BRAIN COMMUNICATIONS

Mild fever as a catalyst for consumption of the ischaemic penumbra despite endovascular reperfusion

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Cerebrovascular ischaemia is potentiated by hyperthermia, and even mild temperature elevation has proved detrimental to ischaemic brain. Infarction progression following endovascular reperfusion relates to multiple patient-specific and procedural variables; however, the potential influence of mild systemic temperature fluctuations is not fully understood. This study aims to assess the relationship between systemic temperatures in the early aftermath of acute ischaemic stroke and the loss of at-risk *penumbral* tissues, hypothesizing consumption of the ischaemic penumbra as a function of systemic temperatures, irrespective of reperfusion status. A cross-sectional, retrospective evaluation of a single-institution, prospectively collected endovascular therapy registry was conducted. Patients with anterior circulation, large vessel occlusion acute ischaemic stroke who underwent initial CT perfusion, and in whom at least four-hourly systemic temperatures were recorded beginning from presentation and until the time of final imaging outcome were included. Initial CT perfusion core and penumbra volumes and final MRI infarction volumes were computed. Systemic temperature indices including temperature maxima were recorded, and pre-defined temperature thresholds varying between 37°C and 38°C were examined in unadjusted and adjusted regression models which included glucose, collateral status, reperfusion status, CT perfusion-to-reperfusion delay, general anaesthesia and antipyretic exposure. The primary outcome was the relative consumption of the penumbra, reflecting normalized growth of the at-risk tissue volume $\geq$10%. The final study population comprised 126 acute ischaemic stroke subjects (mean 63 $\pm$ 14.5 years, 63% women). The primary outcome of penumbra consumption $\geq$10% occurred in 51 (40.1%) subjects. No significant differences in baseline characteristics were present between groups, with the exception of presentation glucose (118 $\pm$ 26.6 without versus 143.1 $\pm$ 61.6 with penumbra consumption, $P = 0.009$). Significant differences in the likelihood of penumbra consumption relating to systemic temperature maxima were observed [37°C (interquartile range 36.5 – 37.5°C) without versus 37.5°C (interquartile range 36.8 – 38.2°C) with penumbra consumption, $P = 0.001$]. An increased likelihood of penumbra consumption was observed for temperature maxima in unadjusted (odds ratio 3.57, 95% confidence interval 1.65 – 7.75; $P = 0.001$) and adjusted (odds ratio 3.06, 95% confidence interval 1.33 – 7.06; $P = 0.009$) regression models. Significant differences in median penumbra consumption were present at a pre-defined temperature maxima threshold of 37.5°C [4.8 ml (interquartile range 0 – 11.5 ml) versus 21.1 ml (0 – 44.7 ml) for subjects not reaching or reaching the threshold, respectively, $P = 0.007$]. Mild fever may promote loss of the ischaemic penumbra irrespective of reperfusion, potentially influencing successful salvage of at-risk tissue volumes following acute ischaemic stroke.

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Received March 12, 2020. Revised May 18, 2020. Accepted June 23, 2020. Advance Access publication July 28, 2020
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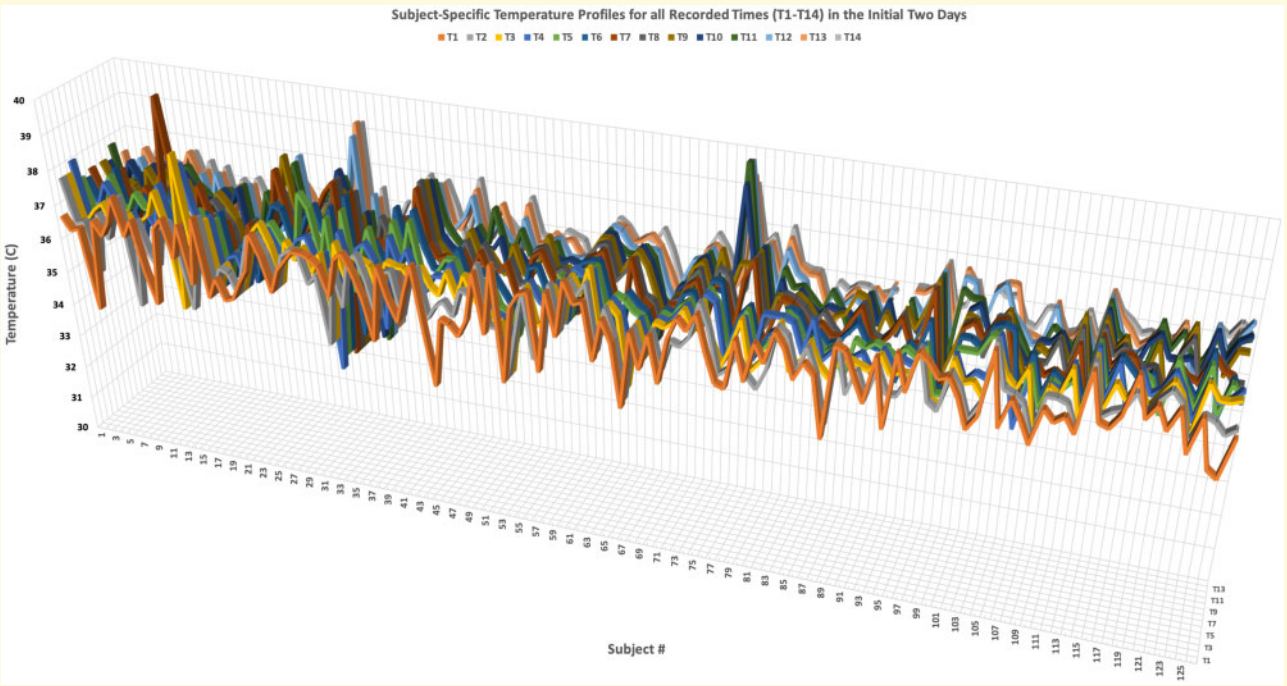
Keywords: cerebral ischaemia; endovascular therapy; CT perfusion; fever; penumbra

