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REVIEW ARTICLE

State of the art in cerebral venous sinus thrombosis animal models

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Abstract

Cerebral venous sinus thrombosis (CVST) is an uncommon venous thromboembolic event accounting for less than 1% of strokes resulting in brain parenchymal injuries. Diagnosis and prognosis are still challenging due to highly variable clinical course and etiologies. Beyond thrombosis, different CVST-related parenchymal injuries may occur and include edema, ischemic strokes, and intra-cerebral hemorrhage (ICH; i.e., parenchymal/subdural hematomas, and subarachnoid hemorrhages), which are identified in 40%–60% of patients without clearly identified mechanisms. In this perspective, experimental animal models contribute to the understanding of initiation, propagation, and resolution of thrombosis, as well as brain-related damages. Last but not least, animal models may be useful to study new therapeutic approaches. In this review, we provide a comprehensive overview of CVST experimental models, focusing on their strengths, limits, and contribution to the current knowledge.

KEYWORDS
brain edema, cerebral venous thrombosis, experimental animal model, inflammation, intra-cerebral hemorrhage, stroke

1 | INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is an uncommon location of venous thromboembolism (VTE) that represents a distinct cause of stroke primarily affecting young adults.[1,2] Predisposing factors for CVST are multiple, including those described in VTE and specific local causes (regional infections, brain tumors, and cranial trauma).[3] Beyond thrombosis, CVST-related parenchymal injuries include edema, ischemic strokes, and intra-cerebral hemorrhage (ICH; i.e., parenchymal/subdural hematomas and subarachnoid hemorrhages), which are identified in 40%–60% of patients.[4–7] Diagnosis and prognosis are still challenging due to the non-specificity and high variability of clinical course. Clinical symptoms result from increased intracranial pressure due to impaired venous drainage and CVST-related brain injury. Clinical symptoms range from isolated headaches (the most frequent one at presentation ≈90%), to focal deficits, seizure, and coma.[4,5,7,8] Recent studies reported higher incidence of CVST than previously estimated (13–17.5 vs. 3–5 million per year, respectively) with an overall death and dependency rate ≈15%.[1,2,6] This may be the result, at least in part, of the improved

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availability and performance of brain imaging, leading to better diagnosis and identification of less severe cases over the past decades.

Based upon the limited evidence available compared to common VTE,[9] initial anticoagulation with either unfractionated heparin (UFH) or low molecular weight heparin (LMWH) bridging with vitamin K antagonists (VKAs) is currently recommended, regardless of the presence of ICH.[1,2] Despite intensive anticoagulation treatment, ≈20% of patients with CVST will experience clinical deterioration or maintain their disability.[4] Endovascular therapy (EVT) through local injection of fibrinolytic drugs (e.g., altepase, urokinase), mechanical thrombectomy, or both have been proposed to improve clinical outcome.[1,2,10] EVT aims to achieve rapid recanalization compared to anticoagulants, which mainly prevent extension, embolization of the existing thrombus, and thrombosis recurrence. To date, no study has demonstrated any additional benefit of EVT over heparin in the setting of the acute phase.[11] Recently, direct oral anticoagulants (DOACs; i.e., dabigatran, rivaroxaban, apixaban) were introduced providing new perspectives for CVST treatment. Although guidelines do not currently support DOACs treatments during CVST, different reports suggested their sufficient safety and efficacy compared to VKAs, which require validation in larger studies.[12,13]

Thus, a major preclinical approach for deciphering the pathophysiology of CVST and testing new therapeutics is the goal of animal models. In contrast to arterial ischemic stroke, only few experimental CVST models have been developed, mostly including small animals, such as rodents, rather than large animal models.[14] Considering differences between the cerebral venous network in rodents and humans and numerous anastomosis, it remains difficult to obtain models relevant clinically.[15,16] Still, the use of rodents allows use of genetically modified strains, which offer the opportunity to elucidate the potential role of genes involved in CVST.

Herein, we review different common rodent models, their strengths and limitations, as well as, their contribution over the last decades to the knowledge of CVST.

