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The prognostic role of iron deficiency in acute ischemic stroke patients: A prospective multicentric cohort study

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ABSTRACT

Background and aims: Iron deficiency (ID) is a prognostic factor in heart failure and acute coronary syndrome. However, its role in cerebrovascular diseases is controversial. We aimed to determine the impact of ID on the functional outcome of acute ischemic stroke patients.

Methods: This was an observational prospective multicentric cohort study. From January to December 2023, we enrolled acute ischemic stroke patients admitted to the stroke units of four comprehensive stroke centers. Venous blood samples were collected at admission to determine the iron status (serum iron, ferritin, transferrin). ID was defined as a serum ferritin concentration < 100 ng/mL or 100–299 ng/mL with transferrin saturation (TSAT) <20 %. The primary endpoint was the poor functional outcome at 90 days defined as modified Rankin Scale (mRS) 3–6. We used binary logistic regression models including confounding factors to test the association between ID and the primary outcome.

Results: The analysis included 442 patients (mean age 73 ± 13, 47.5 % female, median NIHSS 7 [IQR 3–15], 61.3 % treated with intravenous thrombolysis and/or endovascular treatment). ID prevalence was 65.6 %. In all binary logistic regression models, ID predicted poor functional outcome at 3 months irrespective from demographics, stroke severity and characteristics, anemia, risk factors, signs/symptoms of heart failure, glucose at admission, and inflammatory biomarkers (aOR 2.328, 95 % CI 1.272–4.263, $p = 0.006$).

Conclusions: ID was strongly associated with poor functional outcome at 90 days in acute ischemic stroke patients. Further research is required to explore whether iron supplementation could be a potential therapeutic strategy to improve patient outcomes.

1. Introduction

Iron deficiency (ID) is a well-defined prognostic factor in heart failure and acute coronary syndrome, but its role in cerebrovascular diseases remains controversial [1]. While some studies link iron overload to poor functional outcomes following an ischemic stroke [2], there is a paucity of research examining the impact of ID on stroke prognosis [3]. Notably, intravenous iron supplementation has been shown to improve outcomes in various cardiovascular conditions and has been included in the current European Society of Cardiology (ESC) guidelines for heart failure management [4].

Iron is essential for cellular life, playing a critical role in numerous metabolic processes, including DNA repair and muscle strength [5]. Therefore, ID could be a significant prognostic factor in cerebrovascular diseases, similar to its role in other cardiovascular conditions.

This study aimed to determine the impact of ID on the functional outcome of acute ischemic stroke patients.

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2. Methods

2.1. Patients

This was a prospective multicentric observational cohort study conducted in four comprehensive stroke centers.

We included all patients admitted to the stroke units with a clinical and neuroradiological diagnosis of acute ischemic stroke from January to December 2023, and follow-up until April 2024. We collected data regarding demographics, time of stroke onset, functional status before the index event defined using the modified Rankin Scale (mRS) [6], site of vessel occlusion, stroke severity measured by the National Institutes of Health Stroke Scale (NIHSS) [7] at admission, acute recanalization treatment performed (intra-venous thrombolysis [IVT], endovascular treatment [EVT] or both), home medical therapy, cerebrovascular and cardiovascular risk factors, left ventricular ejection fraction (LVEF, assessed via echocardiography), New York Heart Association (NYHA) classification, signs and/or symptoms of heart failure, stroke etiology defined by the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification.

Our study was conducted according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria for observational studies. Written informed consent was obtained from the patients or their family members for their anonymized information to be published in this article. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethics approval was obtained from the institutional review board of "Sapienza" University of Rome (Rif. 7060 Prot. 0149/2023).

2.2. Outcome

The primary outcome was functional dependence or death, defined as mRS score of 3 to 6 at 90 ± 10 days from stroke onset, assessed by a physician blinded to the patient's iron status. Assessments were conducted during an outpatient visit or through a structured telephone interview.

Secondary outcomes included:

- Symptomatic Intracerebral Hemorrhage (sICH): defined as an intracerebral hemorrhage associated with an increase of ≥4 points on the NIHSS at 24 h or resulting in death.
- Remote Intracerebral Hemorrhage: any hemorrhage occurring in a location distinct from the initial stroke site.
- Any Intracerebral Hemorrhage: any detected presence of intracerebral bleeding.
- NIHSS Scores recorded at 24 h after treatment and at 7 days or at discharge, whichever occurred first.
- In-Hospital death: death occurring during the hospital stay.
- Discharge to home: patients discharged directly to their home rather than to a rehabilitation or care facility.
- Excellent outcome: defined as mRS 0–1 at 90 ± 10 days
- Death at 90 Days

