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RECOMMENDATIONS AND GUIDELINESS

Strengths and weaknesses of a new mouse model of thrombosis induced by inferior vena cava stenosis: communication from the SSC of the ISTH

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Deep vein thrombosis (DVT) is a significant cause of morbidity and mortality throughout the world [1]. Risk factors for DVT include major surgery, immobilization, trauma, and cancer, among others [1]. Many mouse models of thrombosis have been used to study mechanisms of thrombosis, including those involving small and large veins. In this report from the Animal Models Subcommittee of the ISTH, we summarize the strengths and weaknesses of the inferior vena cava (IVC) stenosis mouse model of venous thrombosis.

History

In this model, the lumen volume of the IVC is reduced by ~ 90% [2,3]. This model was adapted from a similar rat model called the 'St Thomas Model', involving IVC stenosis followed by endothelial damage with vascular clamps upstream of the stenosis site [4]. Many variants of the St Thomas model have been described. The IVC stenosis mouse model described in this article was first reported in 2011, and does not involve a vascular damage step [2]. Since then, several groups have used the model, with slight variations [3,5–9]. Thrombosis in this new model is thought to be initiated by the combination of endothelial activation, a reduction in blood flow velocity, and disturbed blood flow upstream of the stenosis site [2,3].

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Methods

In this model, a midline laparotomy is performed on anesthetized male mice that are at least 8 weeks of age and 22 g in weight [2,3,6]. The IVC is exposed by atraumatic blunt dissection, and the IVC is carefully separated from the abdominal aorta. A spacer is placed on top of the exposed IVC, and a non-reactive permanent narrowing ligature (7.0 or 8.0 monofil polypropylene) is secured around both the IVC and spacer directly below the renal veins. The spacer is then removed, resulting in a ~ 90% reduction in IVC lumen size at the stenosis site [2]. Injury to the IVC wall is avoided during the procedure. Mice with any bleeding from the IVC are excluded. The surgical site is then closed, and mice are allowed to recover under the influence of appropriate analgesia.

Sources of variability

The spacers used for this model range from 0.26 to 0.36 mm in diameter, and consist of guide wires, sutures, or blunted needles [2,3,5–9]. The most common spacers used to date are a 0.36-mm guide wire [3,9] and a blunted 30-gauge needle [2,6,7]. The effect of spacer diameter and length on thrombosis in this model has not been evaluated. The method of surgical anesthesia also varies, with an isoflurane/oxygen mixture being the most common [2,3,5–9]. There is some debate as to whether side branches should be ligated to control for IVC anatomy variation. A concern with side branch ligation is that it may result in endothelial damage and increase the duration of surgery. One group compared the size of thrombi in mice with and without side branch ligation, and found

no difference between the two groups [9]. This same study, however, found that side branches that were < 1.5 mm from the stenosis site resulted in smaller thrombi [9].

Quantification of thrombus size

Thrombus size in this model is typically quantified by measuring thrombus mass or size at a given time point after the induction of stenosis. In order to obtain these measurements, mice are euthanized and the IVC is harvested. We and others have extended the utility of this model by using high-frequency ultrasonography to image blood flow and quantify IVC thrombus growth *in vivo* (Fig. 1A–C) [9].

Strengths and weaknesses of the new model

It has been well documented that human DVT is most often initiated within the valve sinuses of large veins on an intact endothelium [1]. A strength of this model is that thrombosis is initiated in a large vein in the mouse in the absence of major vessel damage. Furthermore, although there are no valves in the IVC, the disturbed blood flow occurring around the stenosis site may mimic changes in blood flow that occur over valves and within valve pockets in large veins in humans (Fig. 1D). The thrombi that develop in this model are also histologically similar to human venous thrombi [2,3].

The major concern with this model is that thrombus development is variable. The reported incidence of

