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Preoperative collateral circulation state predicts prognosis in patients with acute ischemic stroke undergoing mechanical thrombectomy

Stav kolaterálního oběhu před výkonem predikuje prognózu pacientů s akutní ischemickou cévní mozkovou příhodou podstupujících mechanickou trombektomii

Abstract

Background: Acute ischemic stroke (AIS) patients tend to have rapid disease progression, with collateral circulation gradually failing over time. **Aim:** We aimed to investigate the preoperative collateral circulation state in AIS patients undergoing mechanical thrombectomy and its association with clinical prognosis. **Methods:** Subjects (N = 112) were recruited from AIS patients receiving mechanical thrombectomy during January 2019 and December 2022. The preoperative collateral circulation state in ischemic regions was assessed. Analyses were carried out on different occlusion sites and potential compensation pathways of collateral circulation. **Results:** Subjects presenting poor preoperative collateral circulation had a significantly raised prognostic modified Rankin Scale (mRS) score compared with those exhibiting good preoperative collateral circulation ($P < 0.05$). The preoperative collateral circulation state displayed a significantly positive correlation with prognosis ($r = 0.834$; $P < 0.05$). The baseline National Institutes of Health Stroke Scale score, time from admission to recanalization, modified Thrombolysis in Cerebral Infarction score, and preoperative collateral circulation state were risk factors in terms of prognosis ($P < 0.05$). Sensitivity, area under the curve, and specificity of preoperative collateral circulatory state for predicting the prognosis reached 89.60%, 0.879 (95% CI = 0.800–0.945), and 86.53%, respectively. **Conclusion:** Collateral circulation before mechanical thrombectomy has a significant relationship to AIS patients' prognosis, and a poor one serves as a risk factor independently influencing the prognosis.

Souhrn

Východiska: U pacientů s akutní ischemickou cévní mozkovou příhodou (iCMP) je tendence k rychlému postupu onemocnění, přičemž kolaterální oběh v průběhu času postupně selhává. **Cíl:** Naším cílem bylo vyšetřit stav kolaterálního oběhu před výkonem u pacientů s iCMP podstupujících mechanickou trombektomii a jeho souvislost s klinickou prognózou. **Metody:** Subjekty ($n = 112$) byly rekrutovány z pacientů s iCMP podstupujících mechanickou trombektomii v období od ledna 2019 do prosince 2022. Byl hodnocen stav kolaterálního oběhu v ischemických oblastech před výkonem. Analýzy byly prováděny na různých místech okluze a v potenciálních kompenzačních cestách kolaterálního oběhu. **Výsledky:** Subjekty se špatným kolaterálním oběhem před výkonem měly významně zvýšené prognostické skóre modifikované Rankinovy škály (mRS) ve srovnání s těmi, které vykazovaly dobrý kolaterální oběh před výkonem ($p < 0,05$). Mezi stavem kolaterálního oběhu před výkonem a prognózou byla pozorována významná pozitivní korelace ($r = 0,834$; $p < 0,05$). Mezi rizikové faktory z hlediska prognózy patřily skóre National Institutes of Health Stroke Scale, doba od přijetí pacienta do rekanalizace, modifikované skóre Thrombolysis in Cerebral Infarction a stav kolaterálního oběhu před výkonem ($p < 0,05$). Senzitivita, plocha pod křivkou a specifita stavu kolaterálního oběhu před výkonem pro predikci prognózy dosáhly hodnot 89,60 %, 0,879 (95% CI = 0,800–0,945) a 86,53 %. **Závěr:** Kolaterální oběh před mechanickou trombektomií má významný vztah k prognóze pacientů s iCMP a špatný kolaterální oběh slouží jako rizikový faktor, který nezávisle ovlivňuje prognózu.

