Venous thromboembolism swine model with reflux-induced venous hypertension

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ABSTRACT

Objective: This study describes a novel swine model of venous thromboembolism (VTE) with reflux-induced venous hypertension.

Methods: Six pigs underwent disruption of the tricuspid chordae tendineae to create reflux and venous hypertension in the femoral vein. The vein was traumatized 2 to 3 weeks later by repeated withdrawal of a slightly overinflated occlusion balloon across the lumen, followed by balloon occlusion of the outflow. A small amount of thrombin was injected into the traumatized vein segment immediately after outflow occlusion. Thrombosis of the traumatized vein evolved into an organized thrombus seven weeks later. The histological features of the harvested post-thrombotic femoral vein were studied with hematoxylin and eosin and Trichrome stains.

Results: In all six pigs, initial disruption of the chordae tendineae was successfully performed to create tricuspid reflux and venous hypertension. After two-stage sequential procedures, a thrombus formed in the target femoral vein segment. Histology of the harvested thrombotic vein showed features of an organizing thrombus with collagen formation and fibrosis.

Conclusions: The novel swine VTE model may serve as a platform for developing and testing human-sized therapeutic procedures and devices in translational venous research.

Clinical Relevance: This study describes a swine model of VTE created by incorporating all three elements of Virchow's triad. The model uniquely incorporates reflux-induced venous hypertension, which may be used in studying venous insufficiency and VTE in those with systemic venous hypertension. Likewise, this model may serve as a platform for development and evaluation of diagnostic imaging or therapeutic procedures and devices in subjects with systemic venous hypertension. (JVS—Vascular Science 2024;5:100200.)

Keywords: Hypertension; Large animal model; Reflux; Thrombosis; Venous thromboembolism

Venous thromboembolism (VTE) is a major health problem. The average annual incidence of VTE in the United States is more than 100 cases per 100,000 people. VTE includes deep vein thrombosis (DVT) and pulmonary embolism (PE).[1,2] DVT can further result in post-thrombotic syndrome, a complex disease whose pathology may vary from reflux, obstruction, or, more commonly, a combination. The resulting venous hypertension may result in limb pain, swelling, stasis dermatitis, and even skin ulceration. The treatment costs are estimated to consume 2% of total health care budgets in Western societies.[3,4] Recent advances in diagnosis (eg, Duplex) and treatments (eg, endovascular interventions) have led to a rapid burgeoning of the population seeking treatment. VTE research has not kept pace, however, with the ballooning clinical interest. Much of our current understanding of their pathophysiology comes from autopsy material and surgical specimens collected in the last century. An effective translational animal model to study the disease in depth is much needed. For instance, venous-specific drugs and instruments are being developed to treat VTE. Because of the lack of a suitable animal platform, the industry has to rely largely on costly and often unpredictable clinical trials for regulatory approval.

The goal of this study is to develop a VTE swine model that may serve as a translational animal model for drug and device testing. In a companion paper, we used the present animal model to determine the changes in the mechanical properties of the veins under multiple conditions, including reflux-induced venous hypertension.[5]

METHODS

Animals. Six Yorkshire swine (weight, 40-60 kg) were obtained from a certified vendor. All animal experiments were performed at the California Medical Innovations Institute in accordance with all national and local ethical guidelines and an approved Institutional Animal Care and Use Committee protocol.[6]

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The right femoral veins of six swine were assigned to the experimental vein (tricuspid regurgitation and femoral vein injury), and contralateral (left) femoral veins can serve as control (tricuspid regurgitation and no femoral vein injury). The animal model involved a two-step process with procedures separated by 2 to 3 weeks. At the baseline procedure, experimental animals were sedated with TKX (4.4 mg/kg telazol, 2.2 mg/kg ketamine, and 2.2 mg/kg xylazine) intramuscularly given for 30 minutes before anesthesia induction. Animals were intubated and maintained on surgical anesthesia with isoflurane (1%-3%) and oxygen. Body temperature was maintained by placing the animal on a heated pad and monitoring rectal temperature. The electrocardiogram was monitored using standard limb leads. The blood pressure cuff was placed at appropriate positions to measure arterial pressures. The ventrolateral neck was shaved and scrubbed. Under sterile conditions, a 9F introducer sheath was placed percutaneously in the right jugular vein for the administration of drugs and the introduction of devices and catheters.