2 | RODENT MODELS OF CVST

In experimental models, CVST is induced either by vascular wall injury, blood flow restriction, or coagulation activation, defined as cornerstones of thrombosis in the Virchow's triad.[17] Cerebral cortical veins and dural venous sinuses are commonly targeted to trigger thrombosis. Particularly, the superior sagittal sinus (SSS) is an interesting target, as it constitutes the main location of CVST in human.[4,18] An endovascular approach is rarely used, as it requires available intravascular imaging techniques to obtain a reproducible location of thrombus and is rather performed in large animal models. In rodents, almost all models require invasive approaches with prior exposure of sinuses and veins by craniotomy, which may induce iatrogenic parenchymal damages. Thus, some issues have to be considered by researchers as experimental CVST models present several technical challenges and display different features depending on each technical approach (Table 1, Figure 1).

TABLE 1 Main characteristics of CVST rodent models

Type of model	Species	Sex	Thrombosis location	Injury pattern				Neurological function	Spontaneous recanalization	Timepoint	Ref.
				Hemorrhage	Ischemia	Hemorrhage	Edema				
Photothrombosis	Rat	M	CtV	+		-	+	⇓	ND	1–7 days	20–25
Electrocoagulation	Mouse	M	CtV	+		+	+	⇓	NA	48 h	26
$FeCl_3$	Mouse, rat	M	SSS	±		-	+	=	≈50% on day 7	1–6 weeks	30–37
Permanent ligation	Gerbil	M	SSS+CtV	+		±	+	ND	NA	5 days	40
Thrombogenic substance (Kaolin)	Rat	M	SSS+CtV	+		-	+	ND	≈40% on week 4	4 weeks	49
Thread-embolism	Rat	M/F	SSS	-		-	+	ND	NA	5–14 days	46–48
Combined[a]	Rat	M	SSS+CtV	+		±	+	⇓	NA	2–7 days	39,41–44
Thromboembolic											
Endovascular	Rat	M	TS±SSS	+		+	+	ND	ND	2 h	52
In situ clot-injection	Mouse	F	SSS+CtV	+		±	+	⇓	No recanalization on day 7	7 days	53

Abbreviations: CtV, cortical veins; CVST, cerebral venous sinus thrombosis; F, female; M, male; NA, not adapted; ND, not determined; SSS; superior sagittal sinus; TS, transverse sinus.

[a]Combined models include those using photothrombosis or permanent ligature associated with an injection of thrombogenic substance into SSS.

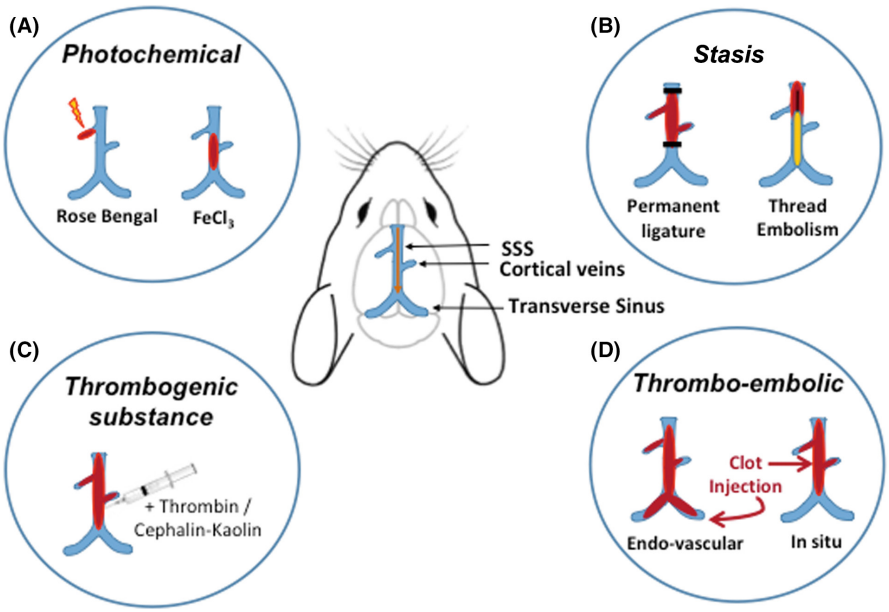


FIGURE 1 Schematic representation of the different injury techniques to induce cerebral venous sinus thrombosis (CVST) in animal including photochemical (A), stasis (B), thrombogenic (C), and thromboembolic (D) models. SSS, superior sagittal sinus.

