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Research article

The combination model of serum occludin and clinical risk factors improved the efficacy for predicting hemorrhagic transformation in stroke patients with recanalization

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ABSTRACT

Background: and Purpose: Hemorrhagic transformation (HT) is one of the severe complications in acute ischemic stroke, especially for the patients who undergo recanalization treatment. It is crucial to screen patients who have high risk of HT before recanalization. However, current prediction models based on clinical factors are not ideal for clinical practice. Serum occludin, a biomarker for cerebral ischemia-induced blood-brain barrier disruption, has potential for predicting HT. This study was to investigate whether the combination of serum occludin and clinical risk factors improved the efficacy of predicting HT.

Methods: This was a single-center prospective observational study. Baseline clinical data and blood samples of recanalization patients were collected upon admission to our hospital. The level of serum occludin was measured using enzyme-linked immunosorbent assay. The diagnosis of HT was confirmed by CT scans within 36 h post recanalization.

Results: A total of 324 patients with recanalization were enrolled and 68 patients presented HT occurrence. HT patients had the higher level of baseline occludin than patients without HT ($p < 0.001$). Multivariate regression analysis showed that serum occludin level, Alberta Stroke Program Early CT Scores and endovascular therapy were independent risk factors ($p < 0.05$) for HT after adjusting potential confounders. The combination of serum occludin and clinical risk factors significantly improved the accuracy of predicting HT [area under the curve (AUC, 0.821 vs 0.701, $p < 0.001$), and net reclassification improvement (31.1 %), integrated discrimination improvement (21.5 %), $p < 0.001$] compared to a model employing only clinical risk factors. The modified AUC (0.806) of combined model based on 10-fold-cross-validation was still higher than clinical risk model (0.701).

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Conclusion: The combination of serum occludin and clinical risk factors significantly improved the prediction efficacy for HT, providing a novel potential prediction model to screen for patients with high risk of HT before recanalization in acute ischemic stroke.

1. Introduction

Stroke is reported to be the second leading causes of mortality in the world, and ischemic stroke accounts for 62.4 % of all incident strokes in 2019 [1]. HT characterized by secondary bleeding in the infarction region is the most dangerous complication in ischemic stroke patients, leading to rapid neurological deterioration at the early phase of stroke [2,3].

Recanalization treatments have been widely applied to ischemic stroke in clinic, including intravenous thrombolysis and endovascular therapy. However, studies have shown that recanalization treatment significantly increased the occurrence of HT complications (1.4-fold in intravenous thrombolysis and 6.6-fold in endovascular therapy, compared to non-recanalization treatment), mainly due to restored blood leaking across the damaged vessels during ischemia [4–7]. Therefore, it is crucial to screen for patients who have high risk of HT before recanalization application. Unfortunately, no ideal method or model has been developed for predicting HT in the clinic at present. Studies have shown that some clinical factors are associated with HT, such as older age, high National Institute of Health Stroke Scale (NIHSS) scores, massive cerebral infarction, low Alberta Stroke Program Early CT Scores (ASPECTS), cardiac cerebral embolism, therapy methods, and other factors [8,9]. The prediction models based on these clinical factors alone are not ideal for clinical practice [10–13], mainly due to the lack of meaningful objective indicators.

Blood-brain barrier (BBB) is a physical barrier that existing blood and cerebrospinal fluid, and is composed of endothelial cells, tight junction protein, pericyte, astrocyte end-foot and basal lamina [14]. Studies have shown that disruption of the BBB is crucial for the generation and development of HT [15,16]. Occludin, a tight junction protein involved in maintaining BBB integrity, was reported to degrade into fragments and enter into circulation in response to cerebral ischemia [17]. Our previous studies indicated that the concentration of occludin in blood was correlated with the extent of BBB permeability in cerebral ischemic animals and was greatly elevated in cerebral ischemic patients [18], suggesting that occludin may be a potential biomarker to predict HT complication in cerebral ischemic patients.

In the present study, we performed a prospective observational study to investigate whether the combination of serum occludin and clinical risk factors improved the efficacy for predicting HT after recanalization.

