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Stroke 2007, 38:90-95: originally published online November 30, 2006

doi: 10.1161/01.STR.0000251798.25803.e0

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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ISSN: 1524-4628

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Increased Body Iron Stores Are Associated With Poor Outcome After Thrombolytic Treatment in Acute Stroke

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Background and Purpose—Iron overload has been associated with greater oxidative stress and brain injury in experimental cerebral ischemia and reperfusion. This study investigates whether high serum ferritin levels, as an index of increased cellular iron stores, are associated with poor outcome, hemorrhagic transformation, and brain edema after treatment with tissue plasminogen activator in patients with acute ischemic stroke.

Methods—A total of 134 consecutive patients treated with intravenous tissue plasminogen activator were prospectively studied in four centers. Serum ferritin levels were determined at baseline, 24 and 72 hours after treatment. Cranial computed tomography was performed on admission and at 24 to 36 hours after tissue plasminogen activator infusion. Stroke severity and outcome were evaluated by using the National Institute of Health Stroke Scale and the modified Rankin Scale.

Results—Computed tomography showed hemorrhagic transformation in 27 patients (hemorrhagic infarction in 15 and parenchymal hematoma in 12; symptomatic in four) and brain swelling with midline shift in 15. Poor outcome (modified Rankin Scale >2) at 90 days was observed in 54.5% of patients. Ferritin levels at baseline were higher in patients with poor outcome at 90 days (median [quartiles], 165 [98,307] versus 17 [12,37] ng/mL; $P<0.001$) and in those who developed parenchymal hematoma ($P=0.006$), symptomatic hemorrhagic transformation ($P=0.008$), and severe brain edema ($P<0.001$). Serum ferritin levels higher than 79 ng/mL before tissue plasminogen activator treatment were independently associated with poor outcome (OR, 117 [95% CI, 25 to 557]).

Conclusions—Increased body iron stores are associated with poor outcome, symptomatic hemorrhagic transformation, and severe edema in patients treated with tissue plasminogen activator after ischemic stroke. These findings suggest that iron overload may offset the beneficial effect of thrombolytic therapies. (*Stroke*. 2007;38:90-95.)

Key Words: ferritin ■ iron overload ■ ischemic stroke ■ outcome ■ oxidative stress ■ thrombolysis

Thrombolysis with tissue plasminogen activator (t-PA) is an effective therapy for acute ischemic stroke. Partial or complete early recanalization of the middle cerebral artery occurs in more than 50% of patients who receive intravenous t-PA treatment; however, early reperfusion does not avoid ischemic stunning, neurologic worsening, or delayed injury in over one-third of these patients.¹⁻³ One of the reasons for deleterious outcomes after t-PA administration may be linked to the neurotoxicity of the drug on the neurovascular unit.⁴ In animal models, t-PA promotes the disruption of the blood-brain barrier that results in edema and cerebral hemorrhage.^{5,6} Furthermore, t-PA-induced reperfusion after cerebral ischemia may enhance the generation of reactive oxygen species, which may aggravate the disruption of microvascular integrity and the cellular functions on the neurovascular unit.⁷

An important source of reactive oxygen species is linked to disturbances in brain iron homeostasis. During cerebral ischemia, free iron released from intracellular stores such as ferritin catalyzes the conversion of superoxide and hydrogen peroxide into the highly reactive toxic hydroxyl radical.^{8,9} Experimental data support a causal role of iron overload in ischemic brain and endothelial damage. Iron intake has been associated with larger infarct volumes, higher oxidative stress, glutamate release, and inflammatory response after permanent middle cerebral artery occlusion in the rat,¹⁰ whereas iron depletion or chelation reduces infarct size, brain edema, and metabolic failure in ischemia/reperfusion experimental stroke models.^{11,12} In patients with acute ischemic stroke not treated with thrombolytic drugs, high serum ferritin values and high cerebrospinal fluid ferritin concentrations deter-

Received June 6, 2006; final revision received July 27, 2006; accepted August 22, 2006.

