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Original Article

Evaluating the Safety and Efficacy of Developed Restora™ Clot Retrieval Device in a Porcine Model

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Abstract

The study evaluated the safety and performance of a new Clot Retrieval Device in a porcine model using three female swine. These swine were fasted overnight without water and kept NPO (nil per os) for six hours post-recovery. They received Aspirin and Clopidogrel for three days before the procedure, continuing with a reduced dose of aspirin post-implantation until sacrifice. Physical examination ensured that the swine met study criteria, followed by anesthesia and monitoring with Ketamine, Xylazine, Propofol, and inhalation anesthesia. Areas for femoral vein access and ECG lead application were shaved, and Atropine was given to control respiratory discharges. On the first day, two swine (P1 and P2) had clots implanted and retrieved using the Clot Retrieval Device. The third pig (P3) also had clots implanted, with retrieval on the procedural day and extended monitoring to day 30. Two types of clots (autologous and synthetic) were placed in each pig and retrieved under heparinization, with angiography and measurements conducted before and after implantation. Histopathological evaluations were done on harvested veins: P1 and P2 on day 0 and P3 on Day 30. The autologous clot was successfully retrieved in all cases, while the synthetic clot remained but did not cause clinical signs. Results demonstrated that the clot retrieval device effectively and safely retrieved autologous clots without injuring targeted arteries, and no clot-related lesions were found in the internal carotid arteries. Swine were monitored for health parameters such as body weight, morbidity, and mortality, with no significant changes or adverse effects observed. Pathological examinations included clinical pathology, necropsy, and histopathology. The study concluded that the clot retrieval device was effective and safe for retrieving autologous clots in a porcine model, suggesting its potential safe use in clinical settings.

Keywords: ***Clot Retrieval Device, Porcine Model, Femoral Vein Access, Autologous Clot, Angiography& Histopathological Evaluation.***

Introduction

In this study, we aimed to assess the safety and performance of the developed Restora™ clot retrieval device in a porcine model. Three female swine were selected for the study and fasted overnight with water withheld before the procedure. The animals were kept NPO (nil per os or nothing by mouth) for 6 hours post-recovery. Prior to the surgery, the animals were kept on anticoagulant treatment with Aspirin 300 mg/animal and Clopidogrel 75 mg/animal (PO) for at least three days before the procedural day. Post-implantation, the anticoagulant treatment continued with a reduced dose of Aspirin (150 mg/animal) until sacrifice. Each animal underwent physical examination to ensure they met the study's acceptance criteria and were anesthetized, instrumented, and monitored using Ketamine 15 mg/kg (IM), Xylazine 2.5 mg/kg (IM), Propofol 0.5 mg/kg (IV bolus), followed by inhalation anesthesia (1-3%) through a facemask. The neck, chest, and thigh areas were clipped free of hair for femoral vein access and ECG lead application. Atropine (0.05 mg/kg IM) was administered to control respiratory tract discharges that could block the endotracheal tube. The procedures were performed under proper analgesia and anesthesia. On day 0, two animals (P1 and P2) were deployed with clots, and the clots were retrieved using the clot retrieval device. The third animal (P3) was also deployed with clots, but the retrieval occurred on the procedural day, with monitoring extended to day 30 [1,2].

The study involved placing two types of clots (autologous and synthetic occluder Menox) [3], in each animal and retrieving them using the test item under heparinization. Angiography and measurements were conducted before and after implantation. On day 0, veins from animal P1 and P2 and on day 30 from P3 for histopathological evaluation. The autologous clot was successfully retrieved in all cases, while the synthetic clot could not be retrieved but did not cause any clinical signs. The study's results demonstrated that the clot retrieval device could effectively and safely retrieve autologous clots without causing injuries to the target arteries. No clot-related lesions were found in the internal carotid arteries, and there were no significant clinical signs observed due to the synthetic clots. The test item proved to be safe for clot retrieval, and the findings support its potential use in clinical settings [4].

The study also assessed the animals for health parameters such as body weight, morbidity, and mortality. There were no significant changes in body weight, and no morbidity or mortality was observed. Pathological examinations, including clinical pathology, necropsy, and histopathology, were performed to identify any adverse effects. The comprehensive evaluation of the clot retrieval device included inserting the device via standard femoral

access, retrieving the clot, and performing aspiration through the guide catheter. Necropsy and histopathological evaluations were conducted to assess any local lesions due to clot retrieval.

Overall, the study concluded that the clot retrieval device is effective and safe for retrieving autologous clots in a porcine model, with no significant adverse effects observed in the tested animals. The findings indicate that the device can be used safely in clinical settings for clot retrieval [5].

Materials and Methods

Medication Details

This section delineates the drugs administered to the animals prior to, during, and following surgery. Details of medications used are mentioned in Table 1.

Table 1: Details of medications used in the study

Medication	Dosage	Route of Administration
Aspirin	300 mg/animal (pre-surgery), 150 mg/animal (post-surgery)	Oral
Clopidogrel	75 mg/animal	Oral
Ketamine	15 mg/kg	Intramuscular (IM)
Xylazine	2.5 mg/kg	Intramuscular (IM)
Propofol	0.5 mg/kg	Intravenous (IV)
Atropine	0.05 mg/kg	Intramuscular (IM)
Tramadol	4 mg/kg	Intramuscular (IM)
Isoflurane	1-3% (inhalation)	Inhalation

Materials Required

- Implant
- Marker
- Tip Coil
- Introducer Sheath
- Delivery Wire

Device Description

The device is a nitinol self-expanding, fully retrievable mesh design intended for clot retrieval in occluded vessels following acute ischemic stroke. It features a gap structure that allows overlap or underlap for enhanced clot retrieval in small-diameter intracranial vessels. The distal portion of the device, made of nitinol, includes platinum-iridium (Pt-Ir) radiopaque markers for optimal visualization of the proximal, and distal ends, and the body of the device. The device is supplied sterile and is for single use only by physicians trained in interventional neuroradiology and ischemic stroke management.

Device Design: The device incorporates a self-expanding nitinol structure, allowing effective clot retrieval and navigation in occluded vessels after acute ischemic stroke. The strategically designed gap enables overlap or underlap, which improves clot retrieval in small-diameter intracranial vessels. Platinum-iridium radiopaque markers enhance visibility during procedures to ensure precise placement. The device is designed with a "Giant-Baby" cell combination: Baby cells enhance expansion and clot holding during deployment, while Giant cells ensure effective clot retention during retrieval. The delivery wire is hydrophilically coated to reduce surgery time by improving lubricity when in contact with body fluids. Stent sizes range from 3-6 mm in diameter with a usable length of 20-40 mm and microcatheter compatibility of 1.7F-2.7F. Sterilization is achieved through Ethylene Oxide (ETO). Size specifications are provided in Table 2, and the product is illustrated in Figure 1.