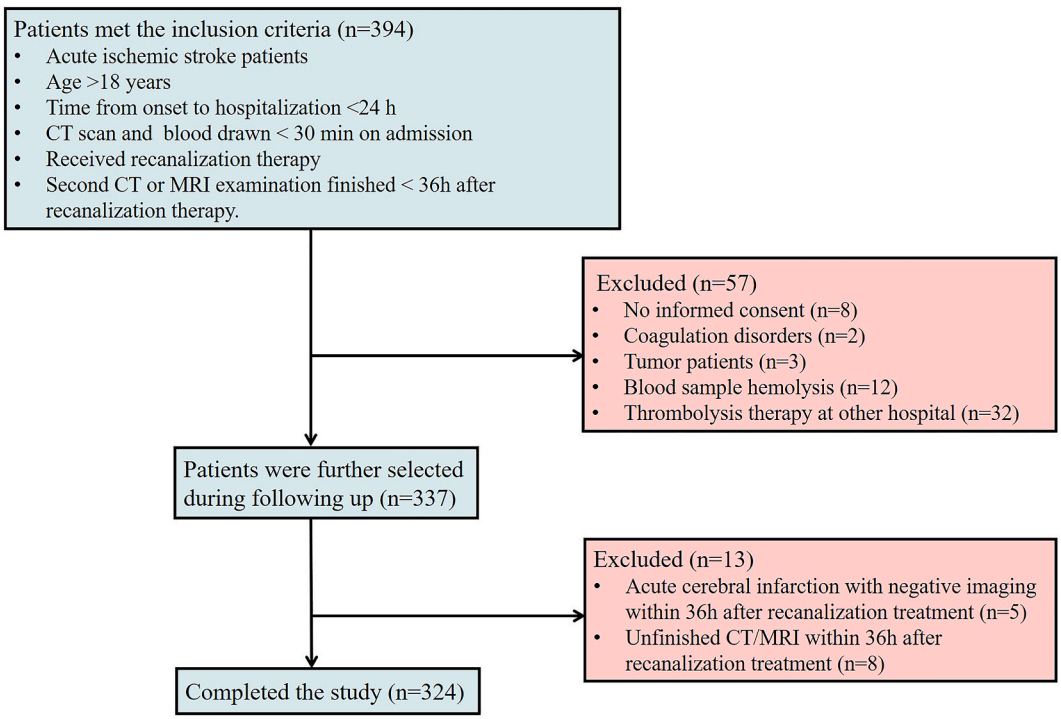

Fig. 1. The flow chart of patients screening. According to the inclusion criterion, a total of 394 acute ischemic stroke patients were screened. Based on clinical and imaging of exclusion criteria, 324 patients were finally included in the study after excluding 70 cases.

2. Methods

2.1. Study design and case enrollment

This single-center prospective cross-sectional study was conducted between February 2021 and December 2021. Consecutive patients were enrolled at the emergency department of Xuanwu Hospital, Capital Medical University.

Inclusion criteria: 1) acute ischemic stroke patient, 2) age >18 years, 3) time from stroke onset to hospitalization <24 h, 4) received recanalization therapy including intravenous thrombolysis, endovascular therapy or bridging therapy, 5) cranial CT scan and blood sample collection were completed within 30 min of admission, 6) cranial CT or MRI examination was reviewed within 36 h after recanalization therapy.

Exclusion criteria: 1) intracranial tumor, 2) severe cardiac, liver or kidney dysfunction, coagulation dysfunction, hemorrhagic disease, malignant tumors or pregnancy, 3) intracranial artery dissection or vasculitis, 4) patient suspected of having an immune system condition or being treated for such a condition, 5) started thrombolysis before arriving at Xuanwu Hospital, 6) blood sample showed hemolysis or turbidity, 7) informed consent was not obtained, 8) data case was incomplete, and 9) no cerebral infarction observed in diffusion-weighted imaging sequence (negative for cerebral infarction).

Finally, a total of 324 patients were recruited from 394 cases and completed this study (Fig. 1).

Table 1
The comparison of baseline characteristics between HT and Non-HT groups in acute ischemic stroke with recanalization treatment.

Variables	Total patients (n = 324)	Non-HT (n = 256)	HT (n = 68)	*p* value
Demographic Data				
Male, n (%)	245 (75.6 %)	193 (75.4 %)	52 (76.5 %)	0.854
Age (years), mean ± SD	63.85 ± 12.57	63.41 ± 12.39	65.51 ± 13.18	0.219
Medical history, n (%)				
Hypertension	210 (64.8 %)	164 (64.1 %)	46 (67.6 %)	0.582
Diabetes	89 (27.5 %)	72 28.1 %)	17 (25.0 %)	0.608
Coronary heart disease	50 (15.4 %)	34 (13.3 %)	16 (23.5 %)	0.038
Hyperlipemia	81(25.0 %)	64 (25.0 %)	17 (25.0 %)	1.000
Atrial fibrillation	57 (17.6 %)	41 (16.0 %)	16 (23.5 %)	0.148
Stroke	68 (21.0 %)	51 (19.9 %)	17 (25.0 %)	0.361
Antithrombotic therapy	81 (25.0 %)	57 (22.3 %)	24 (35.3 %)	0.027
Current smoking	164 (50.6 %)	131 (51.2 %)	33 (48.5 %)	0.698
Clinical data measured, median (IQR)				
SBP (mmHg)	156 (138–171)	154 (138–169)	159.5 (141–178.75)	0.103
DBP (mmHg)	84 (75–95)	84 (75.25–94.75)	86.5 (75–98)	0.441
NIHSS	10 (5–17)	8.5 (4–15)	15 (10–20)	<0.001
mRS	4 (3–4)	4 (3–4)	4 (4–4)	0.004
ASPECTS	9 (8–10)	9 (8–10)	8 (6.25–10)	0.001
Onset to sampling (min)	186 (104.50–348.75)	164 (93.25–315.00)	304(172.75–448.75)	<0.001
Onset to therapy (min)	223 (137.5–463.5)	201 (127.5–413.25)	400 (208.75–585.75)	<0.001
Stroke etiologic subtypes, n (%)				0.067
Large-artery atherosclerosis	210 (64.8 %)	170 (66.4 %)	40 (58.8 %)	
Cardioembolism	76 (23.5 %)	53 (20.7 %)	23 (33.8 %)	
Small-artery occlusion	26 (8.0 %)	24 (9.4 %)	2 (2.9 %)	
Stroke of undetermined cause	12 (3.7 %)	9 (3.5 %)	3 (4.4 %)	
Therapy methods, n (%)				<0.001
Intravenous thrombolysis	159 (49.1 %)	144 (56.3 %)	15 (22.1 %)	
Endovascular treatment	120 (37.0 %)	80 (31.3 %)	40 (58.8 %)	
Bridgy therapy	45 (13.9 %)	32 (12.5 %)	13 (19.1 %)	
Lesion location, n (%)				0.078
Anterior circulation	243 (75 %)	185 (72.3 %)	58 (85.3 %)	
Posterior circulation	78 (24.1 %)	68 (26.6)	10 (14.7 %)	
Both	3 (0.9 %)	3 (1.2 %)	0	
Laboratory test, median (IQR)				
Blood glucose (mmol/L)	6.88 (5.82–8.28)	6.70 (5.78–8.10)	7.19 (5.98–8.97)	0.230
Cholesterol (mmol/L)	4.60 (4.03–5.31)	4.61 (4.11–5.31)	4.44 (3.60–5.19)	0.09
LDL (mmol/L)	2.73 (2.23–3.33)	2.75 (2.29–3.46)	2.65 (1.82–3.22)	0.065
WBC ($\times 10^9$/L)	7.98 (6.27–9.82)	7.63 (6.07–9.39)	8.94 (7.03–11.35)	0.001
Platelet ($\times 10^9$/L)	207.5 (175.0–250.75)	207.5 (174.00–248.75)	210.5 (178.25–265)	0.424
APTT (s)	33.6 (31.4–36.98)	33.55(31.23–36.48)	33.7 (31.90–37.48)	0.298
Fibrinogen (g/L)	3.45 (2.97–3.96)	3.44(2.98–3.91)	3.46 (2.87–4.71)	0.314
D-dimer (mg/L)	0.30 (0.13–0.78)	0.29 (0.12–0.64)	0.67 (0.28–1.30)	<0.001

