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Functional outcome predictors and recanalization in cerebral venous thrombosis: A single-center cross-sectional study

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Abstract:

OBJECTIVES: Cerebral venous thrombosis (CVT) is a rare type of stroke. Functional outcome (FOC) in CVT can be affected by different factors, and recanalization is an important determinant. This study aimed to identify the FOC predictors including recanalization in patients with CVT.

METHODS: This retrospective study reviewed electronic charts of patients with CVT admitted to the King Fahd Hospital of the University, a teaching and referral hospital between January 2012 and March 2023. Demographic and other clinicoradiological characteristics were noted. Factors affecting FOC were investigated using the Chi-square test, odds ratios (ORs), and 95% confidence interval (CI).

RESULTS: Out of 64 patients, 78% achieved good FOC. Age was significantly higher in patients having poor FOC as compared to those having good FOC ($P \leq 0.001$). Women had more likelihood to be functionally independent as compared to men (OR = 1.35, 95% CI = 1.01–1.80, $P = 0.02$). Altered consciousness (OR = 2.94, 95% CI = 1.23–6.99, $P = 0.01$), venous hemorrhagic infarction (OR = 3.26, 95% CI = 1.36–7.82, $P = 0.008$), and nonrecanalization (OR = 1.44, 95% CI = 0.97–2.14, $P = 0.02$) were significantly associated with poor FOC. Hereditary thrombophilia (OR = 0.60, 95% CI = 0.31–1.12, $P = 0.03$) and infections (OR = 0.59, 95% CI = 0.31–1.12, $P = 0.01$) were associated with less likelihood of good FOC. Age ≥50 years ($P = 0.01$) and illness of more than 1-month duration ($P = 0.01$) were associated with nonrecanalization.

CONCLUSION: Older age, male sex, presence of venous hemorrhagic infarction, and nonrecanalization can predict poor FOC in CVT. The recanalization process can be affected by late presentation, and the plausible reason could be a delay in therapeutic anticoagulation. Further prospective and multicenter studies are needed to determine the predictors of FOC and to understand the process of recanalization in CVT.

Keywords:

Cerebral veins, infarction, stroke, venous thrombosis

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Introduction

Cerebral venous thrombosis (CVT) comprises 0.5%–1% of all strokes and affects particularly younger population.[1] Literature reports that CVT is more prevalent than mentioned previously.[2] The prognosis is more favorable in CVT compared to other strokes.[3] A study from Saudi Arabia (SA) has reported complete recovery in 72% of patients.[4] However, it is noteworthy that despite adequate medical treatment, mortality, or significant functional disability occurs in nearly 15% of the patients.[5] Coma, focal findings, cerebral edema, age,

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Box-ED Section

What is already known on the study topic?

- Cerebral venous thrombosis (CVT) is a rare cause of stroke
- Headache is the most common presenting feature. These patients frequently present to the emergency department and are not always evaluated by appropriate neuroimaging.

What is the conflict on the issue? Has it importance for readers?

- Every patient with headache is not evaluated by neuroimaging
- Factors affecting outcomes and recanalization in patients with CVT are debatable and need further evidence.

How is this study structured?

- This was a single-center, retrospective study and included data from 64 patients.

What does this study tell us?

- Delay in diagnosis by not having appropriate neuroimaging on time may affect the recanalization and outcomes adversely
- Age, sex, etiology, and radiological features can predict outcomes
- Recanalization is an important predictor of outcome which itself can be affected by age, duration of illness, and site of thrombosis.

