Original research

Three-month functional outcomes following endovascular thrombectomy during the first wave of the COVID-19 pandemic: a Canadian single-center cohort study

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/neurintsurg-2021-017664).

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Received 14 April 2021
Accepted 23 April 2021

To cite: Neves Briard J, Dufort G, Jacquin G, *et al*. *J NeuroIntervent Surg* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/neurintsurg-2021-017664

ABSTRACT

Background The COVID-19 pandemic has disrupted acute stroke care logistics, including delays in hyperacute management and decreased monitoring following endovascular therapy (EVT). We aimed to assess the impact of the pandemic on 90-day functional outcome among patients treated with EVT.

Methods This is an observational cohort study including all patients evaluated for an acute stroke between March 30, 2020 and September 30, 2020 (pandemic cohort) and 2019 (reference cohort) in a high-volume Canadian academic stroke center. We collected baseline characteristics, acute reperfusion treatment and management metrics. For EVT-treated patients, we assessed the modified Rankin score (mRS) at 90 days. We evaluated the impact of the pandemic on a 90-day favourable functional status (defined as mRS 0–2) and death using multivariable logistic regressions.

Results Among 383 and 339 patients included in the pandemic and reference cohorts, baseline characteristics were similar. Delays from symptom onset to evaluation and in-house treatment were longer during the early first wave, but returned to reference values in the subsequent months. Among the 127 and 136 EVT-treated patients in each respective cohort, favourable 90-day outcome occurred in 53/99 (53%) vs 52/109 (48%, p=0.40), whereas 22/99 (22%) and 28/109 (26%, p=0.56) patients died. In multivariable regressions, the pandemic period was not associated with 90-day favourable functional status (aOR 1.27, 95% CI 0.60 to 2.56) or death (aOR 0.74, 95% CI 0.33 to 1.63).

Conclusion In this single-center cohort study conducted in a Canadian pandemic epicenter, the first 6 months of the COVID-19 pandemic did not impact 90-day functional outcomes or death among EVT-treated patients.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has disrupted usual acute stroke care logistics worldwide.[1] We previously reported that the early pandemic was associated with increased delays from symptom onset to hospital presentation and to reperfusion treatment in our high-volume comprehensive stroke center (CSC) located in Montreal, a Canadian pandemic epicentre.[2] This observation has also been documented in other, although not all, CSCs around the globe.[3 4] In response to COVID-19, most CSCs adjusted their hyperacute stroke management procedures and their post-reperfusion monitoring protocols to minimize contact between healthcare professionals and potentially infected patients, as suggested by professional society recommendations and various experts.[5–7] It is currently unknown whether increased delays to presentation and treatment, as well as decreased neurological monitoring in the hours after reperfusion treatment, translate into worse long-term functional outcomes for patients suffering an acute stroke during the COVID-19 pandemic. Our primary objective was to assess the impact of the early pandemic on 90-day functional outcomes for patients with stroke treated with endovascular therapy (EVT) at our CSC. Our secondary objective was to evaluate whether the characteristics of patients with acute stroke and treatment metrics changed during the first wave (spring 2020) and its subsequent tail (summer 2020).

METHODS

We conducted an observational cohort study at our high-volume CSC for which exposure was defined as the emerging COVID-19 pandemic. Our CSC covers an urban population of approximately 2 million people and performs over 300 acute reperfusion therapies per year, including the majority of EVTs in our catchment area. Patients from our immediate catchment area with a Cincinnati Prehospital Stroke Screen score ≥1/3 and patients in an extended region with a score of 3/3 are brought directly to our CSC.[8] Patients evaluated at other adjacent centers who are eligible for EVT are also transferred as needed.