The *p* values were calculated using the χ^2 test, two independent-sample *t*-test and Mann-Whitney *U* test, as appropriate. SD, standard deviation; IQR, interquartile range; SBP, systolic blood pressure; DBP, Diastolic blood pressure; NIHSS, National Institutes of Health Stroke Scale; mRS, modified-Rankin-Scale; ASPECTS, Alberta Stroke Program Early CT Score; LDL, Low Density Lipoprotein; WBC, white blood cells; APTT, activated partial thromboplastin time.

2.2. Data collection

The clinical and imaging data of patients (Table 1) were collected using an electronic medical system and recorded separately by two investigators. Data administrators were responsible for double-checks. The investigators who collect the clinical data were blind to the grouping information and the result of serum occludin.

2.3. Outcome assessment

Based on the European Cooperative Acute Stroke Study (ECASS II) [19], HT complication was diagnosed if intracranial hemorrhage was not observed on the first CT scan (within 30 min of admission), but on the second set of CT/MRI images (within 36 h after recanalization therapy). The types of HT were defined as hemorrhagic infarction (HI-1, HI-2) or parenchymal hematoma (PH-1, PH-2), based on the presence of space-occupying effects. Patients were considered to show symptomatic HT when the NIHSS score increased $\geq$4 within 24 h. Imaging data were double-checked by senior neuroradiology specialists, who were blinded to other clinical data and serum occludin results.

2.4. Serum samples collection

Blood samples were collected by nurses in the emergency room using 3 mL tubes with no anticoagulant or coagulant within 30 min after admission. After standing for 2 h at room temperature, blood samples were centrifugated (4 °C, 3000 rpm, 10 min). Then the supernatants were collected and stored at −80 °C.

2.5. Measurement of occludin level in serum using ELISA

The level of occludin in serum was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (LSBio Company) according to the product instructions.

2.6. Statistical analysis

Data with normally distribution were expressed as means ± standard deviation and analyzed using independent samples *t*-tests. Data with non-normally distribution were expressed as medians ± interquartile range and analyzed using Mann-Whitney U tests. Data in categorical variables were expressed as frequencies (%) and analyzed using Chi-square tests or Fisher exact tests. The statistically significance was set at $p < 0.05$.

Univariate and multivariate logistic regression were performed to find risk factors of HT. The potential risk factors in univariate analysis ($p < 0.05$) were included in multivariate logistic regression analysis. A variance inflation factor >10 and tolerance <0.1 were employed to check the multicollinearity among variables.

Receiver operating characteristic (ROC) curves and AUC were analyzed to evaluate the performance of different models. In addition to comparing the AUCs of models, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to quantitatively evaluate the efficiency of model improvement [20]. Moreover, Moreover, 10-fold cross-validation was performed to get an unbiased estimate model for prediction accuracy. DeLong's test was used to compare the differences between AUCs [21]. The goodness-of-fit of models was qualitatively analyzed using Akaike information classification (AIC) and Bayesian information classification (BIC) [22]. The clinic usefulness of the models was compared using the decision curve analysis (DCA), which is a method to calculate a clinical net benefit [23].

Table 2
Blood pressure measurements after recanalization therapy at different time points in HT patients (n = 68).