Size Matrix

Table 2: Size matrix of Clot Retrieval Device

Diameters	Lengths
3.00mm	20mm
4.00mm	20mm, 30mm, 40mm
6.00mm	24mm, 40mm

Product Image

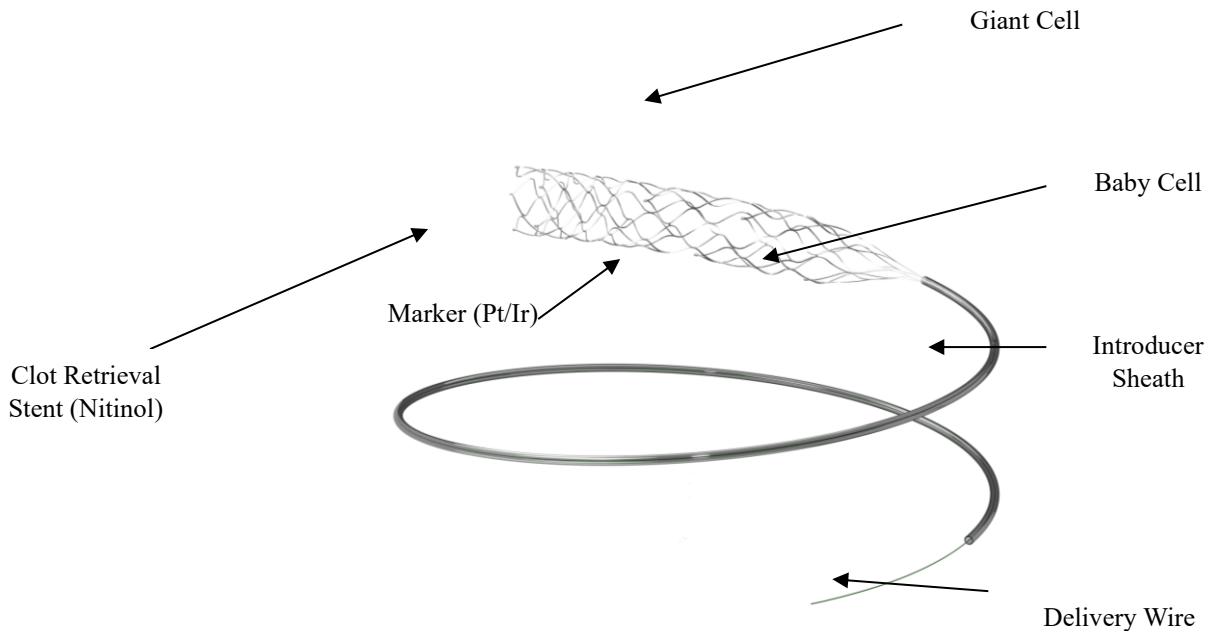


Figure 1: Restora™ Clot Retrieval Device

Device Specification

- Clot retrieval utilizes laser cut technology.
- Designed as self-expanding stents with a mesh structure.
- Captures and traps the blood clot.
- Clot retriever is deployed into the affected blood vessel.
- Once in position, it is expanded to engage the clot.
- The entire system is then withdrawn, bringing the trapped clot along with it.

Device Component Description

Table 3: Device components description

Implant material	Super elastic Nitinol Tube
Radiopaque Marker	Pt/Ir (90%/10%) coil Marker and Pt/Ir (90%/10%) Proximal Marker
Device Design	Stratified meshed structure design
Type of Cells in Device	Giant-baby Cells Combination
Delivery wire	Nitinol Tapered wire
Delivery Wire coating	Nitinol Tapered wire
Delivery wire Length	2000 mm
Introducer Sheath Material	PTFE Liner
Introducer Sheath Length	700mm

Experimental Procedures

Fasting

The animals were fasted, with water withheld overnight before the procedure, and kept NPO for 6 hours after recovering from the procedure.

Animal Preparation

The protocol includes prior to the surgical procedure, animals received anticoagulant treatment with Aspirin (300 mg/animal) and Clopidogrel (75 mg/animal) orally for at least three days, continuing post-surgery with a reduced Aspirin dosage of 150 mg/animal until sacrifice. Anesthesia was administered using Ketamine (15 mg/kg IM), Xylazine (2.5 mg/kg IM), and Propofol (0.5 mg/kg IV bolus), followed by inhalation anesthesia at 1-3% through a facemask. The neck, chest, and thigh areas were shaved to facilitate femoral vein access and ECG lead application. Additionally, Atropine (0.05 mg/kg IM) was given to control respiratory tract secretions that could obstruct the endotracheal tube for inhalation anesthesia. The animals were prepared and draped for aseptic procedures, with all relevant surgical preparation and anesthesia information documented for the study. Body weight measurements of the animals were recorded on Day 0 and Day 30, as detailed in Table 4.

Table 4: Animal P1, P2, P3 body weights (Day 0 to Day 30)

Animal Number	Day 0	Day 30
P1	42.4 kg	NA
P2	43.1 kg	NA
P3	40.2 kg	42.2 kg

Experimental Design or Animal Trial

DAY 0 - The right groin of the animal was shaved to facilitate access. A percutaneous approach utilizing the Seldinger method was employed to insert a 7F sheath into the right femoral artery. Activated Clotting Time (ACT) measurements were taken both before and after heparinization, which aimed to maintain ACT values between 250 and 550 seconds. The initial bolus dose of heparin was administered at 100 IU/kg intravenously, with subsequent doses adjusted based on the ACT readings. A stiff 150 cm, 0.035-inch guide wire was then used in conjunction with a JR guide catheter to access the branches of the carotid artery. Angiography of the carotid arteries was performed using the angiography guide catheter, which included baseline angiography and quantitative vascular angiography (QVA) to identify the target regions in the medial and lateral branches of the carotid artery. Based on the QVA results and the Reference Vessel Diameter (RVD), the appropriate vessel size was selected according to the compliance chart for the Clot Retrieval Device. After removing the guide wire, a clot was injected through the guide catheter into the selected vessel, followed by pre- and post-angiography assessments. The guide wire was then removed, and a microcatheter was advanced to the target region. The Clot Retrieval Device was passed through the microcatheter to remove the clot, with ELL and LLL angiographies conducted to evaluate the results.

Type of clot was used

Autologous whole blood clots were utilized to simulate fresh thrombi. Blood was collected into tubes containing 3.2% sodium citrate at a ratio of 1:9 (blood to citrate). The collected blood was centrifuged at 350 g for 10 minutes at 22 °C to separate the components. The plasma layer was then carefully collected, and calcium chloride ($CaCl_2$) was added to the platelet-rich plasma at a ratio of 1:10 mL of plasma to neutralize the sodium citrate. The recalcified plasma was incubated at 37 °C for 2.5 hours, after which the white thrombi were extracted for use.

Clot Placement Procedure

The clot placement procedure began with the selection of an appropriate clot based on the diameter of the target vessel. A stiff 150 cm, 0.035-inch guide wire was utilized alongside a guide catheter to access the branches of the carotid artery, specifically the medial and lateral side branches. Angiography of the carotid and cerebral arteries was performed using the angiography catheter to identify the optimal site for clot deployment. Baseline angiography and quantitative vascular analysis (QVA) were conducted to pinpoint the target region for implantation within the carotid artery branches. A clot of suitable size was then directed to the target area to achieve either total or partial occlusion of the artery. On Day 0 clot was inserted into animals P1, P2 and P3. The animal P1 and P2 were euthanized on day 0 and Animal P3 was euthanized on day 30. Based on the QVA results and the Reference Vessel Diameter (RVD), the appropriate size of the retrieval device was selected. The clot retrieval device was inserted through the guide catheter to the target site, where the clots were removed to restore blood flow in the affected region. High-resolution cine angiographies were recorded post-retrieval to assess the effectiveness of the clot removal.

DAY 30: The duration of the study was determined by the performance of the deployed stents. One animal was observed for a total of 30 days while receiving anticoagulant treatment throughout this period. The animal's health status was closely monitored through daily cage-side observations; ensuring that feed and water intake were adequate and recorded.

From Day 1 to Day 30, the animal was critically monitored for any signs of ill health, with observations documented once daily. On Day 30, follow-up angiography and quantitative vascular analysis (QVA) were performed on the surviving animal, including an assessment of antegrade flow. Following these evaluations, the animal was euthanized to facilitate the harvesting of the Clot Retrieval Device from the arteries for subsequent photography, gross examination, and histopathological evaluation.

