# A higher body temperature is associated with haemorrhagic transformation in patients with acute stroke untreated with recombinant tissue-type plasminogen activator (rtPA)

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## ABSTRACT

Higher body temperature is a prognostic factor of poor outcome in acute stroke. Our aim was to study the relationship between body temperature, HT (haemorrhagic transformation) and biomarkers of BBB (blood–brain barrier) damage in patients with acute ischaemic stroke untreated with rtPA (recombinant tissue-type plasminogen activator). We studied 229 patients with ischaemic stroke <12 h from symptom onset. Body temperature was determined at admission and every 6 h during the first 3 days. HT was evaluated according to ECASS II (second European Co-operative Acute Stroke Study) criteria in a multimodal MRI (magnetic resonance imaging) at 72 h. We found that 55 patients (34.1 %) showed HT. HT was associated with cardioembolic stroke (64.2 % against 23.0 %; $P < 0.0001$), higher body temperature during the first 24 h (36.9 °C compared with 36.5 °C; $P < 0.0001$), more severe stroke [NIHSS (National Institutes of Health Stroke Scale) score, 14 (9–20) against 10 (7–15); $P = 0.002$], and greater DWI lesion volume at admission (23.2 cc compared with 13.2 cc; $P < 0.0001$). Plasma MMP-9 (matrix metalloproteinase 9) (187.3 ng/ml compared with 44.2 ng/ml; $P < 0.0001$) and cFn (cellular fibronectin) levels (16.3 $\mu$g/ml compared with 7.1 $\mu$g/ml; $P = 0.001$) were higher in patients with HT. Body temperature within the first 24 h was independently associated with HT {OR (odds ratio), 7.3 [95 % CI (confidence interval), 2.4–22.6]; $P < 0.0001$} after adjustment for cardioembolic stroke subtype, baseline NIHSS score and DWI lesion volume. This effect remained unchanged after controlling for MMP-9 and cFn. In conclusion, high body temperature within the first 24 h after ischaemic stroke is a risk factor for HT in patients untreated with rtPA. This effect is independent of some biological signatures of BBB damage.

**Key words:** biomarkers, blood–brain barrier (BBB), haemorrhagic transformation (HT), ischaemic stroke, temperature.

**Abbreviations:** BBB, blood–brain barrier; BP, blood pressure; cFn, cellular fibronectin; CI, confidence interval; CT, computed tomography; DBP, diastolic BP; DWI, diffusion-weighted imaging; END, early neurological deterioration; FLAIR, fluid-attenuated inversion recovery; HI-1, haemorrhagic infarction type 1; HT, haemorrhagic transformation; MMP-9, matrix metalloproteinase-9; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; PH-1, parenchymal haemorrhage type 1; mRS, modified Rankin Scale; rtPA, recombinant tissue-type plasminogen activator.

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## INTRODUCTION

The effect of pyrexia on cerebral ischaemia has been extensively studied in animal models. It has been shown that increased temperature after the ischaemic insult exacerbates the neuronal damage and worsens functional outcome [1–4], whereas induced hypothermia seems to be neuroprotective [5–8] especially when it is applied up to 1 h after focal permanent ischaemia [6]. Hypothermia limits ischaemic damage by decreasing metabolism, suppressing BBB (blood–brain barrier) breakdown [9] and reducing inflammation [9] as well as free radical formation [10]. In humans, most of studies suggest that pyrexia after stroke onset is associated with a marked increase in the morbidity and mortality [11–18].

The risk of HT (haemorrhagic transformation) after cerebral ischaemia is of great concern to the clinician. Delayed reperfusion can lead to negative effects such as the breakdown of BBB and the development of HT and oedema. Although it may develop as part of the natural evolution of ischaemic brain injury [19], HT frequently occurs as a result of the use of anti-coagulants or thrombolytic therapy in the acute phase of stroke [20,21]. Factors such as age and the severity of stroke [22], hypertension [23], the dosage of the thrombolytic agent administered [23–25] and the presence of early ischaemic changes on the cranial CT (computed tomography) at admission [22] have been related to HT after the ischaemic event. However, the underlying mechanisms that mediate the development of HT of an ischaemic brain area are not completely understood.