Time Points	Systolic blood pressure (mean ± SD)	Diastolic blood pressure (mean ± SD)
2h	139.18 ± 21.81	75.47 ± 11.08
4h	136.09 ± 18.39	73.69 ± 10.56
6h	137.96 ± 18.65	74.96 ± 10.78
8h	136.49 ± 17.31	73.49 ± 9.54
10h	135.71 ± 17.30	73.84 ± 9.90
12h	133.90 ± 15.98	72.34 ± 9.51
16h	133.25 ± 16.00	74.30 ± 10.81
20h	134.06 ± 16.73	74.13 ± 9.82
24h	135.35 ± 13.60	73.43 ± 8.75
30h	134.15 ± 14.89	72.12 ± 7.14
36h	128.91 ± 12.02	71.04 ± 8.25

3. Results

3.1. Patient characteristics

The baseline characteristics of 324 recruited cases are presented in Table 1. Among them, 159 cases (49.1 %) received intravenous thrombolysis, 120 patients (37.0 %) received endovascular treatment and 45 patients (13.9 %) received bridging therapy (intravenous thrombolysis plus endovascular treatment).

According to the previous medical history, 210 patients in the present study had a history of hypertension, accounting for 64.8 % in all recruited cases (n = 324). Patients suffering from hypertension disease account for 64.1 % in non-HT patients and 67.6 % in HT patients, respectively (Table 1). Based on the blood pressure at admission, there was no significance in blood pressure between HT and Non-HT patients (Table 1). For patients with recanalization therapy, blood pressure was measured every 2h within the first 12 h, every 4h within 12–24 h, and every 6h within 24–36 h. The mean blood pressure after recanalization therapy demonstrated that HT patients (n = 68) maintained stable blood pressure within 36h (Table 2).

Based on imaging data, 68 patients suffered HT complications within 36 h after treatment, accounting for 21 % of total recruited cases. The proportion of each subtype were: HI-1 (n = 19, 27.9 %), HI-2 (n = 28, 41.2 %), PH-1 (n = 10, 14.7 %) and PH-2 (n = 11, 16.1 %). Compared with patients without HT, HT patients were more likely to have a history of coronary heart disease, have received antithrombotic therapy, have elevated NIHSS, modified Rankin Scale, onset to sampling or therapy, white blood cell counts and D-dimers, but had lower ASPECTS scores.

3.2. The association of baseline serum occludin and HT in acute ischemic stroke patients

To investigate the relationship between baseline occludin level and HT occurrence in recanalization patients, baseline serum occludin was measured using the ELISA method. The results showed that the level of serum occludin in HT patients was higher than that in non-HT patients [median (interquartile range) 0.40 (0.28–0.66) ng/mL vs 0.20 (0.13–0.30) ng/mL, $p < 0.001$, Fig. 2A].

We made further analysis to explore the level of serum occludin in symptomatic or asymptomatic HT patients. Based on the definitions of symptomatic HT [19], intracranial HT patients after recanalization were divided into two groups: asymptomatic HT (n = 51); symptomatic HT (n = 17). The results showed that the symptomatic HT patients had a higher serum occludin levels than asymptomatic HT patients [0.64 (0.36–1.72) vs 0.39 (0.24–0.63) ng/mL, $p < 0.05$, Fig. 2B].

These results suggested a potential link between baseline occludin level and HT occurrence in recanalization patients.

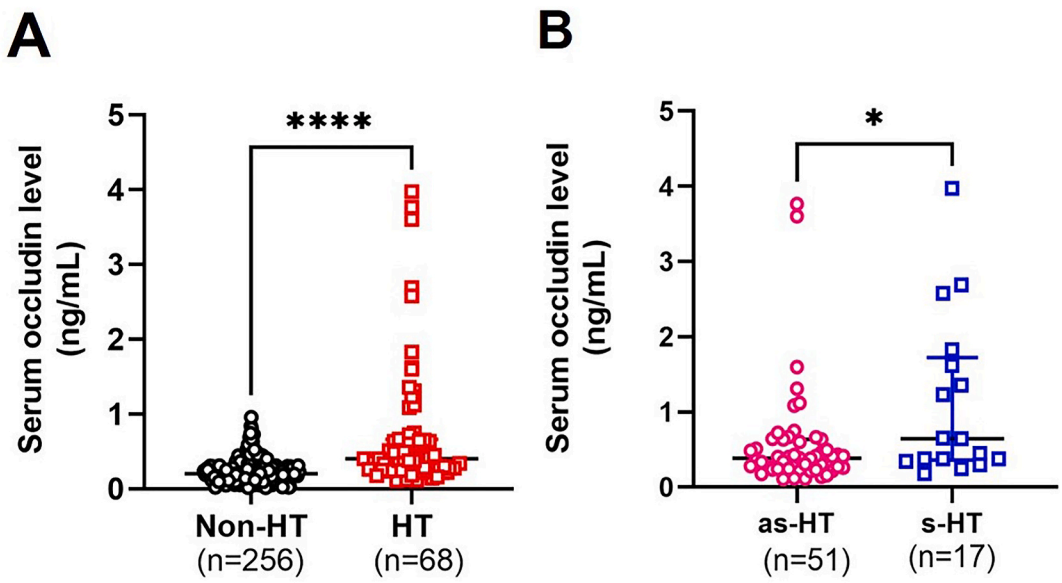

Fig. 2. The association between baseline serum occludin levels and HT in acute ischemic stroke patients. Baseline sera were collected in the emergency department room within 30 min after admission and the level of serum occludin was measured using ELISA. **A.** Acute ischemic stroke patients with HT occurrence had a higher level of serum occludin than non-HT cases [0.40 (0.28–0.66) ng/mL vs 0.20 (0.13–0.30) ng/mL, $p < 0.001$]. **B.** Compared to asymptomatic HT patients, symptomatic HT patients also had a higher serum occludin levels [0.64 (0.36–1.72) ng/mL vs 0.39 (0.24–0.63) ng/mL, $p = 0.029$] $^{*}p < 0.05$, $^{***}p < 0.001$, Data was expressed as median (interquartile range), and the p value was calculated using Mann-Whitney U test. HT, hemorrhagic transformation; ELISA, enzyme linked immune sorbent assay; asymptomatic HT, as-HT; symptomatic HT, s-HT.