Since the outbreak of COVID-19 in Canada, Montreal has consistenly documented over one-quarter of the country's cases, making it the

pandemic's national epicentre. The first wave of the pandemic in Montreal occurred between March and June 2020, during which 27 378 COVID-19 cases were recorded, including 3761 hospitalizations and 3459 deaths (notably, 2620 deaths among residents of long-term care institutions).[9] A subsequent drop in the number of new cases (6883), hospitalizations (305) and deaths (30) due to COVID-19 was documented during the tail of the first wave, between July and September 2020. In the early first wave we implemented our modified code stroke protocol, which includes use of personal protective equipment during all acute stroke evaluations, as well as systematic COVID-19 laboratory screening of all patients evaluated for suspected acute stroke with a nasopharyngeal swab and PCR technology. We did not modify our procedure regarding airway management during EVT, reserving general anesthesia for cases with airway, respiratory, or hemodynamic compromise. To minimize contact between healthcare professionals and potentially infected patients, we modified our post-reperfusion monitoring protocol by decreaseasing the intensity of neurological and vital sign surveillance.[6]

In this study we used data from the Montreal Neurovascular and Stroke Data Repository (MONSTER), a routine electronic medical record tool allowing systematic guideline-based quality assurance, to compare a prospective cohort of consecutive adult patients evaluated for acute stroke between March 30 (date of formal implementation of our modified code stroke protocol) and September 30, 2020 with a retrospective reference cohort of patients evaluated for stroke during the corresponding time period of 2019. We excluded in-hospital strokes. We collected baseline characteristics, pre-stroke modified Rankin scale (mRS) and National Institutes of Health Stroke Scale (NIHSS) scores, delay from symptom onset to hospital presentation, diagnosis (ischemic stroke, including site of large vessel occlusion (LVO) when pertinent, hemorrhagic stroke or stroke mimic), Alberta Stroke Program Early CT score (ASPECTS), reperfusion treatment (thrombolysis and/or EVT), stroke management metrics (door-to-imaging, door-to-needle, door-to-puncture, and door-to-recanalization delays), and results from systematic COVID-19 PCR screening at admission. For patients treated with EVT, we also collected final angiographic recanalization (using the modified Thrombolysis In Cerebral Infarction (mTICI) scale, with mTICI 2b or 3 considered as favourable recanalization) and procedural complications (including arterial dissection or perforation, arterial reocclusion during the procedure and groin hematoma). Finally, a mRS-certified research coordinator assessed 90-day mRS by means of a standardized telephone interview with the patient or their proxy.

For data analysis, we stratified both cohorts into two seasonal subcohorts to reflect the dynamic nature of the pandemic: the spring subcohorts included patients evaluated between March 30 and June 31 (coinciding with the first wave) whereas the summer subcohorts included patients assessed between July 1 and September 30 (first wave tail). Univariate statistical testing was performed using t tests (continuous parametric data), Wilcoxon rank sum tests (continuous non-parametric data), and χ^2 tests (dichotomous data). We used logistic multivariable regressions to estimate the effect of the pandemic on a favorable 90-day functional outcome (defined as mRS 0–2) and on death at 90 days, controlling for potential confounders: patient age, sex, pre-stroke mRS, NIHSS, ASPECTS, treatment with thrombolysis, favourable mTICI and EVT procedural complications. We performed a sensitivity analysis by including an additional dummy variable in these regressions to account for the season during which the patient was evaluated for stroke (spring vs summer). We decided to exclude patients with missing 90-day outcomes from statistical analyses as the majority of these patients were lost to follow-up due to referral from another institution specifically for EVT. As these patients are likely to have more severe strokes and longer delays from symptom onset to recanalization, these data are not missing at random and do not qualify for statistical inference. Statistical analyses were performed with R-Studio (R version 4.0.2, Boston, Massachusetts, USA). The project was approved by our institutional Research Ethics Board (#20.013).

RESULTS

Patient characteristics and stroke management metrics

Overall, 383 and 339 patients were included in the pandemic and reference cohorts, respectively. Patient demographic and clinical characteristics were similar in the four subcohorts (table 1). The proportion of patients meeting standard eligibility criteria for thrombectomy (LVO, NIHSS ≥6, pre-stroke mRS 0–1, ASPECTS ≥6, delay from symptom onset to hospital presentation <6 hours) treated with EVT was 76/81 (94%) in the pandemic cohort and 71/73 (97%) in the reference cohort (p=0.47). Among these EVT-treated patients, subcohorts had similar baseline characteristics, although the 2020 spring cohort had slightly better baseline functional status (median (IQR) pre-stroke mRS 0 (0–1)) compared with the 2019 spring cohort (1 (0–2), p=0.01; table 2).