Gross necropsy and photography were conducted on all arteries where stents were deployed. Functional safety evaluations included parameters such as usability, safety, and effectiveness.

Study Duration and Monitoring

The study duration was 30 days, with the animal under anticoagulant treatment starting 3 days before the procedure and continuing throughout the study. Cage-side observations for health status deviations and feed/water intake were recorded daily.

During the procedure, the animal was critically monitored. Early electives (moribund animals) were euthanized based on humane endpoints, followed by gross and histopathological evaluation to determine the cause of mortality/morbidity.

Upon study completion, follow-up angiography and QVA were performed on the surviving animal to assess antegrade/retrograde flow. The animal was then euthanized, and the vessels used for clot retrieval were harvested for local lesion evaluation and stored in formalin for histopathology. Due to the unpredictable nature of the procedure, not all situations and responses could be accounted for. All findings were recorded in the raw data. In assessing stroke severity and functional outcomes, the NHSS score and Barthel index criteria were applied, as detailed in Table 5.

Table 5: NHSS score criteria and Barthel index criteria

Score	Stroke severity
0	No stroke symptoms
0	No symptoms at all

Table 6: Animal Pl, P2, P3 ACT Values (Day 0 to Termination Day

Sr No	Animal Number	Day 0		Day 30		Termination Day
		Baseline	Post heparin dose	Baseline	Post heparin dose	
1	P1	95	259	Animal euthanized	Animal euthanized	On day 0
2	P2	89	286	Animal euthanized	Animal euthanized	On day 0
3	P3	86	275	98	294	On Day 30

Monitoring During Procedure: Day 1 to 30 days

During the procedure, vital signs including Electrocardiogram (ECG), respiration rate, heart rate, and oxygen saturation were continuously monitored and recorded, along with details of the drug administered, including its name, dosage, and route of administration. Anticoagulation monitoring was conducted using Activated Clotting Time (ACT) values as shown in Table 6.

Pre-operative

Before anesthesia induction, each animal received an intramuscular injection of Tramadol (4 mg/kg1M) as an analgesic prior to anesthetic induction. Additionally, Atropine (0.05 mg/kg, IM) was administered as a pre-anesthetic agent. Following this, sedation was achieved using Ketamine (15 mg/kg, IM), Xylazine (2.5 mg/kg, IM), and Propofol (0.5 mg/kg, IV). Anesthesia was then maintained with Isoflurane inhalation at a concentration of 1-3% delivered via a face mask.

Table 7: Angiographic quantitative vessel analysis of test items

Sr No.	Target Vessel Animal ID	Baseline diameter (mm)	Stent Diameter (mm)	Stent to artery ratio
1	Lt-Carotid artery medial side branch (P1)	2.92	4.0	1.3698630
2	Lt-Carotid artery medial side branch (P2)	3.19	4.0	1.2539184
3	Rt-Carotid artery lateral side branch (P2)	3.79	4.0	1.0554089
4	Lt-Carotid artery lateral side branch (P2)	3.31	4.0	1.2084592
5	Lt-Carotid artery lateral side branch (P3)	3.45	4.0	1.1594202
6	Lt-Carotid artery medial side branch (P3)	2.73	4.0	1.4652014

**Note: following cases we have used 4.0X40 mm Clot Retrieval Device.*

Table 8: Individual and Summary Data of Clinical Chemistry (Day 0)

Animal No.	P1 (Pre)	P2 (Pre)	Mean	SD
Aspartate amino transferase (U/L)	49	42	45.50	4.95
Calcium (mmol/L)	2.43	2.47	2.45	0.03
Creatine Kinase (U/L)	2634	2545	2589.50	62.93
Creatinine (µmol/L)	113	107	110.00	4.24
Lactate dehydrogenase (U/L)	853	750	801.50	72.83
Blood Urea Nitrogen (mmol/L)	2.76	2.75	2.76	0.01
Sodium (mmol/L)	141.2	141.0	141.10	0.14
potassium (mmol/L)	3.93	3.90	3.92	0.02
Chloride (mmol/L)	100.4	102.8	101.60	1.70

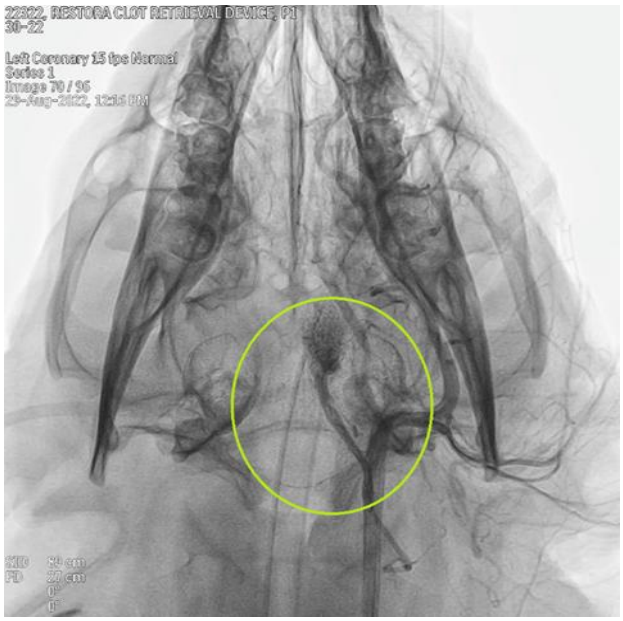
Table 9: Individual and Summary Data of Clinical Chemistry (Terminal Day)

Animal No.	P1 (Post)	P2 (Post)	Mean	SD
Aspartate amino transferase (U/L)	26	30	28.00	2.83
Calcium (mmol/L)	2.22	2.42	2.32	0.14
Creatine Kinase (U/L)	2037	2014	2025.50	16.26
Creatinine (µmol/L)	93	76	84.50	12.02
Lactate dehydrogenase (U/L)	613	735	674.00	86.27
Blood Urea Nitrogen (mmol/L)	3.46	2.53	3.00	0.66
Sodium (mmol/L)	142.8	143.0	142.90	0.14
potassium (mmol/L)	4.36	3.35	3.86	0.71
Chloride (mmol/L)	103.9	104.8	104.35	0.64

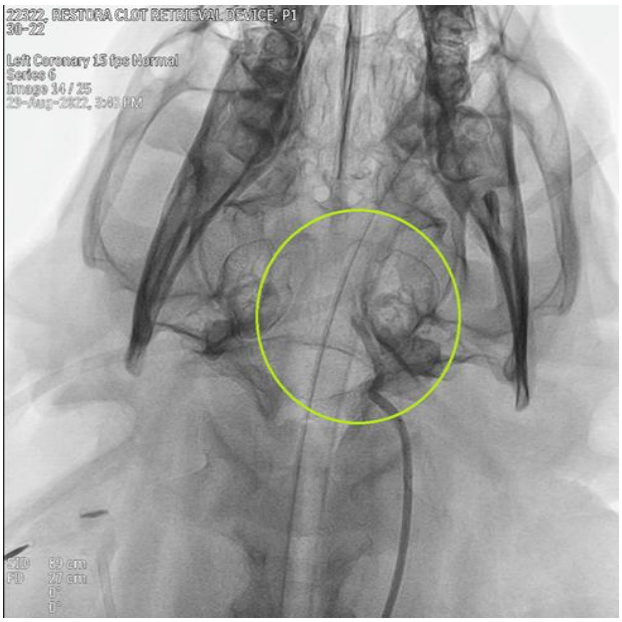
Table 10: Individual and Summary Data of Clinical Chemistry (Day0 and Day30 follow up)