3.3. Subgroup analysis of baseline serum occludin in acute ischemic stroke patients

To clarify the relationship between baseline serum occludin and clinical factors, we made the subgroup analysis (Table 3). The levels of baseline serum occludin in subgroups showed significant difference, based on baseline NIHSS score, stroke etiological subtypes and therapy methods.

3.4. Baseline serum occludin level was an independent risk factor for HT occurrence after recanalization therapy

We further investigated whether baseline serum occludin level (before recanalization) was a risk factor for HT in recanalization patients using univariate and multivariate logistic regression analysis (Fig. 3, Table 4). Univariate logistic analysis showed that 11 factors were associated with HT occurrence within 36 h after recanalization treatment, including baseline occludin [odds ratio (OR) = 105.530, 95 % CI (22.210–501.421), $p < 0.001$]. There was no multicollinearity among the possible risk factors due to variance inflation factor <10 in the multiple liner regression. Multivariate logistic regression demonstrated that besides the two clinical risk factors of endovascular treatment [reference to intravenous thrombolysis, OR = 4.289, 95 % CI (1.479–12.437), $p = 0.007$] and lower ASPECTS on initial CT [OR = 0.834, 95 % CI (0.697–0.999), $p = 0.049$], serum occludin level was an independent predictor [OR = 108.392, 95 % CI (19.734–595.352) $p < 0.001$)] for HT occurrence, even after adjusting for other potential confounding factors. These

Table 3
Subgroup analysis the baseline serum occludin levels in acute ischemic stroke patients.

Parameters	n	Occludin level (ng/mL), median (IQR)	*p* value
Age			0.208
Total	324	0.22 (0.16–0.30)	
≤40y	19	0.21 (0.14–0.33)	
41-60y	95	0.24 (0.14–0.39)	
61-80y	184	0.25 (0.18–0.46)	
≥80y	26		
Sex			0.536
Total	324		
Male	245	0.23 (0.14–0.35)	
Female	79	0.21 (0.14–0.35)	
Hypertension			0.715
Total	324		
Yes	210	0.23 (0.14–0.37)	
NO	114	0.22 (0.15–0.34)	
Diabetes			0.820
Total	324		
Yes	235	0.23 (0.15–0.35)	
No	89	0.23 (0.14–0.36)	
Therapy methods			
Total	324		0.013[a]
Intravenous thrombolysis	159	0.21 (0.13–0.31)	
Endovascular treatment	120	0.23 (0.15–0.40)	
Bridge therapy	45	0.27 (0.19–0.43)	
Onset to sampling			
Total	324		0.118
<4.5h	212	0.22 (0.13–0.33)	
4.5–6h	34	0.23 (0.16–0.55)	
>6h	78	0.26 (0.16–0.40)	
Baseline NIHSS score			
Total	324		0.001[b]
Mild stroke (1–4)	76	0.20 (0.12–0.29)	
Moderate stroke (5–15)	161	0.24 (0.14–0.34)	
Severe stroke (≥16)	87	0.25 (0.17–0.43)	
Stroke etiological subtypes			0.002[c]
Total	324		
Large-artery atherosclerosis	210	0.23 (0.15–0.35)	
Cardiogenic cerebral embolism	76	0.30 (0.18–0.45)	
Small vessel occlusion	26	0.19 (0.10–0.23)	
Stroke of undetermined cause	12	0.18 (0.11–0.25)	

IQR, Interquartile range; NIHSS, National Institutes of Health Stroke Scale. The *p* values were calculated using the χ^2 test, Mann-Whitney *U* test and Kruskal-Wallis test, appropriately.

[a] The difference of serum occludin levels among three groups was significant ($p = 0.013$), while the difference between intravenous thrombolysis and bridge therapy was still significant by Bonferroni correction for multiple tests ($p = 0.017$).

[b] The difference of serum occludin levels among four groups was significant ($p = 0.001$), while the difference between mild stroke and moderate or severe stroke respectively was still significant by Bonferroni correction for multiple tests ($p = 0.012$ and $p < 0.001$).

[c] The difference of serum occludin levels among four groups was significant ($p = 0.002$), while the difference between cardiogenic cerebral embolism and small vessel occlusion was still significant by Bonferroni correction for multiple tests ($p = 0.004$).

