = 0.88), suggesting that intracellular glutathione oxidation may be a particularly sensitive marker of systemic oxidative burden [7,8,12,16]. A comparable oxidative shift in both the thiol-disulfide and glutathione couples has also been reported in the subacute and rehabilitation phase of stroke, where both the disulfide/native thiol and GSSG/GSH ratios were higher in patients than in controls and moved toward normal with recovery,[20] indicating that the disturbance we captured acutely persists into later phases. This oxidative shift has a mechanistic correlate in controlled experimental ischemia-reperfusion: in a rat model of transient middle cerebral artery occlusion, the plasma GSSG/GSH ratio rose significantly after reperfusion, mirroring the direction of change we observed in patients [17].

The redox markers were not significantly associated with NIHSS or infarct volume in this cohort. This finding should be interpreted cautiously, because a non-significant association in a sample of this size does not establish the absence of a graded relationship; it may indicate that the systemic redox disturbance reflects a whole-body stress response rather than a direct readout of lesion size. A similar dissociation between systemic oxidative markers and radiological severity has been noted before [11,19]. This pattern is consistent with earlier thiol-disulfide studies in ischemic stroke: Bektaş and colleagues likewise found lower native and total thiol in stroke patients than controls but no significant difference in the disulfide concentration, together with a negative correlation between native thiol and infarct volume [9]. Our native and total thiol results were concordant with these earlier reports, whereas we observed a higher rather than an unchanged disulfide concentration. This difference is unlikely to be biologically fundamental; it more plausibly reflects differences between cohorts in the time from symptom onset to presentation, in the timing of blood sampling, and in admission stroke severity, all of which influence the dynamic thiol-disulfide balance in the early hours after ischemia.

The association of the GSSG/GSH ratio with poor 90-day outcome was the most clinically oriented finding, but it should be interpreted cautiously. The ratio was associated with outcome in univariable analysis and after adjustment for age, yet the association was attenuated and lost significance once admission NIHSS—the strongest established prognostic factor in acute stroke—was included, and the small number of events (16) leaves the study underpowered to demonstrate independence from clinical severity [19]. The graded rise in poor-outcome frequency across ratio tertiles is consistent with a prognostic signal, but these data cannot establish the ratio as an independent predictor. The lack of predictive value of the

individual GSH and GSSG concentrations, in contrast to their ratio, is compatible with the integrated index capturing the balance between antioxidant reserve and oxidative load more faithfully than either component alone [6,8]. The ratio may therefore be a candidate prognostic marker that warrants confirmation in larger studies with adequate event numbers and prespecified adjustment for established prognostic factors.

Our findings should be interpreted alongside the negative report of Cournot and colleagues, who found that circulating redox biomarkers did not improve cardiovascular risk prediction in a general population [14]. The difference in setting is important: in the acute ischemic state, redox disturbance is pronounced and, at least for the erythrocyte glutathione ratio, tracks short-term functional recovery, whereas in stable populations the signal may be too small to add predictive value. The prognostic use of redox markers may therefore be specific to the acute phase.

This study has limitations. It was conducted at a single center, and the sample size, although adequate to detect the large differences between-group reported here was modest for the secondary outcome analysis, in which the number of poor-outcome events limited the complexity of the multivariable model. The control group was younger and included more women than the stroke group, reflecting the demographics of acute ischemic stroke; we addressed this by adjusting the marker comparisons for age and sex, but residual confounding cannot be excluded. Infarct volume was measured retrospectively by a single reader on de-identified imaging without reference to the biomarker results; the reader was not formally masked to clinical outcome, and neither intra- nor inter-observer reliability was assessed, so the absence of a correlation between the redox markers and infarct volume should be interpreted with this measurement uncertainty in mind. Erythrocyte glutathione measurements were available for a subset of participants, which reduced the sample for the glutathione analyses. The outcome cutoff we report is exploratory and derived in the same sample, so it requires prospective external validation before any clinical use. Finally, the observational design precludes causal inference; whether the redox disturbance contributes to poor recovery or merely marks it cannot be resolved here.

4.1. Conclusions: In acute ischemic stroke, the serum thiol-disulfide and erythrocyte glutathione systems both shift toward oxidation, with the erythrocyte glutathione couple most affected. The redox disturbance does not scale with stroke severity or infarct volume. The erythrocyte GSSG/GSH ratio was associated with poor 90-day functional outcome in univariable and age-adjusted analyses, although this association did not persist after adjustment for admission NIHSS in this small subgroup. The GSSG/GSH ratio may be a candidate prognostic marker; if confirmed in larger, multicenter cohorts with adequate event numbers and prospective validation of a decision threshold, it could help identify stroke patients at risk of poor recovery.

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Conflict of Interest: *The authors declared no conflict of interest.*

Ethical Statement: *The protocol was approved by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University (approval no. 2022/37; 22 February 2022), and written informed consent was obtained from all participants or their legal representatives.*

Patient Consent Statement

Written informed consent was obtained from all participants prior to enrollment in the study.

Data Availability Statement: *The de-identified data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional and ethical approval.*

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