hormonal etiology, and active malignancy can affect outcome in CVT.[6,7] Furthermore, in critically ill cases, the mortality rate can reach up to 30%.[8] The mainstay of management is anticoagulation even in the presence of intracerebral bleeding.[9] It is important to realize that successful recanalization is an important predictor of functional outcome (FOC) in CVT and is related to 3.3-fold higher odds of functional independence.[10] The complete recanalization rate in CVT ranges between 30% and 66%.[11] Understanding the factors that could affect the recanalization process is of pivotal importance as some of these can be addressed and could significantly affect the outcomes in patients with CVT such as anticoagulation.[11,12] It is reported that the recanalization often starts within 8 days of starting therapeutic anticoagulation[13] and successful recanalization occurs within 4 months of oral warfarin therapy.[14] Nevertheless, the process of complete recanalization may take 11 months and affect FOC.[15] Interestingly, the benefit of anticoagulation in CVT is not independent of the recanalization process.[16] The goal of anticoagulation in patients with CVT is not only halting the extension of thrombosis but also reducing the risk of recurrent venous thrombotic events (VTEs) and achieving cerebral venous recanalization.[16] Moreover, the role of novel oral anticoagulants (NOACs) in CVT is debatable.[17] A recent multicenter study reported NOACs to have efficacy comparable to and safety better than warfarin in CVT.[17] A study from SA has also reported NOACs to be equivalent to conventional anticoagulants in CVT patients.[18] Various studies have discussed the predictors of FOC and recanalization in CVT worldwide; however, literature from SA is scarce. Evidence from the region is therefore needed to understand the predictors of FOC and determine the rate of recanalization and its effect on outcomes. We, therefore, sought to identify different factors that could predict the outcomes in CVT.

Methods

This retrospective study was carried out in the Department of Neurology at King Fahd Hospital of the University, a tertiary care teaching hospital in the Eastern Province of SA. Ethical approval was obtained from the Imam Abdulrahman Bin Faisal University Institutional Review Board (IRB) Vide Letter No. IRB-2023-01-213 dated May 16, 2023, with a waiver of informed consent since there was no direct relation with human subjects in this study.

Electronic data of all patients aged ≥14 years admitted with a diagnosis of CVT between January 2012 and March 2023 were included. Data were collected by performing a systematic review of electronic medical records (EMRs). EMRs of patients admitted before 2012 were deficient and therefore not included. Demographic and clinical characteristics such as presenting symptoms and duration of symptoms before presentation were collected. Two age categories were defined for the patients: <50 years and ≥50 years. All patients had complete blood counts and prothrombotic parameters worked up when needed. Underlying etiology and provoking factors were identified based on the history (puerperium, Oral contraceptive pills use (OCPs), herbal medications, and opiates), routine blood laboratories (polycythemia, thrombocytosis, and diabetic ketoacidosis), and results of tests for hypercoagulable states (hereditary thrombophilia; protein C, protein S, antithrombin III, factor V Leiden, homocysteine, acquired thrombophilia; antinuclear antibodies, anticardiolipin antibodies, antiphospholipid antibodies, lupus anticoagulant, and antibodies to double-stranded DNA). Diagnosis of central nervous system infections was made according to cerebrospinal fluid analysis. Diagnosis of systemic disorders such as ulcerative colitis and Behcet's disease was made by an internal medicine physician. The type of anticoagulation therapy given, including the duration, was noted.

All patients underwent neuroimaging on admission, like computed tomography (CT), and/or magnetic resonance imaging (MRI) brain. Diagnosis of CVT was

made by vascular imaging such as CT venogram/MR venogram (MRV)/digital subtraction angiogram. The location of thrombosis (transverse, SSS, sigmoid, internal jugular vein [IJ], and straight) and the number of sinuses affected were noted. Follow-up studies were reviewed to assess recanalization. Grading of recanalization was based on the Qureshi classification.[19] Recanalization of venous sinuses was defined as successful for complete/partial recanalization and unsuccessful for nonrecanalization based on follow-up imaging at ≥1-month duration. Patients having follow-up imaging in less than a month duration were not assessed for recanalization.

FOC was assessed by a modified Rankin score.[20] Patients achieving scores 0–2 were considered having good/favorable, whereas those who died or achieved scores 3–6 were considered having poor/unfavorable outcomes.