2.3. Laboratory assessment

Iron status and routine laboratory tests were collected by peripheral venous blood samples from all patients at stroke unit admission. Anemia was defined as a hemoglobin level < 12 g/dL in women and < 13 g/dL in men. Iron status was measured using ferritin (ng/mL), serum iron (mg/dL), and transferrin (mg/dL). Transferrin saturation (TSAT) was calculated using the formula: [(serum iron/transferrin) × 70.9] [8]. ID was defined as a ferritin level < 100 ng/mL or ferritin 100 to 299 ng/mL in combination with a TSAT <20 % [9]. We also defined ID as TSAT <20 %, or considering TSAT as a continue variable. Serum concentrations of C- reactive protein (CRP) (mg/dL) were assessed at each institution using standard methods. We also collected glycated hemoglobin (HbA1c), white blood cells (WBC), red blood cells (RBC), Neutrophils, Lymphocytes, serum glucose (mg/dL), creatinine (mg/dL), total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglycerides.

2.4. Statistical analyses

Continuous variables were expressed as means ± Standard Deviation (SD) when normally distributed and as medians with interquartile range (IQR) when non-normally distributed. Categorical variables were reported as numbers and percentages. Intergroup differences were tested using the Student's *t*-test, Mann-Whitney *U* test, Pearson $\chi 2$ test, or Fisher's test when appropriate. Missing data were indicated in the tables and excluded in the analysis. Probability values of <0.05 were considered to be of statistical significance.

To establish the prognostic value of iron deficiency (ID) on functional outcomes, three binary logistic regression models were constructed. Each model included a different set of confounding variables based on their clinical relevance and previous literature. In the model 1 we included basic demographic variables and clinical factors. In model 2 we added cardio-cerebrovascular risk factors and medical history. In model 3 we also included inflammatory biomarker and blood tests. For all models, we employed a stepwise backward selection method, retaining variables with a univariate *p*-value <0.10. Variables that did not have at least 10 observations of the outcome events were excluded from the models.

Subgroup analysis was adjusted for age, sex, anemia, LVO, admission NIHSS, and CRP. *P*-value for interaction were calculated to determine whether the effect of ID on poor functional outcomes differs between subgroups.

All statistical analyses were performed using SPSS version 28.0.1.1 and R studio version 2024.04.0 + 735.

2.5. Sample size

To determine the sample size (n) needed to compare ischemic strokes with ID and those without ID, we used this formula:

sample size = [[(Zα/2 + Zβ)2·[P1(1 − P1) + P2(1 − P2)]]/(P1-P2)2 where Zα/2 is Z value for α = 0.05, Zβ is Z value for 80 % power, P1 is the expected incidence of the outcome in the ID group (0.50) and P2 is the expected incidence of the outcome in the non-ID group (0.35). To obtain a representative cohort, 338 patients were needed to be included in the study..

3. Results

3.1. Baseline characteristics

We recruited 442 patients (mean age 73 ± 13 years, 47.5 % female). We reported in Table 1 the patients' characteristics and differences between patients with ID and patients without ID at univariate analysis.

Median NIHSS was 7 (IQR 3–15),m 61.3 % treated with IVT and/or EVT. ID prevalence was 65.6 %. Patients with ID were older (75 ± 14 vs 70 ± 12, $p = 0.001$), more often female (57.6 % vs 31.7 %, $p < 0.001$), anemic (43.9 % vs 31.9 %, $p = 0.018$), less frequently current/past smokers (27.3 % vs 38.7 %, $p = 0.017$), with higher admission NIHSS (8 IQR 4–16 vs 6 IQR 3–11, $p = 0.001$), had more frequently LVO occlusion (60.5 % vs 46.5 %, $p = 0.006$), were more frequently treated with EVT (46.1 % vs 32.4 %, $p = 0.007$) or EVT + IVT (21 % vs 10.6 %, $p = 0.008$). They had higher use of antihypertensive (67.5 % vs 53.5 %, $p = 0.005$), antiplatelets (36.9 % vs 24.6 %, $p = 0.012$), and beta-blockers (38 % vs 25.4 %, $p = 0.010$), had lower hemoglobin levels (12.49 ± 2.03 vs 13.37 ± 2.16, $p < 0.001$), LDL (93 ± 36 vs 102 ± 41, $p = 0.021$), and triglycerides (98 ± 36 vs 119 ± 48, $p < 0.001$).

Table 1
Overall patient's characteristics and differences between patients with iron deficiency (ID) and patients without ID.