HT after cerebral ischaemia seems to be related to the disruption of vascular endothelium [26]. The association between high levels of MMP-9 (matrix metalloproteinase 9) or cFn (cellular fibronectin) and HT have been previously reported, both in patients treated [27–29] and not treated with rtPA (recombinant tissue-type plasminogen activator) [30,31].

The aim of the present study was to investigate the relationship between body temperature, HT and biomarkers of BBB damage in patients with acute ischaemic stroke untreated with rtPA.

## MATHERIALS AND METHODS

### Subjects

This is a secondary analysis of a prospective study [32] including 229 patients with acute ischaemic stroke of less than 12 h from stroke onset without previous disability [mRS (modified Rankin Scale) score $\leqslant$1]. Patients included in clinical trials ($n = 5$), as well as those who have been treated with rtPA ($n = 47$) were excluded. Patients with chronic inflammatory diseases ($n = 8$), infectious disease ($n = 6$) in the 15 days before inclusion or during hospitalization ($n = 2$) were also excluded, so a total of 161 patients with acute stroke were finally included in the present study. The Local Ethical Committees approved the clinical protocol and patients or relatives gave signed informed consent.

Medical history recording potential stroke risk factors, clinical examination, blood and coagulation test, 12–lead electrocardiogram and chest radiography were performed at admission. Stroke subtype was classified according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [33]. NIHSS (NIHSS (National Institutes of Health Stroke Scale) was performed at admission, 24, 48 and 72 h, day 7 and at 3 months to evaluate stroke severity. END (early neurological deterioration) was defined as an increase equal to or greater than 4 points in NIHSS within the first 72 h with respect to baseline NIHSS score. Stroke functional outcome was evaluated by mRS at 3 months.

BP (blood pressure) and tympanic temperature were determined at admission and every 6 h during the first 3 days. Tympanic temperature was measured using an infrared thermometer (ThermoScan 07; Braun). We considered the mean tympanic temperature obtained in the first 24 h for posterior analyses.

Only patients with an SBP (systolic BP) $\geqslant$220 mmHg or a DBP (diastolic BP) $\geqslant$120 mmHg received anti-hypertensive drugs within the first 48 h following admission. Treatment with insulin for hyperglycaemia (blood glucose >160 mg/dl) and with 2 g of intravenous metamizol or 1 g of oral acetaminophen for hyperthermia (tympanic temperature >37.5 °C) was initiated early after hospitalization, following Spanish Neurological Society Guidelines for the treatment of stroke. Patients with a temperature $\geqslant$37.5 °C are protocolized to rule out infections. These patients undergo a chest radiograph, blood tests and urinalysis; likewise, intravenous catheters are reviewed and the urinary catheter is changed. Subcutaneous low-dose heparin as prophylaxis against pulmonary thromboembolism and antiplatelet drugs were prescribed. Anti-coagulants were given to patients with a major cardioembolic source but not as a treatment for END.

A multimodal MRI (magnetic resonance imaging) was performed at admission and at 72 h. The MRI protocol included DWI (diffusion-weighted imaging), T2-weighted and FLAIR (fluid-attenuated inversion recovery) imaging. Lesion volume was evaluated in DWI sequences performed at admission using the manual planimetric segmentation method.