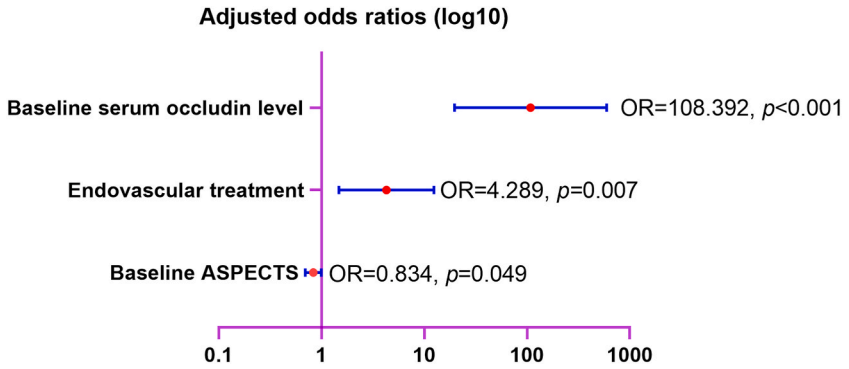

Fig. 3. Forest plot of risk factors for HT. After adjusting for confounding variables, forest plot showed that baseline serum occludin level (OR = 108.392, $p < 0.001$), endovascular therapy (reference to intravenous thrombolysis, OR = 4.289, $p = 0.007$) and baseline ASPECTS (OR = 0.834, $p = 0.049$) were the risk factors for HT in multivariate regression analysis, respectively. OR, odds ratio; ASPECTS, Alberta Stroke Program Early CT Score; HT, hemorrhagic transformation.

Table 4
Univariate and multivariate logistic to identify the risk factors associated with HT in acute cerebral infarction patients with recanalization treatment.

Variables	Unadjusted Odds Ratio	*p* value[a]	Adjusted Odds Ratio	*p* value[b]
	(95 % CI)		(95 % CI)	
NIHSS	1.062 (1.032–1.093)	<0.001	0.993 (0.942–1.047)	0.786
ASPECTS	0.751 (0.655–0.862)	<0.001	0.834 (0.697–0.999)	0.049
mRS	1.666 (1.192–2.330)	0.003	0.939 (0.573–1.539)	0.802
Onset to sampling	1.001 (1.001–1.002)	0.005	–	–
Onset to therapy[c]	1.001 (1.001–1.002)	0.001	1.000 (0.999–1.001)	0.683
WBC	1.120 (1.037–1.210)	0.004	1.033 (0.927–1.150)	0.558
LDL	0.735 (0.544–0.992)	0.044	0.835 (0.589–1.185)	0.313
Coronary heart disease	2.009 (1.032–3.913)	0.040	1.977 (0.823–4.752)	0.128
Antithrombotic therapy	1.904 (1.068–3.394)	0.029	0.984 (0.440–2.202)	0.969
Therapy methods		<0.001		0.022
Intravenous thrombolysis	Reference			
Endovascular treatment	4.800 (2.498–9.225)	<0.001	4.289 (1.479–12.437)	0.007
Bridging therapy	3.900 (1.691–8.995)	0.001	1.965 (0.597–6.471)	0.266
Serum occludin	105.530 (22.21–501.421)	<0.001	108.392 (19.734–595.352)	<0.001

NIHSS, National Institutes of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT Score; mRS, modified-Rankin-Scale; WBC, white blood cells; LDL, Low Density Lipoprotein.

[a] The *p* values were calculated using univariate regression analysis.

[b] The *p* values were calculated using multivariate regression analysis.

[c] Onset to sampling and onset to therapy had the multicollinearity (variance inflation factor >10 and Tolerance <0.1), and the latter was included into the multivariate logistic regression analysis based on the smaller *p* value.

results suggested that baseline occludin may be incorporated into the clinical risk factor model for prediction of HT as an important variable.

3.5. The combination of serum occludin and clinical risk factors improved the predicative efficiency for HT occurrence after recanalization

To investigate whether the combination of serum occludin and clinical risk factors improved the predictive efficiency for HT occurrence after recanalization, three prediction models were constructed: Model I [clinical factors (ASPECTS and endovascular treatment)]; Model II: occludin alone; and Model III: clinical factors + occludin.

The prediction accuracy of the three models was evaluated by using AUCs. The results showed that the AUC of Model III was

Table 5
The comparison of AUC differences among different models.

Variables	AUC difference	95 % CI		*p* value
		Lower limit	Upper limit	
Model III vs Model II	0.017	−0.026	0.059	0.441
Model III vs Model I	0.119	0.067	0.171	<0.001
Model II vs Model I	0.099	0.018	0.179	0.016

Model I, clinical risk factors (ASPECTS and endovascular treatment); Model II, occludin alone; Model III, clinical risk factors + occludin.

significantly higher than Model I (0.821 vs 0.701, $p < 0.001$, Table 5 and Fig. 4), while the AUC of Model III showed no significantly difference, compared with that of Model II (0.821 vs 0.804, $p = 0.441$), suggesting that the combination of serum occludin and clinical risk factors improved the predictive efficiency for HT occurrence.

Based on the significant difference of AUCs between Model I and Model III, Improvement rate of combination model (Model III) for prediction HT was quantitatively evaluated using NRI and IDI. The statistical analysis results showed that NRI and IDI in Model III were significantly increased by 31.1 % ($p < 0.001$) and 21.5 % ($p < 0.001$), respectively, when referenced to Model I (Table 6).

To evaluate the potential stability of Model III, 10-fold cross-validation was further performed to obtain an unbiased estimation for Model III (Fig. 5). The adjusted AUC of Model III after calibration (0.806) maintained higher than Model I (0.701).

To evaluate the goodness-fit of the Model III, AIC and BIC were qualitatively analyzed. Smaller AIC and BIC mean better model. The Model III using occludin combined clinical factors (AIC = 249.41, BIC = 268.31) was better than Model I (clinical factors, AIC = 309.94, BIC = 325.07) or Model II (occludin alone, AIC = 266.76, BIC = 274.32).