Data was entered and analyzed using Statistical Package for the Social Sciences (version 20.0, SPSS Inc, Chicago, IL, USA). Descriptive statistics in terms of means with standard deviation (SD), median, and interquartile range (IQR) were calculated for continuous variables. Frequency and percentages were calculated for categorical variables. Pearson Chi-square tests or Fisher's exact tests were performed for comparison of categorical variables such as factors associated with recanalization and FOC. Independent *t*-tests were performed for comparison of continuous variables such as age associated with recanalization and FOC. The difference in distribution of categorical variables was ascertained by estimating odds ratios (ORs) and 95% confidence intervals (CI) using univariate analysis. In all cases, a two-sided $P < 0.05$ was considered statistically significant.

Results

Descriptive findings

In total, 64 patients (35 women and 29 men) with a confirmed diagnosis of CVT were admitted during the study period. The age ranged between 15 and 92 years. All patients except one were >18 years old. The mean age of patients at the time of diagnosis was 37.9 ± 16 years (mean ± SD). Three-fourths of patients presented within 1 month of symptoms onset which were new-onset headache, double vision, blurred vision, hemiparesis, seizure, and altered level of consciousness. The baseline clinicodemographic characteristics are summarized in Table 1. Headache was the most common symptom. Altered consciousness was the manifestation in 20% of the patients. Hereditary thrombophilia and female sex-related hormonal etiologies (puerperium/oral contraceptive use) were the frequently identified etiologies. The median treatment duration in months with IQR was 9 (6–12), whereas the timing to follow-up imaging in days was 180 (135–356). All patients received treatment for more than 3 months of duration. Follow-up imaging to assess recanalization accurately was available in 46 patients only. Four patients died, 6 had repeat imaging at <1 month duration, and 8 patients were lost to follow-up.

Table 1: Demographic and clinical characteristics of all patients

Variables	*n* (%)
Demographic characteristics	
Age, median (IQR)	34.5 (27–46.8)
Male	29 (45.3)
Female	35 (54.7)
Duration of illness <1 month	49 (77)
Clinical features	
Headache	51 (80)
Visual symptoms	11 (17.2)
Impaired consciousness	13 (20.3)
Hemiparesis	19 (30)
Seizures	11 (17.2)
Etiology	
Hereditary thrombophilia	11 (17.2)
Antiphospholipid syndrome	7 (10.9)
Thrombocytosis/polycythemia	3 (04.7)
Puerperium/oral contraceptive use	11 (17.2)
Infections	10 (15.6)
Dehydration and diabetic ketoacidosis	5 (07.8)
Ulcerative colitis	2 (3.12)
Behcet's disease	6 (9.37)
Sickle cell disease	5 (7.8)
Herbal medications and opiates	2 (3.12)
Malignancy	2 (3.12)
Undetermined	3 (4.7)
Radiological variables	
Transverse sinus thrombosis	50 (78.1)
Sigmoid sinus thrombosis	42 (65.6)
Internal jugular vein thrombosis	30 (46.9)
Superior sagittal sinus thrombosis	27 (42.2)
Straight sinus thrombosis	4 (6.25)
Inferior sagittal sinus thrombosis	3 (4.7)
Hemorrhagic venous infarcts on neuroimaging	15 (23.4)
Treatment and outcomes	
Warfarin	20 (31.3)
New oral anticoagulants	23 (35.9)
Heparin/enoxaparin	17 (26.6)
No anticoagulation	4 (6.25)
Follow-up imaging ≥1-month duration (46)	46 (72)
Successful recanalization achieved	31 (67.4)
No recanalization	15 (32.6)
Good outcome	50 (78)

IQR: Interquartile range

Overall mortality was 6.3%. Of four patients who died, three of them had venous hemorrhagic infarctions ($P = 0.01$) with significant edema on presentation. Transverse sinus was affected in all four, sigmoid in two, SSS, and IJ in one patient. Three

patients (4.7%) had recurrent events. Two had recurrent CVT (one with Behcet's disease and other protein S deficiency), and both were advised to continue lifelong anticoagulation. One patient with antiphospholipid syndrome (APS) had deep venous thrombosis in the leg and pulmonary embolism.