Stroke management metrics by subcohort are shown in table 3. The delay from symptom onset to hospital presentation was longer during the pandemic's first wave than the previous spring (142 (62–348) vs 101 (59–212) min, p=0.03), as were door-to-needle (34 (26–41) vs 24 (21–30) min, p=0.01) and door-to-recanalization times (98 (89–123) vs 80 (62–95) min, p=0.01) for patients directly evaluated at our institution. The door-to-needle delay improved in summer 2020, but remained longer for patients directly evaluated at our CSC (29 (26–39) vs 24 (21–32) min, p=0.03); other summer metrics were similar between pandemic and reference summer subcohorts. Figure 1 shows the median monthly reperfusion metrics for patients directly evaluated at our CSC, in addition to the number of individuals testing positive for COVID-19 in the community and the number of patients hospitalized for COVID-19 in the entire Montreal area. The most important differences between the 2019 and 2020 metrics appear to have occurred in the spring, corresponding to the first wave timeline.

COVID-19 status

Data on COVID-19 screening was available for 354/383 (92%) of patients in the pandemic cohort. Four patients (1%; two with an ischemic stroke and two with septic encephalopathy) tested positive at admission; these patients all presented between March and May 2020. Between June and September 2020, three additional patients (two with ischemic strokes, one with a stroke mimic) were evaluated for stroke after having tested positive in the community (25, 42, and 102 days, respectively, prior to presentation). Among this total of seven patients testing positive for COVID-19 on admission or during the weeks prior, one was treated with thrombolysis but none had LVO or underwent EVT. There were no cases of nosocomial COVID-19 infections in the pandemic cohort. Eight patients tested positive after discharge during follow-up in the clinic or a subsequent visit at the emergency department (median 100 days after admission for acute stroke (range 30–151)).

Outcomes in EVT-treated patients

Regarding short-term EVT outcomes, there were no differences between cohorts with respect to favourable final angiographic

Table 1 Clinical characteristics of the four subcohorts

	2020 pandemic cohort: spring (n=206)	2019 reference cohort: spring (n=178)	P value	2020 pandemic cohort: summer (n=177)	2019 reference cohort: summer (n=161)	P value
Demographic data						
Age, mean (SD)	69.9 (16.3)	72.1 (15.1)	0.18	69.9 (16.9)	68.9 (16.2)	0.67
Sex, n (%) male	99 (48)	84 (47)	0.87	96 (54)	75 (47)	0.16
Baseline comorbidities, n (%)						
Hypertension	114 (55)	107 (60)	0.35	93 (53)	94 (58)	0.28
Dyslipidemia	85 (41)	68 (38)	0.54	68 (39)	65 (40)	0.71
Diabetes mellitus	47 (23)	37 (21)	0.63	36 (20)	40 (25)	0.32
Atrial fibrillation or flutter	22 (11)	33 (19)	0.03	24 (14)	30 (19)	0.20
Prior ischemic stroke or TIA	43 (21)	34 (19)	0.67	22 (13)	27 (17)	0.26
Prior intracranial hemorrhage	6 (3)	2 (1)	0.22	3 (2)	2 (2)	0.91
Coronary or peripheral artery disease	26 (13)	29 (16)	0.31	36 (20)	27 (17)	0.40
Tobacco use	36 (17)	33 (19)	0.79	30 (17)	26 (16)	0.84
Prior antithrombotic treatment, n (%)						
Antiplatelet	65 (32)	54 (30)	0.79	53 (30)	43 (27)	0.51
Direct anticoagulant	18 (9)	15 (8)	0.91	25 (14)	22 (14)	0.90
Vitamin K antagonist	8 (4)	3 (2)	0.20	2 (1)	3 (2)	0.58
Patient flow, n (%)						
Direct evaluation	157 (76)	114 (64)	0.01	125 (71)	109 (68)	0.56
Transfer from another institution	49 (24)	64 (36)		52 (29)	52 (32)	
ED assessment, median (IQR)						
Pre-stroke mRS	1 (0–2)	1 (0–2)	0.74	1 (0–2)	1 (0–2)	0.47
NIHSS	10 (4–19)	12 (5–19)	0.52	12 (5–19)	10 (3–19)	0.21
ASPECTS	10 (8–10)	10 (8–10)	0.36	10 (8–10)	10 (9–10)	0.19
Diagnosis, n (%)						
Ischemic stroke	136 (66)	124 (70)	0.45*	120 (68)	116 (72)	0.39*
Anterior circulation LVO	61 (45)	79 (64)		76 (63)	61 (53)	
Posterior circulation LVO	10 (7)	4 (3)		5 (4)	9 (8)	
Hemorrhagic stroke	23 (11)	22 (12)		13 (7)	11 (7)	
Stroke mimic	47 (23)	32 (18)		22 (25)	34 (21)	
Ischemic stroke treatment, n (%)						
Thrombolysis	54 (40)	70 (56)	0.01	55 (45)	52 (45)	0.88
Thrombectomy	54 (40)	74 (60)	<0.01†	73 (61)	62 (53)	0.25†