	Missing values n (%)	Overall n: 442	Iron Deficiency n: 271	No Iron Deficiency n: 142	p-value
Sex (Female)	0 (0.0 %)	210 (47.5 %)	156 (57.6 %)	45 (31.7 %)	**<0.001**
Age (years)	1 (0.2 %)	73 (±13)	75 (±14)	70 (±12)	**0.001**
Pre-mRS > 2	4 (0.9 %)	23 (5.3 %)	18 (6.7 %)	5 (3.5 %)	0.186
Unwitnessed stroke	0 (0.0 %)	150 (33.9 %)	96 (35.4 %)	46 (32.4 %)	0.538
Posterior circulation stroke	1 (0.2 %)	73 (16.6 %)	44 (16.3 %)	22 (15.5 %)	0.900
Large vessel occlusion	0 (0.0 %)	248 (56.1 %)	164 (60.5 %)	66 (46.5 %)	**0.006**
Site large vessel occlusion					
Middle Cerebral Artery-M1	1 (0.2 %)	95 (22.4 %)	62 (23.8 %)	27 (19.7 %)	0.052
Middle Cerebral Artery-M2	1 (0.2 %)	63 (14.8 %)	45 (17.2 %)	16 (11.7 %)	
Anterior Cerebral Artery	1 (0.2 %)	2 (0.5 %)	0 (0.0 %)	2 (1.5 %)	
Carotid-T	1 (0.2 %)	34 (8.0 %)	20 (7.7 %)	10 (7.3 %)	
Tandem	1 (0.2 %)	32 (7.5 %)	23 (8.8 %)	7 (5.1 %)	
Basilar Artery	1 (0.2 %)	11 (2.6 %)	6 (2.3 %)	3 (2.2 %)	
Posterior Cerebral Artery	1 (0.2 %)	10 (2.4 %)	7 (2.7 %)	1 (0.7 %)	
NIHSS admission	2 (0.5 %)	7 (3–15)	8 (4–16)	6 (3−11)	**0.001**
Recanalization treatment					
Intra-venous thrombolysis	0 (0.0 %)	89 (20.1 %)	106 (39.1 %)	50 (35.2 %)	0.437
Endovascular treatment	0 (0.0 %)	105 (23.8 %)	125 (46.1 %)	46 (32.4 %)	**0.007**
Combined treatment	0 (0.0 %)	77 (17.4 %)	57 (21.0 %)	15 (10.6 %)	**0.008**
TICI 2b-3	6 (1.4 %)	142 (80.7 %)	94 (79.0 %)	37 (82.2 %)	0.645
Etiology					
Atherosclerosis	3 (0.7 %)	69 (15.6 %)	39 (14.4 %)	22 (15.6 %)	0.840
Cardioembolic	3 (0.7 %)	169 (38.2 %)	112 (41.5 %)	51 (36.2 %)	
Lacunar	3 (0.7 %)	34 (7.7 %)	20 (7.4 %)	12 (8.5 %)	
Other causes	3 (0.7 %)	30 (6.8 %)	16 (5.9 %)	11 (7.8 %)	
Undetermined	3 (0.7 %)	137 (31.0 %)	83 (30.7 %)	45 (31.9 %)	
Comorbidities					
Hypertension	0 (0.0 %)	343 (77.6 %)	213 (78.6 %)	106 (74.6 %)	0.363
Atrial Fibrillation	0 (0.0 %)	139 (31.4 %)	92 (33.9 %)	42 (29.6 %)	0.367
Coronary artery disease	0 (0.0 %)	68 (15.4 %)	46 (17.0 %)	17 (12.0 %)	0.179
Previous stroke or TIA	0 (0.0 %)	85 (19.2 %)	49 (18.1 %)	31 (21.8 %)	0.360
Diabetes	0 (0.0 %)	110 (24.9 %)	49 (18.1 %)	31 (21.8 %)	0.328
Heart Failure (NYHA III-IV)	0 (0.0 %)	12 (2.7 %)	7 (33.3 %)	5 (41.6 %)	0.587
Chronic kidney disease	0 (0.0 %)	28 (6.3 %)	16 (5.9 %)	11 (7.7 %)	0.472
Chronic liver failure	0 (0.0 %)	3 (0.7 %)	2 (0.7 %)	1 (0.7 %)	1.000
Current/Past smokers	0 (0.0 %)	140 (31.7 %)	74 (27.3 %)	55 (38.7 %)	**0.017**
Alcohol abuse	0 (0.0 %)	12 (2.7 %)	5 (1.8 %)	7 (4.9 %)	0.119
Atheromasia ≥50 %	0 (0.0 %)	57 (12.9 %)	34 (12.5 %)	18 (12.7 %)	0.970
Peripheral artery disease	0 (0.0 %)	20 (4.5 %)	15 (5.5 %)	5 (3.5 %)	0.365
Hyperlipemia	0 (0.0 %)	236 (53.4 %)	150 (55.4 %)	67 (47.2 %)	0.114
Anemia	1 (0.2 %)	171 (38.7 %)	119 (43.9 %)	45 (31.9 %)	**0.018**
Treatment (medical hystory)					
Antihypertensive	0 (0.0 %)	273 (61.8 %)	183 (67.5 %)	76 (53.5 %)	**0.005**
Antiplatelets	0 (0.0 %)	145 (32.8 %)	100 (36.9 %)	35 (24.6 %)	**0.012**
Direct oral anti-coagulants	0 (0.0 %)	51 (11.5 %)	36 (13.3 %)	14 (9.9 %)	0.311
Iron supplements	0 (0.0 %)	2 (0.5 %)	2 (0.7 %)	0 (0.0 %)	0.548
Beta blockers	0 (0.0 %)	148 (33.5 %)	103 (38.0 %)	36 (25.4 %)	**0.010**
Statins	0 (0.0 %)	120 (27.1 %)	78 (28.8 %)	34 (23.9 %)	0.293
Insulin	0 (0.0 %)	24 (5.4 %)	20 (7.4 %)	4 (2.8 %)	0.060
Iron Deficiency defined as:					
Ferritin <100 ng/mL or 100–299 and TSAT <20 %	29 (6.6 %)	271 (65.6 %)			
TSAT <20 %	25 (5.7 %)	265 (63.5 %)			
Ferritin <100 ng/mL or CPR > 0.5 mg/dL and TSAT<20 %	17 (3.8 %)	301 (70.8 %)			
Ferritin <100 ng/mL or TSAT <20 %	13 (2.9 %)	322 (72.9 %)			