2.1 | Photothrombosis and electrocoagulation CVST models

Photothrombosis and electrocoagulation models have been used to induce isolated thrombosis of cortical cerebral veins (Figure 1A). Photothrombosis models use photoreactive dye (i.e., rose bengal), which, after intravenous injection, accumulates in membranes of vascular endothelial cells. It promotes formation of reactive oxygen species and endothelial injury when exposed to 543 nm-wavelength light.[19] Type, intensity, duration of light illumination source, and rose bengal concentration must be optimized to achieve thrombosis without damaging cortical parenchyma in off-target areas. High-intensity excitation light on the brain can lead to endothelial damage, but also adverse thrombosis in nearby and deeper structures.[19] It is likely due to the non-specificity of dye distribution and nature of one-photon reaction. Depth-targeted vessel photothrombosis by multiphoton excitation could represent an ongoing evolution to avoid unintended photodamage.

However, photothrombosis models are minimally invasive and avoid intracranial vessel dissection compared to electrocoagulation models.[20–26] Some authors have demonstrated that occlusion of two adjacent cortical veins is required to induce early and reproducible ischemic lesion, with rare hemorrhagic events within 2 h.[23] These models support the hypothesis that cortical vein involvement may be critical in the occurrence of brain injury during compromised venous circulation. However, isolated cortical vein thrombosis represents a rare condition in humans (<5%) as it is most often combined with thrombosis of a major cerebral sinus.[4,5] In fact, isolated cortical vein thrombosis results more frequently in cortical subarachnoid hemorrhage than common CVST.[27] Thus, these experimental models do not strictly mirror the main location of CVST and its pathophysiology.

2.2 | Ferric chloride injury model

Ferric chloride ($FeCl_3$), an oxidizing agent, has been widely used to induce VTE as well as CVST in rodents.[28] This model is based on topical application of $FeCl_3$-soaked filter paper to vessel adventitia resulting in thrombosis of SSS (Figure 1A). Clot initiation is attributed to free iron-induced denudation of endothelial cells and exposure of subendothelium, which trigger platelet and coagulation activation.[19] Recently, it has been shown that $FeCl_3$ also induces nonspecific charge-based aggregation of different blood components involved in vessel occlusion.[29] Experimental models for CVST using $FeCl_3$ are characterized by transient thrombosis of SSS. Spontaneous and early recanalization has been reported within 1 week depending on studies, species, length, and concentrations of $FeCl_3$-soaked filter paper (from 10% to 40%).[30–37] Of note, concentrations used were higher than previously reported in extra-cerebral venous models of thrombosis (<10%).[28] Vessel structure and thickness can impact the diffusion of $FeCl_3$ in the vascular space and can therefore vary the $FeCl_3$-related thrombosis burden.[29] Although $FeCl_3$ achieves successful SSS thrombosis,[31,36,37] Stolz et al demonstrated that $FeCl_3$-related occlusion led to the congestion of bridging cortical veins towards SSS but not to thrombosis.[33] Therefore, $FeCl_3$ model leads inconsistently to ischemic lesions with rare hemorrhagic transformation in contrast to 12% to 44% of cases reported in humans.[1] Of note, the site of $FeCl_3$ application should be thoroughly irrigated to avoid uncontrolled $FeCl_3$ diffusion to parenchyma, which could contribute to cortical injury unrelated to SSS thrombosis.

Together, these results underline that $FeCl_3$-induced SSS thrombosis does not tend to spread to the cortical veins or other sinuses resulting in good prognosis without symptomatic injury. Thus, this model does not strictly reproduce CVST pathophysiology observed in humans. This model allows the investigation of acute thrombus

formation and its resolution at different time points and can be easily implemented in mice.

2.3 | Stasis-associated CVST models

Stasis-associated CVST models include permanent ligation or insertion of a thread-embolism into the SSS (Figure 1B). These models seek to achieve complete stasis in the SSS, to produce occlusive thrombi with high reproducibility.