Animal No.	P3 (0 Day)	P3 (30 Day)
Aspartate amino transferase (U/L)	29	30
Calcium (mmol/L)	2.83	2.60
Creatine Kinase (U/L)	487	1445
Creatinine (µmol/L)	107	134
Lactate dehydrogenase (U/L)	483	639
Blood Urea Nitrogen (mmol/L)	4.5	3.32
Sodium (mmol/L)	142.3	143.1
potassium (mmol/L)	3.50	3.40
Chloride (mmol/L)	102.6	106.8

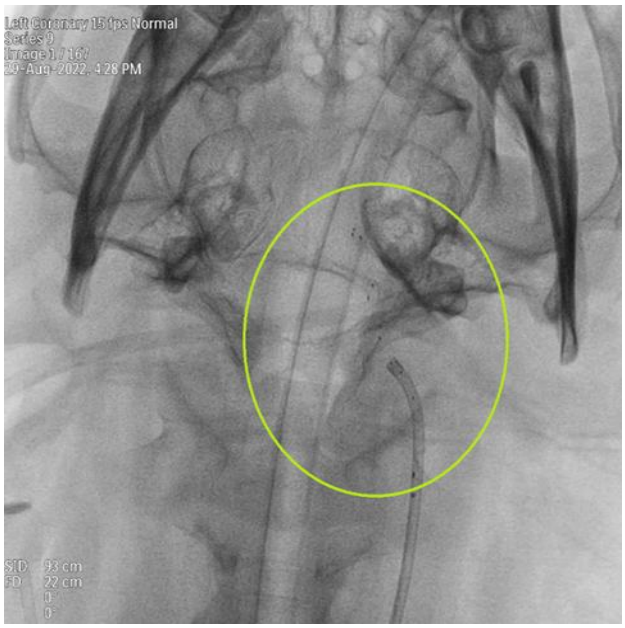
Angiography images



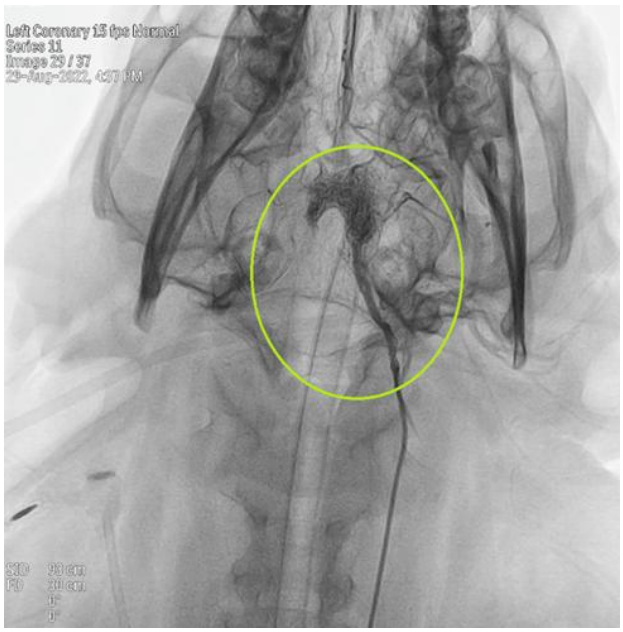
On day 0 baseline angiography of left carotid artery medial side branch.



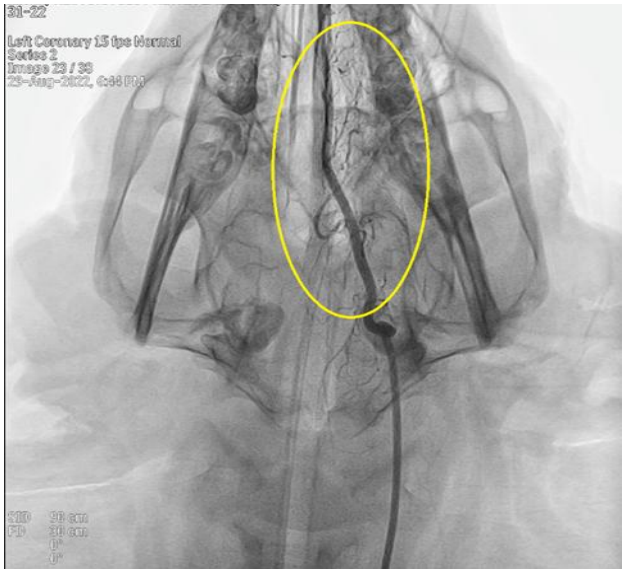
On day 0 clots injected into the left carotid artery of medial side branch and artery was totally occluded.



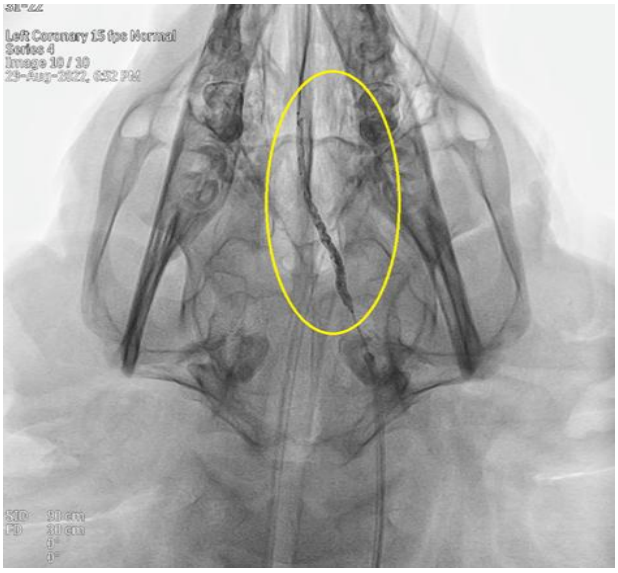
Animal P1, on day 0 clot was retrieved in left carotid artery of medial side branch with clot retrieval 4.0X40mm stent.



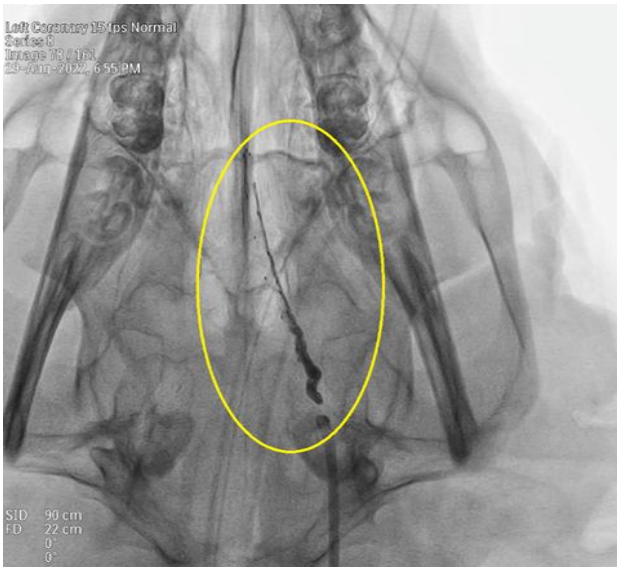
Animal P1, on day 0 after retrieved clot post angiography of left carotid artery of medial side branch TIMI III flow was established.