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Introduction

Cardiovascular diseases have now witnessed increasing prevalence and mortality rates as the aging problem is getting worse in the world. Stroke refers to damage to focal or overall brain tissues owing to cerebrovascular injuries caused by various factors, among which acute ischemic stroke (AIS) has the highest prevalence. In the case of AIS, patients mainly have focal neurological deficit symptoms such as sensory disturbance and hemiplegia, and the prognosis of mild stroke is usually good, but patients with severe stroke experience coma, shock, and other serious adverse events, which are life-threatening [1,2]. Over one-third of AIS cases are attributed to large vessel occlusion, which is often associated with rapid disease progression and poor outcomes if not treated promptly [3]. Therefore, timely vascular recanalization is of great importance for saving the life of these patients. For AIS patients with onset time < 12 h, mechanical thrombectomy exhibits better efficacy and higher vascular reperfusion rate than intravenous and intra-arterial thrombolysis, which has been recommended as the preferred regimen with the highest benefit in relevant guidelines from the Chinese Society of Neurology of the Chinese Medical Association, American Stroke Association, and European Stroke Organisation [4].

Despite the increasing success in restoring vessel patency through mechanical thrombectomy, a considerable number of patients still experience poor clinical outcomes [5]. Therefore, factors affecting the prognosis of AIS patients undergoing mechanical thrombectomy have attracted particular attention, one of the most critical being collateral circulation. Collateral circulation refers to a network of pre-existing or newly developed vascular anastomoses that compensate for reduced cerebral blood flow by redirecting perfusion to ischemic brain regions [6]. These pathways can help

preserve the penumbra, limit infarct expansion, and maintain metabolic function during vessel occlusion. The extent and quality of collateral circulation are key determinants of clinical outcome in AIS. Good collateral flow is associated with a smaller infarct core, slower infarct progression, higher rates of successful reperfusion, and better functional recovery after mechanical thrombectomy. Conversely, poor collateral status may lead to rapid infarct growth, incomplete reperfusion, and higher rates of hemorrhagic transformation or mortality, even when large vessels are successfully recanalized [7].

Given these considerations, the evaluation of preoperative collateral circulation status may serve as a valuable prognostic indicator in AIS patients undergoing mechanical thrombectomy. In this study, we aimed to assess preoperative collateral circulation in patients with AIS treated by mechanical thrombectomy and to analyze its relationship with the prognosis. We also explored the differences across individual occlusion sites and potential compensatory pathways, with the goal of contributing to individualized therapeutic strategies and improving patient outcomes.

Materials and methods

Subjects

Subjects (112 in total) were sourced from AIS patients in Xing'an League People's Hospital undergoing mechanical thrombectomy during January 2019 and December 2022, including 67 males and 45 females with an age of 35–80 years (65.65 ± 5.98 years on average).

The following inclusion criteria were used:

1. patients diagnosed with AIS based on standard clinical and imaging criteria consistent with national guidelines from 2019 to 2022; for reference, the diagnostic criteria aligned with those described in the 2023 Chinese Guidelines on the Dia-

gnosis and Treatment of Acute Ischemic Stroke [8];

2. those with an onset time ≤ 6 h prior to diagnosis;
3. those with indication for mechanical thrombectomy;
4. those with clearly identified occlusion sites;
5. those with diameters of ≥ 2 mm on the blood vessels of identified occlusion sites;
6. those with a National Institutes of Health Stroke Scale (NIHSS) score ≥ 6 points;
7. those with a modified Thrombolysis in Cerebral Infarction (mTICI) grade 2b or 3;
8. those with normal cognitive functions before disease onset;
9. those with normal behavioral functions before disease onset;
10. those with a modified Rankin Scale (mRS) score < 2 points prior to stroke onset;
11. those who and whose families provided the informed consent form with a signature after being informed of this study.

Exclusion criteria involved:

1. patients with extensive infarct lesions (exceeding 1/3 of the blood supply territory of the middle cerebral artery) confirmed by non-contrast CT or diffusion-weighted MRI;
2. those with AIS caused by traumatic brain injuries or intracranial malignant tumors;
3. those with unsuccessful recanalization after mechanical thrombectomy;
4. those with an mTICI grade 0–2a;
5. those with a history of brain surgery;
6. those with dysfunction of vital organs or uncontrolled infections;
7. those with mental disorders.

Assessment of the preoperative collateral circulation state

Before mechanical thrombectomy, all patients underwent DSA examination to assess the collateral circulation state in ischemic regions by adhering to the guidelines from

Tab. 1. Evaluation criteria for collateral circulation state in ischemic regions.