Ligature models are based on permanent rostral and caudal ligatures of the SSS. However, patterns of branching cortical veins along SSS and location of ligature could introduce variability in these models.[38,39] Only one model, performed in gerbils, is based on an isolated SSS ligation. This model resulted in occlusion of both SSS and cortical veins with ischemia and petechial hemorrhages.[40] All of other ligation-based models are combined models, performed in rats, which associate with stasis and hypercoagulability. Compared to gerbils, rats have extensive collateral veins draining into different sinuses to overcome SSS thrombosis and prevent parenchymal injury. Thus, rat CVST models require an additional injection of thrombogenic substance (e.g., kaolin–cephalin, thrombin) into the SSS after ligation, in order to increase thrombosis burden and induce parenchymal damage.[39,41–44] These results underline that, beyond using the same model, characteristics can change according to variation of cerebral venous network of each individual and each species.[44]

Ligation-based models result in permanent occlusion of SSS that is not suitable for venous recanalization process investigation. Nevertheless, these models offer the opportunity to study cellular mechanisms involved during persistent thrombosis despite anticoagulant therapy as observed in 15% of patients.[45]

In contrast, thread-embolism models (silicone, plastic) consist in a transient occlusion of the SSS.[46–48] These models allow studying mechanisms involved during occlusion and recanalization (after thread removal) while potentially varying the occlusion time. The latter models induce brain edema and angiogenesis following SSS occlusion, but they do not involve occlusion of other draining veins (i.e., cortical veins), which may account for the absence of observed ischemic lesions or ICH. Thread-embolism models resulted in a mechanical occlusion and not in a thrombus formation into the SSS that could also change CVST phenotype compared to ligation-based models.

Currently, stasis-related models have mainly been developed in rats. Experiments in larger animals, such as rats, allow easier surgery, and greater blood and tissue collection. Despite these advantages, the use of rats has lagged behind that of mice in the development of genetically engineered strains that could be useful to study specific risk factors in CVST models.

2.4 | Thrombogenic substance-induced CVST

Thrombogenic substance-induced CVST models are based on local delivery of a high dose of thrombin (50–100 UI/ml), cephalin/kaolin suspension, in situ into the SSS (Figure 1C). These models aim to induce a more physiological thrombus. With the exception of the model developed by Wang et al,[49] most of these are combined models. They include ligation[39,41–44] or photothrombosis[50] around the SSS to limit uncontrolled dispersion of the thrombogenic solution into venous circulation, as well as in other organs. In these models, thrombosis involves a large part of the SSS, which in most cases extends into cortical veins. This model induces a more acute CVST with parenchymal edema, reproducible ischemic lesions, and a high rate of ICH lesions (40%–60%) that occur within 24 hours.[39,42,43] Cortical parasagittal infarcts disclose variable lesion volume depending on thrombosis extension and measurement method (e.g., *in vivo* imaging, histology). Consistently, neurological assessments after CVST demonstrated an early worsening of motor activity.[41,42,50] Due to the venous ligation, improvement of infarct lesions, edema, and neurological functions after 1 week are more likely related to the initiation of venous collateral circulation and angiogenesis rather than a decrease in thrombosis burden.[50]

Thrombogenic substance-induced CVST models are useful to study mechanisms involved in hemorrhagic transformation following CVST as observed in humans.[4] A major shortcoming of the SSS ligation is the limitation of their application for therapeutic evaluation.[41,43] In fact, opening the venous sinus is required to monitor thrombosis size reduction during treatment. To overcome this drawback, Rahal et al suggested using temporary ligatures to improve this model.[51]

2.5 | Thromboembolic CVST models

Two different thromboembolic CVST models have been developed using an endovascular approach or a clot injection into the SSS (Figure 1D). Compared to ligation-based models, thromboembolic CVST models associate the injection of an *ex vivo* standardized preformed clot, as pro-coagulant surface, with the reduction of cerebral venous blood flow to induce CVST.