3.2. Iron deficiency and outcome

Patients with ID more frequently reached poor functional outcome (mRS 3–6) at 90 days (51.9 % vs 28.1 %, $p < 0.001$) and had higher rates of 90-day mortality (20.5 % vs 11.7 %, $p = 0.032$) as compared to patients without ID (Table 2). Symptomatic ICH occurred more frequently in ID patients (8.2 % vs 0.7 %, $p = 0.002$). Variables associated with poor functional outcome at univariate analysis are showed in Supplemental material Table 1.

To identify predictors of 3-month poor outcome (mRS 3–6), we performed three binary logistic regression models (Table 3 and Supplemental material Table 3) using three definitions of ID: (1) ferritin level < 100 ng/mL or ferritin 100 to 299 ng/mL in combination with a TSAT <20 %, (2) TSAT <20 %, or (3) TSAT as a continuous variable. In all models, ID predicted poor functional outcome at 3 months irrespective of age, sex, witnessed stroke, LVO, NIHSS at admission, EVT, anemia, etiology, hypertension, atrial fibrillation, Coronary Artery Disease (CAD), current/past smokers, signs/symptoms of heart failure, beta blockers, CRP, and glucose at admission.

Fig. 1 shows the boxplot of TSAT values across mRS at 90 days. Fig. 2 reports the relationship between TSAT and the predicted probability of poor outcome obtained by a binary logistic regression including age, sex, admission NIHSS, LVO, anemia, and CRP as confounding factors.

3.3. Subgroup analysis

We performed a subgroup analysis to test the association between ID and poor functional outcome throughout different subgroups including sex, age, baseline NIHSS, anemia, and LVO (Fig. 3). The association between ID and poor functional outcome was consistent across all subgroups tested. No statistically significant interactions were observed, suggesting that ID's association with functional outcomes is stable across subgroups.

Table 2
Outcome differences between patients with Iron Deficiency (ID) and patients without ID.