Induction of tricuspid reflux. Tricuspid regurgitation was accomplished by graded disruption of tricuspid chordae with the custom avulsion device previously described (Fig 1, *A*).[7,8] Intracardiac echo (ICE) (Siemens Acuson SC2000 with ACUSON AcuNav Ultrasound Catheter 8 French × 90 cm) was applied to assess the presence and degree of tricuspid regurgitation (reflux velocity) by color flow Doppler (Fig 1, *B*). The number of chordae avulsed is progressively increased until the targeted level of tricuspid reflux is achieved. The targeted reflux level is reached when right atrial and right ventricular pressures are nearly equalized (≤2 mmHg, pressure difference as measured via 7F selective catheter). The animals were sent back to animal rooms for routine care and monitoring after recovery from anesthesia. The target vein to develop the thrombus was the right common femoral vein because catheterization is easier on the right side due to the natural vascular anatomy.

Induction of fibrotic venous changes. A period of 2 to 3 weeks was allowed to lapse for endothelial dysfunction to occur before proceeding to the next stage. At the second stage, thrombosis of the right femoral vein was induced in the following manner: Animals were prepared as described above. A 12F introducer sheath was placed percutaneously in the right jugular vein. A commercially available standard occlusion balloon catheter (Equalizer 7 French 27 mm × 65 cm, Boston Scientific) was tracked to the right femoral vein over a guidewire.

The diameter of the target vein segment (7.4 ± 0.4 mm) was measured by injecting contrast agent through the balloon catheter. The balloon was then inflated manually by hand through a pressurized syringe under fluoroscopy to fill the vein segment (approximately 5 cm long) and overdistended slightly without rupture. A 10% to 15% larger than target vein diameter is adequate. Scoring of the endothelium of the femoral vein is achieved by repeated (3 times) pulling of the inflated balloon across its length. Blood flow to the traumatized segment is stopped for about 2 hours by balloon occlusion of femoral vein outflow. Administration of 0.5 mL of contrast (50% saline and 50% contrast) through the tip of the balloon catheter confirms complete femoral vein occlusion. The site of balloon occlusion should be at or below any tributaries visualized on contrast injection. Injection of 5000 US units of D-Stat Flowable Hemostat Thrombin (diluted into 5 mL of buffered water, Vascular Solution, Inc) immediately after outflow occlusion facilitates thrombosis of the traumatized segment. Successful induction of thrombosis is confirmed by gentle contrast injection, which delineates the thrombus. The occlusion catheter and sheath were removed, and then the animal was allowed to recover from anesthesia. Routine daily care was provided with daily observation to confirm good health without signs of potential complications.

Confirmation of venous thrombosis. After 7 weeks, the venous thrombosis was detected in the injured vein segment, confirmed by injecting contrast into the outflow site of the right common femoral vein through the transducer sheath placed in the jugular vein. At the end of the studies, animals were sacrificed in an ethically compliant manner with a bolus injection of super-saturated potassium chloride after 5 minutes of deep isoflurane (5%) anesthesia. Then the surrounding tissue of femoral veins was dissected in situ to expose the thrombosed vein segment. The vein was ligated 2 to 3 cm away from the thrombosed vein segment and transected along the outside of the ligation sites. The harvested thrombosed vein segment was fixed with 10% formalin. The left femoral veins were also harvested to perform histological examinations. Blood samples were collected and tested at baseline, 2 to 3 weeks, and 7 weeks after tricuspid valve injury. White blood cell count, platelet count, prothrombin

ARTICLE HIGHLIGHTS

- **Type of Research**: Large animal model
- **Key Findings:** This study describes a swine model of venous thromboembolism created by incorporating all three elements of Virchow's triad. It uniquely features reflux-induced venous hypertension.
- **Take Home Message:** The novel swine venous thromboembolism model may serve as a platform for developing and testing human-sized therapeutic procedures and devices in translational venous research.

