



Short report

Circadian rhythm of ischaemic core progression in human stroke

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ABSTRACT

Introduction Experimental stroke studies suggest an influence of the time of day of stroke onset on infarct progression. Whether this holds true after human stroke is unknown, but would have implications for the design of randomised controlled trials, especially those on neuroprotection.

Methods We pooled data from 583 patients with anterior large-vessel occlusion stroke from three prospectively recruited cohorts. Ischaemic core and penumbra volumes were determined with CT perfusion using automated thresholds. Core growth was calculated as the ratio of core volume and onset-to-imaging time. To determine circadian rhythmicity, we applied multivariable linear and sinusoidal regression analysis adjusting for potential baseline confounders.

Results Patients with symptom onset at night showed larger ischaemic core volumes on admission compared with patients with onset during the day (median, 40.2 mL vs 33.8 mL), also in adjusted analyses ($p=0.008$). Sinusoidal analysis indicated a peak of core volumes with onset at 11pm. Core growth was faster at night compared with day onset (adjusted $p=0.01$), especially for shorter onset-to-imaging times. In contrast, penumbra volumes did not change across the 24-hour cycle.

Discussion These results suggest that human infarct progression varies across the 24-hour cycle with potential implications for the design and interpretation of neuroprotection trials.

INTRODUCTION

Understanding the determinants of acute infarct progression is crucial for delivering optimal treatments to patients with large-vessel occlusion (LVO) stroke and for the design of randomised controlled trials. The time of day of stroke onset was recently found to influence infarct progression and the treatment effect of neuroprotective strategies after experimental stroke. Specifically, strokes induced in the inactive phase of rodents, that is, during the day, showed faster infarct progression and more pronounced neuroprotective treatment benefits compared with induction in the active phase, that is, at night.¹ Considering the opposing rest-activity cycles of rodents and humans this might explain the limited efficacy of neuroprotective strategies in clinical trials, which mostly recruited patients during daytime, that is, in their active phase. However,

whether the time of day of onset indeed influences acute infarct progression in human stroke is unknown. Such knowledge would allow adapting the design of acute stroke trials, especially those on neuroprotection.² Here, we explored whether ischaemic core and penumbra volumes as well as core growth show circa-24 hour ('circadian') rhythms by meta-analysing data from three independent and prospectively recruited cohorts of patients with anterior LVO stroke.

METHODS

Study sample

Study approval was acquired by the respective institutional review boards according to the Declaration of Helsinki of 2013 and requirement for written consent was waived. Patients with acute ischaemic stroke due to anterior circulation LVO were selected from three prospectively recruited cohorts (Munich, Basel, Goettingen) if they had (1) occlusions of the internal carotid or proximal middle cerebral artery, (2) complete multiparametric CT imaging including non-contrast CT, single-phase CT angiography and CT perfusion (CTP) imaging and (3) known time from symptom onset or last seen well. For Munich, we included 316 of 748 consecutive patients enrolled between 2015 and 2020 in the German Stroke Registry (NCT03356392). For Basel, we included 157 of 393 consecutive patients enrolled in the Swiss Stroke Registry between 2015 and 2020. For Goettingen, we included 120 of 576 consecutive patients enrolled between 2015 and 2018. If symptom onset was unknown, we calculated the average time between last seen well and time of recognition.

Neuroimaging

CT imaging on admission was performed using SOMATOM Definition Force, AS + and Flash scanners (Siemens Healthineers, Forchheim, Germany). CTP data were processed using syngo Neuro Perfusion CT (Siemens Healthineers, Forchheim, Germany) including threshold-based calculation of ischaemic core (cerebral blood volume <1.2 mL/100 mL) and penumbra volumes (cerebral blood flow <35.1 mL/100 mL per minute).³ The mismatch ratio was calculated as the ratio of the total and core volume. In the absence of multiple measurements at different time points per patient,

we assumed a linear function for core growth and calculated it as the ratio of core volume and onset-to-imaging time (in mL/hour).⁴ ASPECTS on non-contrast CT⁵ and regional leptomeningeal collateral score on CT angiography⁶ were assessed by experienced radiologists at all sites (WGK, PR and MP).