Spring: March 30 to June 31.
Summer: July 1 to September 30.
*P value for the proportion of ischemic strokes among all patients.
†P value for the proportion of thrombectomies among ischemic stroke patients.
ASPECTS, Alberta Stroke Program Early CT Score; ED, emergency department; LVO, large vessel occlusion; mRS, modified Rankin score; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack.

result (mTICI 2b–3: 92% vs 85%, p=0.08) or procedural complications (17% vs 18%, p=0.69). Data for 90-day mRS were available for 99/127 (78%) patients in the pandemic cohort and for 109/136 (80%) in the reference cohort. The proportion of favourable 90-day outcome (mRS 0–2) was 53/99 (53%) vs 52/109 (48%, p=0.40), whereas 22/99 (22%) and 28/109 (26%, p=0.56) patients died in each respective cohort. Outcome data did not differ by season (online supplemental material). In multivariable logistic regressions, the pandemic period was not independently associated with favourable 90-day functional outcome (aOR 1.27, 95% CI 0.60 to 2.56, p=0.56) or death (aOR 0.74, 95% CI 0.33 to 1.63, p=0.46). Addition of a dummy season variable did not alter the regression estimate effects (online supplemental material).

DISCUSSION

In a prior study focused on the early impact of the COVID-19 pandemic at our CSC, we observed increased delays between symptom onset, evaluation, and treatment for acute stroke.[2] This was in line with findings from other stroke centers worldwide.[3 4] However, the impact of increased delays, in addition to decreased neurological monitoring after reperfusion treatment, on 90-day functional outcomes for EVT-treated patients has not yet been described. In this study we did not observe a difference in short or long-term outcomes between patients treated with EVT in 2020 compared with 2019, despite longer delays to presentation and recanalization during the first wave of the pandemic. This may in part be explained by the fact that treatment times remained within recommended ranges despite