Abbreviations: AIS = acute ischaemic stroke; CIconfidence interval; CTP = CT perfusion; IQR = interquartile range; mTICI = modified treatment in cerebral ischaemia; OR = odds ratio; $Temp_{avg}$ = systemic temperature average; $Temp_{max}$ = systemic temperature maxima; T_{max} = time-to-maximum of the deconvolved tissue residue function

Graphical Abstract



Introduction

The neurovascular unit exhibits remarkable sensitivity to hyperthermia, which is known to potentiate ischaemic injury and influence viability even when mild (Wolfe, 1960; Busto *et al.*, 1987; 1989; Dehkharghani and Qiu, 2020). Approximately half of acute ischaemic stroke (AIS) patients develop fevers in the early stroke aftermath compelling exploration of underlying mechanisms, treatments and therapeutic targets; however, neither the interactions between temperature homeostasis, perfusion and viability, nor the influence of brain-to-systemic and intracerebral temperature gradients are fully understood (Yablonskiy *et al.*, 2000; Sukstanskii and Yablonskiy, 2006; Dehkharghani *et al.*, 2017; Fleischer *et al.*, 2017). Notwithstanding, correlations linking fever with stroke severity and infarction progression have been documented in numerous human and animal studies, and a heightened morbidity and mortality in febrile stroke patients irrespective of fever origin has been shown (Azzimondi *et al.*, 1995; Reith *et al.*, 1996; Castillo *et al.*, 1998; Hajat *et al.*, 2000; Kammersgaard *et al.*, 2002).

We previously reported upon the association between mild fever and imaging progression of infarction *vis-à-vis* consumption of at-risk penumbral tissues following full reperfusion in endovascularly treated AIS patients (Dehkharghani *et al.*, 2016). Direct mechanistic insights into the interplay of fever and those factors driving the at-risk penumbra to succumb to ischaemic injury are lacking and not easily tested empirically in living human subjects; however, the identification of operationally useful and potentially modifiable predictors of stroke progression following reperfusion could nevertheless promote improved outcomes (Wintermark *et al.*, 2008; 2013; Olivot *et al.*, 2014; Haussen *et al.*, 2016b; Guenego *et al.*, 2018; de Havenon *et al.*, 2019).

The effects of fever in stroke are confounded by inconsistencies in the clinical definitions of pyrexia, which vary considerably beginning from conservative thresholds of 37.5°C. While previously examined in binary terms, a *dose effect* for pyrexia as a driver of penumbra loss merits consideration, specifically with respect to early post-ischaemic fevers, during which the intensity of potentiation is believed to be greatest (Dietrich *et al.*, 1990; Baena *et al.*, 1997; Castillo *et al.*, 1998; Wrotek *et al.*, 2011). Further, ostensibly mild systemic fever thresholds may belie the presence of more severe intracerebral hyperthermia developing under the influence of local hemodynamic and immunologic variables (Cabanac and Caputa, 1979; Busto *et al.*, 1987, 1989; Dietrich *et al.*, 1990; Cabanac, 1993; Stone *et al.*, 1995; Wass *et al.*, 1995; Simon, 2007; Dehkharghani *et al.*, 2015b, 2017). The identification of optimal temperature thresholds for prediction of infarction progression motivates this study. The impact of varying temperature thresholds upon the imaging penumbra is assessed in endovascularly treated patients, hypothesizing detectable and significant relationships between early post-ischaemic temperatures and consumption of the putatively at-risk volume measured by standardized CT perfusion (CTP).

Materials and methods

Patient selection

We retrospectively reviewed a prospectively collected endovascular stroke therapy registry of 626 patients presenting to Grady Memorial Hospital, spanning January 2011–September 2014, with the aim of assessing the impact of fever upon infarction progression in endovascularly treated patients. This retrospective study was approved by the research ethics committee of the Institutional Review Board. Patients greater than 18 years of age were included for analysis if they met the following criteria: (i) AIS due to large vessel occlusion involving the intracranial internal carotid artery and/or the middle cerebral artery M1 and/or M2 segments on CT angiography; (ii) time from last seen well to groin puncture $\leq$12 h; (iii) full supratentorial CTP data sets amenable to analysis for ischaemic core and penumbra volumes (see below); (iv) at least four-hourly systemic temperatures available from the medical record, beginning from presentation to the time of final, follow-up MRI; (v) follow-up MRI with diffusion weighted imaging for final infarction volume estimation prior to discharge or final disposition; (vi) absence of positive microbial cultures from systemic sources or other signs of systemic infection during the observational period; (vii) follow-up with 90-day functional outcome (modified Rankin scale) assessment. Eligible patients received intravenous tissue-type plasminogen activator as per standard guidelines. Endovascular device selection was made at the discretion of the neurointerventionalist.

Clinical variables including history of hypertension, congestive heart failure, hyperlipidaemia, atrial fibrillation and type II diabetes mellitus were obtained, as well as smoking history and plasma glucose at presentation.

Imaging protocol

All patients underwent a standardized institutional imaging protocol, including non-contrast CT, CT angiography and CTP. CT imaging was performed on a 40-mm, 64-detector-row clinical system (GE VCT Lightspeed, General Electric Healthcare, Waukesha, WI, USA). Helical non-contrast CT (120 kV, 100 – 350 auto-mA) was obtained from the foramen magnum through the vertex at 5.0 mm slice thickness. In the absence of visible intracranial haemorrhage during real-time evaluation by a radiologist and stroke neurologist, two separate, contiguous axial CTP slabs were obtained for 8 cm combined coverage of the supratentorial brain. Cine mode acquisition (80 kV, 100 mA) permitting high temporal resolution (1 s sampling interval) dynamic bolus passage imaging was obtained following the administration of 35 ml iodinated contrast (Isovue 370, Bracco Diagnostics) power injected at 5 ml/s through 18 gauge or larger antecubital IV access. Contrast administration was followed by 25 ml saline flush at the same rate. Lastly, helical CT angiography (120 kV, 200 – 350 auto-mA) was obtained from the carina to the vertex (slice thickness/interval 0.625 mm/0.375 mm) following IV administration of 70 ml iodinated contrast injected at 5 ml/s, and followed by 25 ml saline flush at the same rate. All images were transferred to a separate workstation for analysis (Apple Mac Pro 16 core, Apple, Cupertino, USA) using a third party DICOM viewer (Osirix Pro 64-bit, Pixmeo, Geneva, Switzerland).