Outcome	Missing values n (%)	Overall n: 442	Iron Deficiency n: 271	No Iron Deficiency n: 142	p-value
mRS 3–6 at 90 days	29 (6.6 %)	181 (43.8 %)	134 (51.9 %)	36 (28.1 %)	**<0.001**
Any intracerebral hemorrhage (ICH)	7 (1.6 %)	115 (26.4 %)	78 (29.2 %)	31 (22.1 %)	0.126
Remote intracerebral hemorrhage	3 (0.7 %)	3 (0.7 %)	1 (0.4 %)	2 (1.4 %)	0.272
Symptomatic intracerebral hemorrhage (sICH)	7 (1.6 %)	24 (5.5 %)	22 (8.2 %)	1 (0.7 %)	**0.002**
NIHSS 24 h	3 (0.7 %)	4 (2–12)	5 (2–14)	3 (1–7)	**<0.001**
NIHSS 7 days/ discharge	19 (4.3 %)	3 (1–8)	3 (1–12)	2 (0–5)	**<0.001**
In Hospital Death	1 (0.2 %)	26 (5.9 %)	16 (5.9 %)	7 (5.0 %)	0.694
Discharge at home	1 (0.2 %)	180 (40.8 %)	98 (36.2 %)	68 (48.2 %)	**0.018**
mRS 0–1 at 90 days	29 (6.6 %)	185 (45.8 %)	96 (37.2 %)	75 (58.6 %)	**<0.001**
Death at 90 days	29 (6.6 %)	62 (15.3 %)	53 (20.5 %)	15 (11.7 %)	**0.032**

Table 3
Binary Logistic Regression for functional dependence or death (mRS 3–6) at 90 days. Values are: aOR (95 % C.I.), *p*-value.

	Ferritin <100 ng/mL or 100–299 and TSAT <20 %	TSAT <20 %	TSAT
Model 1	2.184 (1.261–3.780), 0.005	2.994 (1.709–5.246), <0.001	0.931 (0.902–0.960), <0.001
Model 2	2.105 (1.199–3.695), 0.010	2.998 (1.678–5.357), <0.001	0.931 (0.901–0.962), <0.001
Model 3	2.328 (1.272–4.263), 0.006	2.714 (1.435–5.131), 0.002	0.935 (0.902–0.969), <0.001

Model 1: Age, Sex, Witnessed stroke, LVO, NIHSS admission, EVT, Anemia, Etiology.
Model 2: Age, Sex, Witnessed stroke, LVO, NIHSS admission, EVT, Anemia, Etiology, Hypertension, Atrial Fibrillation, CAD, Current/past smokers, signs/symptoms HF, Beta blockers.
Model 3: Age, Sex, Witnessed stroke, LVO, NIHSS admission, EVT, Anemia, Etiology, Hypertension, Atrial Fibrillation, CAD, Current/past smokers, signs/symptoms HF, Beta blockers, CRP, Glucose.

4. Discussion

The main finding of this study was that ID represented a strong predictor of poor outcome in ischemic stroke patients admitted to stroke units. Our prospective multicentric cohort study, encompassing 442 patients, demonstrated that ID was prevalent, affecting 65.6 % of the ischemic stroke population, and was strongly associated with poor functional outcomes at 90 days post-stroke.

In cardiovascular disease, ID has been proved to be an independent prognostic factor, particularly in heart failure patients with reduced ejection fraction [1,10]. The ESC guidelines on heart failure recommend screening for ID and anemia and provide evidence for intravenous iron supplementation to alleviate symptoms, improve exercise capacity, quality of life, and reduce risk of hospitalization [4]. Similar evidence, although fragmentary, has been found for patients with coronary artery disease [11], aortic stenosis [12], and atrial fibrillation [13], stressing that ID is a significant prognostic factor in various cardiovascular diseases.

In cerebrovascular diseases, the role of ID is still controversial, especially as to its prognostic role after an ischemic stroke. Most studies have focused on the risk of incident stroke and the iron status, suggesting a U-shaped relationship between iron biomarker levels and stroke risk [14,15]. Few studies investigated the role of ID as a prognostic factor after an ischemic stroke. Doehner W. et al. in 2022 found that ID and anemia are independently related to lower functional capacity after acute stroke, to poor functional outcome after rehabilitation [3], and to lower muscle strength in acute stroke patients [16]. Recently, in a large retrospective cohort study, ID was found to be associated with a higher risk of all-cause mortality over a 5-year period [17]. Conversely, other studies found that iron overload can also have a detrimental effect on the prognosis of acute ischemic stroke [2,18]. In our study we confirmed the hypothesis that ID is a prognostic factor for poor functional outcome at 90 days, and the risk of poor outcome increases with the decrease of TSAT levels.