Table 2 Clinical characteristics of EVT-treated patients in the four subcohorts

	2020 pandemic cohort: spring (n=54)	2019 reference cohort: spring (n=74)	P value	2020 pandemic cohort: summer (n=73)	2019 reference cohort: summer (n=62)	P value
Demographic data						
Age, mean (SD)	68.2 (16.8)	73.4 (12.4)	0.06	71.1 (11.9)	71.0 (12.5)	0.95
Sex, male n (%)	28 (52)	34 (46)	0.51	37 (51)	31 (50)	0.94
Baseline comorbidities, n (%)						
Hypertension	25 (46)	43 (58)	0.19	41 (56)	40 (65)	0.32
Dyslipidemia	14 (26)	28 (38)	0.16	31 (42)	23 (37)	0.53
Diabetes mellitus	6 (11)	10 (14)	0.68	16 (22)	16 (26)	0.60
Atrial fibrillation or flutter	9 (17)	21 (28)	0.12	8 (11)	14 (23)	0.07
Prior ischemic stroke or TIA	8 (15)	14 (19)	0.54	7 (10)	8 (13)	0.54
Prior intracranial hemorrhage	0 (0)	1 (1)	0.39	1 (1)	0 (0)	0.36
Coronary or peripheral artery disease	4 (7)	10 (14)	0.27	16 (22)	11 (18)	0.55
Tobacco use	7 (13)	14 (19)	0.37	13 (18)	6 (10)	0.18
Prior antithrombotic treatment, n (%)						
Antiplatelet	13 (24)	18 (24)	0.97	23 (32)	18 (29)	0.76
Direct anticoagulant	4 (7)	9 (12)	0.38	6 (8)	10 (16)	0.16
Vitamin K antagonist	2 (4)	3 (4)	0.92	2 (3)	1 (2)	0.66
Patient flow, n (%)						
Direct evaluation	14 (26)	22 (30)	0.63	25 (34)	20 (32)	0.81
Transfer from another institution	40 (74)	52 (70)		48 (66)	42 (68)	
ED assessment, median (IQR)						
Pre-stroke mRS	0 (0–1)	1 (0–2)	0.01	1 (0–1)	1 (0–1)	0.79
NIHSS	18 (12–25)	18 (14–23)	0.90	17 (12–24)	18 (23–23)	0.68
ASPECTS	9 (8–10)	9 (8–10)	0.76	9 (8–10)	10 (9–10)	0.20
Ischemic stroke treatment, n (%)						
Thrombolysis	32 (59)	47 (64)	0.62	39 (53)	37 (60)	0.47
Thrombectomy	54 (100)	74 (100)		73 (100)	62 (100)	
Under general anesthesia	7 (13)	7 (9)	0.53	5 (7)	9 (15)	0.15
ICA	7 (13)	3 (4)		10 (14)	3 (5)	
MCA (M1 segment)	28 (52)	36 (49)		42 (57)	38 (61)	
MCA (proximal M2 segment)	3 (6)	18 (24)		4 (6)	5 (8)	
Tandem occlusion (ICA +M1/M2)	9 (17)	13 (18)		14 (19)	8 (13)	
Vertebrobasilar artery	6 (11)	3 (4)		4 (5)	8 (13)	
Other occlusion combination	0 (0)	1 (1)		0 (0)	0 (0)	

ASPECTS, Alberta Stroke Program Early CT Score; ED, emergency department; ICA, internal carotid artery; MCA, middle cerebral artery; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

these increased delays. Comparable outcomes between groups also suggest that reduced intensity of clinical monitoring did not negatively affect outcomes, as we previously anticipated.[6] Furthermore, in-house acute stroke metrics normalized during the tail of the first wave—that is, the summer months following the first wave—except door-to-needle delays which improved without reaching reference cohort levels. This improvement in most acute stroke metrics is reassuring and likely reflects a learning curve as stroke teams adjusted to new pandemic protocols. As our stroke team receives prehospital prenotification for all suspected acute stroke cases, door-to-imaging delays were not disturbed by the early pandemic. However, door-to-needle and door-to-puncture times were longer during the first wave, likely due to delays in secure patient transportation between the neuroimaging suite in the emergency department to the resuscitation bay and finally the angiography suite. We hypothesize that the learning curve was, for the most part, associated with improvement in patient flow between these different units within the hospital. Strict bans on family visitors enforced early during the pandemic could also have contributed to longer door-to-needle and door-to-puncture times by making the collection of past medical history and contraindications to reperfusion treatment more time consuming.

Whereas some centers have reported worse short-term EVT outcomes during the early pandemic,[10 11] possibly due to increased use of periprocedural general anesthesia, our data are concordant with other previous reports that did not observe a significant impact of the early pandemic on short-term EVT outcomes.[12 13] The current paper contributes additional evidence on this phenomenon as it is the first, to our knowledge, to report on the long-term outcomes of patients treated with EVT during the first wave of the pandemic and its subsequent tail.