HT was evaluated in the second MRI in FLAIR sequences and was analysed according to the ECASS II (second European Co-operative Acute Stroke Study) criteria [34]. HI-1 (haemorrhagic infarction type 1) was defined as small petechiae along the margins of the infarct, and HI-2 (HI type 2) was defined as more confluent petechiae within the infarct area but without a space-occupying effect. PH-1 (parenchymal

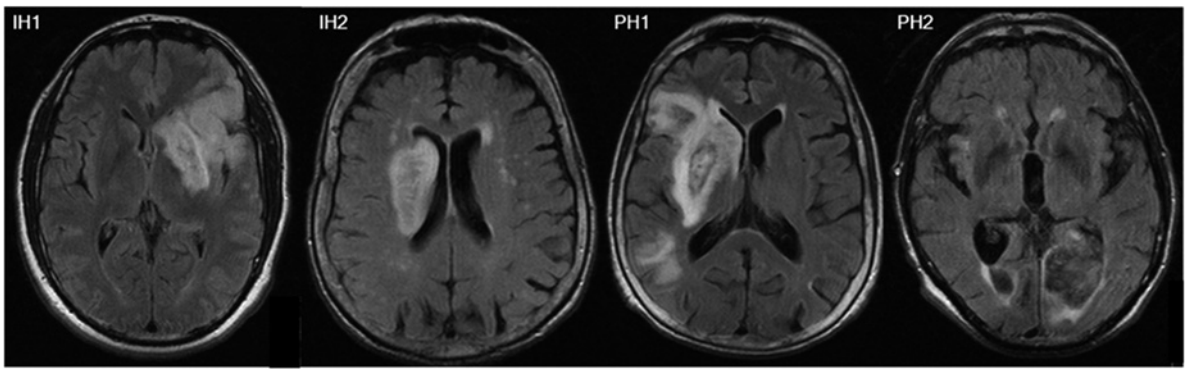


**Figure 1 Types of HT in MRI–FLAIR according to ECASS criteria**

HI-1, small petechiae along the margins; HI-2, confluent petechiae; PH-1, $\leqslant$30 % of the infarcted area with space occupying effect; PH-2, >30 % of the infarcted area with space occupying effect.

haemorrhage type 1) was defined as blood clots in $\leqslant$30 % of the infarcted area within some light space-occupying effect, and PH-2 (PH type 2) as blood clots in >30 % of the infracted area with substantial space-occupying effect (Figure 1). We considered symptomatic HT as being associated with neurological deterioration. All imaging studies were performed by neuroradiologists blinded to clinical and analytical data in a single scanner, GE 1.5 T.

## Laboratory tests

Blood samples were obtained at admission and collected in crystal tubes containing EDTA. Plasma was obtained by centrifugation (3000 *g* for 15 min) and stored at − 80 °C. Plasma MMP-9 and cFn levels were measured with commercially available quantitative sandwich ELISA kits obtained from Biotrack (GE Healthcare) and Adeza Biochemical respectively. Determinations were performed according to manufacturer's instructions by researchers blinded to clinical and neuroimaging data. The intra-assay and inter-assay coefficients of variation were <5 % for both MMP-9 and cFn determinations.

## Statistical analysis

Results are expressed as numbers (percentages) for categorical variables and as means ± S.D. or medians (quartiles) for the continuous variables, depending on the normal or not-normal distribution of data. Proportions were compared using the $\chi^2$ test, and the Student's *t* test or the Mann–Whitney test were used to compare continuous variables between groups. Spearman analysis was used for bivariate correlations.

The influence of body temperature, MMP-9 and cFn on the development of HT was assessed by logistic regression analysis, after adjusting for the main baseline variables related to outcome in the univariate analyses (enter approach and probability of entry $P < 0.05$). Results are expressed as adjusted OR (odds ratio) with the corresponding 95 % CI (confidence interval). The results of the OR corresponding to each unit of the corresponding variable (e.g. per °C of temperature, per unit of NIHSS, per ng of MMP-9, etc.). The statistical analysis was conducted using SPSS 16.0 for Windows XP.