The discrepancies among studies may at least in part arise from the different definitions of ID. Most cardiovascular studies define ID as a serum ferritin concentration < 100 ng/mL, or a ferritin concentration 100–299 ng/mL in combination with a TSAT <20 %. This definition is based on its good specificity and sensitivity for identifying ID, [9] compared to the gold standard of bone marrow histology. However, study methodologies vary, with some using only single iron status biomarkers such as serum iron, transferrin, TSAT, or ferritin [19]. In our study, we used the definition utilized by ESC and by randomized controlled trials conducted to determine the impact of iron

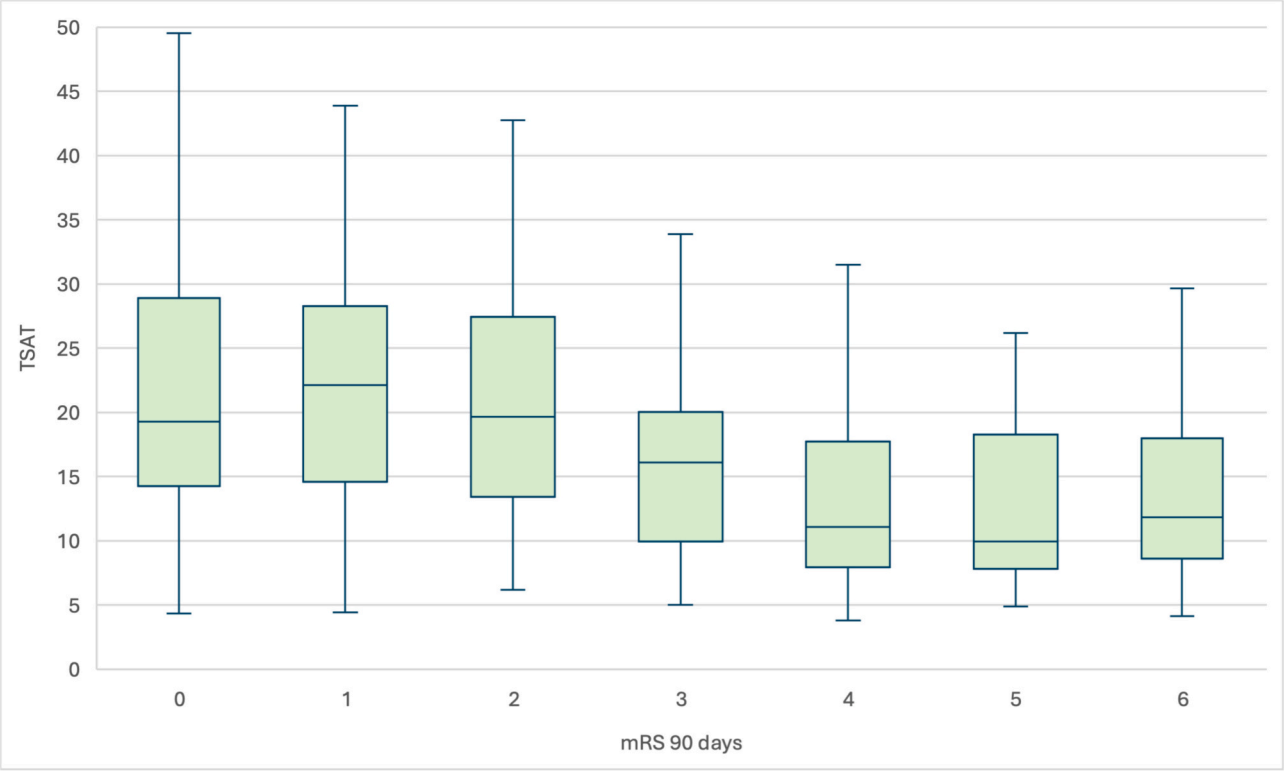

Fig. 1. TSAT value by 90-day mRS.