Predictors of functional outcome

A significant association of certain variables with FOC was observed. The association of different demographic and clinicoradiological variables with FOC is detailed in Table 2. Less than one-fourth of patients in the study (22%) had poor outcomes which was significantly associated with increased age ($P \leq 0.001$). The mean SD age was 55.2 ± 18.6 years for patients having a poor outcome. A significant association between female sex and good FOC was found as women had increased numbers of good outcomes as compared to men (OR = 1.35, 95% CI = 1.01–1.80, $P = 0.02$). Out of 29 men, 10 had poor outcomes. Altered consciousness at presentation was significantly associated with poor outcome (OR = 2.94, 95% CI = 1.23–6.99, $P = 0.01$) and headache with good outcome (OR = 1.56, 95% CI = 0.93–2.62, $P = 0.01$). The percentage-wise distribution of different factors in patients with poor outcomes is shown in Figure 1. Regarding underlying etiology, hereditary thrombophilia (OR = 3.0, 95% CI = 1.26–7.09, $P = 0.03$) and infections (OR = 3.06, 95% CI = 1.26–7.43, $P = 0.01$) were significantly associated with poor outcomes, whereas female sex-related hormonal etiology (OR = 1.35, 95% CI = 1.15–1.59, $P = 0.05$) was associated with good outcomes. Patients with other systemic diseases, Behcet's disease (OR = 1.34, 95% CI = 1.14–1.57, $P = 0.10$), APS (OR = 1.32, 95% CI = 1.13–1.54, $P = 0.16$), and sickle cell disease (OR = 1.31, 95% CI = 1.13–1.53, $P = 0.21$) had higher likelihood of achieving functional independence as suggested by higher odds but could not attain significant P value which could be attributed to small number of patients. Venous hemorrhagic infarction (OR = 3.26, 95% CI = 1.36–7.82, $P = 0.008$) and nonrecanalization (OR = 1.44, 95% CI = 0.97–2.14, $P = 0.02$) of venous sinuses were significant predictors

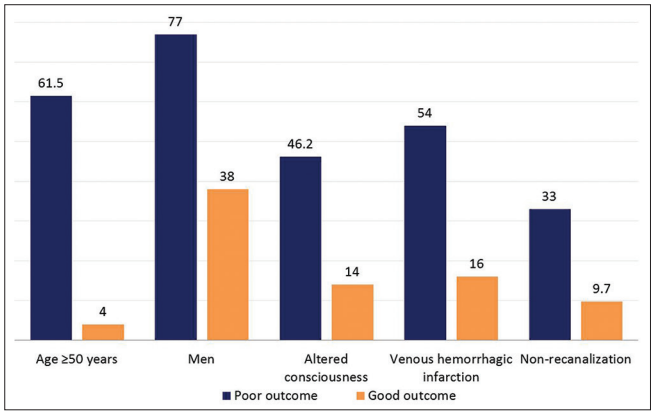

Figure 1: Percentage-wise distribution of variables in patients with different outcomes

Table 2: Association of different demographic and clinical variables with outcome

Variables	Good outcome		Poor outcome		*P*
	OR	95% CI	OR	95% CI	
Age (years), means±SD	33.4±11.9		54±18.4		<0.01
Age (years)					
>50	0.22	0.06–0.78	7.2	3.18–16.2	<0.01
<50	4.44	1.28–15.4	0.13	0.06–0.31	<0.01
Sex					
Female	1.35	1.01–1.80	0.33	0.11–0.94	0.02
Male	0.74	0.55–0.98	3.0	1.05–8.6	0.02
Headache	1.56	0.93–2.62	0.34	0.14–0.80	0.01
Loss of consciousness	0.63	0.38–1.07	2.9	1.23–6.99	0.01
Hemiparesis	0.74	0.51–1.07	2.36	0.96–5.82	0.06
Hereditary thrombophilia	0.60	0.31–1.12	3.0	1.26–7.09	0.03
Puerperium/oral contraceptive use	1.35	1.15–1.59	-	-	0.05
Infections	0.59	0.31–1.12	3.06	1.26–7.43	0.01
Warfarin	1.23	0.98–1.56	0.36	0.09–1.48	0.12
New oral anticoagulants	1.04	0.78–1.37	0.86	0.30–2.47	0.98
Heparin/enoxaparin	0.77	0.53–1.13	2.11	0.83–5.38	0.11
Duration of treatment	9.6±5.2		5.9±4.7		0.04
Superior sagittal sinus	0.99	0.76–1.29	1.02	0.40–2.61	0.95
Transverse venous sinus	0.99	0.72–1.35	1.02	0.33–3.18	0.96
Sigmoid venous sinus	1.01	0.77–1.34	0.94	0.36–2.47	0.90
Internal jugular vein	0.96	0.74–1.25	1.13	0.44–2.18	0.79
Venous hemorrhagic infarction	0.62	0.38–1.01	3.26	1.36–7.82	0.008
Successful recanalization	1.44	0.97–2.14	0.25	0.07–0.89	0.02