# RESULTS

A total of 55 (34.1 %) of the 161 patients included in the study developed HT. Twenty-seven patients (16.7 %) had HI-1, 18 (11.1 %) had HI-2, seven (4.3 %) had PH-1 and three (1.8 %) had PH-2. Table 1 shows the main clinical characteristics and laboratory parameters of patients classified according to each of the types of HT. Table 2 shows the main characteristics of patients with and without HT. HT was associated with a cardioembolic source of stroke (69.8.2 % compared with 24.0 %; $P < 0.0001$), higher body temperature during the first 24 h (36.9 °C compared with 36.5 °C; $P < 0.001$), higher DBP [80 (68–91) mmHg against 76 (68–84) mmHg; $P = 0.001$], more severe stroke [NIHSS score, 14 (9–20) compared with 10 (7–15); $P = 0.002$], and greater DWI lesion volume at admission [23.2 (15.6–92.5) cc compared with 13.2 (4.5–22.6) cc; $P < 0.0001$). Plasma levels of MMP-9 [187.3 (100.2–235.4) ng/ml compared with 44.2 (23.6–123.4) ng/ml; $P < 0.0001$] and cFn [16.3 (5.4–42.7) $\mu$g/ml compared with 7.4 (2.1–20.4) $\mu$g/ml; $P = 0.001$] were significantly higher in patients with HT than in those without HT.

Mean tympanic temperature in the first 24 h was higher in patients with PH ($P < 0.0001$). Moreover, the higher the temperature in the first 24 h the greater is the severity of HT (Figure 2).

Twelve patients showed a mean tympanic temperature in the first 24 h higher than 37.5 °C, and other 49 patients had also some determination higher than 37.5 °C. The analysis of this group of patients was similar to the total group (results not shown).

**Table 1 Clinical characteristics and laboratory parameters corresponding to each of the types of HT**

Results are expressed as numbers for categorical variables and as means ± S.D. or medians (quartiles) for the continuous variables.

| Parameter | HT1 ($n = 27$) | HT2 ($n = 18$) | PH1 ($n = 7$) | PH2 ($n = 3$) |
|---|---|---|---|---|
| Age (years) | 70.2 ± 10.1 | 70.6 ± 8.9 | 70.2 ± 4.2 | 75.0 ± 5.3 |
| Time from stroke onset (min) | 231.6 ± 141.7 | 185.2 ± 116.9 | 137.2 ± 63.2 | 351.0 ± 138.8 |
| Alcohol consumption (*n*) | 2 | 4 | 1 | 1 |
| Hypertension (*n*) | 13 | 9 | 4 | 3 |
| Diabetes (*n*) | 6 | 5 | 3 | 2 |
| Atrial fibrillation (*n*) | 14 | 14 | 6 | 3 |
| Hyperlipidaemia (*n*) | 4 | 2 | 1 | 1 |
| Leucocytes ($\times 10^3$ cells/dl) | 7.5 (6.1 — 10.1) | 9.7 (6.2 — 12.1) | 12.1 (6.3 — 13.2) | 11.4 (7.8 — ) |
| Platelets ($\times 10^3$ cells/dl) | 217.0 (165.0 — 265.0) | 191.0 (135.5 — 274.0) | 215.5 (119.0 — 296.2) | 266.0 (242.0 — ) |
| Fibrinogen levels at admission (mg/dl) | 356.0 (309.0 — 423.0) | 438.0 (259.0 — 508.5) | 552.5 (289.5 — 583.7) | 523.0 (496.0 — ) |
| Glucose levels at admission (mg/dl) | 130.0 (116.0 — 169.0) | 155.0 (93.0 — 195.0) | 148.5 (121.0 — 205.2) | 169.0 (91.0 — ) |
| Median SPB in the first 24 h (mmHg) | 147.0 (139.0 — 180.0) | 153.0 (136.0 — 161.5) | 149.0 (133.7 — 201.0) | 140.0(123.0 — ) |
| Median DBP in the first 24 h (mmHg) | 84.0 (70.0 — 90.0) | 83.0 (73.0 — 100.0) | 77.5 (65.0 — 108.7) | 85.0 (68.0 — ) |
| Temperature at admission (°C) | 36.5 (36.3 — 36.7) | 37.1 (36.4 — 37.3) | 36.0 (35.8 — 37.0) | 37.6 (36.5 — ) |
| Median temperature in the first 24 h (°C) | 36.8 (36.5 — 37.0) | 37.1 (36.6 — 37.2) | 37.6 (37.3 — 37.6) | 37.6 (37.5 — ) |
| Median temperature within 24–48 h (°C) | 36.6 (36.4 — 37.0) | 36.8 (36.4 — 36.9) | 36.8 (36.4 — 37.0) | 36.6 (35.8 — ) |
| Median temperature within 48–72 h (°C) | 36.5 (36.4 — 36.8) | 36.7 (36.4 — 36.9) | 36.8 (36.3 — 37.0) | 36.1 (35.7 — ) |
| NIHSS score at admission | 14 (10 — 18) | 17 (12 — 20) | 15 (12 — 21) | 9 (6 — ) |
| DWI infarct volume at admission (cc) | 19.7 (14.3 — 58.8) | 68.7 (15.1 — 171.5) | 19.3 (14.5 — 107.8) | 37.9 (13.5 — ) |
| MMP-9 levels (ng/ml) | 130.2 (80.6 — 168.1) | 115.4 (69.6 — 202.5) | 150.4 (48.0 — 236.3) | 172.9 (150.2 — ) |
| cFn levels ($\mu$g/ml) | 17.2 (4.9 — 27.1) | 30.2 (14.1 — 57.1) | 36.1 (11.8 — 44.1) | 48.5 (5.2 — ) |