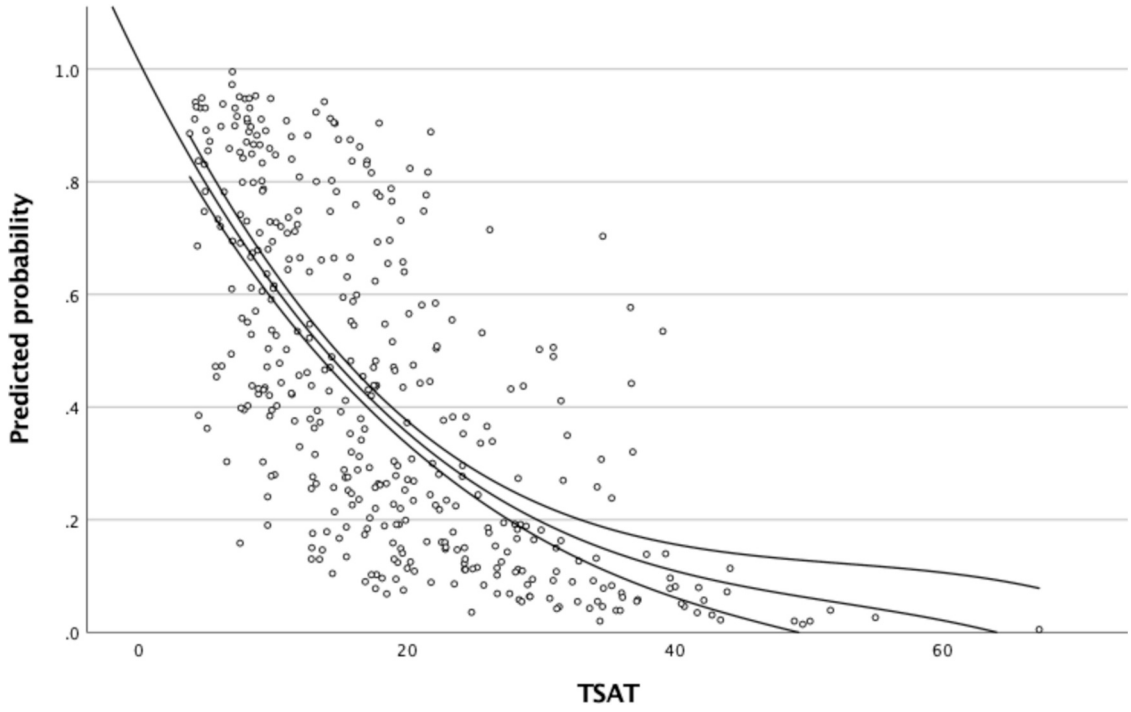

Fig. 2. Predicted probability of poor functional outcome at 90 days (mRS 3–6) and TSAT value.

supplementation in heart failure patients [20,21]. In addition, we used TSAT <20 % and TSAT as a continuous variable and found it strongly related with predicted probability of poor functional outcome at 3 months (Fig. 1 and Fig. 2).

Iron deficiency (ID) is associated with numerous confounding factors in stroke patients. It is important to note that iron is closely related to inflammation through hepcidin pathway, which reduces iron absorption and the release of ferritin from cells [22]. ID is a significant cause of anemia, a well-known risk factor for adverse outcomes in stroke. Moreover, ID has also been linked to poor nutritional status. In our study, we collected key confounding factors, including anemia, hemoglobin, lipid panel, C-reactive protein (CRP), and other inflammatory