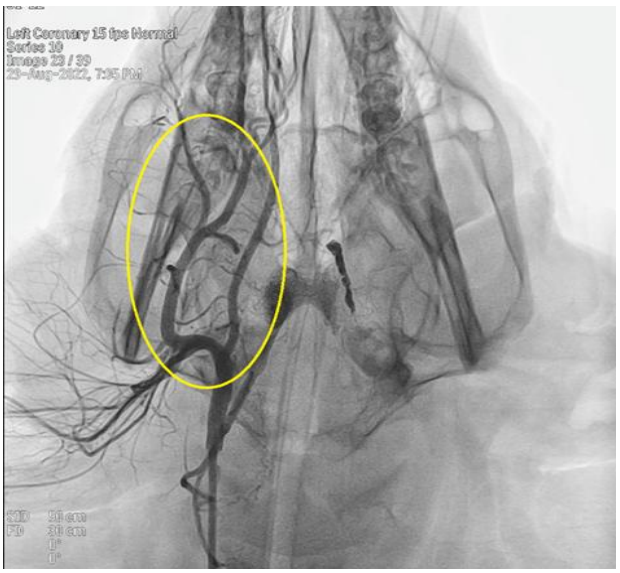
Animal P2, on day 0 baseline angiography of left carotid artery medial side branch.



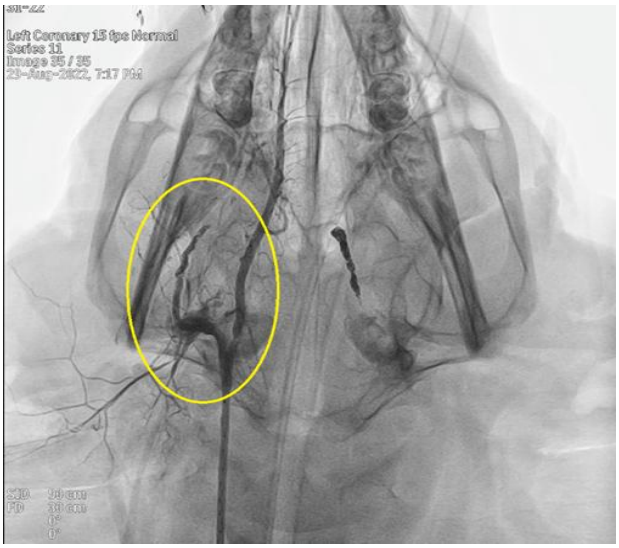
Animal P2, on day 0 clot injected into the left carotid artery of medial side branch, and artery was totally occluded.



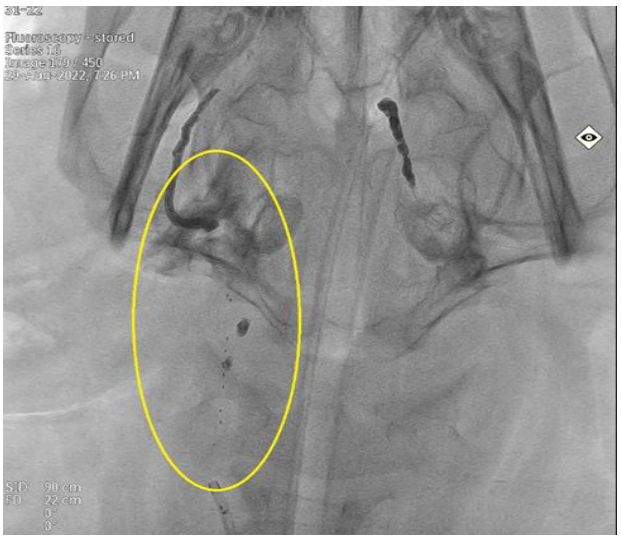
Animal P2, on day 0 clot was retrieved in left carotid artery of medial side branch with clot retrieval 4.0X40mm stent.



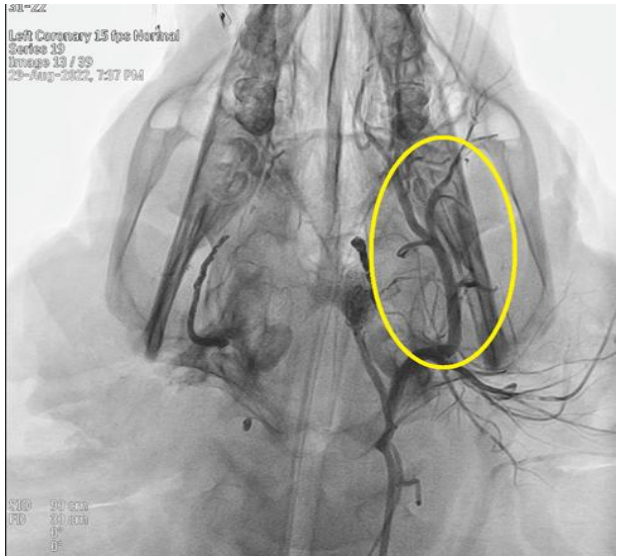
Animal P2, on day 0 baseline angiography of right carotid artery lateral side branch.



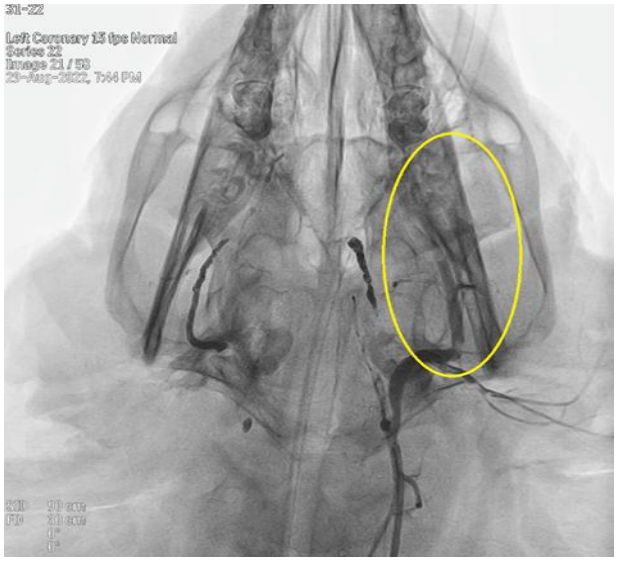
Animal P2, on day 0 clot injected into the right carotid artery of lateral side branch, and artery was occluded.



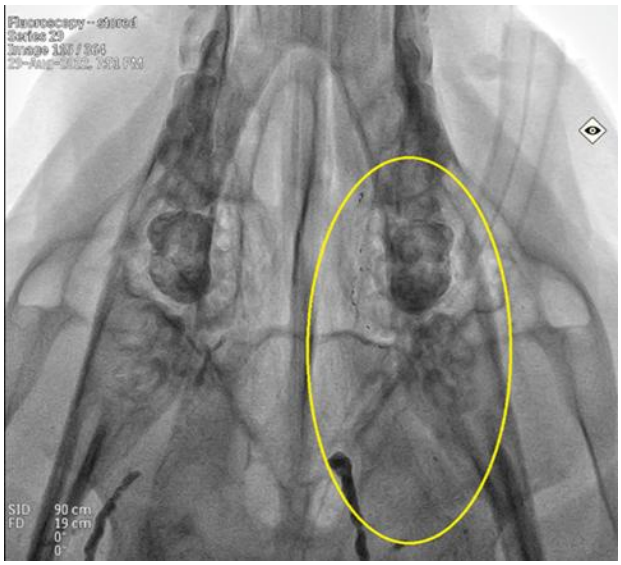
Animal P2, on day 0 clot was retrieved in right carotid artery of lateral side branch with clot retrieval 4.0X40mm stent.