CTP analysis

The details of the perfusion protocol and post-processing pipeline were elaborated previously (Dehkharghani *et al.*, 2015a, 2016; Lima *et al.*, 2016; Nogueira *et al.*, 2016; Haussen *et al.*, 2016a); briefly, all perfusion imaging was processed using a commercial version of a vendor-independent software platform (RAPID version 4.5, iSchemaView, Menlo Park, CA, USA). Voxel-wise thresholding of tissue parametric perfusion maps was performed, including the time-to-maximum (T_{max}) of the tissue residue function, which was computed to estimate the critically hypoperfused tissue volume at 6 s delay; cerebral blood flow (CBF, expressed in ml/100g/min) maps at default vendor thresholds of relative (r)CBF <30% of contralateral normal tissues were used for estimation of the irreversibly infarcted *core*, and the balance of the $T_{max} > 6$s volume used as the putatively at-risk ischaemic penumbra volume.

The hypoperfusion intensity ratio, derived from the ratio of the CTP $T_{max} > 10$ s to the CTP $T_{max} > 6$ s lesion volumes has been recognized as a surrogate of collateral integrity, and was recorded in each subject from the initial CTP examination (Olivot *et al.*, 2014; Guenego *et al.*, 2018; de Havenon *et al.*, 2019).

Imaging outcomes were quantified in terms of relative infarction growth, reflecting a normalized percent consumption of the at-risk penumbra, i.e. [(final infarction volume−*initial CTP core*)/*initial CTP penumbra*] and expressed as percent growth. The primary outcome examined was relative loss of the at-risk penumbra ≥10%. We considered negative values for penumbral consumption to equate clinically with fully rescued at-risk tissue volumes and technically reflective of the small volumetric error inherent to the use of CTP at presentation; all negative values for consumed penumbra were therefore treated as zero percent growth (Wheeler *et al.*, 2013; Albers *et al.*, 2016; Cereda *et al.*, 2016).

Temperature analysis

Systemic temperatures were recovered from the subject medical records, beginning from the time of initial presentation and through the initial 2 days of hospitalization at a recorded frequency of no less than every 4 h. Temperature indices included maximal ($temp_{max}$) and average ($temp_{avg}$) temperatures for each subject over that sampling period. The source of systemic temperatures reflected local practice as detailed previously (Dehkharghani *et al.*, 2016) but comprised primarily tympanic thermometry. In addition to the preceding temperature indices described above, individual patient temperature maxima were thresholded at the following a priori defined levels: (i) ≥37°C; (ii) ≥37.5°C; (iii) ≥37.8°C; and ≥38°C to reflect varying fever thresholds in practice (Hindfelt, 1976; Castillo *et al.*, 1994; Reith *et al.*, 1996; Axelrod and Diringer, 2008; Dehkharghani *et al.*, 2016). Anti-pyretic administration during the initial 48 h was noted in all cases. Patients with positive microbial cultures from bodily specimens during the temperature reporting period were not included to eliminate potential confounding effects of non-neurologic fevers.

Endovascular therapy

All subjects underwent endovascular therapy at the discretion of the vascular neurologist and neurointerventionalist. The use of general anaesthesia, also administered at the discretion of the operator, and final modified treatment in cerebral ischaemia (mTICI) status (0 – 3) were recorded. Delay in time from initial CTP to the mTICI 2b/3 reperfusion (or CTP to groin puncture in non-successfully reperfused patients) was recorded to correct for potential infarct expansion in the interval preceding reperfusion which could confound determination of infarction growth and penumbra loss.

Statistical analysis

Continuous variables are reported as mean±SD if normally distributed. Non-normally distributed variables were reported by median and interquartile ranges (IQRs). Categorical variables are reported as proportions. Non-parametric tests and t-tests were used for continuous variables and Fisher's exact test was used to compare categorical variables. A two-tailed P-value <0.05 was considered significant.

Patients were divided into two categories based in the primary radiologic outcome of penumbra consumption ≥10%. We compared patient demographics (age and sex), clinical variables (hypertension, diabetes, hyperlipidaemia, atrial fibrillation, smoking), presentation National Institute of Health Stroke Scale score, general anaesthesia administration during angiography and treatment, antipyretic administration, glucose level, imaging variables (CTP infarction core volume, hypoperfusion intensity ratio, degree of mTICI reperfusion, and time from CTP imaging to reperfusion), and IV tissue-type plasminogen activator administration between groups in univariate analysis, and any variables differing significantly were selected in subsequent binary logistic regression in addition to pre-specified covariates of interest described below.

Average and maximal temperatures in the first 2 days were treated as continuous variables. Further, the four a priori thresholds of temperature maxima (see Temperature analysis section) were examined in logistical regression models to compare the relative consumption of the penumbra between groups. Binary logistic regression analyses were performed with and without adjustment for all significant variables in addition to pre-specified factors in two separate models: Model 1 adjusted for glucose, successful reperfusion, hypoperfusion intensity ratio and general anaesthesia; and Model 2 adjusted for Model 1 + antipyretic use + time from CTP to reperfusion.

Associations with temperatures on the first, as well as in the initial two hospital days were examined separately to recapitulate time of fever onset in previously reported studies of fever following stroke. Receiver operating characteristics were used to determine the prediction profile of temperature maxima for penumbra consumption ≥10%, and to identify the optimal threshold of $temp_{max}$. All statistical analysis was performed with SPSS (Chicago, IL) version 25.0.