**Table 2 Clinical characteristics and laboratory parameters by HT groups**

Results are expressed as numbers (percentage) for categorical variables and as means ± S.D. or medians (quartiles) for the continuous variables.

| Parameter | HT | | *P* value |
|---|---|---|---|
| | No ($n = 106$) | Yes ($n = 55$) | |
| Age (years) | 69.5 ± 9.4 | 71.2 ± 8.3 | 0.561 |
| Time from stroke onset (min) | 190.7 ± 96.3 | 224.5 ± 138.9 | 0.312 |
| Alcohol consumption (*n*) | 11 (10.4%) | 8 (14.5%) | 0.417 |
| Hypertension (*n*) | 59 (55.7%) | 29 (52.7%) | 0.169 |
| Diabetes (*n*) | 33 (31.1%) | 16 (30.2%) | 0.608 |
| Atrial fibrillation (*n*) | 24 (22.6%) | 37 (69.8%) | <0.0001 |
| Hyperlipidaemia (*n*) | 28 (26.4%) | 8 (14.5%) | 0.071 |
| Leucocytes ($\times 10^3$/dl) | 8.2 (5.5–11.4) | 8.1 (5.6–12.5) | 0.411 |
| Platelets ($\times 10^3$/dl) | 226 (190–301) | 218 (181–265) | 0.113 |
| Fibrinogen levels at admission (mg/dl) | 408 (293–491) | 391 (312–515) | 0.408 |
| Glucose levels at admission (mg/dl) | 110 (96–133) | 116 (97–141) | 0.109 |
| Median SPB in the first 24 h (mmHg) | 150 (138–164) | 146 (130–163) | 0.218 |
| Median DBP in the first 24 h (mmHg) | 80 (68–91) | 76 (68–84) | 0.001 |
| Temperature at admission (°C) | 36.5 (36.3–36.7) | 36.6 (36.2–36.9) | 0.209 |
| Median temperature in the first 24 h (°C) | 36.5 (36.3–36.9) | 36.9 (36.6–37.3) | <0.0001 |
| Median temperature within 24–48 h (°C) | 36.6 (36.4–36.9) | 36.7 (36.5–37.0) | 0.112 |
| Median temperature within 48–72 h (°C) | 36.6 (36.4–37.0) | 36.7 (36.5–37.1) | 0.191 |
| NIHSS score at admission | 10 (7–15) | 14 (9–20) | 0.002 |
| DWI infarct volume at admission (cc) | 13.2 (4.5–22.6) | 23.2 (15.6–92.5) | <0.0001 |
| MMP-9 levels (ng/ml) | 44.2 (23.6–123.4) | 187.3 (100.2–235.4) | <0.0001 |
| cFn levels ($\mu$g/ml) | 7.1 (2.1–20.4) | 16.3 (5.4–42.7) | 0.001 |