Table 3 Ischemic stroke treatment metrics

	2020 pandemic cohort: spring (n=206)	2019 reference cohort: spring (n=178)	P value	2020 pandemic cohort: summer (n=177)	2019 reference cohort: summer (n=161)	P value
Stroke evaluation metrics						
Symptom onset to hospital presentation delay						
All patients	142 (62–348)	101 (59–212)	0.03	110 (52–256)	97 (61–233)	0.73
All ischemic stroke patients	169 (63–353)	116 (62–229)	0.12	110 (50–261)	97 (58–233)	0.91
Door-to-imaging						
Direct evaluation	18 (13–23)	16 (10–22)	0.13	19 (14–24)	18 (11–23)	0.19
Transfer from another institution	29 (20–46)	21 (15–31)	0.04	21 (13–34)	21 (14–41)	0.78
Reperfusion metrics						
Door-to-needle						
Direct evaluation	34 (26–41)	24 (21–30)	0.01	29 (26–39)	24 (21–32)	0.03
Transfer from another institution	52 (43–79)	61 (45–76)	0.55	61 (47–78)	48 (39–65)	0.09
Door-to-puncture						
Direct evaluation	68 (60–90)	56 (43–70)	0.03	73 (56–83)	74 (52–79)	0.99
Transfer from another institution (door: referring ED)	147 (133–174)	153 (121–184)	0.88	149 (137–177)	149 (130–185)	0.61
Transfer from another institution (door: CSC ED)	20 (15–24)	16 (11–24)	0.10	18 (16–21)	16 (12–24)	0.18
Door-to-recanalization						
Direct evaluation	98 (89–123)	80 (62–95)	0.01	98 (82–115)	104 (90–117)	0.78
Transfer from another institution (door: referring ED)	190 (160–228)	189 (154–230)	0.97	190 (161–206)	183 (162–217)	0.94
Transfer from another institution (door: CSC ED)	50 (37–69)	42 (32–64)	0.25	50 (41–71)	53 (37–69)	0.64

CSC, Comprehensive Stroke Center; ED, emergency department.

During the first 6 months of the pandemic our CSC did not experience some of the concerning changes that were observed in other regional and international cohorts. For instance, we did not observe a higher incidence of LVO stroke in young patients during the first wave, as was the case in New York City, another major North American pandemic epicenter.[14 15] We also did not document a decrease in acute stroke evaluation and treatment volumes.[15–18] These differences likely highlight the local