Data availability

Clinical and neuroimaging data are available upon reasonable request.

Table 1 Baseline characteristics for study cohort and univariate analysis for relative penumbra consumption ≥10%[a]

	No (n = 75)	Yes (n = 51)	P-value
Age	64.5 ± 13.4	61.7 ± 15.5	0.770
Sex (% men)	44	27	0.585
Hypertension	54	31	0.245
Hyperlipidaemia	26	13	0.328
Atrial fibrillation	20/74	14	1.000
Diabetes	16	12	0.829
Smoker	13	6	0.455
Glucose (mean ± SD)	118.0 ± 27.6	143.1 ± 1.6	0.009*
National Institute of Health Stroke Scale (median, IQR)	18 (9)	18 (8)	0.912
IV tissue-type plasminogen activator	29	22	0.712
CTP infarct core volume (ml)	20.3 ± 24.4	17.1 ± 26.5	0.510
Hypoperfusion intensity ratio	0.47 ± 0.23	0.40 ± 0.23	0.126
General anaesthesia	24	18	0.701
Successful reperfusion (%, mTICI2b/3)	83	75	0.652
Antipyretic use	50	37	0.431
Time from CTP to reperfusion [minutes, median (IQR)]	166.0 (100.7)	166.8 (74.8)	0.965
Presentation temperature (°C)	36.1 (1.3)	36.4 (1.0)	0.236
$Temp_{max}$ Days 1 − 2 [°C, median (IQR)]	37.0 (0.5)	37.5 (0.7)	0.001*
$Temp_{avg}$ Days 1 − 2 [°C, median (IQR)]	36.5 (0.5)	36.7 (0.8)	0.029*
$Temp_{max} \geq 38$°C	3	7	0.089
$Temp_{max} \geq 37.8$°C	8	11	0.128
$Temp_{max} \geq 37.5$°C	13	26	<0.001*
$Temp_{max} \geq 37$°C	49	41	0.074
Favorable outcome (90 days 0 − 2 mRS)	55	21	0.001*

[a]Penumbra consumption reflecting percent, relative infarction growth.
*Statistical significance at two-tailed $P < 0.05$.

Results

A total of 126 endovascular AIS cases met the inclusion criteria. Details of the study population are summarized in Table 1. The mean age was 63 ± 14.5 years and 63% were women. The main reason for exclusion was the lack of CTP ($n = 442$) related to the less frequent use of CTP-based selection early in the study period. Twelve subjects had insufficient entries of systemic temperatures for analysis on the basis of the inclusion criteria above. Among the study population, 51 (40.1%) subjects were positive for the primary imaging outcome of relative penumbra consumption ≥10%.

Median baseline National Institute of Health Stroke Scale was 18 (IQR 13 − 22). Median CTP infarction core volume was 9.3 ml (IQR 0 − 23) and the median mismatch (i.e. penumbra) volume was 108 ml (IQR 72 − 173). General anaesthesia was administered in 42 (33.3%) patients. A total of 121 (96.0%) patients achieved full (mTICI2b/3) reperfusion, with a median time from CTP to reperfusion of 142 min (IQR 109 − 185). Eighty-three (65.9%) patients achieved a good clinical outcome as characterized by 90-day mRS ≤ 2. There were no positive systemic cultures in the study population during the initial 2 days of hospitalization.

The median (IQR) of the maximal temperature reached ($temp_{max}$) in the study population was 37.2°C (36.9 − 37.6°C). Figure 1 shows the time−temperature profile per subject over the observation period, including all available time points. The median (IQR) of the population average temperature ($temp_{avg}$) was 36.5°C (36.3 − 36.9°C). Thirty-nine patients (40.0%) reached a maximum temperature of at least 37.5°C. Anti-pyretic administration during the observation period was recorded in 87 patients (69.0%).

Univariate analysis (Table 1) demonstrated no significant differences in baseline clinical and demographic characteristics between those subjects with and those without loss of the at-risk penumbra ≥10%, with the exception of presentation glucose (143.1 ± 61.6 versus 118.0 ± 27.6, $P = 0.009$). Neither presentation National Institute of Health Stroke Scale ($P = 0.912$), rates of successful reperfusion ($P = 0.652$), presentation CTP infarction core volume ($P = 0.510$) nor time from CTP to full reperfusion ($P = 0.965$) differed significantly between the two groups. Rates of favourable clinical outcomes (90-day mRS 0 − 2) differed significantly between those subjects with and those without penumbra loss ≥10% ($P = 0.001$). There was no statistically significant difference in systemic temperature at the time of initial presentation between groups (36.3°C with versus 36.2°C without penumbra loss, $P = 0.461$).

Significant differences in both $temp_{avg}$ [36.5°C (IQR 36.3 − 36.8°C) without loss versus 36.7°C (IQR 36.3 − 37.1°C) with loss, $P = 0.029$] and $temp_{max}$ [37.0°C (IQR 36.8 − 37.3°C) without loss versus 37.5°C (IQR 37.2 − 37.9°C) with loss, $P = 0.001$] were present when comparing populations with and without significant penumbra loss. Unadjusted and adjusted multivariable