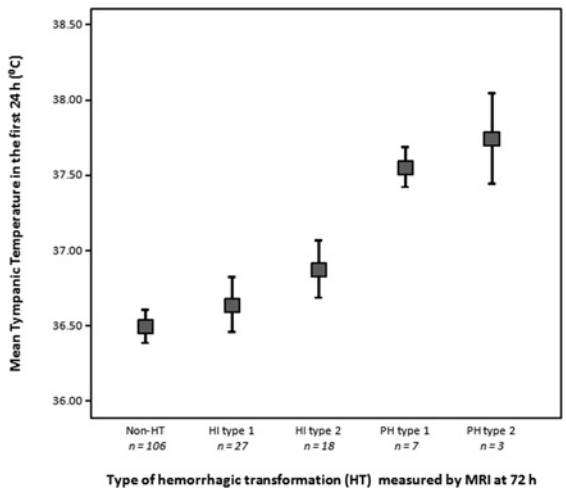


**Figure 2** Mean tympanic temperature in the first 24 h by HT subtype

**Table 3** Adjusted OR of HT for median temperature within the first 24 h, including MMP-9 (model 1) and cFn (model 2) levels

| | OR (95 % CI) | *P* value |
|---|---|---|
| Model 1 | | |
| Median temperature in the first 24 h | 6.7 (1.9–20.6) | 0.001 |
| Cardioembolic stroke subtype | 5.0 (1.8–12.7) | 0.001 |
| NIHSS score at admission | 1.0 (0.9–1.1) | 0.37 |
| Median DBP in the first 24 h | 0.9 (0.9–1.0) | 0.10 |
| DWI infarct volume at admission | 1.0 (0.9–1.0) | 0.75 |
| MMP-9 levels at admission | 3.7 (1.6–8.3) | <0.0001 |
| Model 2 | | |
| Median temperature in the first 24 h | 6.9 (2.0–22.7) | <0.0001 |
| Cardioembolic stroke subtype | 5.7 (2.6–14.6) | 0.001 |
| NIHSS score at admission | 1.0 (0.9–1.1) | 0.24 |
| Median DBP in the first 24 h | 0.9 (0.9–1.0) | 0.18 |
| DWI infarct volume at admission | 1.0 (0.9–1.0) | 0.51 |
| cFn levels at admission | 4.3 (1.0–9.8) | <0.0001 |

Body temperature within the first 24 h was independently associated with HT [OR, 7.3 (95 % CI, 2.4–22.6); $P < 0.0001$] after adjustment for cardioembolic stroke subtype, baseline NIHSS score, DBP and DWI lesion volume at admission. The OR of body temperature for HT did not substantially change after the inclusion of MMP-9 levels [OR, 6.7 (95 % CI, 1.9–20.6); $P = 0.001$], or cFn levels [OR, 6.9 (95 % CI, 2.0–22.7); $P < 0.0001$] in the multivariate model (Table 3). On the other hand, MMP-9 levels [OR, 3.7 (95 % CI, 1.6–8.3); $P < 0.0001$], and cFn levels [OR, 4.3 (95 % CI, 1.0–9.8); $P < 0.0001$] were also independently associated with HT after adjustment for median temperature in the first 24 h, cardioembolic stroke subtype, baseline NIHSS score, DBP, and DWI lesion volume at admission (Table 3).

## DISCUSSION

The results of the present study show that higher body temperature within the first 24 h from stroke onset is associated with HT in patients with acute ischaemic stroke untreated with rtPA, independently to biomarkers of BBB disruption, such as MMP-9 and cFn.