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Stroke is available at <http://www.strokeaha.org>

DOI: 10.1161/01.STR.0000251798.25803.e0

mined early after symptom onset have been associated with subsequent neurologic worsening, poor neurologic outcome, large infarct volume, and elevated concentrations of glutamate in blood.^{13–15} Serum ferritin levels are thought to be directly proportional to cellular iron stores and can be used to assess iron overload in the absence of inflammation, cancer, and infectious diseases.^{16,17}

Taken together, these findings suggest that iron overload might offset the beneficial effect of t-PA, although this hypothesis has never been tested. The aim of this study was to investigate whether high serum ferritin concentrations before treatment, as an index of increased cellular iron stores, are associated with poor outcome, hemorrhagic transformation (HT), and brain edema in patients with ischemic stroke treated with intravenous t-PA.

Patients and Methods

We studied consecutive patients with acute ischemic stroke treated with intravenous t-PA within 3 hours from symptom onset in four university hospitals. All patients were treated according to the SITS-MOST registry (<http://acutestroke.org>) and were prospectively evaluated by using cranial computed tomography, neurologic and functional scales during a follow up of 90 days. For the purpose of this investigation, patients with prior disability (modified Rankin score [mRS] >1), alcohol consumption ≥ 40 g/day, current treatment with iron, and known infectious, inflammatory, or cancer diseases at the time of treatment were excluded. Cotreatment with NXY-059 or placebo into the SAINT I trial was allowed,¹⁸ but the present investigation was not a substudy of SAINT I. No other investigational drugs were used. The protocol was approved by the ethics committees of the participating centers and informed consent was signed by patients or their relatives.

Clinical Variables

Baseline characteristics and stroke subtype were recorded. Patients were continuously monitored in an acute stroke unit. Stroke severity was quantified just before t-PA administration and at 24 hours by using the National Institute of Health Stroke Scale (NIHSS). Early neurologic deterioration or improvement was diagnosed when the NIHSS worsened or improved ≥ 4 points between baseline and 24 hours. Patients were classified into two outcome groups according to the mRS evaluated at 90 days: Poor outcome (mRS score >2) and good outcome (mRS score ≤ 2).

Neuroimaging Variables

Computed tomography scans were carried out immediately before treatment and at 24 to 36 hours after thrombolytic therapy. Early computed tomography signs of infarction were evaluated on admission and infarct volume, hemorrhagic transformation, and brain edema were assessed at 24 to 36 hours. HT was classified in hemorrhagic infarction type 1 (HI1) and 2 (HI2) and parenchymal hematoma type 1 (PH1), type 2 (PH2), and remote (rPH). HT was defined as symptomatic when it was associated with early neurologic deterioration. Severe brain edema was diagnosed if extensive swelling caused any shifting of the structures of the midline. Infarct volume was calculated by using the formula $0.5 \times a \times b \times c$, where a and b are the largest perpendicular diameters and c is the slice thickness. Computed tomography scans were evaluated by investigators who were blind to the laboratory determinations and clinical outcome.

Laboratory Determinations

Serum samples were taken immediately after admission (within 3 hours from stroke onset and before the t-PA treatment), at 24 hours, and at 72 hours after the t-PA bolus, and stored at -80°C . Ferritin was determined by electrochemiluminescence immunoassay by using an analyzer ELECSYS 2010 (Roche Diagnostics GmbH). Clin-

ical investigators were unaware of the laboratory results until the end of the study, once the database was closed.

Statistical Analyses

Proportions between two groups were compared by using the χ^2 test or Fisher exact test. Continuous variables have been expressed as the mean and SD, or median and quartiles in the case of distribution that was not normal, and compared by the Student *t* test or the Mann-Whitney test as appropriate. Median ferritin values in different hospital populations were compared by the Kruskal-Wallis test. Spearman coefficient was used to analyze the correlation between ferritin concentrations and infarct volume. The linearity of ferritin for outcome was assessed. Receiver operating characteristic curves were configured to establish cutoff points of ferritin level that optimally predicted the occurrence of poor outcome. Accordingly, the impact of serum ferritin levels on outcome (primary objective), HT, and brain edema formation (secondary objectives) was assessed by logistic regression analysis. Results were adjusted for age, stroke severity, and other baseline variables related to outcome in the bivariate analysis with a probability value < 0.1 . The sensitivity and specificity of the predictive model were calculated and potential interactions between confounders and ferritin levels were checked. A sensitivity analysis was performed in patients not randomized in the SAINT I trial.