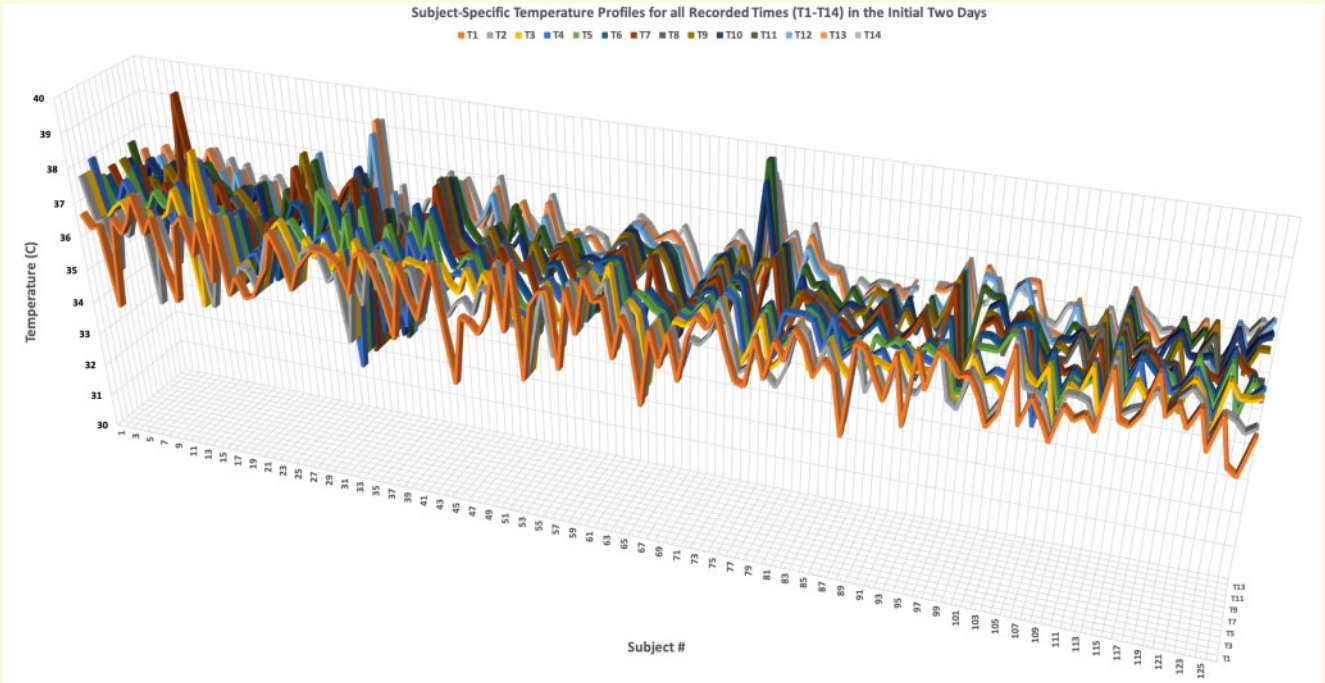


Figure 1 Subject-specific temperature profiles: individual subject time−temperature profiles for the entire study population over the temperature observation period.

Table 2 Relative penumbra consumption ≥10%: unadjusted and adjusted binary logistic regression[a]

	Unadjusted	Model 1[b]	Model 2[c]
$Temp_{max}$ (Day 1)	1.45 (0.90–2.32)	1.34 (0.81–2.21)	1.33 (0.80–2.20)
	$P = 0.124$	$P = 0.249$	$P = 0.277$
$Temp_{avg}$ (Day 1)	1.40 (0.83–2.37)	1.33 (0.75–2.34)	1.35 (0.76–2.40)
	$P = 0.206$	$P = 0.327$	$P = 0.314$
$Temp_{max}$ (Days 1 and 2)	3.57 (1.65–7.75)	3.02 (1.35–6.79)	3.06 (1.33–7.06)
	$P = 0.001^*$	$P = 0.007^*$	$P = 0.009^*$
$Temp_{avg}$ (Days 1 and 2)	1.89 (0.94–3.79)	1.74 (0.86–3.49)	1.73 (0.85–3.51)
	$P = 0.073$	$P = 0.122$	$P = 0.128$

[a]Odds ratios and confidence intervals for model parameters shown.
[b]Model 1: adjusted for pre-specified covariates glucose, successful reperfusion, collaterals (hypoperfusion intensity ratio) and general anaesthesia.
[c]Model 2: adjusted for Model 1 + antipyretic + time from CTP to reperfusion.
*Statistical significance at two-tailed $P < 0.05$.

models assessing the relationship between temperatures in the initial 2 days and penumbra loss ≥10% are reported in Table 2. The unadjusted model found significant associations for $temp_{max}$ [odds ratio (OR) 3.57, 95% confidence interval (CI) 1.65 – 7.75; $P = 0.001$] which persisted in both adjusted Model 1 (OR 3.02, 95% CI 1.35 – 6.79; $P = 0.007$) and Model 2 (OR 3.06, 95% CI 1.33 – 7.06; $P = 0.009$). No significant differences were identified for $temp_{avg}$ in unadjusted or either of the adjusted models, with respect either to Day 1 or combined Day 1 and 2 average temperatures (Table 2). A secondary sensitivity analysis incorporating initial absolute mismatch volume to Model 1 variables revealed that the association between $temp_{max}$ over the initial 2 days and penumbral consumption remained significant (OR 2.93, 95% 1.29–6.68; $P = 0.01$), while the remaining associations remained non-significant ($temp_{max}$ Day 1: OR 1.26, 95% CI 0.76–2.09; $P = 0.370$; $temp_{avg}$ Days 1 – 2: OR 1.70, 95% CI 0.83–3.47; $P = 0.147$; $temp_{avg}$ Day 0: OR 1.27, 95% CI 0.71–2.29; $P = 0.418$).

Among the study population, the highest quartile of penumbral consumption represented the highest median $temp_{max}$, as follows: Q1 37.2°C (0.4), Q2 37.0°C (0.5), Q3 37.2°C (0.7) and Q4 37.6°C (0.7); $P = 0.005$. Similarly, comparing the lower (36.9°C) and upper (37.6°C) quartiles of $temp_{max}$ revealed significant differences in relative infarction growth ($P = 0.008$). A dose effect from the slope of regression indicates an ∼20% increase