Finally, to evaluate the potential clinical usefulness and net benefit of Model III, DCAs of the three models was further analyzed (Fig. 6). The Model III (the combination of occludin with clinical factors) was farther way from the slanted dashed line and the horizontal black line than Model I or Model II, indicating the Model III had a best clinical benefit among the three models.

These results indicated that the model using baseline serum occludin combined with clinical risk factors may be effective for predicting early HT occurrence in ischemic stroke patients who undergo recanalization therapy.

4. Discussion

The present study reported that the combination of baseline occludin in serum with clinical risk factors could improve the efficacy for HT prediction in acute cerebral ischemic stroke patients with recanalization treatment, providing a new effective prediction model to screen for patients who had a high risk of HT before recanalization administration.

HT is one of the severe complications of ischemic stroke especially after recanalization, leading to early neurological deterioration and poor prognosis [2,3]. Until now, no method has been applied to predict HT in clinical practice. Many clinical risk factors have been reported to be associated with HT, including higher NIHSS, lower ASPECTS, massive cerebral infarction, cardiogenic cerebral embolism, treatment methods, and others [8,9]. In addition, multimodal CT and MRI methods have been developed to predict HT, including permeable-/perfusion-related parameters [24–26]. However, the traditional prediction models based on these clinical risk factors or imaging methods are not ideal (AUC<0.7) for clinical practice [10–13], suggesting that finding novel objective biomarkers and including them into the prediction model may be a better way to improve prediction efficacy.

Studies have shown that BBB disruption during cerebral ischemia is closely associated with HT occurrence [15,16]. As a key protein of tight junctions, occludin plays a crucial role in keeping the physical functions of the BBB [27]. Our previous studies showed that cerebral ischemia induced degradation of occludin protein into fragments in an animal model of cerebral ischemia [17,18]. The level of occludin in the circulation was correlated with the extent of BBB damage, and the present study also found that the high levels of baseline serum occludin in HT patients may be responsible for the disruption of BBB integrity. These results indicated that occludin in serum may serve as an ideal biomarker for BBB damage, and may improve HT prediction in stroke patients.

The prediction model in the present study incorporated the level of baseline serum occludin into the traditional model based on clinical factors, and the results showed that the calibrated AUC of the new model was significantly elevated compared to the traditional model (0.821 vs 0.701), and the new model had a better good-fit test (the smaller value of AIC and BIC) and net benefit in clinical

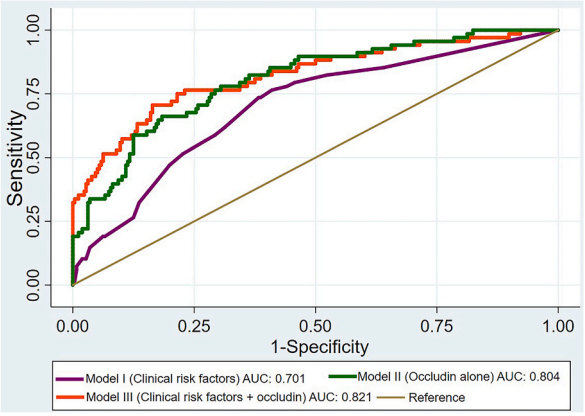

Fig. 4. ROC curves of three prediction models. To investigate whether serum occludin improved the predicative efficiency of clinical risk factors model for HT occurrence after recanalization, ROC curves of three prediction models were made: Model I [clinical factors (ASPECTS and endovascular treatment)]; Model II: occludin alone; Model III: clinical factors + occludin. The AUC of Model III was significantly higher than Model I (0.821 vs 0.701, $p < 0.001$), suggesting that the combination of serum occludin and clinical risk factors improved the predictive efficiency for HT. The p value was calculated using delong test. AUC, area under the curve; HT, hemorrhagic transformation; ROC, receiver operator characteristic curve.

Table 6
Reclassification of HT by adding the values of serum occludin in ischemic stroke patients with recanalization treatment.

Models	NRI		IDI	
	Estimate (95 % CI)	*p* value	Estimate (95 % CI)	*p* value
Model I	Reference	–	Reference	–
Model III	0.311 (0.195–0.427)	<0.001	0.215 (0.146–0.284)	<0.001

Model I, clinical risk factors (ASPECTS and endovascular treatment); Model III, clinical risk factors + occludin.
NRI, net reclassification improvement; IDI, integrated discrimination improvement.

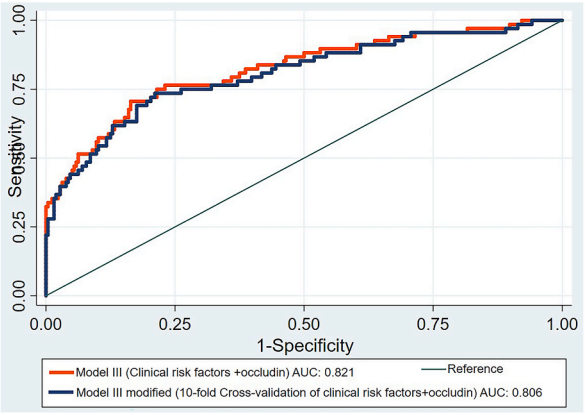

Fig. 5. 10-fold Cross-validation of the combination model. To evaluate the potential stability of Model III, 10-fold cross-validation was further performed to obtain an unbiased estimation for Model III. The AUC from a 10-fold cross validation on predicting HT, based on Model III (ASPECTS, endovascular treatment plus serum occludin levels) in multivariate logistic regression model, was 0.806. The adjusted AUC of Model III after calibration (0.806) maintained higher than Model I (0.701). AUC, area under the curve; ASPECTS, Alberta Stroke Program Early CT Score; HT: hemorrhagic transformation.