Results

Of 151 patients treated with intravenous t-PA during the study period (the length of the inclusion period ranged from 5 to 26 months in the four centers), 134 were included in this study. Reasons for exclusion were unavailability of blood samples at baseline in 11 patients and infectious, cancer, or inflammatory diseases in six patients. No differences were found between the included population and the total patients treated with t-PA regarding age, NIHSS at baseline, time from onset to treatment, and stroke subtype (data not shown).

Clinical Outcome

At 24 hours, neurologic deterioration was found in 6% of patients, stable course in 51%, and neurologic improvement in 43%. Poor outcome at 90 days was recorded in 73 (54.5%) patients. Eighteen patients (13.4%) died during the study period and none was lost of follow up. Causes of mortality were HT in two patients, malignant brain edema in five, other stroke related causes in two, and nonvascular causes in nine patients.

Table 1 shows the baseline characteristics in the total patients and by outcome groups. Carotid territory involvement, cardioembolic subtype, and early computed tomography signs of infarction were more frequent and stroke severity was greater in the poor outcome group. Twenty-three patients were cotreated with NXY-059 or placebo into the SAINT I study,¹⁸ eight patients with good outcome and 15 patients with poor outcome ($P=0.256$).

At baseline, ferritin concentrations in blood were significantly higher in patients with poor outcome than in those with good outcome (Table 1). There was a lack of linearity of serum ferritin for outcome. The receiver operating characteristic curve showed that ferritin levels over 79 ng/mL had the highest sensitivity (86%) retaining good specificity (93%) to predict poor outcome; this value corresponded to the median value. The area under the curve was 0.944 ($P<0.001$). There was a relationship between the distribution of the Rankin scores and baseline levels of ferritin categorized in quartiles:

TABLE 1. Baseline Characteristics in the Total Series and by Outcome Groups

	Total Patients (n=134)	Good Outcome Group (n=61)	Poor Outcome Group (n=73)	<i>P</i>
Age	67 (12)	64.7 (12.6)	69.3 (10.9)	0.017
Sex, male	65.7	68.9	63.0	0.478
History of vascular risk factors				
Hypertension	49.3	47.5	50.3	0.528
Diabetes	18.7	13.1	23.3	0.132
Smoking habit (current)	14.9	19.7	11	0.328
Dyslipidemia	32.1	32.8	31.5	0.874
Stroke	8.2	9.8	6.8	0.362
Prior antiplatelet therapy, %	28.4	26.3	30.2	0.777
Vital signs and laboratory parameters				
Systolic blood pressure, mm Hg	152 (23)	152.4 (21.5)	151.4 (23.6)	0.842
Diastolic blood pressure, mm Hg	82 (13)	82.2 (13.1)	81.1 (12.3)	0.787
Body temperature, °C	36.2 (0.5)	36.2 (0.4)	36.2 (0.6)	0.910
Serum glucose, mg/dL	137 (52)	136 (51)	137 (53)	0.666
Platelet count (×1000)	241 (92)	241 (70)	242 (107)	0.689
Activated partial thromboplastin time, s	28 (10)	28 (8)	29 (11)	0.930
Serum ferritin, ng/mL	78.8 [17.5, 208.3]	17.5 [11.9, 36.8]	165.1 [98.4, 307.6]	<0.001
Time from onset to treatment, minutes	152 [125, 170]	160 [125, 174]	143 [122, 165]	0.102
NIHSS at baseline	14 [9, 19]	10 [7, 15]	18 [12, 20]	<0.001
Arterial territory				
Carotid		84	95	
Vertebrobasilar		16	5	
TOAST classification				
Cardioembolic		32.8	52.1	0.001
Atherothrombotic		13.1	24.7	
Lacunar		13.1	1.4	
Undetermined		41.0	21.9	
Early computed tomography signs	33.6	16.4	47.9	<0.001
Hypoattenuation >33% middle cerebral artery	6.0	1.7	9.6	0.056