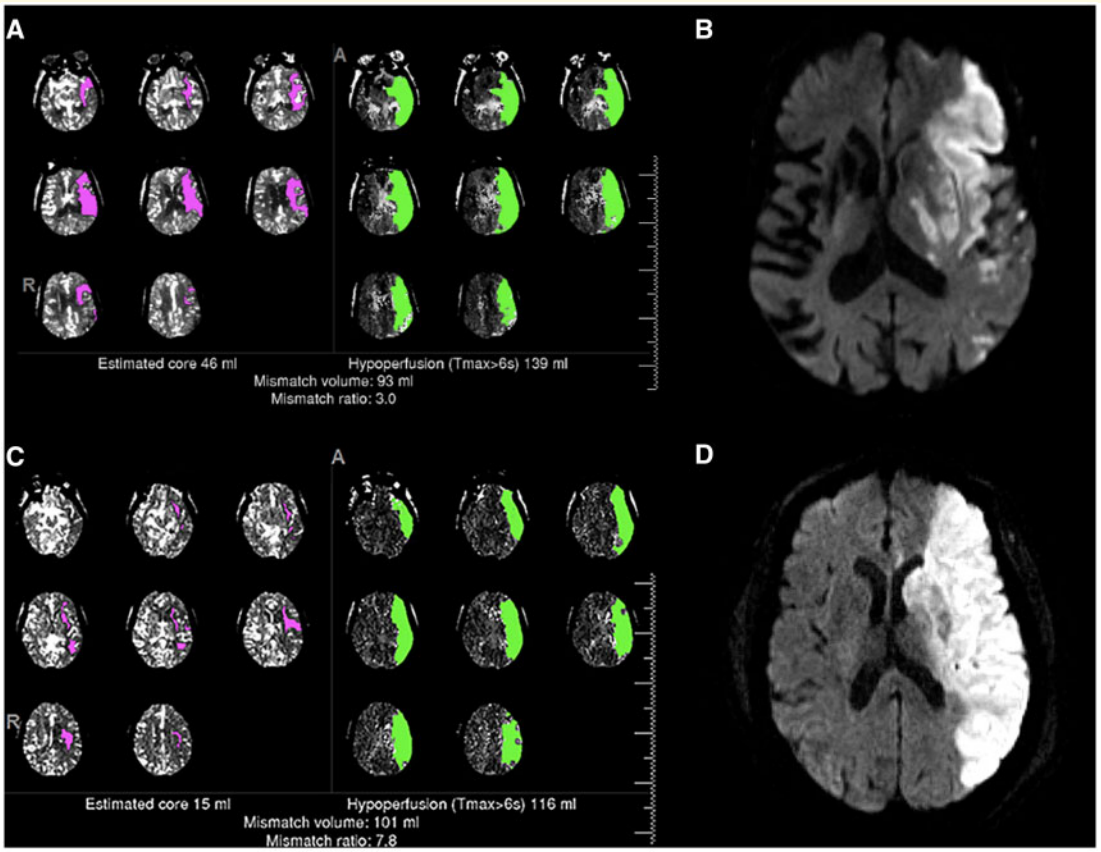


Figure 2 Temperature and relative penumbral salvage following reperfusion: presentation CTP (**A**, **C**) and respective, follow-up DWI (**B**, **D**) from two subjects both achieving full angiographic reperfusion for large vessel occlusion acute ischaemic stroke. CTP in both subjects shows initial penumbra mismatch volumes derived from relative cerebral blood flow (infarction core, magenta overlay) and T_{max} (critical hypoperfusion, green overlay) parametric maps. The subject in **A** reached maximal systemic temperatures ($temp_{max}$) of 36.3°C (<10th percentile population $temp_{max}$) in the initial 2 days with a relative penumbra consumption of 0.0% on follow-up DWI following mTICI2b reperfusion. The subject in **B** reached $temp_{max}$ of 38.2°C (>90th percentile population $temp_{max}$) with relative penumbra consumption ~77% despite mTICI3 reperfusion.

Table 3 Body temperature and penumbra consumption: multi-threshold analysis of fever and infarction growth

	Median (IQR) percentage penumbra consumption		
	Temperature threshold *negative*	**Temperature threshold *positive***	***P*-value**
$Temp_{max} \geq 38.0$°C (Days 1 and 2)	7.1 (19.6)	27.7 (54.9)	0.323
$Temp_{max} \geq 37.8$°C (Days 1 and 2)	6.6 (19.4)	10.3 (39.8)	0.319
$Temp_{max} \geq 37.5$°C (Days 1 and 2)	4.8 (13.4)	21.1 (47.1)	0.007*
$Temp_{max} \geq 37$°C (Days 1 and 2)	5.2 (12.3)	8.5 (38.5)	0.554
$Temp_{max} \geq 38.0$°C (Day 1)	7.6 (19.7)	56.2 (63.8)	0.361
$Temp_{max} \geq 37.8$°C (Day 1)	8.2 (19.7)	4.9 (56.2)	1.000
$Temp_{max} \geq 37.5$°C (Day 1)	7.8 (18.1)	12.1 (43.6)	0.814
$Temp_{max} \geq 37$°C (Day 1)	7.2 (19.4)	8.3 (33.6)	0.858

*Statistical significance at two-tailed $P < 0.05$.

in likelihood of penumbra loss for each 0.1°C increase in $temp_{max}$ during Days 1 and 2 ($P = 0.009$). Figure 2 shows two representative AIS subjects with full angiographic reperfusion (Subject 1, mTICI2b; Subject 2, mTICI3). While both subjects presented with large mismatch volumes and favourable CTP profiles for reperfusion, large differences in absolute and relative salvage of the at-risk penumbra are noted, concordant with greater (90th percentile) $temp_{max}$ in Subject 2 in whom mTICI3 reperfusion was achieved, versus 10th percentile $temp_{max}$ in Subject 1 in whom mTICI2b reperfusion was achieved.