The first model consists of an endovascular retrograde injection of an autologous blood clot through the external jugular vein in rats.[52] Clot injections have been combined with ligature of contralateral jugular vein resulting in venous flow reduction to promote thrombosis. Compared to previous models, this one leads to a higher thrombotic burden characterized by systematic thrombosis of the transverse sinus and the SSS, as well as the deep venous system. This model was associated with severe ischemic lesions and ICH (with a pattern ranging from intra-parenchymal to intra-ventricular hemorrhage) within 2 h. The latter model mirrors severe CVST with involvement of multiple sinuses and veins. The main advantage of this model is that the surgical approach does not require a craniotomy or direct manipulation of intracranial vessels. These two points are critical to prevent iatrogenic injuries. In addition, composition of injected thrombi could be modulated using clots derived from whole blood or selected blood components. The main difficulty of this model remains the standardization of thrombus location during its injection.

In contrast, we have recently developed a thrombus-injected CVST mouse model.[53] The clot was pre-formed in a tube using thrombin and incubated overnight before injection directly into the SSS. Injection was combined with bilateral external jugular vein ligation. With this model, a persistent SSS occlusion over 7 days is obtained, associated with extensive thrombotic occlusion of cortical veins. Symptomatic ischemic and hemorrhagic damages were observed in 100% and 30% of animals, respectively, closely mimicking the human condition. Although this model does not allow the study of *in vivo* thrombus formation, it is suitable for the evaluation of thrombosis extension and its resolution.

3 | RELEVANT ENDPOINTS FOR CVST MODELS

No recommendations are available regarding the methodological requirements to accurately assess CVST in experimental models. A wide range of *ex vivo* and *in vivo* methods are currently used, making it difficult to compare the different studies. In contrast to deep vein thrombosis (DVT) models, beyond the evaluation of thrombosis the assessment of brain damag and clinical impact needs to be considered.

The evaluation of thrombosis in CVST models still remains challenging. Immunohistology is mainly used to confirm thrombosis on whole brain analysis although reliable thrombosis quantification represents a significant limitation. In contrast to DVT, thrombus weight is rarely measured in CVST models. In fact, excision of thrombus from SSS without damaging the brain can be challenging as SSS is triangular in cross-section and adjoins dura mater. In addition, some models provide small thrombi, like photochemical models, requiring ultrasensitive microbalance.[54]

Imaging of veins and brain represent a cornerstone of CVST models, including micro- and nanocomputed tomography imaging to study contrast-perfused vessels and occluded veins on whole brain after removal.[55,56] To evaluate *in vivo* thrombosis processes, different techniques have been developed such as laser Doppler flowmetry, magnetic resonance venography (MRV), and real-time intravital microscopy. Laser Doppler flowmetry has been commonly used to evaluate variation in local cerebral blood flow as an indirect measure of thrombosis burden.[57] MRV is a contrast-free and non-invasive technique whose benefits are to assess thrombus size after its formation and recanalization rate over weeks.[49] Intravital microscopy can be used to confirm *in vivo* thrombosis using fluorescein,[41,50] or imaging probe targeting thrombus.[36] In fact, intravital microscopy offers the opportunity to monitor thrombosis over time using injection of labeled fluorescent thrombus.[53] This technique allows us not only to assess thromboinflammatory processes in large vessels but also in microcirculation by labeling neutrophils, platelets, or fibrin (Figure 2B).[40,53]

Regarding parenchymal damages evaluation, histological analyses are commonly used to quantify ischemic/hemorrhagic lesions and edema using hematoxylin–eosin, Nissl, and Evans blue staining, respectively.[32,43,53] For infarct volumes, the percentage of injured (pyknotic) neurons may be determined to reflect the brain damage related to venous ischemia (Figure 2C). Triphenyltetrazolium chloride (TTC) staining is also used to determine ischemic volumes but is less sensitive than Nissl staining. *In vivo* techniques include tissue impedance, electro-encephalogram, and magnetic resonance imaging (MRI).[37,41] MRI constitutes a useful tool to evaluate and monitor