Note: Values are presented as proportions, mean (standard deviation), or median [quartiles].

the higher the ferritin quartile, the worse the distribution of mRS scores (Figure 1). Regarding the early clinical course, serum concentrations of ferritin before treatment were significantly higher in patients with subsequent neurologic worsening (254 [86,375] versus 59 [16,190] ng/mL), whereas they were significantly lower in patients who improved 4 or more points in the NIHSS at 24 hours (21 [13,86] versus 132 [54,302] ng/mL).

Figure 2 shows the serum ferritin profile during the first 72 hours after treatment. Ferritin levels showed a parallel slight increase in both outcome groups. No differences were found in ferritin levels between sexes and between hospital populations.

Logistic regression analysis showed that serum ferritin levels at baseline higher than 79 ng/mL had an odds of 117 [95% CI, 25 to 557] of poor outcome after adjusting for age, carotid territory, cardioembolic subtype, time from onset to treatment, NIHSS score, and early signs of infarction on computed tomography (Table 2). The model provided good sensitivity (95.1%) and specificity (89.0%). The inclusion of

hypoattenuation >33% of middle cerebral artery territory, or of hypodensity volume at 24 to 36 hours, in the model did not change the effect. After exclusion of the 23 patients randomized in the SAINT I, the adjusted OR of poor outcome was 226 [95% CI, 30 to 1700]. No biologic and plausible interactions were found between ferritin levels and the other confounding factors.

Cranial Computed Tomography Findings

Computed tomography at 24 to 36 hours showed no signs of infarction in 10% of patients. Median hypodensity volume was 19 [3,80] cc. In 91% of patients, it was located in the middle cerebral artery territory. HT was observed in 27 (20%) patients (HI1 in 7, HI2 in 8; PH1 in 7, PH2 in 4, and rPH in 1; symptomatic in 4, all PH), whereas severe cerebral edema was found in 15 (11.2%) patients.

There was a significant correlation between serum ferritin level and infarct volume ($r=0.663$; $P<0.001$). Ferritin concentrations at baseline were significantly higher in patients with PH (Figure 3) and in the four patients with symptomatic

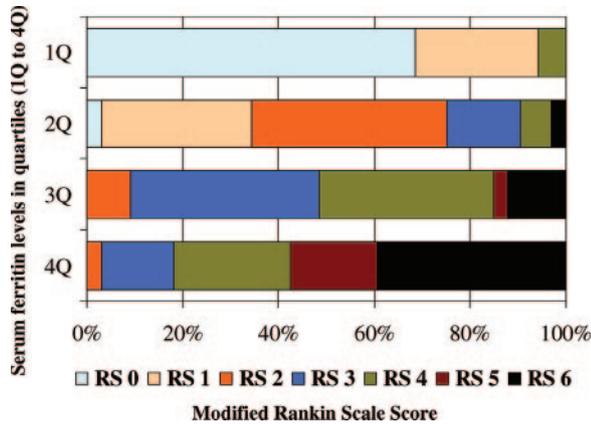


Figure 1. Distribution of mRS scores at day 90 by serum ferritin levels categorized in quartiles. The size of the color bars indicates the proportion of patients with a particular score for each ferritin quartile (Q). Ferritin levels for the first Q, <17 ng/mL; second Q, 18 to 79 ng/mL; third Q, 80 to 208 ng/mL, fourth Q >208 ng/mL.

HT ($P=0.008$). Ferritin levels were also higher in patients who developed severe brain edema at 24 to 36 hours (313 [109,470] versus 55 [16,144] ng/mL; $P<0.001$). Values higher than 79 ng/mL were associated with a 6.4 [1.3 to 32] -fold increase in the risk of severe brain edema and with a nonsignificant 4.2 [0.8 to 22] -fold increase in the risk of PH after adjustment for age, baseline NIHSS score, and cardioembolic subtype.