Several studies have investigated the prognostic role of temperature in stroke patients. Increased temperature has been associated with END, poor functional outcome and increased mortality at short and long term [13–17]. We have demonstrated that hyperthermia was associated with higher plasma levels of pro-inflammatory markers in acute ischaemic stroke, suggesting that these deleterious effects of hyperthermia may be mediated by the effect of pro-inflammatory cytokines [32]. On the other hand, regarding ischaemic stroke patients treated with rtPA, our group has also demonstrated that there was an association of high temperature at 24 h with lack of arterial recanalization and greater hypodensity volume, as well as a trend of higher body temperature in patients who showed symptomatic HT [35]. Surprisingly, the results of the present study and the study cited above suggest that the association between increased body temperature and HT is higher in patients with ischaemic stroke untreated with rtPA, in which the association between body temperature and HT was independent after adjusting for the confounder factors.

The study results showed an association between HT and a higher proportion of atrial fibrillation, greater neurological deficit measured by the NIHSS and larger infarct volumes. These associations were previously described in several studies [28,30,36–39].

In experimental focal cerebral ischaemia a significant loss of basal lamina components of the cerebral microvessels have been demonstrated. This loss in vessel wall integrity is associated with the development of petechial haemorrhage. The mechanisms of this microvascular damage may include plasmin-generated laminin degradation, MMPs activation, transmigration of leucocytes through the vessel wall and other processes [40]. The relationship between hyperthermia and BBB disruption has been demonstrated in animal models by morphological [41], and functional studies [42]. It has been shown that hyperthermic preconditioning prevents disruption of BBB, resulting in amelioration of hypoxic-ischaemic neuronal damage in newborn rat [43]. Hypothermia limits ischaemic damage by decreasing metabolism, suppressing BBB breakdown [9] and reducing inflammation [9], and free radical formation [10]. Recently, it has been shown that hyperthermia masks the neuroprotective effects of rtPA treatment after ischaemic injury, probably by increased BBB permeability, increased oedema volume, and early progression of ischaemic penumbral region to irreversibly damaged tissue as shown by pro-

gressively increasing perfusion deficits in hyperthermic rats [44].

According to previous studies [27–31], we have showed that HT was associated with high MMP-9 and cFn plasma levels in patients with acute ischaemic stroke. MMP-9 has been implicated in various deleterious mechanisms after cerebral ischaemia, such as brain oedema, HT and increased cell death. The molecular mechanism of HT in the ischaemic brain seems to be related to increased activation of MMP-9. Findings obtained from studies in animal models indicate that MMP-9 is up-regulated soon after ischaemia (3–4 h) and that it plays a role in BBB breakdown [45]. The intracerebral injection of collagenases results in disruption of the BBB, producing intracerebral haemorrhage and brain oedema in experimental models of cerebral ischaemia [46].

On the other hand, the relationship between MMP-9 and hyperthermia has been studied in animal models. Mild hypothermia attenuates BBB disruption, decreases MMP-2 and MMP-9 expression and suppresses MMP activity [47].

However, the present study has some limitations. First, although we have demonstrated the accuracy of higher body temperature for the prediction of HT, these results were obtained from a *post-hoc* analysis, so they should be confirmed in a prospective study including a large number of patients, especially in the subgroup with PH-2, which has been reported to experience neurological deterioration more often. The small number of patients in our study prevented us from obtaining conclusive data for the prediction of this particular type of HT. Secondly, we use MRI to evaluate HT using ECASS II criteria (where CT was used to evaluate HT). We have defined these HT criteria for MRI. Finally, the main limitation of the present study is its observational rather than experimental nature. The difference in temperatures in the first 24 h between patients with and without HT is very small (36.5 °C compared with 36.9 °C), and these changes could be easy to generate with simple interventions. The statistical adjustment does not adjust for unmeasured confounders, a problem that could be overcome by randomization.

In conclusion, we have shown that higher body temperature within the first 24 h was associated with HT in ischaemic stroke patients untreated with rtPA. This effect remained unchanged after controlling for some biological signatures of BBB damage (MMP-9 and cFn).

## AUTHOR CONTRIBUTION

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