Grade	Evaluation criteria
0	no collateral blood flow in ischemic regions
1	low-rate collateral blood flow around ischemic regions, presenting continuous perfusion deficits
2	high-rate collateral blood flow around ischemic regions, showing only partial blood flow flowing into ischemic regions
3	low-rate but complete blood flow into ischemic regions in late venous stages
4	retrograde perfusion of blood flow, exhibiting high-rate and complete perfusion of blood flow to the entire ischemic region

Tab. 2. Analyses of different occlusion sites and possible compensatory pathways of collateral circulation in acute ischemic stroke patients.

Occlusion site	Collateral circulation pathway
middle cerebral artery	ipsilateral anterior and posterior cerebral artery leptomeningeal vascular networks branches adjacent to the ipsilateral middle cerebral artery as well as ipsilateral anterior and posterior cerebral artery leptomeningeal vascular networks
distal part of the internal carotid artery	anterior communicating artery, together with ipsilateral posterior cerebral artery leptomeningeal vascular network
proximal part of the internal carotid artery	external-internal carotid anastomosis, anterior communicating artery, and ipsilateral posterior cerebral artery leptomeningeal vascular network
internal carotid artery – ipsilateral middle cerebral artery or anterior cerebral artery in tandem	branches adjacent to the ipsilateral middle cerebral artery plus ipsilateral anterior and posterior cerebral artery leptomeningeal vascular networks or contralateral anterior cerebral artery branches

Tab. 3. Prognostic modified Rankin Scale scoring criteria.

Score	Evaluation criteria
0 point	completely asymptomatic
1 point	symptomatic, with no apparent functional impairment, with ability to accomplish daily work as well as living activities independently
2 points	mildly disabled, with inability to perform all activities prior to the onset of the disease but ability to look after themselves
3 points	moderately disabled, requiring care from others, and able to walk on their own
4 points	seriously disabled, requiring care from others, and unable to walk on their own
5 points	bedridden, completely dependent on the care from others, and unable to control bladder and bowel movements

the American Society of Interventional and Therapeutic Neuroradiology (ASITN)/Society of Interventional Radiology (SIR) [9], and the evaluation criteria are stated in Tab. 1. Collateral circulation was considered poor if the grade was 0–2 or good if the grade was 3–4. Analysis on possible compensatory pathways of collateral circulation was carried out among patients with different occlusion sites. With middle cerebral artery occlusion present, the secondary collateral circulation was involved. When the internal carotid artery was occluded in the distal part, collateral circulation was triggered for blood perfusion maintenance. The primary and secondary collateral circulation was involved when the internal carotid artery experienced occlusion of the proximal part, and for occlusion of the internal carotid artery-ipsilateral middle cerebral artery or anterior cerebral artery in tandem, the secondary collateral circulation was involved (Tab. 2).

Follow-up following mechanical thrombectomy

Patients were followed up for 90 days after mechanical thrombectomy, and their prog-

nosis was evaluated by the mRS score, with corresponding evaluation criteria shown in Tab. 3. A poor prognosis group (mRS score: 3–5 points) plus a good prognosis group (mRS score: 0–2 points) were established for patient assignment on the basis of their prognostic outcomes after mechanical thrombectomy. The following data were collected from the two groups of patients: sex, age, past medical history, baseline NIHSS score, cause of stroke (large artery atherosclerotic [LAA] or cardioembolic infarction [CE]), onset-to-admission time, occlusion site, admission-to-revascularization time, number of thrombectomy passages, mTICI grade, and preoperative collateral circulation state.

Statistical analysis

Statistical analysis was accomplished using SPSS 25.0 software (IBM, Armonk, NY, USA). The expression format of mean \pm standard deviation ($x \pm s$) together with the independent samples t-test for intergroup comparison was employed for measurement data. Description of percentage (%) and comparison by the χ^2 test between the two

groups were used for enumeration of the data. Spearman analysis was implemented to explore the correlation between preoperative collateral circulation state and prognosis in AIS patients receiving mechanical thrombectomy. Risk factors contributing to the prognosis were identified through a multivariate logistic regression analysis. Performance of collateral circulation before mechanical thrombectomy in AIS patients regarding prognosis assessment was determined through a receiver operating characteristic (ROC) curve analysis, with the area under the ROC curve (AUC) of > 0.7 indicating high predictive efficiency. $P < 0.05$ indicated a difference in statistical significance.