Discussion

The present study shows that high serum ferritin concentration before t-PA infusion predicts poor outcome at 90 days in patients with acute ischemic stroke. Importantly, the higher the ferritin level quartile, the worse the distribution of mRS scores. Furthermore, ferritin levels on admission were higher in patients who developed early neurologic worsening, greater infarct volume, symptomatic hemorrhagic transformation, and severe brain edema after t-PA treatment. These results replicate previous findings in stroke patients not treated with reperfusion therapies,¹³⁻¹⁵ and suggest a biologic

TABLE 2. Adjusted Odds Ratios of Poor Outcome for Baseline Characteristics

Independent Variables	OR	95% CI	P
Age	1.07	1.01 to 1.14	0.014
Carotid territory	1.03	0.14 to 7.40	0.972
Cardioembolic subtype	1.00	0.27 to 3.65	0.999
Time from onset of treatment	0.98	0.96 to 1.00	0.187
Baseline NIHSS	1.07	0.96 to 1.21	0.199
Early signs of brain infarction	2.25	0.52 to 9.60	0.273
Baseline serum ferritin >79 ng/mL	117.0	24.4 to 559.5	<0.0001

Reference categories are: serum ferritin levels ≤ 79 ng/mL, no early signs of brain infarction, and vertebrobasilar arterial territory. Age, time from onset of treatment, and baseline NIHSS are continuous. There were no patients with missing data.

deleterious effect of iron overload on ischemic brain and endothelial injury in patients who receive intravenous t-PA.

Serum ferritin is a suitable index of the amount of cellular iron stores and, consequently, might be related to the availability of iron in the infarcted area.^{16,17} In brain tissue, most of the nonheme iron is in the form of ferritin, which is localized in astrocytes and microglia.¹⁹ Ferritin synthesis in brain cells may be induced in hypoxic acidosis²⁰ or in response to oxidative stress to reduce the accumulation of reactive oxygen species.²¹ Therefore, increased ferritin could be in part the result of a neuroprotective mechanism with the aim of sequestering toxic-free iron in the ischemic brain. However, this effect has only been demonstrated several hours after the insult in brain cells, but not in the peripheral blood and within 3 hours after the onset of ischemia as shown in our patients. Serum ferritin levels remained substantially within normal values in our country (20 to 300 ng/mL for men and 15 to 200 ng/mL for women),²² thus indicating that there was not a sudden increase like in other stress response phenomena. In addition, previous studies have shown stability of serum ferritin levels during the first 48 hours after stroke onset and a lack of correlation between ferritin and acute-phase reactants in blood such as cortisol and C-reactive protein.^{15,23} Iron

