Supplemental Material

Supplemental Methods

Study Design and Data Sources
Inclusion dates were 2015-2016 for center 1, 2018 for center 2 and 2015-2020 for center 3, the time-period when post-transfer MRI scans were routinely performed. During the study period, the post-transfer imaging was required by protocol for all patients in center 1 but only for patients with long transfer times at centers 2 and 3 (*i.e*. patients with short transfer times were usually directly transferred to the angiosuite, unless there was a clinical change during the transfer). To limit the selection bias induced by clinically driven post-transfer imaging, we have restricted the inclusions to patients with long transfer times in these centers. Center 1 collected data on all patients, regardless of whether thrombectomy was attempted following the control MRI, whereas centers 2 and 3 collected thrombectomy-treated patients only (*i.e.* data from patients who did not undergo groin puncture was not available).

Radiological data
All patients underwent MRI on admission at the primary center, and upon arrival at the comprehensive center, MRI being the routine first-line imaging technique for acute stroke in all centers. All MRIs were performed with a standardized protocol at each institution, which systematically included diffusion-weighted imaging (DWI), T2*, and intracranial MR-angiography. Perfusion imaging was rarely performed in the primary center but was routinely performed in the comprehensive center unless decided otherwise by the physician in charge.

One stroke neurologist with 10-years of clinical expertise in stroke imaging (PS) reviewed all MRIs, with several variables collected on both time-points. First, the topography of infarct on baseline DWI, categorized as absent, involving exclusively deep or superficial regions, or both deep and superficial. Second, infarct volumes on DWI, manually outlined based on DWI signal intensity encompassing the entire area of bright DWI signal intensity. Areas of decreased apparent diffusion coefficient with subtle DWI signal changes were also segmented. To ensure unbiased segmentations, infarct on the primary and comprehensive stroke center DWIs were outlined several days apart, blinded to all clinical and radiological data (including time-of-day). Third, perfusion imaging was processed using RAPID software (iSchemaView, Menlo Park, CA) v.5.0, with segmentation artifacts removed manually whenever necessary. The severity of hypoperfusion was assessed using the Hypoperfusion Intensity Ratio (HIR) defined as the proportion of Tmax>6s volume with Tmax>10s (i.e., Tmax>10s volume / Tmax>6s volume), low HIR indicating milder hypoperfusion and better collaterals. Fourth, occlusion site on MR-angiography, divided into intracranial ICA, M1 and M2 was assessed; the M1 segment being defined as the first portion of the middle cerebral artery up to the main bifurcation. Last, arterial recanalization occurring during the transfer, evaluated on the comprehensive stroke center MR-angiography, using the revised Arterial Occlusion Lesion scale score.

Considering the proof-of-concept aim of this study, the patients with arterial recanalization occurring during the transfer, defined as a revised Arterial Occlusion Lesion scale score ≥2a, were excluded. Patients with large (>70 mL) infarcts at the time of initial imaging were excluded to limit selection bias, since those patients may have been less frequently transferred at night considering that the benefit of thrombectomy was uncertain.

Statistical analyses
Univariable comparisons between day vs night transfers and baseline characteristics were assessed using the Mann-Whitney U test for continuous variables, and the Chi-square test of Fisher's exact test for categorical variables, as appropriate. The association between inter-hospital IG rate and nighttime was assessed through β coefficient and its 95% Confidence Interval, calculated in multivariable linear regression analysis, with log-transformed IG rate as the dependent variable to keep the linear

assumption valid. As the HIR was not available in the entire dataset and was typically unavailable at the primary center (*i.e*. perfusion was primarily performed at the comprehensive center MRI), two different multivariable models were constructed. The first model was adjusted for baseline occlusion site, NIHSS score, and infarct topography (on the primary center MRI), and the second for HIR, occlusion site and infarct topography (*i.e.,* the variables independently associated with faster inter-hospital IG in our former publication,[1] save for initial infarct volume, as explained thereafter). Both models were deliberately not adjusted for initial infarct volume since it may induce a spurious correlation between time-of-day and inter-hospital IG rate, *i.e.* a portion of the time-of-day effect is potentially mediated through initial infarct volume.[2,5] Statistical analyses were performed using SPSS 29.0 (IBM, Armonk, NY). Two-tailed P<0.05 was considered significant.

Supplemental Results

Sensitivity analyses limited to Center 1
Among the 95 patients transferred to Center 1, 16 (17%) were transferred at night and 79 (83%) during the day. Median inter-hospital IG rate was higher when occurring at night (6.9 mL/hr, IQR 1.7-11.3) as compared to the day (1.5 mL/hr, IQR 0.4-5.8, *P*<0.001). Nighttime transfer was independently associated with log-transformed inter-hospital IG rate following adjustment for baseline occlusion site, NIHSS score and infarct topography (β coefficient=0.21, 95%CI 0.06-0.37, *P*=0.007), and there was a trend in the alternative model adjusted for HIR, occlusion site and infarct topography (n=87 patients with perfusion imaging; β coefficient=0.11; 95%CI -0.03 ; 0.24, *P*=0.110).

Sensitivity analyses with further adjustment on initial infarct volume in Model 1
Nighttime transfer was independently associated with log-transformed inter-hospital IG rate following adjustment for baseline occlusion site, NIHSS score, infarct topography and infarct volume (β coefficient=0.12, 95%CI 0.02-0.21, *P*=0.021).