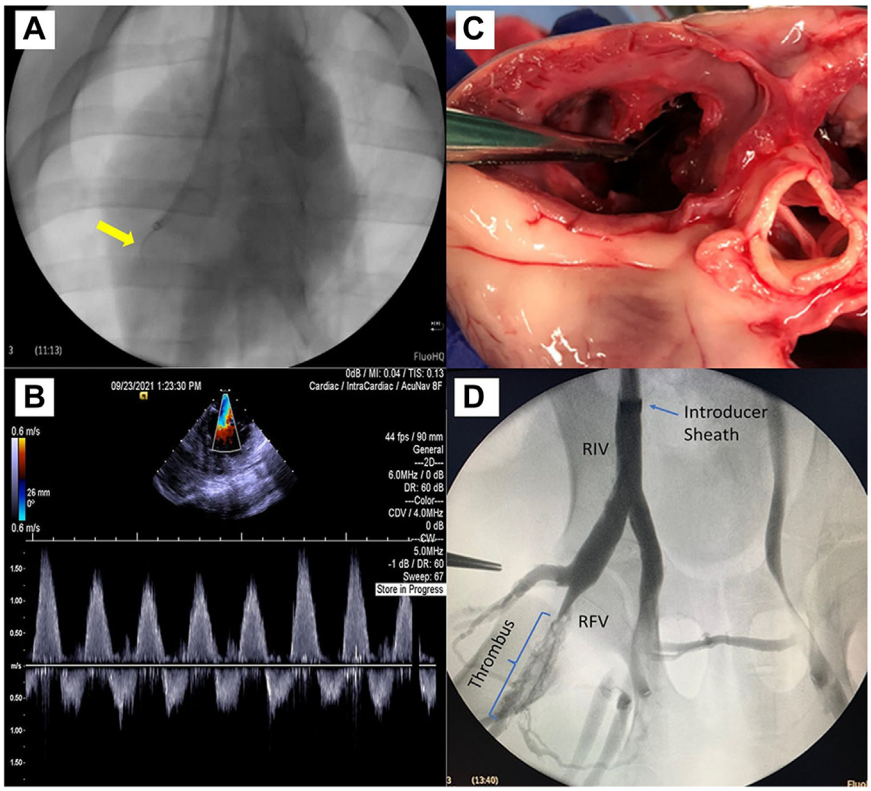

Fig 1. Induction of tricuspid regurgitation. **A,** Fluoroscopic image showing a custom avulsion device rotated to cut the tricuspid chordae in the right ventricle. The *yellow arrow* indicates the avulsion device. **B,** Representative intracardiac echo image indicating the reflux in the right atrium (ie, tricuspid regurgitation) by graded disruption of tricuspid chordae. **C,** A representative image of disruption of tricuspid chordae and free jagged edges of leaflets in postmortem gross examination. The chordae of tricuspid leaflets were disrupted with the custom avulsion device, resulting in tricuspid reflux and elevated venous hypertension. **D,** A representative image of the thrombus formed successfully in the right femoral vein (*RFV*) segment, demonstrated by injecting contrast agent through the transducer sheath under fluoroscopy into the outflow end of the injured segment of *RFV*. Intravenous angiography shows that the contrast agent filling in the venous lumen is not continuous and uniform but is characterized by intermittent and uneven filling defects. *RIV,* right iliac vein.

time, activated partial thromboplastin time, fibrinogen, and D-dimer were measured.

Statistical analysis. The comparison of blood sample among the three time points was analyzed using analysis of variance, with (*) *P*-value $< .01$ indicating a significant difference.

RESULTS

There were no arrhythmias, adverse events, or complications during or after the staged procedures. Limb pain, swelling, stasis dermatitis, and skin ulcers of the lower extremities were not observed throughout the study. The animal weight at the terminal procedure was 66.7 ± 6.7 kg, compared with 51.4 ± 2.1 kg at baseline. Disruption of the chordae tendineae was created successfully in all six experimental animals. Postmortem gross examination also showed that 70% to 80% of the chordae tendinea of the tricuspid valve in each heart were disrupted (Fig 1, *C*). In all six animals, thrombus formed successfully in the target femoral vein segment**.** There was no fibrosis on the adventitial surface of the thrombus vein segment, and no adhesion with surrounding tissues. Fig 1, *D* shows a representative image of thrombus formation (approximately 4 cm in length, presence of recanalization) at the target site, confirmed by injection of contrast agent delineating the thrombus under fluorography. The thrombus did not completely occlude the entire vascular lumen, and retrograde venography (Fig 1, *D*) showed that the contrast agent passed through the thrombus and visualized the venous segment distal to the thrombus, suggesting the presence of recanalization (non-occlusive thrombus).