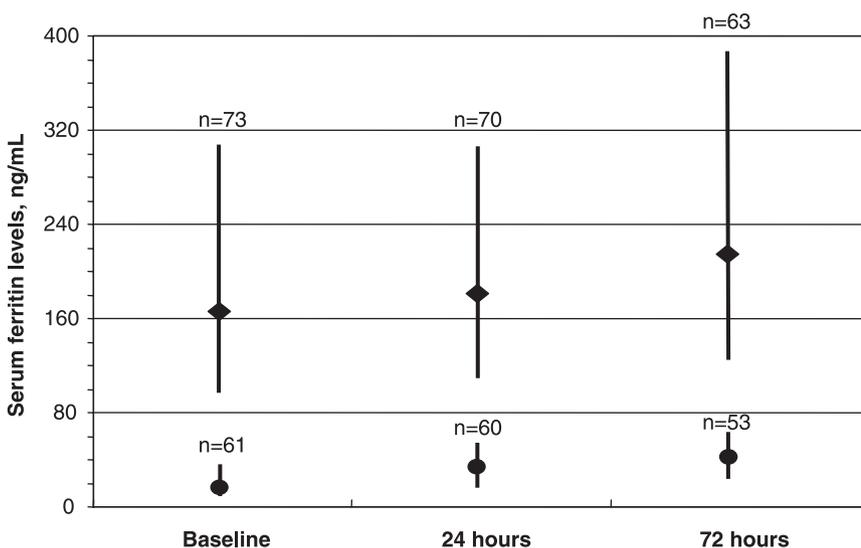


Figure 2. Median values and interquartile range (bars) of serum ferritin concentrations at baseline and at 24 and 72 hours after intravenous t-PA bolus by outcome groups at day 90 (◆ poor outcome; ● good outcome). Numbers indicate patients studied at each time.