Statistical analysis

Imaging data from all three centres were separately log-transformed, standardised (centred by mean and scaled by SD), and subsequently merged. We excluded patients with imaging parameter values deviating more than three SD from the mean (two for Munich and Basel, six for Goettingen). Circadian rhythmicity of imaging parameters was explored using univariable or multivariable linear regression analysis by stratifying the time of day of symptom onset into the groups ‘morning’ (3 am – 9 am), ‘day’ (9 am – 3 pm), ‘evening’ (3 pm – 9 pm) and ‘night’ (9 pm – 3 am) as recently proposed.¹ Analyses were adjusted for age, onset-to-imaging times, the collateral score and centre. To detect circadian rhythmicity across the continuous timescale independent of the selection of time windows, we further performed sinusoidal regression analyses using the ‘R’ package ‘DiscoRhythm’⁷ after linearly regressing out the confounders age, collateral score, onset-to-imaging times and centre. All analyses were performed using ‘R’, V.4.0.2.⁸

RESULTS

We analysed data from 583 patients with anterior LVO stroke from three cohorts (Munich: N=314, Basel: N=155; Goettingen: N=114). Patients had a median age of 76 years, a median National Institutes of Health Stroke Scale (NIHSS) score on admission of 15, and a median onset-to-imaging time of 139 min. Symptom onset occurred in the morning in 19.2 %, during the day in 32.6 %, in the evening in 27.3 %, and at night in 20.9% of patients. Onset-to-imaging time was the only baseline parameter that showed differences between these groups ($p < 0.0001$, table 1).

Patients with symptom onset at night showed larger ischaemic core volumes on admission compared with patients with onset during the day (median, 40.2 mL vs 33.8 mL), also when adjusting for age, collateral score, onset-to-imaging time and centre ($p = 0.008$). In adjusted sinusoidal regression analysis, we observed a similar trend with core volumes peaking in patients with onset at 11 PM ($p = 0.21$, figure 1A). To determine whether this pattern would depend on changes of the total ischaemic volume, we explored potential circadian rhythmicity of the mismatch ratio (total volume divided by core volume). Corresponding to the rhythm observed for core volumes, the mismatch ratio was lowest with onset at night compared with day ($p = 0.006$) with a similar trend in sinusoidal analyses (peak at 11am, $p = 0.08$, figure 1B), indicating a limited association between total and core volumes. Of note, penumbra volumes did not change across the 24-hour cycle (figure 1C).

Considering that core volumes were higher for patients with onset at night independent of the total ischaemic volume, we hypothesised that the ischaemic core would grow faster at night compared with day. Indeed, core growth was faster at night compared with day onset (adjusted $p = 0.01$), especially for shorter onset-to-imaging times (figure 1D).

DISCUSSION

Complementing a recent experimental stroke study, we here provide first evidence for circadian rhythms of both ischaemic core volume and core growth after human stroke. Across different analytical approaches, patients with onset around midnight showed larger core volumes and faster core progression compared with patients with onset around noon. Our results suggest that the treatment window might close faster for patients with night compared with day onset. Accordingly, time to endovascular treatment and intravenous thrombolysis might be even more important in patients with night onset. From a clinical trial perspective, patients with onset at night might need to be recruited shorter after symptom onset compared with day