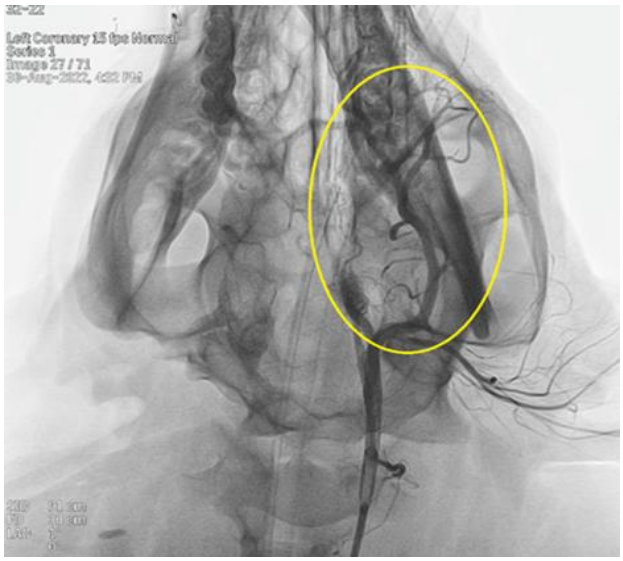
Animal P2, on day 0 baseline angiography of left carotid artery lateral side branch.



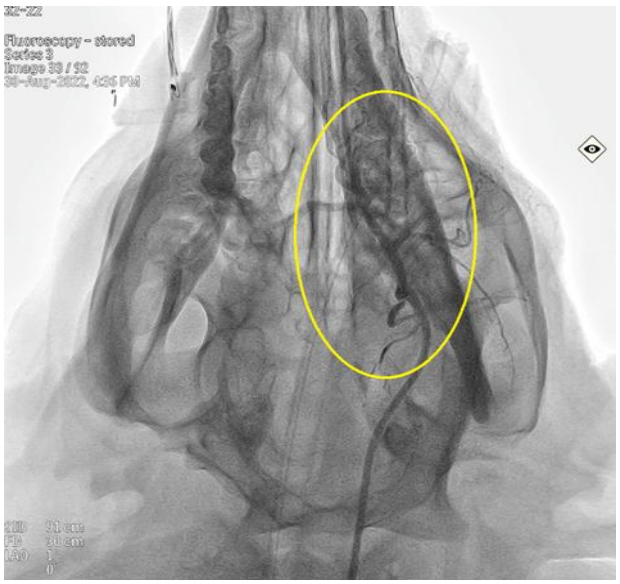
Animal P2, on day 0 clot injected into the left carotid artery of lateral side branch, and artery was totally occluded.



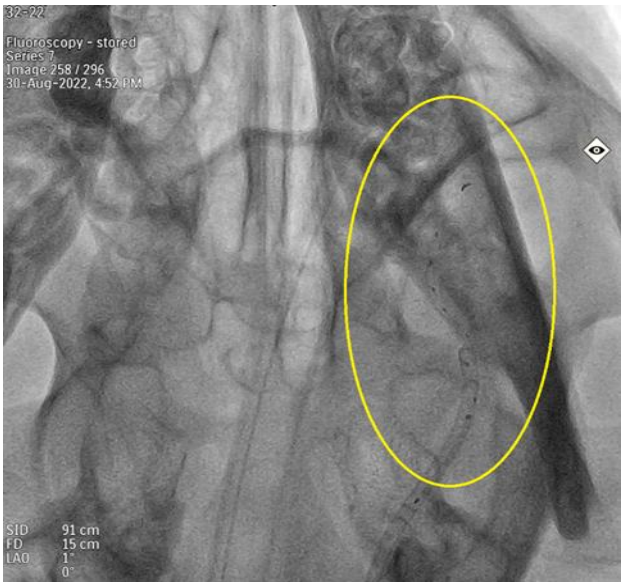
Animal P2, on day 0 clot was retrieved in right carotid artery of lateral side branch with clot retrieval 4.0X40mm



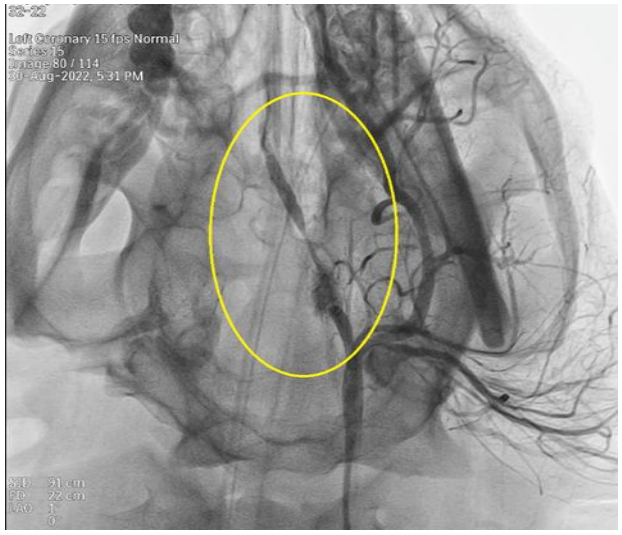
Animal P3, on day 0 baseline angiography of left carotid artery lateral side branch.



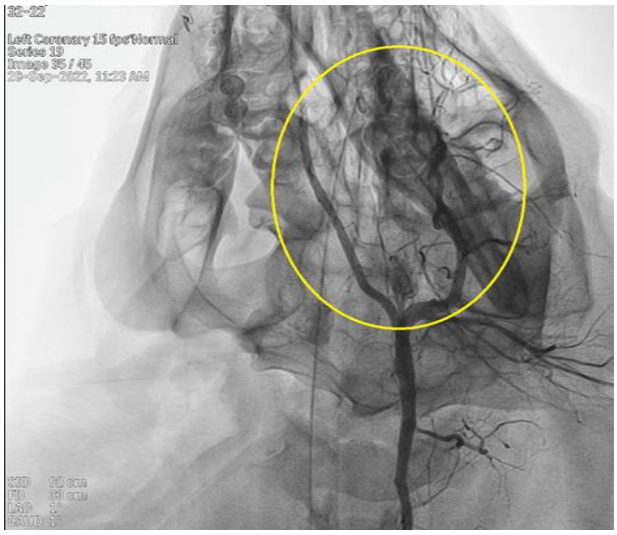
Animal P3, on day 0 clot injected into the left carotid artery of lateral side branch, and artery was occluded.



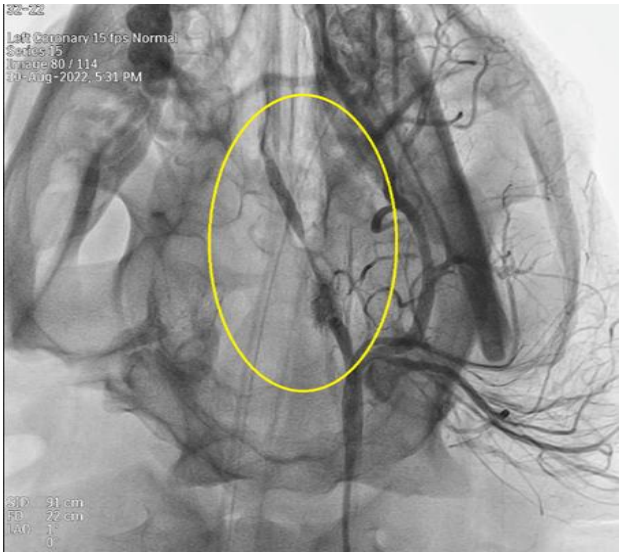
Animal P3, on day 0 clot was retrieved in left carotid artery of lateral side branch with clot retrieval 4.0X40mm stent.