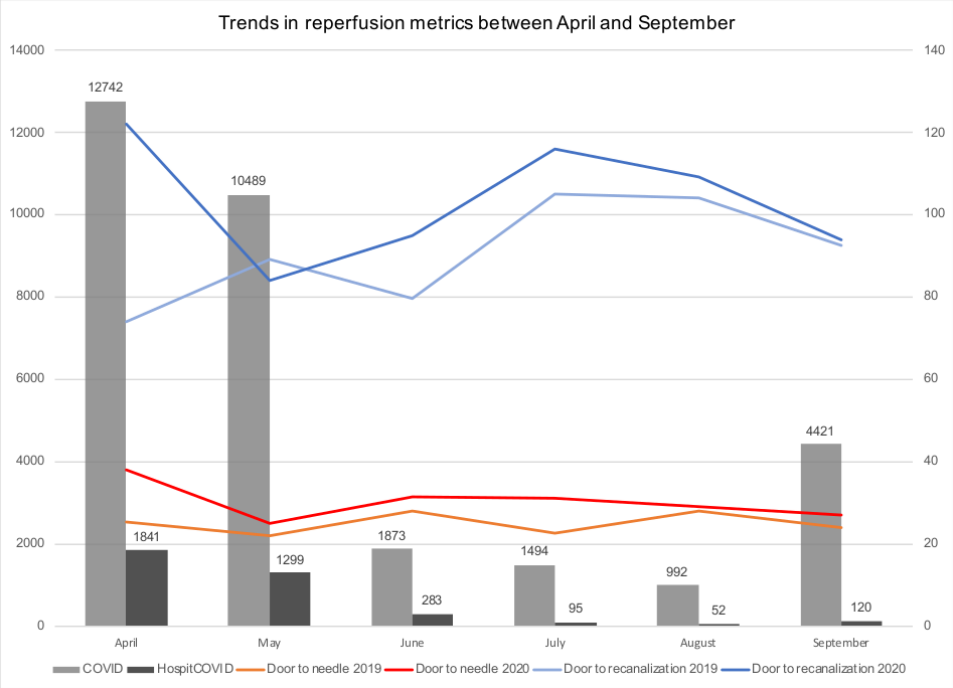

Figure 1 Reperfusion metrics by month. Metrics are presented as median values. COVID-19: total number of individuals testing positive for COVID-19 in the Montreal area. HospitCOVID: total number of individuals hospitalized for COVID-19 in the Montreal area.

heterogeneity of COVID-19 epidemiology and that the impact of the pandemic is likely to differ accordingly.

The major strength of our study is the detailed data captured on all consecutive patients evaluated for a possible acute stroke during the study period and the high proportion (approximately 80%) of EVT-treated patients for which 90-day outcomes were documented.

However, it does have several limitations. As a single-center study of an academic CSC, the results may not be generalizable to all institutions. As mentioned previously, since local COVID-19 epidemiology is highly variable within different jurisdictions, its impact probably differs in consequence. For instance, most individuals in Montreal who were infected with COVID-19 during the first wave resided in long-term care homes. These patients are less likely to be transferred to hospital in the event of an acute medical condition due to their baseline comorbidities and objectives of care, which may explain why we did not observe a single case of LVO stroke in a patient with COVID-19 during the first 6 months of the pandemic despite our high volume of acute stroke evaluations, and our location within a Canadian pandemic epicenter. However, despite variations in local epidemiology, most CSCs around the globe adapted their hyperacute management and post-reperfusion monitoring with protocols similar to ours, following guidance from the Society of NeuroInterventional Surgery and others.[5 19] Therefore, our findings are likely applicable to a broad variety of CSCs. A second limitation of our study is the possibility of bias resulting from stricter patient selection for EVT during the pandemic, reflected by better pre-stroke functional status of EVT-treated patients in the pandemic cohort, which would could skew results towards the null hypothesis. The magnitude of this selection bias, if present, is likely small as the differences between mRS 0 and 1 are often clinically subjective, and since the proportion of patients with ischemic stroke meeting standard eligibility criteria for EVT who were treated with this therapy remained similar between cohorts (94% vs 97%, p=0.47). Finally, a third limitation was the lack of detailed data on socioeconomic patient characteristics. Some studies conducted in the USA have highlighted that the pandemic has worsened disparities in healthcare access and outcomes following acute stroke, although these findings may perhaps be less applicable to countries such as Canada with universal public healthcare coverage.[20 21]

CONCLUSIONS

In this single-center cohort study conducted in a Canadian pandemic epicenter, the first 6 months of the COVID-19 pandemic did not impact 90-day functional outcomes or death among EVT-treated patients. Reassuringly, increased delays between symptom onset, presentation, and treatment returned to reference pre-pandemic values during the summer months following the first wave. Replication of these findings in other settings and with larger datasets will be important.

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Contributors JNB: Conceptualized and designed the study, collected and analyzed the data, drafted the manuscript. CD, GD: Collected and analyzed the data, drafted the manuscript. GJ: Collected and analyzed the data, reviewed the manuscript for intellectual content. WA, OB, WB, ND, YD, JDBD, JE, LG, DEI, CO, JR, DR, CS, AW: Reviewed the manuscript for intellectual content. AYP: Conceptualized and designed the study, analyzed the data, revised the manuscript, supervised the project.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This project was approved by the institutional Research Ethics Board of the Centre hospitalier de l'Université de Montréal (#20.013).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request by a qualified investigator.

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