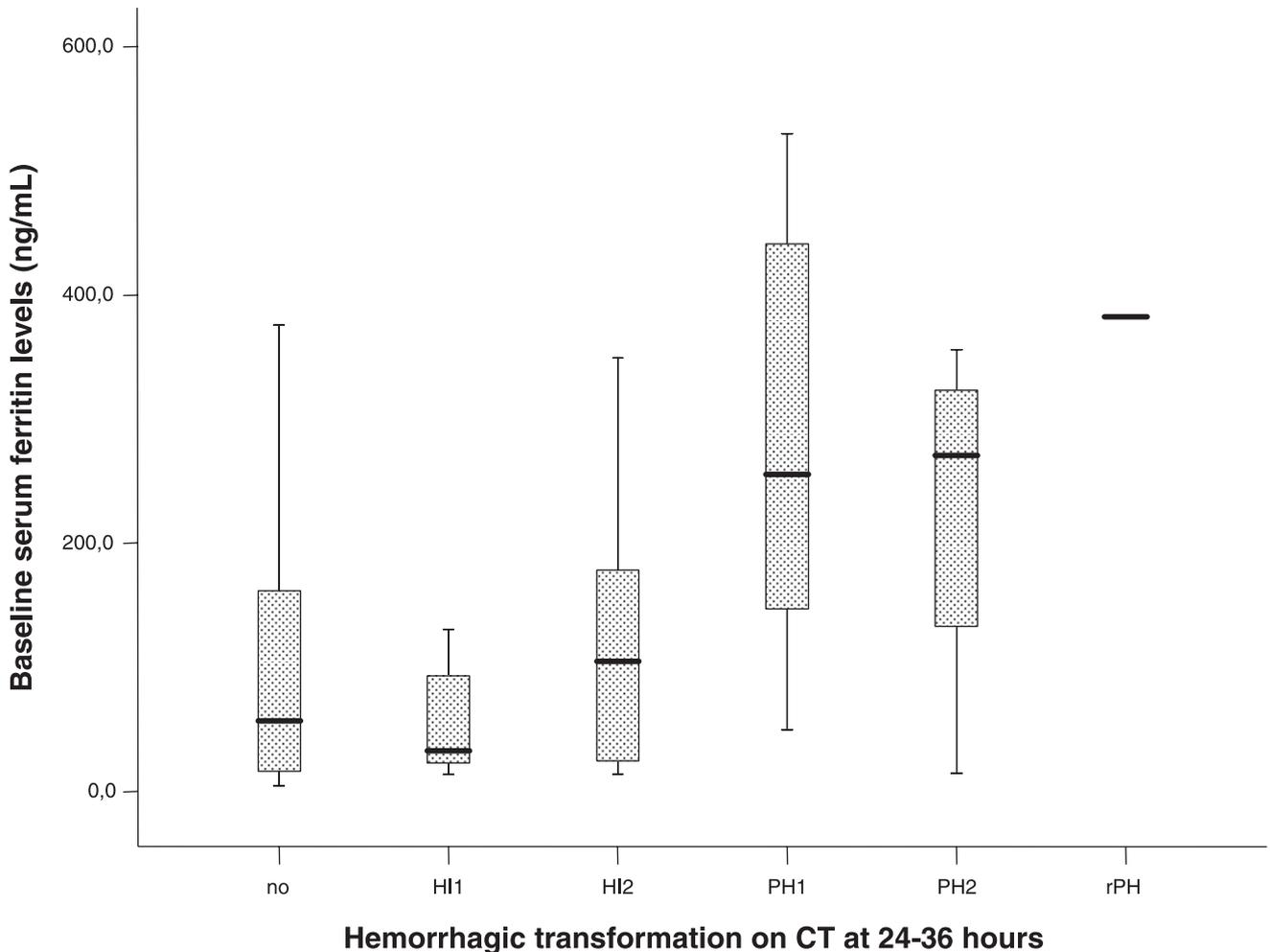


Figure 3. Box plots show median values (horizontal line), interquartile range (box boundaries), and the maximum and minimum observed values (vertical lines) of serum ferritin by HT subtype. Kruskal-Wallis test for comparison of ferritin levels between no HT, HI, and PH groups ($P=0.041$).

overload can be the result of mutations in the hemochromatosis gene. These mutations are frequent in the general population and H63D and C282Y heterozygous carriers can show higher serum ferritin values than noncarriers.²²

The molecular bases for a greater brain injury secondary to iron overload are the generation of radical hydroxyl from oxygen during reperfusion, increased excitotoxic damage, and blood-brain barrier disruption.^{8,10–12} Cellular biology in animal models of cerebral ischemia, and reperfusion has shown a close link between reactive oxygen species generation in microvessels and astrocytic end feet, increase of blood-brain barrier permeability, and matrix metalloproteinase-9 expression.⁷ A hypothesis that arises from these experimental data are whether intravenous t-PA and iron may have a synergistic neurotoxic effect on the neurovascular unit. Free radical-mediated damage in iron overload might be enhanced by the toxic effects of t-PA, because t-PA may increase excitotoxicity and matrix metalloproteinase-9 overexpression.⁶ In this context, blood-brain barrier disruption is substantially reduced by using PBN, a radical scavenger, in combination with t-PA in a rat thromboembolic model of stroke,²⁴ and NXY-059, a free radical trapping agent, given intravenously within 6 hours of acute ischemic stroke, reduced the risk of intracerebral hemorrhage in patients cotreated with

t-PA, and improved outcome in one randomized controlled trial. These novel findings support a role of reactive oxygen species-mediated injury on the neurovascular unit in patients with ischemic stroke treated with intravenous t-PA.

The main strengths of this study are the central and repeated measurement of serum ferritin levels, the use of prespecified imaging definitions on computed tomography and neurologic scales, and the prospective 3-month follow up by using a broadly accepted protocol, the SITS-MOST register. Furthermore, the characteristics of the patients were similar to those of other populations treated with t-PA, the main factors that may influence t-PA response were recorded, and the analysis was adjusted for them. However, this study has a number of limitations that must be acknowledged. First, the adjustment of logistic analysis for baseline characteristics that influenced outcome after stroke does not completely rule out an association of poor outcome and increased ferritin as a result of unknown confounders or as a biomarker of greater stroke severity and infarct volume. However, the serum profile of ferritin in this study does not support a sharp increase within the first 3 hours from symptoms onset in the poor outcome group. Second, there is a lack of information about the time of arterial recanalization, because we did not

prospectively monitor the arterial patency at fixed intervals. Transcranial Doppler monitoring of timing of arterial recanalization will permit the study of the interaction between increased ferritin levels and time to recanalization on stroke outcome. Finally, NXY-059 cotreatment could influence the results because 23 patients were randomized in SAINT I; however, the association of high ferritin levels with poor outcome remained robust after the exclusion of these patients.

In summary, patients with stroke with increased serum ferritin concentrations before treatment with intravenous t-PA have a higher risk of poor clinical outcome, hemorrhagic transformation, and brain edema than patients with low ferritin values. These findings suggest that iron overload may counterbalance the benefits of thrombolytic therapy observed in patients with low ferritin levels. If these results are confirmed in future studies, iron chelators or free radical trapping agents should be used to reduce the neurotoxic effects of iron in patients with acute ischemic stroke who are treated with intravenous t-PA.

Disclosures

None.

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