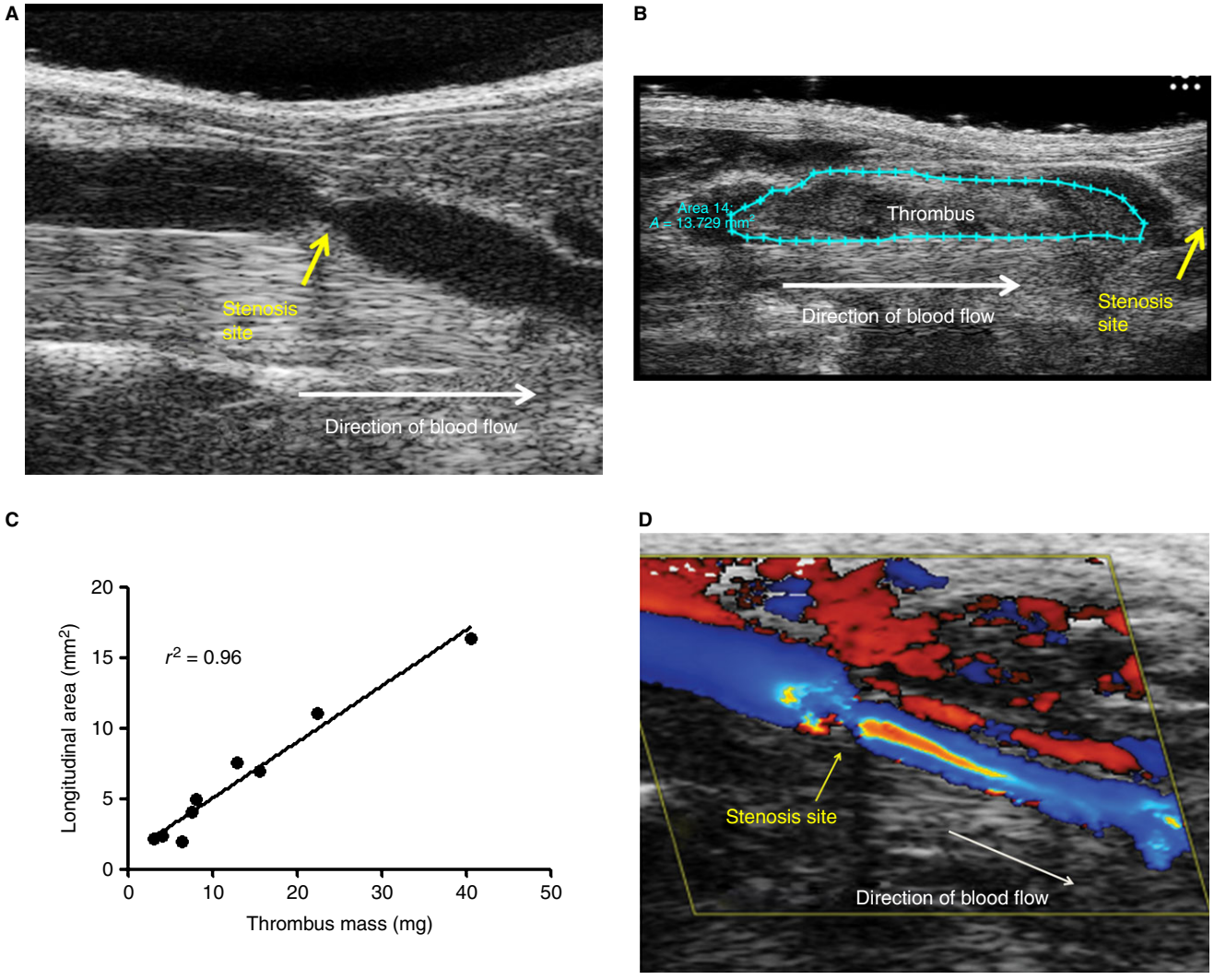

Fig. 1. *In vivo* imaging of the inferior vena cava (IVC) stenosis model. (A, B) IVC stenosis was performed in C57Bl/6J mice, and ultrasonographic imaging of the stenosis site (A) before and (B) after thrombus formation was performed. (C) Longitudinal thrombus area was measured in mice at 24 h, by ultrasonography. Thrombi were then immediately harvested and weighed. Linear regression analysis for longitudinal thrombus area vs. thrombus mass identified a strong correlation (r^2 = 0.96). (D) Color Doppler imaging reveals a pattern of disturbed blood flow around the stenosis site prior to thrombus development.

thrombosis at 48 h in wild-type mice ranges from 44% to 100% [2,5,8,9]. We have a lot of experience with this model in our laboratory, and followed the development and growth of thrombi in 15 C57Bl/6J mice aged 14–18 weeks over a period of 48 h. High-frequency ultrasonography was used to measure thrombus size in each mouse at 3, 6, 9 and 48 h after the initiation of thrombosis (Table S1). As expected, we found that both thrombus size at each time point and time to thrombus initiation were variable, with thombi appearing in the IVC anywhere from 3 to 48 h after stenosis (thrombus area, incidence: 3 h, 0–8.5 mm^2, 13%; 6 h, 0–11.1 mm^2, 33%; 9 h, 0–10.1 mm^2, 40%; 48 h, 0–11.1 mm^2, 53%). Another limitation of this model is that it is performed in a vein that does not have valves.

Conclusions

Like any model, this mouse model of venous thrombosis does not accurately reflect all aspects of human DVT. However, as discussed above, it does have certain strengths over other available models. Primarily, the initiation of thrombosis occurs without the induction of major vascular damage, which more closely mimics human DVT than models triggered by chemical or physical vessel damage. Nevertheless, the high variability in thrombus development is a significant concern that limits its utility for detecting small differences between groups.

Addendum

J. Geddings and N. Mackman wrote the manuscript, with input from M. Aleman, A. Wolberg, M. von Brühl, and S. Massberg.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Size and incidence of thrombosis in the IVC stenosis model.

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