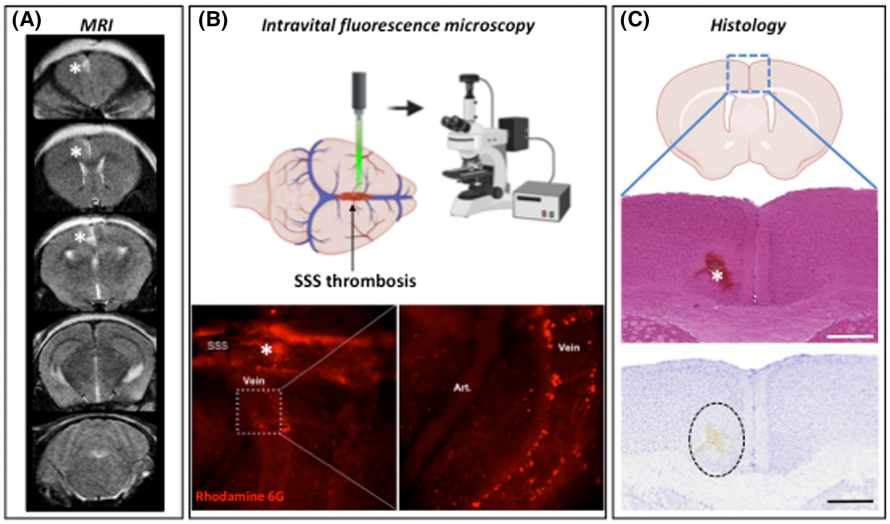


FIGURE 2 Illustration of the different techniques for assessing cerebral venous sinus thrombosis (CVST)-related damage in superior sagittal sinus (SSS)-injected thrombus mouse model (adapted from Bourrienne et al[53]). A, Serial coronal T2-weighted images from mouse 1 day after CVST induction, demonstrating localized ischemia in cortical parenchyma (white asterisk). B, Representative intravital microscopy images showing cerebral vascular network after CVST (created with biorender.com). Leukocyte and platelet staining with rhodamine 6G (red) were recruited both at the thrombosis site into the SSS (asterisk) and at a distance from the thrombus in branching cortical veins (white dotted lines). C, Representative hematoxylin and eosin (top) and Nissl staining (bottom) of coronal brain sections after CVST showing hemorrhagic infarct (white asterisk). Scale bar: 500 μm. MRI, magnetic resonance imaging.

brain injury during CVST model, even though it cannot reach sensitivity of histological analysis (Figure 2A). Both free-contrast and contrast MRI have been used to depict and quantify ischemic, hemorrhagic lesions and edema. Of note, MRI assessments during $FeCl_3$ model should be interpreted with caution as any residual $FeCl_3$ can cause magnetic susceptibility artifacts disturbing magnetic field and suggest the presence of hemorrhagic lesions. $AlCl_3$, a non-ferromagnetic clot inducing-agent has been proposed as an alternative in preclinical studies to overcome this pitfall.[58]

Although rodent models have provided insights in the pathophysiological processes during CVST, neurological functional outcome was not systematically reported. Differences in lesion type and size, time points, and sensitivity of neurological scores can variably affect neurological evaluation. In the acute phase, Bederson scale and rotarod test have been commonly used to assess neurological functions. However, they could be less sensitive than implementation of different sensorimotor tests to detect slight neurological impairment.[26,35,37,48,53] In contrast, long-term models of CVST are rarely implemented, and data on the assessment of neurological function are therefore limited.[33,41,46] Only one study has shown that CVST was associated with long-term locomotor activity deficits using the measurement of wheel running activity.[41]

4 | ADVANTAGES, LIMITATIONS, AND APPLICATIONS OF CVST MODELS

Due to the multifactorial nature of CVST, *in vivo* animal models provide important tools for deciphering thrombosis-mediated mechanisms and testing new therapies. An ideal CVST model should be easily reproducible and provide the spectrum of injury patterns observed in humans. Thus, no single model encompasses CVST at all stages, each model having its advantages and limitations (Table 2). While results from animal models of thrombosis have been widely extrapolated to humans, significant differences in hemostasis and thrombosis processes, as well as variations in vascular network, may limit their relevance for bench-to-bedside translational research. In mice and rats, abundant interconnecting collaterals and higher connections between intracranial and extracranial venous pathways could overcome thrombosis and protect them from cerebral damage compared to humans.[39,59] Thus, using rodents requires careful evaluation of their vasculature and precise occlusion techniques to successfully achieve CVST. Currently, CVST models mainly trigger local thrombosis within healthy vessels unlike human CVST, in which thrombosis is usually associated with systemic pro-coagulant abnormalities or pro-inflammatory states.[4,5] Although components of the coagulation cascade are strongly conserved between mammals, response to pro-coagulant stimuli is highly different between humans and rodents. In particular, several studies have reported a decrease in thrombin generation in plasma from mice and rats compared to humans after tissue factor–dependent activation of coagulation.[60,61] Therefore, thrombus formation pathways also strongly influence thrombosis burden and its resolution, as well as related brain damages.