In blood chemical analyses, there was no statistical difference between 7 weeks post-injury and baseline in white blood cell count ($P = .02$), platelet count ($P = .45$), prothrombin time ($P = .26$), activated partial thromboplastin time ($P = .23$), fibrinogen ($P = .28$), and D-dimer ($P = .97$), which indicates that systemic inflammatory and thrombogenic risks were not significantly increased

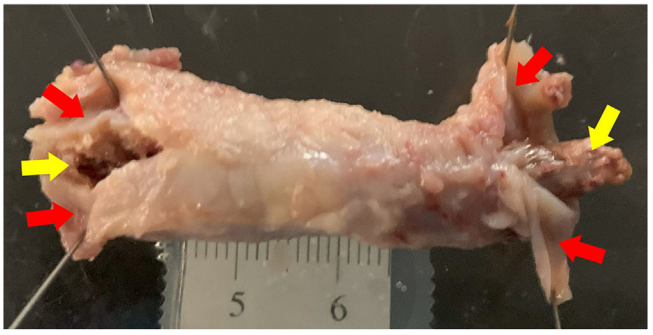

Fig 2. A representative photograph of a thrombosed vein segment ex vivo. A venous thrombus was visible (approximately 5.5 cm long and 0.7 cm in diameter) after both ends of the thrombosed venous segment were cut open. *Red arrows*: venous wall. *Yellow arrows*: thrombus.

by the tricuspid valve injury and regional venous damage in this swine model. In all six animals, histological examination confirmed that the thrombus was in the shape of a 4.0 to 5.5 cm long cord, a length range ideal for compliance and size measurements using our novel intravenous balloon (6 cm long) on this model.[5] Fig 2 is a representative photograph of a thrombosed vein segment ex vivo. Both ends of the thrombosed venous segment were cut open, and a venous thrombus about 5.5 cm in length and 0.7 cm in diameter was visible. All slides with histological stains are carefully reviewed. The thrombotic plugs are observed in all histological slides. Collagen and fibrosis are also observed in all histological slides. Typically, the histological pictures of two samples are represented in Fig 3 and Fig 4. The morphologic integration of the vein with thrombus is represented by hematoxylin and eosin stain. Fig 3, *A* and *C* and Fig 4, *A* and *C* show hematoxylin and eosin stain of the two examples. The thrombotic plugs occupied approximately 70% and 90% cross-sectional area of venous lumens in Fig 3, *A* and *B* and Fig 4, *A* and *B*, respectively. The collagen formation is observed in both examples (Fig 3, *A* and *C* and Fig 4, *A* and *C*). Trichrome stains represented the fibrosis in external edge and developing towards the core of thrombotic plugs in Fig 3, *B* and Fig 4, *B*, respectively. The fibrosis is either loose (Fig 3, *D*) or dense (Fig 4, *D*) around/in the thrombotic plugs. Control (left) femoral veins were composed of intima, media, and adventitia, and the wall thickness was basically uniform. The endothelium was intact, and there were no signs of thrombus.

DISCUSSION

A new VTE animal model in swine is created thought a two-step process. This model exhibited venographic and histologic changes characteristic of a venous thrombotic stenosis (Fig 1, *D*, Fig 3, Fig 4) and has been applied as a valuable tool to successfully test our novel venous balloon for compliance measurements and stent size at three venous states: untreated control, venous hypertension-induced tricuspid injury, and post-thrombotic stenosis.[5]

The swine is the most used large animal model in the study of VTE because of its similar body size, thrombus coagulation cascade, and vascular anatomy to humans.[9,10] Multiple swine models induced by local thrombin administration were reported based on venous stasis and hypercoagulability techniques.[10-13] Balloon catheter occlusion is currently the most popular technique in the swine model to create venous stasis.[10] It is not only easy to perform and reduces harm to the animal, but it also maintains the anatomical integrity of the venous system. In comparison, endovascular occlusion leaving foreign material (suture, stent, coil, clip) in the vessels changes physiological flow conditions and produces artifacts on imaging, making further possibilities of therapeutics and device testing difficult.[10,14] Since first introduced by Roy et al, balloon catheter occlusion plus thrombin administration has become a popular method for creating swine VTE models due to its simplicity and efficiency.[11,12,14-16] The occlusion times and thrombin doses used varied in different studies. Currently, the commonly used occlusion time is 1 to 2 hours. The dose of thrombin varies from 50 to 10,000, and no optimal dose studies have been reported. To ensure the thrombus length (<6 cm), we chose the maximum dose used in the study by Roy et al (ie, 5000 U).[15] The induced thrombus segment was appropriately 4.0 to 5.5 cm long with recanalization, making it an ideal model in terms of length and catheter insertability for testing compliance and size of the thrombosed vein segment using our novel venous balloon (6 cm long).[5]