Results

The preoperative collateral circulation state was further evaluated by ASITN/SIR criteria, and the results showed that 75 patients had poor collateral circulation, accounting for 66.96%.

Subjects presenting with poor preoperative collateral circulation had a significantly raised prognostic mRS score compared with those with a good one ($P < 0.05$) (Tab. 4). It

Tab. 4. Prognostic mRS scores in acute ischemic stroke patients undergoing mechanical thrombectomy with different preoperative collateral circulation states [(x ± s), point].

Collateral circulation state	N	Prognostic mRS score
poor	75	3.78 ± 0.35
good	37	0.87 ± 0.10
t		49.480
P		< 0.001

mRS – modified Rankin Scale; N – number

was revealed by Spearman correlation analysis that the preoperative collateral circulation state had a significant correlation with the prognosis of AIS patients receiving mechanical thrombectomy ($r = 0.834$; $P < 0.05$) (Fig. 1).

Higher baseline NIHSS scores and proportions of subjects with a stroke history, patients having ≥ 3 thrombectomy passages, patients with an mTICI grade 2b, patients with poor preoperative collateral circulation, and longer admission-to-revascularization time were observed more often in the poor prognosis group than in the good prognosis group ($P < 0.05$). Other data in the two groups showed no differences of statistical significance ($P > 0.05$) (Tab. 5).

The dependent variable was set as the prognosis of AIS patients receiving mechanical thrombectomy, while the data of statistical difference between the two groups were determined as independent variables to implement the multivariate logistic regression analysis. According to the findings, the baseline NIHSS score, admission-to-revascularization time, mTICI grade, and preoperative collateral circulation were risk factors impacting the prognosis of AIS patients receiving mechanical thrombectomy ($P < 0.05$) (Tab. 6).

According to the ROC curve analysis, the AUC of the preoperative collateral circulation for prognosis evaluation among AIS subjects undergoing mechanical thrombectomy reached 0.879 (95% CI = 0.800–0.945), with a sensitivity of 89.60% and specificity of 86.53% (Fig. 2).

Discussion

As a major disease resulting in disability and death in humans, AIS progresses rapidly, so taking effective treatment measures in time is particularly important for hindering progressive hypoxic-ischemic injuries in brain

tissues [10]. Currently, the safety and effectiveness of mechanical thrombectomy in clinical treatment of AIS have been demonstrated, which is characterized by rapid removal of thrombi and shortening of the time to recanalization [11,12]. However, some patients undergoing mechanical thrombectomy still have poor prognostic outcomes, which are clinically believed to be attributed to various factors, including collateral circulation state.

There are three compensatory pathways of collateral circulation, namely primary collateral circulation, secondary collateral circulation, and tertiary collateral circulation [13–15]. Under normal circumstances, the posterior and anterior communicating arteries are closed, and the primary collateral circulation, that is the circle of Willis, is triggered with reduced blood flow ascribed to serious internal artery occlusion or stenosis on one side. After the primary collateral circulation is triggered, the aforementioned communicating arteries are open to provide compensatory blood flow to ischemic regions to delay internal injuries. If the primary collateral circulation cannot meet the needs for a blood supply, the secondary collateral circulation, including the ophthalmic artery and primary leptomeningeal collateral circulation, is activated. The ophthalmic artery starts functions when internal carotid artery occlusion or stenosis occurs, enabling blood flow to flow back from the external carotid artery to the internal artery [16]. As the last compensatory pathway, the tertiary collateral circulation is a supplement to the above-mentioned compensatory pathways, and it restores blood flow in ischemic regions by promoting vascularization, which, however, takes several days [17]. In the case of AIS, the perfusion pressure and flow of the cerebrovascular network are in-