Thus, model selection should be based on the specific research question taking into account variation in venous network of selected species, time points, relevant endpoints, available equipment, and techniques.

Photochemical injury (i.e., $FeCl_3$ and rose bengal models) is probably relevant to study mechanisms underlying thrombogenesis during CVST because the: (1) thrombus is formed *in vivo*, (2) without the addition of pro-thrombotic substance, (3) with maintained blood venous flow. However, rose bengal models have been only developed to induce isolated cortical vein thrombosis and do not allow the complete pathophysiology of CVST to be studied because sinuses are not involved (Figure 1A).

Although all models achieve systematic brain damage, injury patterns differ according to the methods chosen to induce thrombosis. Thromboembolic and combined models are the most relevant ones to study parenchymal injuries as they address a broad spectrum of injuries including edema, ischemic lesions, and hemorrhagic transformation. In addition, in these models, brain injury has been associated with neurological deficits (Table 1). However, all of these are invasive and could induce iatrogenic parenchymal damage and bleeding.

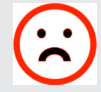
Researchers should also consider thrombus formation pathways used in the different models as it influences the degree of occlusion and related brain damages. Thromboembolic and combined models using thrombin provide fibrin- and erythrocyte-rich clots, which recapitulate CVST features. Advantage of the thromboembolic model over combined ones is the opportunity to characterize the clot before its injection and modulate its composition. In the clot-injected model, thrombus constitutes both pro-coagulant and pro-inflammatory surfaces, as observed with thrombus early growth through new platelet and leukocyte recruitment.[53]

Experimental models constitute also a useful tool for novel therapy testing and require, in this case, a model characterized by thrombosis formed into an open sinus channel. Thrombus composition and time of exposure to anticoagulant therapy also affects recanalization. In the $FeCl_3$ model, pretreatment with heparin delays thrombus formation but does not represent the clinical scenario in which heparin is used to treat CVST within a median of 7 days after the onset of symptoms.[4,36,62] Conflicting results are reported when heparin is administrated after CVST induction with no improvement of recanalization rate.[33,63,64] Pharmacological thrombolysis using tissue-type plasminogen activator was also evaluated and achieved higher recanalization rate than heparin in pre-clinical models.[63] However, this strategy is not currently recommended, as it does not improve functional outcome in patients with CVST.[11]

5 | NEXT CHALLENGES FOR CVST MODELS

Despite their limitations, animal models have significantly increased our understanding of CVST pathophysiology. Currently,

TABLE 2 Advantages and limitations of CVST models according to specific research question

	Specific research question to study					
Type of model	**Thrombogenesis**	**Injury pattern**	**Neurological impairment**	**Recanalization evolution**	**Treatment evaluation**	**Other remarks**
Photothrombosis						Does not involve sinuses thrombosis
Electrocoagulation						
$FeCl_3$						Possible artifacts using MRI technique
Permanent ligation			ND			
Thrombogenic substance (Kaolin)			ND			
Thread-embolism			ND			After withdrawal, recanalisation could be studied
Combined[a]						Useful for studying persistent thrombosis
Thromboembolic						
Endovascular			ND			
in situ clot injection						

Abbreviations: CVST, cerebral venous sinus thrombosis; MRI, magnetic resonance imaging; ND, not determined; SSS, superior sagittal sinus.