In this study, venography showed femoral veins recanalized 7 weeks postoperatively, not unlike patients with DVT. In all six animals, histological examination confirmed that the thrombus was in the shape of a 4.0 to 5.5 cm long cord with recanalization, with thrombotic plugs occupying approximately 70% to 90% of the cross-sectional area of the venous lumen. Collagen formation and fibrosis were observed on histological analysis. These demonstrate the stability of this chronic swine model. A recent chronic study (2022) with a maximum follow-up of 5 weeks reported non-occlusive thrombus on magnetic resonance imaging and terminal autopsy, identical to our results. Additionally, this study found that collagen formation is first noted at 4 to 5 weeks, but the fibrosis formation was not mentioned.[14] In contrast, collagen and fibrosis are observed in all histological slides in our study, suggesting the need for more long-term studies in the future.

The optimal timing between the development of tricuspid regurgitation and the induction of femoral vein thrombosis is unclear. Although we did not perform measurements to confirm venous hypertension and endothelial dysfunction/remodeling in femoral vein in

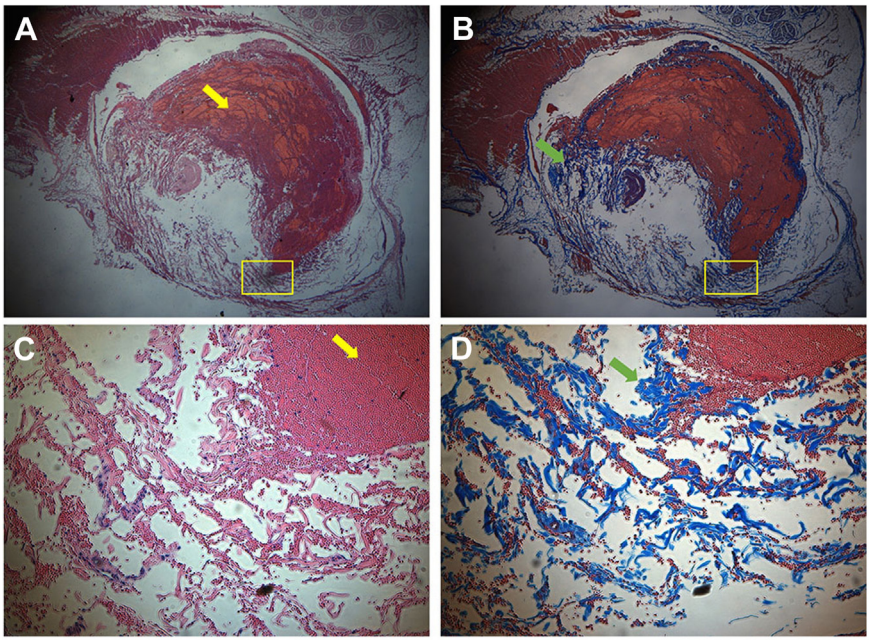

Fig 3. Example 1. Thrombosis in the femoral vein. Hematoxylin and eosin and trichrome stains show collagen formation (*blue; green arrow*) and fibrosis (*pink; yellow arrow*). **A,** Hematoxylin and eosin staining. Objective: 2×. **B,** Trichrome staining. Objective: 2×. **C,** Zooming view (*yellow box*) of hematoxylin and eosin staining in **(A)**. Objective: 20×. **D,** Zooming view (*yellow box*) of trichrome staining in **(B)**. Objective: 20×.