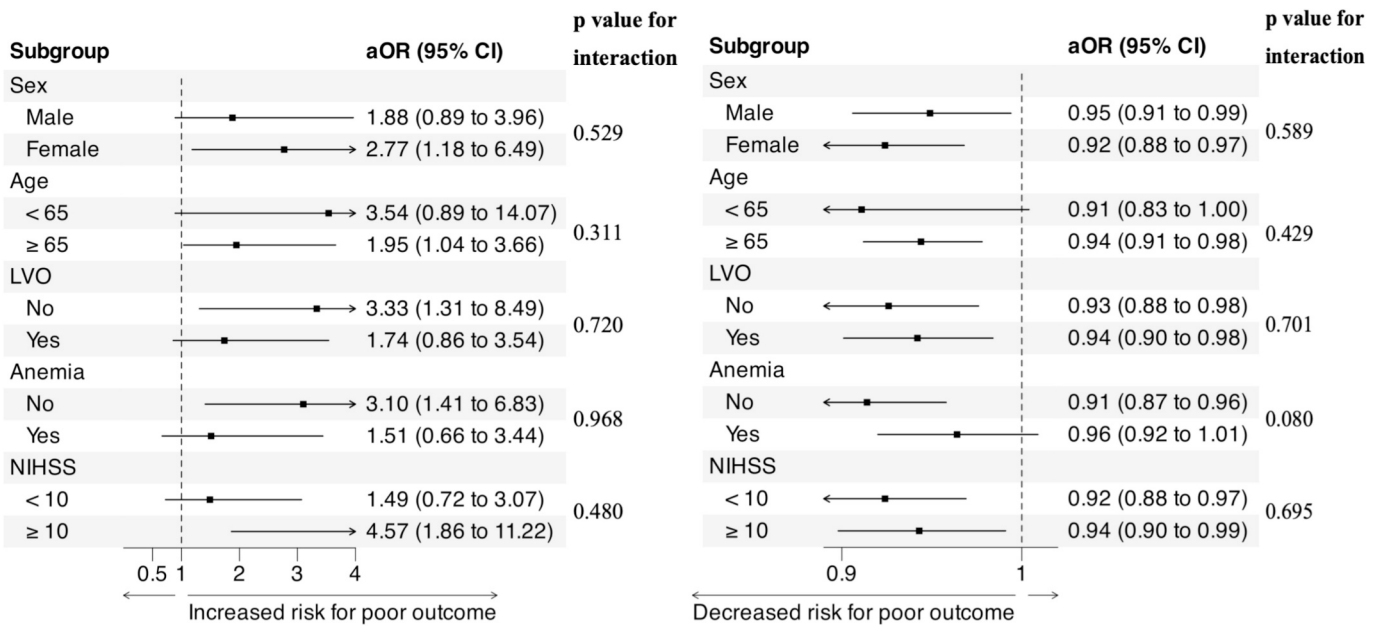

Fig. 3. Subgroup analysis for ID defined as Ferritin <100 ng/mL or 100–299 and TSAT <20 % (left) or for TSAT as a continuous variable (right).

biomarkers such as the neutrophil-to-lymphocyte ratio. We also accounted for conventional stroke severity indicators and other confounders such as large vessel occlusion (LVO) and witnessed stroke severity. These variables were included in the logistic regression models to adjust our findings. Despite these adjustments, ID remained independently associated with poor functional outcomes.

We performed a subgroup analysis to further explore the role of ID in predicting functional outcomes. This analysis was stratified by sex, age, stroke severity, presence of anemia, and LVO, to assess potential differences in the impact of ID on outcomes within these groups. Our results demonstrated that the association between ID and poor functional outcome did not vary significantly across any of these subgroups. Additionally, when TSAT was treated as a continuous variable, the findings remained consistent, further supporting the robustness of our results. These observations suggest that ID may be an independent factor associated with poor outcomes, regardless of anemic status or other clinical and demographic variables. However, the issue remains open to further investigation.

From a pathophysiological perspective, iron is essential in all organ systems and cells as it is involved in key metabolic processes. Iron is crucial for DNA replication and repair, gene expression, mitochondrial metabolism, cellular detoxification and catabolism, and immune system function [5]. Therefore, iron is vital for life, and both its deficiency and overload can lead to metabolic dysregulation. For instance, ID is commonly associated with fatigue and impaired cognitive development. Moreover, ID anemia and biophysical alteration of red blood cells have been demonstrated to directly lead to vasculopathy [23]. Conversely, ferroptosis, a form of cell death dependent on iron, may play a significant role in neuronal death following a stroke [24].

Finally, intra-venous iron supplementation, but not oral supplementation, has been proved to be effective in heart failure patients [20,21,25]. In stroke patients no studies were found on iron supplementation. However, it has been recently published a rationale and design of a multicenter randomized trial on efficacy and safety of oral ferrous iron on the prevention of vascular cognitive impairment among patients with cerebral Infarction or transient ischemic attacks [26].

4.1. Limitations and strengths

The main limitation of this study lay on its observational design encompassing a heterogeneous ischemic stroke cohort, leading to potential selection bias and residual confounding factors that were not accounted for. Furthermore, we did not collect information on patients' previous history of ID or anemia, which may have influenced their baseline iron status and potentially impacted outcomes. Moreover, iron status was not re-evaluated at the 3-month follow-up. This limited our ability to assess the evolution of ID over time and its potential impact on functional recovery beyond the acute phase.

Despite these limitations, our study included a broad range of confounding factors, leading to robust binary logistic regression models. Secondly, the study was conducted across four comprehensive stroke units, enhancing the generalizability of the findings by including diverse patient populations from different institutions. Moreover, data were collected prospectively with a predefined protocol which reduces recall bias.

5. Conclusions

Our study demonstrates that ID is a predictor of poor functional outcomes at 90 days in patients with acute ischemic stroke. ID was prevalent in our cohort, affecting 65.6 % of patients, and remained an independent risk factor for adverse outcomes despite adjustments for multiple confounding variables.

These findings highlight the need for clinical trials investigating the potential therapeutic benefits of iron supplementation in stroke patients to explore possible interventions that may improve patient outcomes.

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CRediT authorship contribution statement

Antonio Ciacciarelli: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anne Falcou:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Ettore Nicolini:** Writing – review & editing, Formal analysis. **Aldobrando Broccolini:** Investigation, Data curation. **Giovanni**

Frisullo: Investigation, Data curation. **Serena Abruzzese:** Investigation, Data curation. **Irene Scala:** Investigation, Data curation. **Sabrina Anticoli:** Investigation, Data curation. **Elisa Testani:** Investigation, Data curation. **Ennio Montinaro:** Investigation, Data curation. **Cristina Ranchicchio:** Investigation, Data curation. **Manuela De Michele:** Writing – review & editing, Validation, Supervision. **Danilo Toni:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing Interest

The Authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2024.123371.

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