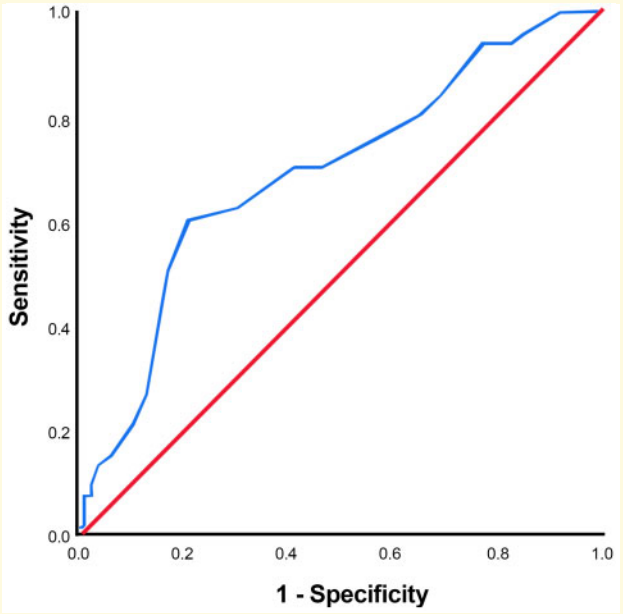


Figure 3 Receiver operating characteristics for prediction of relative penumbra loss ≥10%: Varying thresholds for systemic temperature maxima in the initial 2 days following presentation are represented. Maximal temperatures ≥37.5°C demonstrated the best performance for prediction of infarction growth (sensitivity = 0.61, specificity = 0.79, AUC 0.69, 0.60–0.79; $P < 0.001$).

Additional analysis performed only upon the fully reperfused subjects ($n = 121$), demonstrated similar results, with $temp_{max}$ over the initial 2 days showing significant associations in unadjusted (OR 3.57, 95% CI 1.61–7.91; $P = 0.002$), and both adjusted (Model 1: OR 3.08, 95% CI 1.34 – 7.01; $P = 0.008$; Model 2: OR 3.07, 95% CI 1.30 – 7.23; $P = 0.010$) models.

Examination of varying fever thresholds between 37°C and 38°C (Table 3) demonstrated the most significant differences in median relative penumbra consumption at the pre-defined threshold of 37.5°C [penumbra consumption 4.8% (IQR 0 – 11.5%) for $temp_{max}$ <37.5°C versus 21.1% (IQR 0 – 44.7%) for $temp_{max}$ ≥37.5°C, $P = 0.007$]. Significant differences were not found at the remaining pre-defined temperature thresholds. ROC analysis shown in Fig. 3 demonstrates an optimal operating point at a fever threshold ≥37.5°C (sensitivity 0.61, specificity 0.79; AUC 0.69, 95% CI 0.60 – 0.79; $P < 0.001$).

Discussion

This study reveals a robust relationship between body temperature in the early aftermath of AIS and relative infarction growth as defined by operational paradigms of the ischaemic penumbra. While the direction of this relationship and causality cannot be unambiguously concluded, the findings lend further support to the influence of elevated body temperatures upon ischaemic, including potentially at-risk, tissues. Importantly, the strength of this association even when accounting for full reperfusion underscores the well-recognized impact of temperature on the neurovascular unit in clinically measurable and modifiable terms.

Several aspects of this relationship merit emphasis and may guide our understanding of the impact of fever following AIS. Firstly, while systemic temperature elevations at any time in the acute and subacute phases of ischaemia portend worse outcomes, an especially heightened vulnerability to fever has been described in the earliest phases of ischaemic brain injury (Azzimondi *et al.*, 1995; Kim *et al.*, 1996; Reith *et al.*, 1996; Baena *et al.*, 1997; Castillo *et al.*, 1998; Wrotek *et al.*, 2011). We therefore focussed the investigation herein upon the effects of body temperature in the initial 2 days of presentation to test the hypothesis that early and ostensibly mild temperature elevations predict stroke progression even among reperfused patients using contemporary imaging paradigms. We hasten to add that we elected to use a normalized measure of infarction growth—i.e. relative penumbral consumption—as we reported previously (Dehkharghani *et al.*, 2016). We chose this approach to mitigate, or ideally circumvent potential vagaries related to the use of absolute infarction growth metrics as reported commonly in imaging-based ischaemic stroke trials. Consequently, an established benchmark for the profile of RIG outcomes in large cohorts remains to be established but is under current investigation in our group. We considered 10% to represent a reasonable and conservative first approximation of *expected* progression, in line with coarse estimations from available results in fully reperfused cohorts in past imaging-based endovascular therapy trials (Campbell *et al.*, 2015).

Secondly, the clinical definitions of pyrexia vary widely across medical, physiologic and other bioscientific contexts. In this study, we compared varying thresholds to examine potential dose effects and to capture potentially clinically meaningful prediction thresholds for infarction growth. The finding that infarction progression is best predicted at the seemingly conservative threshold of 37.5°C may be surprising at first inspection, but can be understood when considering systemic temperatures as coarse and often inaccurate surrogates of cerebral temperatures, particularly following ischaemic injury (Stone *et al.*, 1995; Simon, 2007; Dehkharghani *et al.*, 2017); specifically, well-known brain-to-systemic temperature gradients have been shown to further diverge following ischaemia, and in a spatially heterogeneous fashion throughout the brain itself (Karaszewski *et al.*, 2012, 2013; Dehkharghani *et al.*, 2017). Emerging non-invasive brain thermometry techniques reported previously by our group in human and non-human primate (NHP) studies, including after controlled MCA stroke induction in the phylogenetically similar rhesus macaque, could facilitate empirical study of spatially and temporally distributed

brain temperatures in this setting (Cady *et al.*, 2011; Karaszewski *et al.*, 2012, 2013; Dehkharghani *et al.*, 2017). Accordingly, a seemingly mild systemic fever threshold of 37.5°C may belie significantly greater intracerebral hyperthermia as we have observed following NHP stroke induction, in particular in the ischaemic tissue bed wherein secondary thermogenic inflammatory cascades have been proposed to compound the impaired radiative cooling arising from hypoperfusion (DeGraba, 2000; Whiteley *et al.*, 2012; Gauberti *et al.*, 2016). Despite our controlling for antipyretics and our having excluded subjects with positive cultures or signs of systemic infections in this study, we presume such effects to be agnostic to the source of the fever, although this was not specifically tested within the design of our study (Hajat *et al.*, 2000; Greer *et al.*, 2008). The differences observed for average versus maximal temperatures in our study also merit further discussion. We anticipated that average temperatures over the study period might better capture the aggregate effects of systemic temperature as it relates to infarction growth, which was not borne out in our results. While conjectural, the profound fluctuations in individual patient temperatures in combination with the incompletely controlled temperature collection environment may undermine our attempt to accurately capture the footprint of average temperatures as intended. We consider the temporal characteristics of fevers, including precisely when, to what magnitude, and for what duration they exist, as well as its response to any therapeutic measures to be critical to a true mechanistic understanding of this relationship.