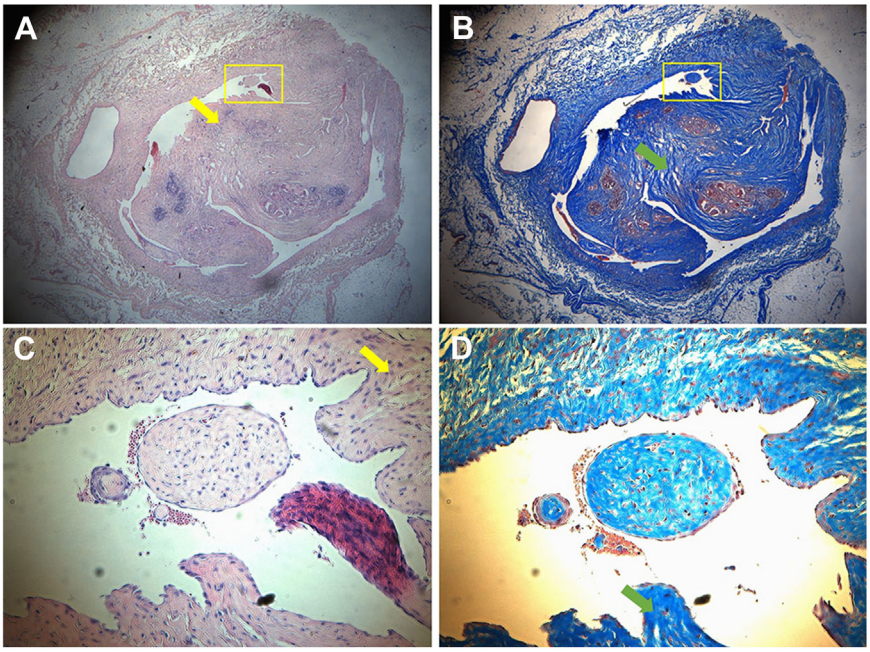

Fig 4. Example 2. Thrombosis in the femoral vein. Hematoxylin and eosin and trichrome stains show collagen formation (*blue; green arrow*) and fibrosis (*pink; yellow arrow*). **A,** Hematoxylin and eosin staining. Objective: 2×. **B,** Trichrome staining. Objective: 2×. **C,** Zooming view (*yellow box*) of hematoxylin and eosin staining in **(A)**. Objective: 20×. **D,** Zooming view (*yellow box*) of trichrome staining in **(B)**. Objective: 20×.

this study, using a large animal model of tricuspid injury, our previous studies demonstrated that tricuspid injury can induce venous hypertension within 8 or 4 weeks, causing growth and remodeling of common iliac vein or the thoracic duct's outward remodeling and thickening.[7,8] We have also found that 2 to 3 weeks of venous

hypertension will lead to venous remodeling/endothelial dysfunction (unpublished data).

Endothelial damage contributes not only to thrombus formation but also to stabilization of thrombi through fibrosis and avoidance of thrombus migration. The current swine model, involving all three components of Virchow's triad (venous stasis, endothelial damage/dysfunction, and hypercoagulability), provides a robust approach to thrombosis. This swine model uniquely incorporates reflux-induced venous hypertension, which may be the focus of some studies. Congestive heart failure (CHF) is a condition associated with a relatively high risk of DVT. Without thromboprophylaxis, clinical imaging confirms that up to 22% of hospitalized patients with CHF will develop DVT.[17] Patients with heart diseases such as CHF and right ventricular dysfunction are often accompanied by elevated venous pressure. This unique animal model can be undoubtedly used in studying venous (eg, pulsatile) insufficiency and VTE in those with systemic venous hypertension/ventricular dysfunction/CHF in the acute and chronic settings. Likewise, this model may serve as a platform for development and evaluation of diagnostic imaging or therapeutic procedures and devices in subjects with systemic venous hypertension.

Study limitations. Although the VTE swine model was successfully established through multiple techniques in this study, the swine venous system remained relatively compensated for 7 weeks postoperatively. It is not unexpectable that the symptoms of post-thrombotic syndrome were not observed in this study. A complete molecular analysis (polymerase chain reaction, Western blot, immunofluorescence) of biomarkers of activated endothelium, platelets, and thrombus as well as immediate postoperative changes in thrombotic biomarkers should be performed in the future. Additionally, noninvasive technique (duplex ultrasound) and invasive venous pressure analysis should be conducted to characterize the time course of the model. To provide a more convincing comparison and further model validation, more group studies should be considered in future studies to make the study more comprehensive and conclusive.

CONCLUSION

A VTE swine model is described, created by incorporating all three elements of Virchow's triad. It uniquely incorporates reflux-induced venous hypertension as a feature. This swine model may be suitable for studying venous insufficiency and VTE with systemic venous hypertension and for developing and testing human-sized therapeutic procedures and devices.

AUTHOR CONTRIBUTIONS

Conception and design: SR, GK
Analysis and interpretation: MW, XL, LH, JD, SR
Data collection: MW, XL, LH
Writing the article: MW
Critical revision of the article: MW, XL, LH, JD, SR, GK
Final approval of the article: MW, XL, LH, JD, SR, GK
Statistical analysis: Not applicable
Obtained funding: Not applicable
Overall responsibility: GK

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DISCLOSURES

GK is the owner of 3DT Holdings LLC.

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