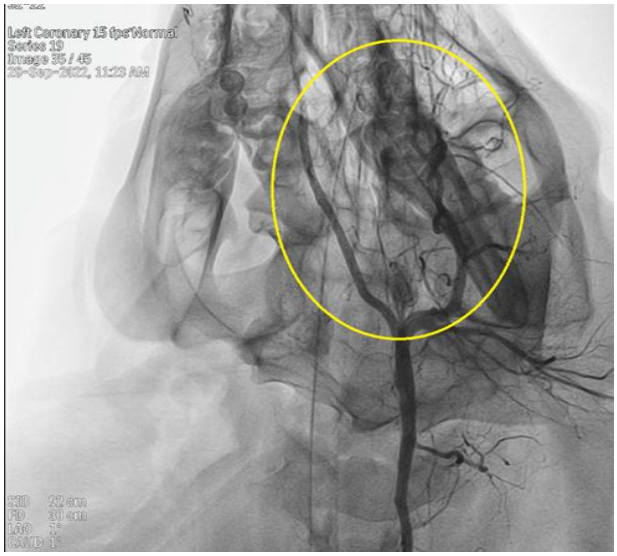
Animal P3, on day 0 after retrieved clot post angiography of left carotid artery of medial side branch TIMI II flow was established.



Animal P3, on day 30 angiography the left carotid artery of medial and lateral side branches TIMI III flow was established.



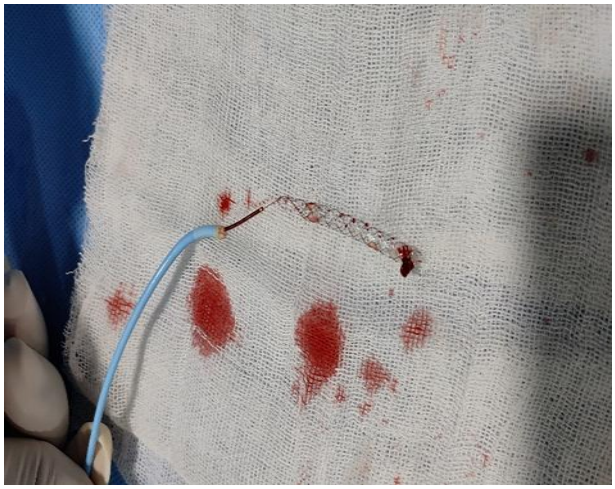
Animal P3, on day 0 after retrieved clot post angiography of left carotid artery of medial side branch TIMI II flow was established.



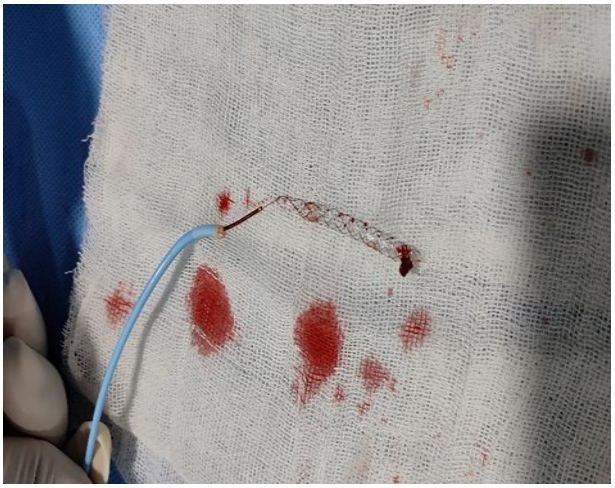
Animal P3, on day 30 angiography the left carotid artery of medial and lateral side branches TIMI III flow was established.

Figure 2: Angiography images of animals P1, P2, and P3

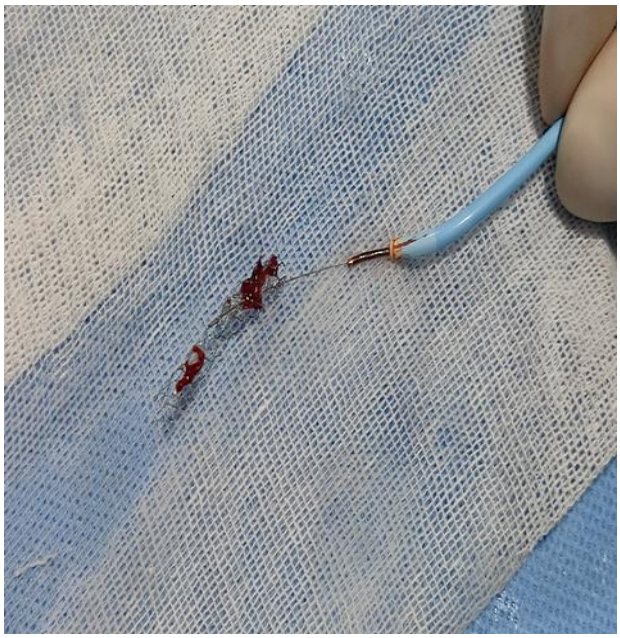
Clot Retrieval Image



Animal no P1: On 0-day Clot retrieval stent was retrieved the clot

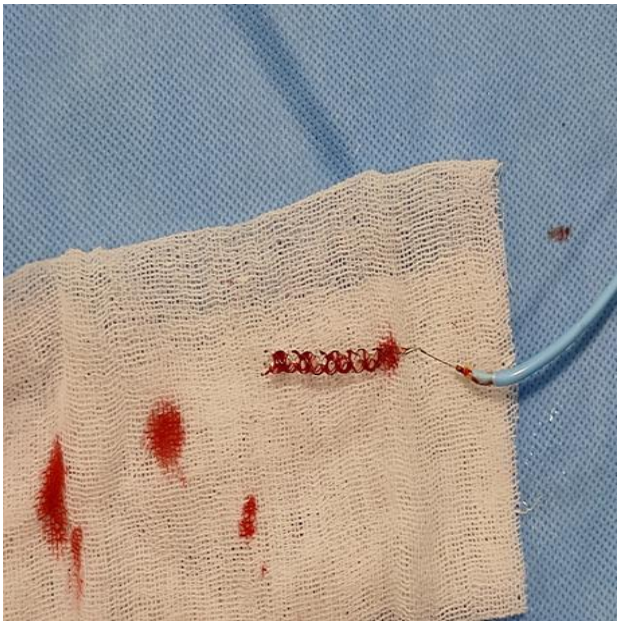


Animal no P1: On 0-day Clot retrieval stent was retrieved the clot



Animal no P2: On 0-day Clot retrieval stent was retrieved the clot

Animal P2 (Zoom version): On day 0, the clot retrieval stent successfully extracted the clot.