Lastly, while the temperature sensitivity of the neurovascular unit and the clinical harms of fever amid ischaemic stroke are well known, our study was designed to quantify this effect at the tissue level based on readily available CTP profiles and final imaging outcomes by MRI. By assessing infarction growth on follow-up MRI relative to the penumbral (mismatch) volume at presentation, the effect can be studied in terms of relative, volumetric infarct growth. In this manner, the fate of at-risk tissues can be expressed practically as it relates to the efficacy of reperfusion, after which the growth of at-risk tissues should be theoretically arrested. Nevertheless, we documented infarction growth which we suspect to occur under the influence of mild fever, and even when correcting for known covariates of failed penumbral salvage following reperfusion. The precision and accuracy of CTP imaging as used in this study, while itself imperfect, has been shown to perform well in terms of volumetric agreement, both in digital phantom studies, human *in vivo* stroke trials and real-world cohorts (Kudo *et al.*, 2013; Dehkharghani *et al.*, 2015a; Cereda *et al.*, 2016).

We acknowledge a number of study limitations, principally those inherent to the retrospective nature of the analysis. Our single-institution, prospectively collected registry of endovascularly treated AIS cases provided some uniformity to the imaging and treatment strategies underpinning the study design; however, the recruitment of cases spanned periods of change in acute stroke care, over which biases could have evolved. Perfusion-based patient selection was performed only ad hoc at the discretion of the care team during the early phase of inclusion, leading to exclusion of many cases and potentially occult biases which cannot be easily corrected. Nevertheless, as neither the perfusion imaging protocol, the post-processing pipeline, nor operator expertise varied, we anticipate the bias relating to this variability to be small. Similarly, the study period spans important epochs of evolution in endovascular technology, most notably the advent of stent-retriever technology, the use of which became standard practice during the study period and is known to impact outcomes (Nogueira *et al.*, 2012; Saver *et al.*, 2012; Haussen *et al.*, 2016b). Because our model accounts for the duration of the endovascular procedure, and in light of the generally high reperfusion rates by consistent endovascular operators, we again anticipate the effect to be small. Further related to the constraints of our retrospective design, follow-up MRI could not be assessed at fixed intervals following presentation and treatment. Per our clinical protocol, confirmatory MRI for those patients able to undergo it safely is generally performed between the second and fifth day, and this variability could introduce unaccounted effects. We anticipate that, for those subjects undergoing earlier follow-up MRI, not all influences of temperature will have manifested in their imputed final infarction volume, while in those imaged beyond the 2-day window, influences of later temperatures outside the interrogation period can be missed. Notwithstanding, we felt that keeping the window of temperature collection consistent was least costly with regards compromising the primary question of interest; however, we would underscore the importance of strict temperature collection and follow-up imaging protocols in future, prospective studies. We attempted to account for the use of antipyretics, and to exclude from analysis those subjects with substantive clinical evidence for infection to the extent possible, although the effects of subclinical infectious processes are not fully controlled. Lastly, while our study population comprised a relatively large number of endovascularly treated cases, it may have been underpowered to capture nuances of the relationship between varying temperature thresholds and infarction progression, in particular using fever thresholds at and above 38°C, which were encountered with insufficient frequency to support examination of extended dose effects from more severe fevers. Whether monitored temperatures or potentially therapeutic modulation of estimated cerebral temperatures will prove efficacious in improving outcomes in endovascularly treated stroke subjects remains to be studied in larger, prospectively recruited and controlled cohorts.

In summary, these findings suggest that potentially subclinical elevations in body temperature could promote consumption of the ischaemic penumbra, even in

reperfused patients. Dedicated investigation to clarify the potentially causative nature of mild fever as a catalyst for infarction progression is encouraged to ameliorate the effects of hyperthermia upon the fate of ischaemic tissues.

Competing interests

S.D.: Dr Dehkharghani reports receipt of travel support from iSchemaview, the developer of RAPID. S.Y. M.B., L.P., E.S.: none. D.C.H.: Dr Haussen reports the following disclosure VizAI stock options; Stryker (consulting); Vesalio (consulting). R.G.N.: Dr Nogueira reports the following disclosures Stryker Neurovascular (DAWN Trial Principal Investigator—no compensation, TREVO Registry Steering Committee—no compensation; Consultant—significant); Cerenovus/Neuravi (ENDOLOW Trial Principal Investigator—no compensation, EXCELLENT Registry Principal Investigator—no compensation, ARISE-2 trial Steering Committee—no compensation, Physician Advisory Board, modest); Phenox (PROST Trial Principal Investigator, Physician Advisory Board, modest); Anaconda (Physician Advisory Board, modest); Genentech (Physician Advisory Board—modest); Biogen (CHARM Trial Steering Committee; Physician Advisory Board—modest); Prolong Pharmaceuticals (Physician Advisory Board—modest); Brainomix (Physician Advisory Board, stock options); Viz-AI (Physician Advisory Board, stock options); Corindus Vascular Robotics (Physician Advisory Board, stock options); Vesalio (Physician Advisory Board, stock options); Ceretrieve (Physician Advisory Board, stock options); Astrocyte (Physician Advisory Board, stock options); Cerebrotech (Physician Advisory Board, stock options); Imperative Care (Imperative Trial Principal Investigator, modest).

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