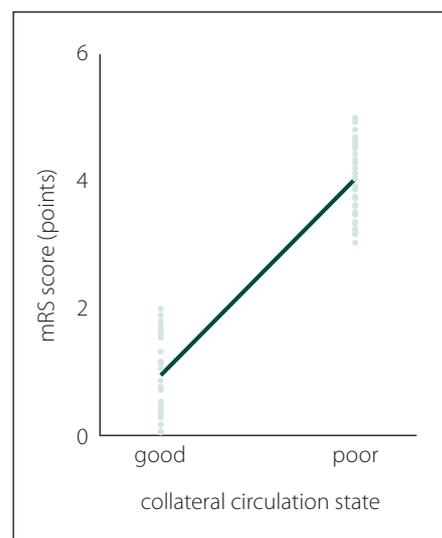


Fig. 1. Correlation of preoperative collateral circulation state with prognosis of acute ischemic stroke patients undergoing mechanical thrombectomy.

mRS – modified Rankin scale

Obr. 1. Korelace stavu předoperačního kolaterálního oběhu s prognózou pacientů s akutní ischemickou cévní mozkovou příhodou podstupujících mechanickou trombektomii.

mRS – modifikovaná Rankinova škála

creased by collateral circulation through various compensatory pathways, so as to relieve ischemic injuries in brain tissues. Besides, collateral circulation through various pathways can also limit infarct core size, avoiding generating large ischemic penumbra, extending the time window for effective treatment, and ultimately avoiding irreversible damages possibly leading to worse clinical outcomes [18]. Cao et al. [19] discovered that AIS patients with worse collateral circulation had worse outcomes. In this study, the occlusion sites and possible collateral circulation sources were analyzed in 112 AIS patients, and the results revealed that there were some differences in the compensatory mechanism of collateral circulation among subjects suffering from occlusion at different sites. Specifically, in middle cerebral artery occlusion, the secondary collateral circulation was mainly involved, and the prognosis was acceptable if the secondary collateral circulation was activated in time. In patients whose internal carotid artery was occluded at the distal part, collateral circulation was triggered to maintain blood perfusion, while in those with the internal carotid artery occluded at the proximal part, the primary plus second-

Tab. 5. Comparisons of clinical data of good and poor prognosis groups.

Clinical data		Poor prognosis group (N = 34)	Good prognosis group (N = 78)	χ^2/t	P
Sex; N (%)	male	20 (58.82)	47 (60.26)	0.020	0.887
	female	14 (41.18)	31 (39.74)		
Age (x ± s, years)	≥ 60	15 (44.12)	28 (35.90)	0.676	0.411
	< 60	19 (55.88)	50 (64.10)		
Smoking; N (%)		10 (29.41)	32 (41.03)	1.363	0.243
Alcohol drinking; N (%)		6 (17.65)	25 (32.05)	2.454	0.117
Arterial hypertension; N (%)		12 (35.29)	19 (24.36)	1.414	0.234
Diabetes mellitus; N (%)		8 (23.53)	26 (33.33)	1.077	0.299
Hyperlipidemia; N (%)		11 (32.35)	24 (30.77)	0.028	0.868
History of stroke; N (%)		5 (14.71)	3 (3.85)	4.210	0.040
Baseline NIHSS score (x ± s, points)		12.65±1.89	10.12±1.78	6.788	<0.001
Cause of stroke; N (%)	LAA	21 (61.76)	35 (44.87)	2.703	0.100
	CE	13 (38.24)	43 (55.13)		
Occlusion site; N (%)	middle cerebral artery	12 (35.29)	27 (34.62)	0.178	0.981
	distal part of the internal carotid artery	9 (26.47)	20 (25.64)		
	proximal part of the internal carotid artery	8 (23.53)	21 (26.92)		
	internal carotid artery – ipsilateral middle cerebral artery or anterior cerebral artery in tandem	5 (14.71)	10 (12.82)		
Onset-to-admission time (x ± s, h)		3.14 ± 0.46	3.20 ± 0.54	0.564	0.574
Admission-to-recanalization time (x ± s, min)		154.45 ± 17.87	105.00 ± 16.93	13.980	< 0.001
Number of thrombectomy passages (x ± s)	≥ 3	21 (61.76)	20 (25.64)	13.315	< 0.001
	< 3	13 (38.24)	58 (74.36)		
mTICI grade; N (%)	grade 2b	12 (35.29)	10 (12.82)	7.577	0.006
	grade 3	22 (64.71)	68 (87.18)		
Preoperative collateral circulation state; N (%)	poor	28 (82.35)	47 (60.26)	5.226	0.022
	good	6 (17.65)	31 (39.74)		