Table 1 Baseline characteristics, imaging data and their circadian rhythms

Characteristics	Munich n=314	Basel n=155	Goettingen n=114	P (circadian)
Age, median (IQR) (years)	76 (67–82)	76 (66–82)	77 (68–83)	0.37
Female, % (n)	46.2 (145)	54.2 (84)	57.0 (65)	0.13
Time of day of symptom onset, % (n)				
Morning (3 am - 9 am)	19.7 (62)	15.6 (24)	22.8 (26)	0.76
Day (9 am - 3 pm)	33.1 (104)	33.5 (52)	29.8 (34)	
Evening (3 pm - 9 pm)	27.4 (86)	27.7 (43)	26.3 (30)	
Night (9 pm - 3 am)	19.7 (62)	23.2 (36)	21.1 (24)	
NIHSS on admission, median (IQR)	14 (9–18)	16 (12–19)	12 (7–18)	0.36
Affected vessel, % (n)				
MCA	67.5 (220)	80.1 (125)	100 (114)	0.20
ICA	32.5 (101)	19.3 (30)	0 (0)	
NCCT ASPECTS, median (IQR)	9 (8–10)	9 (8–10)	8 (7–9)	0.26
Collateral score, median (IQR)	15 (12–18)	10 (6–16)	14 (10–18)	0.75
Onset → imaging, min, median (IQR)				
Morning (3 am - 9 am)	215 (106–368)	118 (93–199)	185 (95–268)	<0.0001
Day (9 am - 3 pm)	130 (77–234)	83 (71–121)	103 (77–134)	
Evening (3 pm - 9 pm)	141 (75–214)	81 (70–100)	122 (75–214)	
Night (9 pm - 3 am)	369 (225–494)	211 (96–427)	361 (256–494)	
Core volume, median (IQR) (mL)	35 (21–61)	36 (21–67)	37 (24–58)	0.008*

Differences between groups (morning, day, evening, night) were assessed using Kruskal-Wallis tests for continuous and Fisher's exact test for categorical data.
*Adjusted for age, onset-to-imaging time, collateral score and centre
ASPECTS, Alberta Stroke Program Early CT Score; ICA, internal carotid artery; MCA, middle cerebral artery; NCCT, non-contrast computed tomography.