SD: Standard deviation, CI: Confidence interval, OR: Odds ratio

of poor outcome. The neuroimaging of two patients having variables that could predict FOC is shown in Figure 2 [Figure 2a-d is from the patient with good FOC. The imaging is showing successful recanalization which is a predictor of good outcome and Figure 2e-h is from the patient with poor FOC. The patient presented with impaired consciousness and the imaging showed hemorrhagic infarction, both features could predict poor FOC]. Significant *P* value was not achieved for treatment with anticoagulation; nevertheless, OR was higher for a good outcome in those who received warfarin and NOACs. Two patients underwent thrombectomy and decompressive craniectomy, and both had poor outcome: one died and the other patient survived with moderate disability. Of two patients who had underlying malignancy, one died and one survived with a good outcome.

Predictors of recanalization

Out of 46 patients assessed for recanalization, 67.4% had successful recanalization. The association of different variables with recanalization is detailed in Table 3. Age ≥50 years (OR = 2.27, 95% CI = 0.88–5.82, *P* = 0.01) and duration of illness more than 1 month before presentation were significantly associated with nonrecanalization. All patients with only SSS involvement had successful recanalization. Failure of recanalization was significantly associated with poor outcomes. Duration of treatment (*P* = 0.83) was not different between patients who had recanalization and those who did not and therefore was not the confounder as all patients received standard care treatment. Nonrecanalization was significantly associated (OR = 2.98, 95% CI = 1.52–5.82, *P* = 0.01) with the development of idiopathic intracranial hypertension (IIH).

Discussion

In this single-center study, certain factors were found to predict outcomes and recanalization in patients with CVT. A higher number of patients had a good outcome in our study than reported previously from SA,[3,4] and this could be because of a higher proportion of patients receiving anticoagulation treatment including NOACs in our study. Nevertheless, some studies have reported good outcomes in similar or even higher numbers of patients than ours.[5,6,21] Age, sex, duration of illness, underlying etiology, presenting clinical features, and radiological findings significantly affected the outcome of our study. Age and altered consciousness predicting the poor outcome is similar to previous observation.[5-7] However, in contrast to Klein *et al*.,[6] we found sex, clot location, and underlying female sex-related hormonal etiology to affect outcomes, which is similar to Salottolo *et al*.'s findings.[7] The presence of hemorrhage on neuroimaging and infectious etiology predicting the poor outcome is consistent with the findings by Ferro *et al*.[5] Hereditary thrombophilia was a frequent cause of CVT in our study and was identified as the most common cause previously.[5] However, in our study, it was associated with poor outcome. Underlying active malignancy has previously been reported to be strongly associated with poor outcome.[5,6] Only two patients in our cohort had underlying malignancy; one of them died and one achieved functional independence, and therefore, it is not plausible to draw any conclusion here. Late presentation, more than 1 month from the onset of symptoms affecting outcome adversely, could be explained by the delay in starting proper anticoagulation influencing recanalization. Therefore, the consequences