CE – cardioembolic infarction; LAA – large artery atherosclerotic; mTICI – modified Thrombolysis in Cerebral Infarction; N – number; NIHSS – National Institutes of Health Stroke Scale

ary collateral circulation was triggered, which provided compensatory blood flow to ischemic regions and enabled patients to have a good prognosis. In patients with tandem occlusion, the secondary collateral circulation got involved. Furthermore, it was discovered that 75 subjects in the present research had unsatisfactory collateral circulation, accounting for 66.96%, which is similar to previous findings that over 60% of AIS patients have poor collateral circulation.

In AIS patients, the collateral circulation state is a vital basis for determining prognostic outcomes [20–22]. In this study, preoperative collateral circulation state was investigated for its role in influencing the prognosis

of AIS patients treated with mechanical thrombectomy based on the analysis of the collateral circulation. Results denoted that the mRS score was evidently increased in subjects manifesting poor preoperative collateral circulation in contrast to those presenting with good preoperative collateral circulation, and that an obvious relation was found between collateral circulation state and mRS score, which are similar to findings of Liu et al. [23]. Moreover, AIS patients were followed up for prognosis after mechanical thrombectomy in this study to further clarify the correlation between preoperative collateral circulation state and prognosis in AIS patients receiving mechanical thrombec-

tomy. It was uncovered that in addition to the baseline NIHSS score, admission-to-recanalization time, mTICI grade, and preoperative collateral circulation state also affected the prognosis of AIS patients, further confirming the correlation between preoperative collateral circulation state and prognosis of AIS patients undergoing mechanical thrombectomy.

Furthermore, ROC curves were plotted, based on which it was found that regarding the prognosis evaluation for AIS patients undergoing mechanical thrombectomy, preoperative collateral circulation state yielded an AUC of 0.879 and a sensitivity of 89.60% and specificity of 86.53%. These re-

Tab. 6. Results of multivariate analysis of risk factors for prognosis of acute ischemic stroke patients undergoing mechanical thrombectomy.

Factor	Assignment	β	S.E.	Wald	P	OR (95% CI)
History of stroke	yes = 1, no = 0	0.149	0.098	1.178	0.126	1.002 (0.786~1.807)
Baseline NIHSS score	original value entered	0.886	0.562	9.203	< 0.001	4.002 (2.276~10.907)
Number of thrombectomy passages	$\geq 3 = 1, < 3 = 0$	0.165	0.113	1.970	0.120	0.979 (0.823~1.980)
Admission-to-recanalization time	original value entered	1.020	0.448	5.674	0.023	2.786 (1.254~5.489)
mTICI grade	grade 2b = 1, grade 3 = 0	0.970	0.410	4.898	0.029	2.172 (1.276~6.891)
Preoperative collateral circulation	poor = 1, good = 0	1.045	0.621	8.798	< 0.001	3.544 (2.189~8.933)

mTICI – modified Thrombolysis in Cerebral Infarction; NIHSS – National Institutes of Health Stroke Scale