[a]Combined models include those using photothrombosis or permanent ligature associated with an injection of thrombogenic substance into the SSS.

therapeutic options for treating CVST are mainly limited to anticoagulant therapy as a standard of care to prevent thrombosis extension. Anticoagulant treatment improves early recanalization rate and recovery of brain injury.[65] While parenchymal lesions are determinant for patient prognosis in the setting of CVST, predictive factors for their development are still largely elusive.[66] Thus, future directions for preclinical animal studies should focus on mechanisms involved in parenchymal injury formation to identify predictive biomarkers and new CVST therapeutic targets.

Experimental studies have demonstrated that CVST leads to parenchymal injuries when collateral venous circulation fails to maintain cerebral blood flow during CVST.[57] Emerging evidence indicates that, beyond thrombosis, an inflammatory process occurs during CVST including recruitment of immune cells and upregulation of pro-inflammatory cytokines.[35] These data raise the possibility that inflammatory response could, at least in part, contribute to brain damage, as described in arterial ischemic stroke.[67] Regardless of the experimental CVST model, early platelet- and leukocyte-endothelial cell adhesion is described in the cerebral vasculature concurrently to blood–brain barrier (BBB) disruption.[34,40,43,53,68] Recruitment of leukocytes is also accompanied by a pro-inflammatory activation of microglial cells, the resident macrophages of the central nervous system.[35] In the $FeCl_3$ injury model, depletion of neutrophils prevented BBB breakdown.[34] These results suggest that neutrophils could contribute to cerebral injury releasing proteolytic enzymes or mediating pro-inflammatory response. Cellular mechanisms involving adhesion, activation, and migration of leukocytes/platelets remain to be determined in the field of CVST. Further studies are needed to assess potential adverse effect of all these inflammatory cells in parenchymal damage. Recently, Aguiar de Sousa *et al* found that higher baseline levels of plasma inflammatory biomarkers (IL-6, protein C reactive) are associated with worse functional prognosis at 3 months but not with early evolution of brain injury.[69] Thus, relationships among inflammation, parenchymal damage, and clinical outcome in patients still need to be elucidated. In a murine model of CVST, inhibition of anticoagulant protein C, mimicking inherited thrombophilia, resulted in higher BBB permeability, leukocyte adherence, and mortality after CVST.[34] These data support that both thrombosis and inflammation are common pathways during CVST. In addition, CVST elicits an acute endothelial dysfunction characterized by: (1) tight junction disruption, (2) basal lamina damages, and (3) upregulation of matrix metalloproteinase 9 activity (MMP-9).[35,46,70] A clinical study has suggested circulating MMP-9 as a predictive biomarker of brain damage in patients with CVST.[71] Nevertheless, impact of chronic endothelial dysfunction on outcome of CVST has been poorly investigated as almost all experimental models trigger local endothelial damage within healthy vessels.

CVST is a multifactorial disorder precipitated by different risk factors such as thrombophilia, use of oral contraceptive, pregnancy, cancer, infection, or inflammatory diseases. Identified in 70–80% of patients with CVST,[4,5] these factors are expected to affect coagulation, the fibrinolytic system, and endothelial function. Challenge remains to develop animal models that address specific risk factors for investigating their potential role in thrombosis and cerebral injury.

6 | CONCLUSIONS

Reproducing all stages of CVST in experimental models to yield translational research remains challenging. Experimental animal models for CVST have provided a basis for studying its physiopathology, prognosis, and treatment. All models highlight that CVST is a complex disease that depends on both venous collateral circulation and risk factors for growing thrombus such as pro-coagulant abnormalities, endothelial dysfunction, and inflammation. Thrombus formation pathways vary according to the CVST models and strongly influence thrombosis burden and its resolution, as well as related brain damage. Ideally, models should recapitulate the pattern of thrombosis, the spectrum of parenchymal injuries, and clinical settings observed in humans and provide a platform for investigating novel therapeutic approaches.

However, none of them resume all stages of human CVST. Therefore, the choice of CVST model should be based on a specific research question to address and considering brain and vessel evaluation.

AUTHOR CONTRIBUTIONS

MCB and JG did the literature research and wrote the first draft of the review. MCB, NA, and MM designed the figures and the tables. NA and MM performed critical revision. All the authors reviewed the manuscript and approved the submitted version.

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

INFORMED CONSENT

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