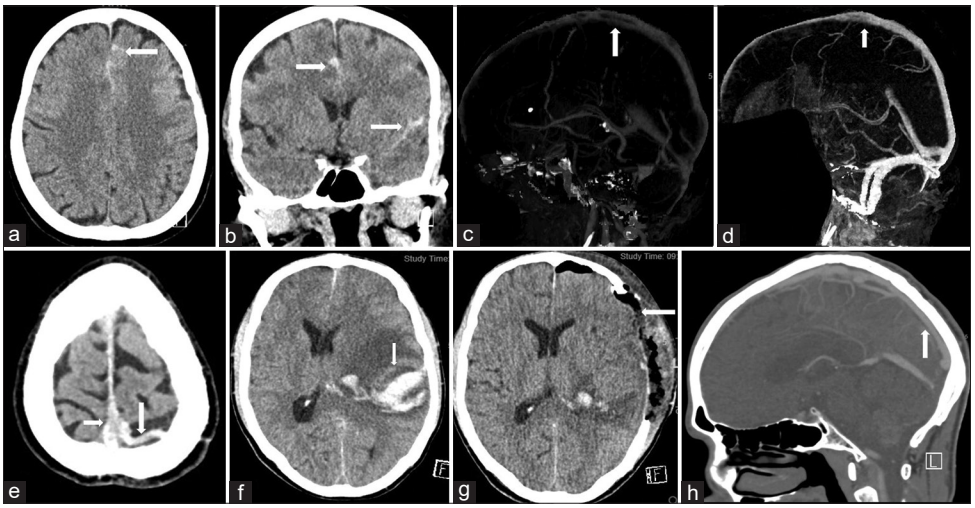

Figure 2: Example of patients with different variables predicting outcome. (a-d) A 45-year-old female with a 1-day history of severe headache and left-sided numbness. The patient was started on enoxaparin. (a) Axial and (b) coronal section of noncontract computed tomography (CT) scan head showing subarachnoid hemorrhage. (c) Maximum intensity projection (MIP) imaging shows a filling defect in the superior sagittal sinus (SSS). (d) MIP at 6 months showed successful canalization, the patient achieved a good outcome. (e-h) A 51-year-old male with severe headache, right-side weakness, and unconsciousness. (e) Axial section noncontract CT shows hyperdense cortical vein and SSS, (f) Left parenchymal hemorrhage with pressure effect. (g) The patient underwent thrombectomy and decompressive craniotomy. (h) A follow-up study at 10 months showed recanalization of SSS, the patient was functionally dependent

Table 3: Variables and their association with recanalization

Variables (*n*)	Successful recanalization (*n*=31)		No recanalization (*n*=15)		*P*
	OR	95% CI	OR	95% CI	
Age younger than 50 years	2.27	0.88–5.82	0.36	0.17–0.75	0.015
Duration of illness >1 month	0.35	0.16–0.76	2.12	0.95–4.73	0.012
Superior sagittal sinus alone	1.62	1.26–2.08	-		0.04
Transverse alone	0.56	0.19–1.68	2.05	0.86–4.84	0.16
Treatment duration: 9.7±5.3	9.8±5.6	-	9.4±4.3	-	0.83
Timing to follow-up imaging: 245.7±139 days	216.5±135	-	325.3±123	-	0.02
Poor outcome	0.50	0.20–1.27	2.37	1.11–5.05	0.04
Idiopathic intracranial hypertension	0.27	0.04–1.59	2.98	1.52–5.82	0.01

Continuous data are represented as mean±SD. CI: Confidence interval, OR: Odds ratio, SD: Standard deviation

of ignoring new-onset neurological symptoms including headaches should be emphasized and those presenting to the emergency department should be evaluated by appropriate imaging. Overall, mortality and poor outcomes are comparable to Feher *et al*.'s findings.[22]