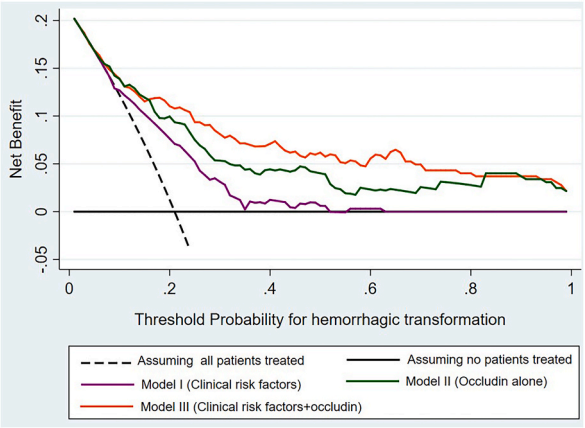

Fig. 6. Decision curve analyses for the three models. To evaluate the potential clinical usefulness and net benefit of Model III, DCAs of the three models was further analyzed. The Model III (the combination of occludin with clinical factors) was farther way from the slanted dashed line and the horizontal black line than Model I or Model II, indicating the Model III had a best clinical benefit among the three models. DCA, decision curve analyses.

practice. Moreover, the prediction ability between Model II (occludin alone) and Model III (clinical risk factors plus occludin) had no significant difference, which is mainly due to the unhomogenized groups and large variations of clinical factors. These results indicated that serum occludin, as an objective indicator, had a significant contribution in improving prediction efficacy of traditional model (Model I, clinical risk factors).

Therefore, it is necessary to explore more new prediction models to improve the prediction efficiency of traditional risk factors model. Due to the variety of study population characteristics, many stratification factors, and some subjective measurement parameters (NIHSS, ASPECTS, mRS) in the traditional factors model, the more objective indicators reflecting BBB injury should be introduced

into the prediction models to improve the efficiency of prediction [28]. The present study provides a potential feasible way or direction to optimize and improve the accuracy of the prediction model by incorporating objective indicators. In addition to occludin, many biomarkers related to brain damage in response to cerebral ischemia have been studied, including cellular fibronectin, matrix metalloproteinase-9, and S100 calcium-binding protein B [29–31]. Further studies are warranted to screen for additional novel objective indicators and to develop combination models with two or more objective indicators.

To clarify the relationship between baseline serum occludin and clinical factors, we made the subgroup analysis. The levels of baseline serum occludin in subgroups showed significant difference, based on baseline NIHSS score, stroke etiological subtypes and therapy methods. The possible reason is that patients, who underwent bridge therapy, moderate-severe stroke or cardiogenic cerebral embolism, might have a worst BBB disruption at admission. However, the levels of baseline serum occludin did not show significant difference in subgroups based on onset to sampling (<4.5h, 4.5–6h, >6h), which is mainly due to the blood samples were not continuously collected from the same individuals. Further studies will be carried out to explore the association of onset to sampling and serum occludin levels.

Developing a prediction model of HT should be of great significance for guiding clinical treatment. For the patients who are evaluated early after arrival at the emergency room with high risks of HT, doctors can then pay more attention and take measures to reduce the risk of HT occurrence, including reducing the dosage of antiplatelet drugs, increasing CT detection after operation, and even discontinuing recanalization therapy after weighting the pros and con.

There are some limitations in the present study. Firstly, the present study was carried out in a single center, and the prediction model developed in the present study needs to further verified in multi-center investigations. Secondly, the predictive ability of the model for different types of HT was not analyzed due to the limitation of sample size. Thirdly, until now, few literatures showed the exact value of half-life of occludin after stroke. According to our previous data in animal and patients, occludin levels in blood dramatically increased within 4.5-h ischemia and kept in a high concentration at 4.5–24 h after ischemia [17,18], the exact value of half-life of occludin is needed to explore in the future study to help determine its usefulness in predicting HT.

In summary, this is the first prospective study to the best of our knowledge, which explores the relationship between serum occludin levels and HT within 36 h after recanalization treatment. Use of serum occludin levels, as an independent risk factor for HT, significantly improves the efficiency of clinical risk factors model for prediction HT in acute cerebral ischemic stroke and provides a novel prediction model to screen for patients with high risk of HT before recanalization administration.

5. Conclusion

Serum occludin combined with clinical risk factors model significantly improved the prediction efficacy for HT occurrence, providing a novel potential prediction model to screen for patients with high risk of HT before recanalization in acute cerebral ischemic stroke patients.

Ethics statement

This study was reviewed and approved by the Ethics Committee of Xuanwu Hospital of Capital Medical University, with the approval number: linyanshen [2020]127.

Informed consent

Written informed consent was obtained from all recruited patients or their legally authorized representative before enrolled.

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Data availability statement

Data in the present study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Shuhua Yuan: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Qingfeng Ma:** Formal analysis. **Chengbei Hou:** Software, Methodology. **Weili Li:** Formal analysis. **Ke Jian Liu:** Writing – review & editing, Conceptualization. **Xunming Ji:** Supervision, Conceptualization. **Zhifeng Qi:** Writing – review & editing, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no knowncompeting financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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