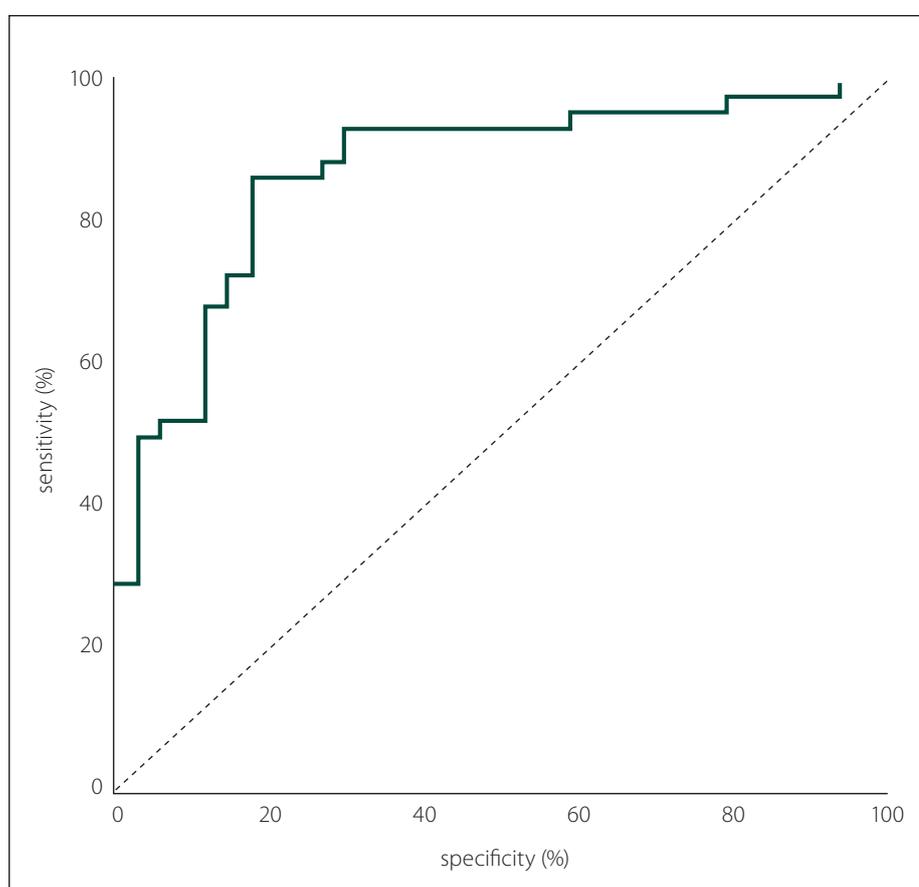


Fig. 2. Receiver operating characteristic curve of preoperative collateral circulation for prognosis evaluation among acute ischemic stroke subjects undergoing mechanical thrombectomy.

Fig. 2. Křivky receiver operating characteristic kolaterální cirkulace před výkonem pro hodnocení prognózy u pacientů s akutní ischemickou cévní mozkovou příhodou podstupujících mechanickou trombektomií.

sults suggest that monitoring the preoperative collateral circulation state is conducive to assessment of the condition and prognosis, rendering a reference for decision-making in the clinic, thus improving the prognosis of patients to the greatest extent.

By exploring occlusion sites and collateral compensation patterns, we herein contribute additional insights beyond collateral grading alone. The practical implication of our results lies primarily in clinical decision-making and prognostic stratification. Spe-

cifically, our findings indicate that a poor preoperative collateral circulation state independently predicts unfavorable outcomes in AIS patients undergoing thrombectomy. Clinicians can utilize collateral circulation assessment for the early identification of high-risk patients, optimizing patient selection, and potentially guiding individualized therapeutic strategies and resources allocation.

Nevertheless, this study also has limitations. Firstly, as a retrospective study, there may be inherent selection and information biases. Secondly, we did not collect follow-up imaging data systematically to evaluate the recanalization status dynamically over time. Thirdly, clinical factors potentially influencing the collateral status, such as baseline blood pressure, glycemic control, or pre-existing vascular conditions, were not comprehensively analyzed. Future prospective multicenter studies are needed to validate our findings and refine the clinical utility of collateral circulation scoring in AIS patients undergoing mechanical thrombectomy.

Conclusion

In conclusion, preoperative collateral circulation before mechanical thrombectomy is significantly correlated with the prognosis of AIS patients, and poor preoperative collateral circulation emerges as a risk factor independently impacting these patients' prognosis. Preoperative collateral circulation can serve as a crucial index for assessing the prognosis of such patients.

Ethics approval and consent to participate

The entire study was conducted in accordance with the Helsinki Declaration of 1975 (as revised in 2004 and 2008). The study has received ethical approval by Xing'an League People's Hospital (No. NMG-

DIH2019-011, date of approval: 5. 1. 2019), date of approval: 5. 1. 2019. All participants signed a written informed consent to participate in the study. All methods in the presented study were performed in accordance with the relevant guidelines and regulations.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Yuzhu Xu designed the study, Songtao Guo conceived and supervised the study, Hui Han and Jian Sun performed and analyzed the experiments, and Ba Dan and Xi Wu drafted the paper.

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Conflicts of interest

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

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