The rate of successful recanalization in our study is similar to previous studies.[11,13,23] However, some studies monitoring patients for a longer duration have reported recanalization in an even higher number of patients than ours.[13,15] Timing of the repeat imaging to assess recanalization varied among the studies. While studies have assessed recanalization based on imaging from 1 week to 3 years, most studies used 3 months or longer duration repeat imaging to assess recanalization.[23-25] A prospective study investigating 68 patients with CVT by MRI/MRV reported evidence of recanalization in 74% of patients within 8 days of anticoagulation which increased to 95% by 90 days.[13] It is worth noting that another recent study observed that the recanalization rate does not change much at 28 days and 3 years of follow-up study.[25] Rezoagli *et al*. reported 19% nonrecanalization with 28-day–3-month follow-up and 20% with 6–12-month follow-up study.[25] No significant differences in CVT recanalization were reported among different timings (overall $P = 0.45$).[25] We did not include a repeat study of <1-month duration showing no recanalization based on the minimum study duration mentioned by Rezoagli *et al*.[25] As 28% of our patients did not have repeat studies to assess recanalization, the actual rate of successful recanalization could be somewhat different. Thrombosis of the SSS is identified as a positive predictor,[11] whereas sigmoid and transverse involvement were identified as negative predictors for recanalization.[12] We also observed SSS involvement to be associated with successful recanalization. A recent systematic meta-analysis has reported the failure of recanalization to be significantly associated with chronic headache, poor FOC, and recurrent CVT.[23] We also observed the patients having an absence of recanalization to develop IIH later, which was statistically significant. Overall, predictors of recanalization and its effect on outcomes in our cohort of patients from a single region in SA were the same as described previously.[13,15,25] Data about anticoagulation in CVT are variable; few studies have reported inconclusive results regarding the effect of anticoagulation on outcome.[21,25] Moreover, due to a lack of highly supportive evidence, the duration of anticoagulation beyond the acute phase has not yet been decided.[26] However, evidence suggests that most of the recanalization occurs within the first 3 months of anticoagulation and therefore continuation of treatment beyond 3 months depends on underlying etiology.[13,25] Importantly, anticoagulation potentially reduces the death and dependency risk in patients with CVT,[27] and the European Stroke Organization recommends 3–12 months of anticoagulation to prevent recurrent venous thromboembolic events including CVT.[26] However, there are no randomized clinical trials for the optimal duration of treatment, and therefore, the decision depends on the underlying etiology and risk of having recurrent VTEs. Patients with VTE or prothrombotic conditions with higher risks of recurrent events need permanent anticoagulation.[26] NOACs and warfarin are considered equally safe and efficacious in the treatment of CVT.[17,18] We also did not find any significant difference between NOACs and warfarin on the FOC.

Limitations

Some limitations must be noted when interpreting the findings described in the study here, one of which is having a retrospective study design; restricting us to including the patients with diagnoses labeled as CVT and therefore, chances of missing patients due to a lack of proper diagnosis cannot be excluded. We could not assess recanalization for all patients as we were dependent only on follow-up imaging available on the EMRs. Second, the sample size was small; however, the number included appears satisfactory considering the prevalence of CVT. Third, as this is a single-center study, the conclusion drawn here does not represent other areas of the country and therefore cannot be generalized. Despite its limitations, factors affecting the outcome and recanalization in CVT have been discussed

and compared with published data here. Furthermore, the results of our study can contribute significantly to the epidemiological data about CVT from SA. Future studies from the region including prospective neuroimaging at uniform intervals are required to understand more about the process of recanalization and its correlation with FOC in patients with CVT.

Conclusion

Less than one-fourth of patients with CVT had poor FOC in our cohort**.** Older age, male sex, hereditary thrombophilia, infections, venous hemorrhagic infarction, and nonrecanalization were predictors of poor outcomes in patients with CVT. Age of more than 50 years and symptom onset of more than 1-month duration adversely affected the recanalization process. The need for appropriate neuroimaging to evaluate patients with headaches having suspicion of CVT in the emergency setting should be emphasized and practiced to avoid any delay in anticoagulation that could otherwise affect the recanalization process and hence FOC.

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Author contributions statement
RS and AZ: Review and editing (equal). AZ: Conceptualization (lead), writing – original draft (lead), formal analysis (lead), and writing – review and editing (equal). AZ: Methodology (lead). AZ and RS: Writing – review and editing (equal).

Conflicts of interest
None declared.

Ethical approval
Ethical approval was obtained from the Imam Abdulrahman Bin Faisal University IRB Vide Letter No. IRB-2023-01-213 dated May 16, 2023, with a waiver of informed consent since there was no direct relation with human subjects in this study.

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