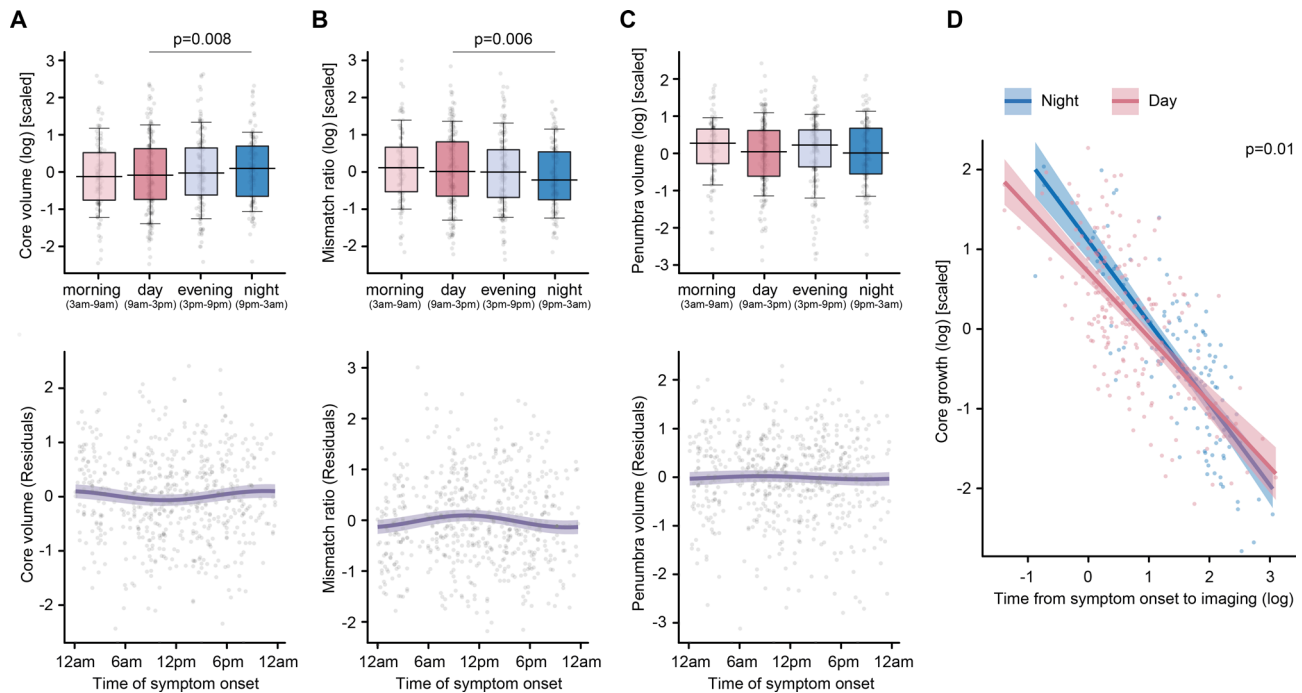


Figure 1 Circadian rhythms of core volume and growth after human ischaemic stroke. (A–C) Night onset was associated with higher core volume (A) and lower mismatch ratio compared with onset during the day (B) in both linear (upper plot) and sinusoidal regression analysis (lower plot) while penumbra volumes did not show differences across circadian times (C). (D) Core growth, calculated as the ratio of core volume and onset-to-imaging time, was higher for night onset compared with day onset especially for shorter onset-to-imaging times. (A–C) Upper plots indicate unadjusted log-transformed and scaled data while significance bars indicate $p < 0.05$ for linear regression analyses adjusting for age, onset-to-imaging time, the collateral score and centre. Lower plots show sinusoidal rhythms of linear regression residuals adjusted for age, collateral score, onset-to-imaging time and centre. (A–D) The sinusoidal and linear fits (continuous lines) and 95% CIs are shown in colour.

onset to show similar treatment efficacy. Further research is needed to determine whether the efficacies of acute stroke treatments indeed show circadian rhythmicity.

Circadian clocks are temporal molecular programmes, which are entrained by environmental cues such as light and feeding, to generate circadian rhythms of behaviour and physiology including sleep-wake cycles, blood pressure and hormone secretion.⁹ Our data now add to a growing body of evidence for how the human circadian clock impacts on disease progression.¹⁰ While core clock genes modify the expression of up to 40% of all genes including drivers of cell death¹¹ and neuronal redox defence,¹² the specific biological underpinnings of the observed phenomenon are unknown.

Our study complements a recent experimental stroke study, which found faster infarct progression when stroke was induced at daytime in rodents, that is, their inactive phase, compared with night.¹ Our results provide first evidence for a similar pattern in human stroke suggesting faster infarct progression and thus higher core volumes on admission in the inactive phase at night. However, while the experimental study also found evidence for larger penumbra volumes in rodents with stroke onset in the inactive phase using laser-speckle imaging, we did not find any trend for circadian rhythmicity of penumbra volumes after human stroke using CTP. Future studies may apply complementary methods such as MRI both after human and experimental stroke to explore whether these differences hold true.

Strengths of our study include the large sample size pooled from three centres that used identical CT scanners and software for CTP quantification, the application of different statistical approaches including sinusoidal regression analysis and the adjustment for important confounders such as collaterals⁴

and the onset-to-imaging time. Our study is limited in its cross-sectional character, which hampers definitive conclusions on core growth over time, which would ideally be assessed using serial imaging. This, however, would be almost impossible to conduct in patients. Further, considering that the large majority of human night-time strokes occur during sleep, the exact time of symptom onset is unknown for these patients. Here, we calculated the average time between last seen well and time of recognition in order to avoid severe bias towards inclusion of day-time strokes, which would have resulted when excluding all patients with unknown symptom onset including all patients with wake-up stroke. Lastly, we defined the ischaemic core using a pre-specified CTP-derived cerebral blood volume threshold and not based on molecular or pathological mechanisms. Thus, our findings could also be interpreted as patients with symptom onset at night showing larger areas with lower cerebral perfusion translating to more severe ischaemia. Future studies should explore whether this tissue still responds to neuroprotectants and whether our findings also translate to other core definitions¹³ and thus discriminate severe ischaemia from tissue death.

In conclusion, we provide evidence for circadian rhythmicity of ischaemic core volumes on admission as well as core growth with faster infarct progression in patients with onset at night. Further research is needed to better understand the underlying pathophysiology and whether these rhythms also impact on the efficacy of acute stroke treatments.

Correction notice This article has been corrected since it appeared Online First. Contributor statement has been updated for minor changes.

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