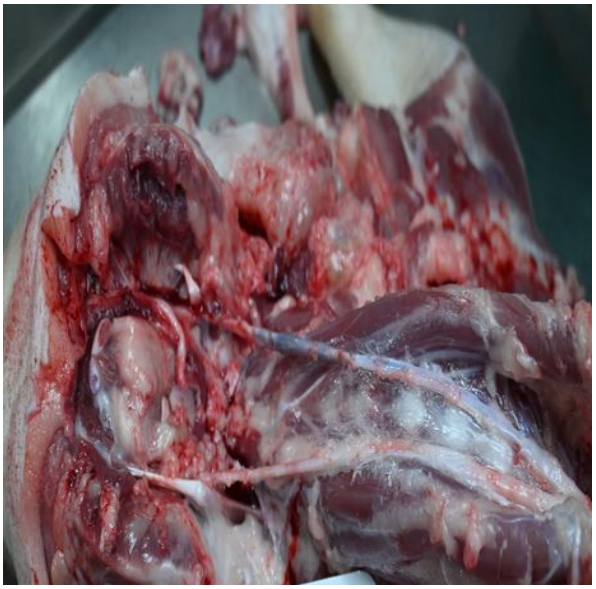
Animal no P3: On 0-day Clot retrieval stent was retrieved the clot

Figure 3: Clot retrieval images of animals P1, P2, and P3

Necropsy

In this animal study, researchers humanely euthanized the animals using a barbiturate overdose at scheduled intervals to minimize distress, allowing for a thorough examination by a pathologist of any external and internal gross pathological changes related to stent implantation. The collected stents were preserved in 10% neutral buffered formalin, and the surrounding arterial tissue was processed for resin embedding. Thin sections, approximately 100-200 microns thick, were prepared using specialized cutting and polishing machines. These sections were stained with hematoxylin and eosin (H&E) to highlight cellular structures, and subsequently examined under a light microscope by a study pathologist to evaluate histopathological lesions. This comprehensive approach provided valuable insights into the biological response of arterial tissue to the implanted stents, contributing to the assessment of their safety and efficacy. Necropsy images are mentioned in Figure 4.

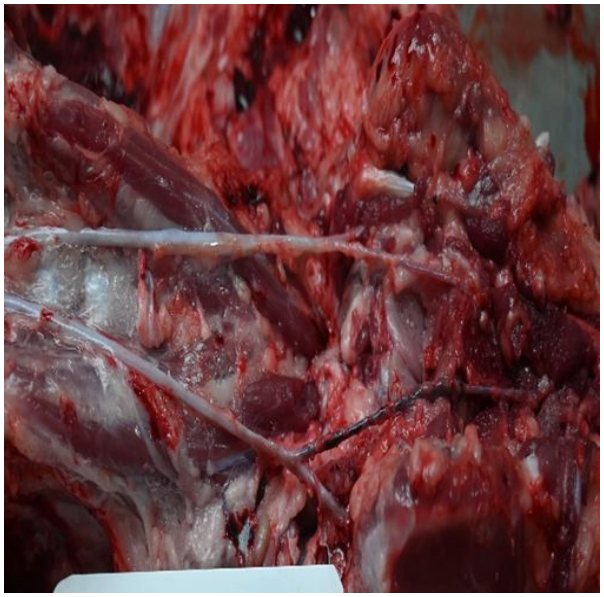
Necropsy Image



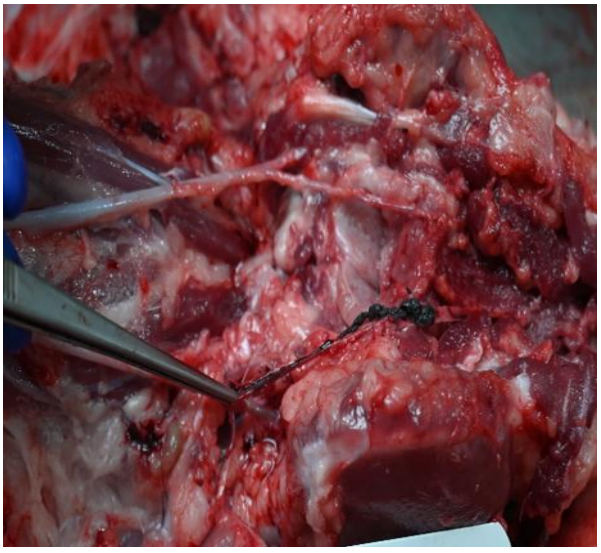
Animal P1, Necropsy Image

Animal P1, Necropsy Image

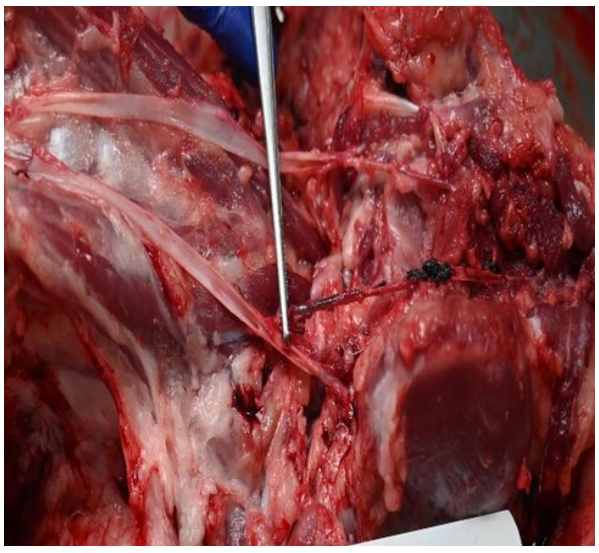
Menox clots harvested from Animal P1 after necropsy



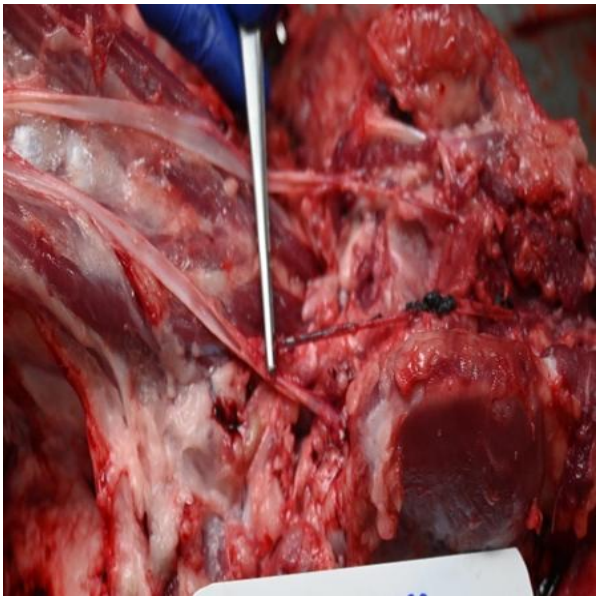
Presence of Menox clots after Necropsy from Animal P2



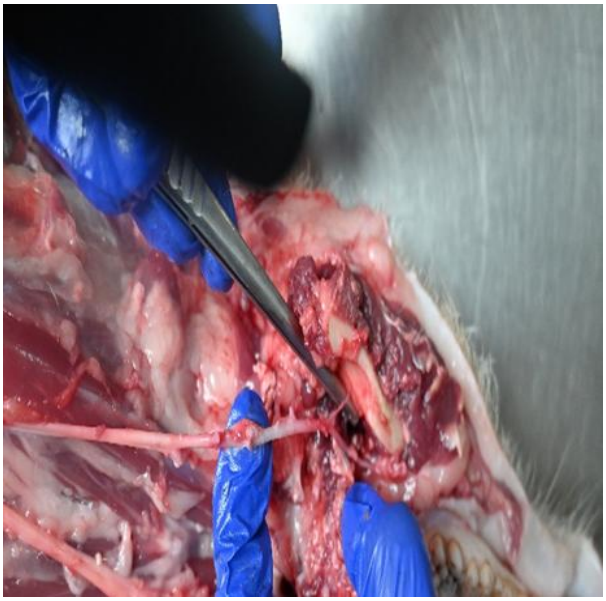
Presence of Menox clots after Necropsy from Animal P2



Presence of Menox clots after Necropsy from Animal P2



Animal P2 Target site for Autologous clot retrieval



Animal P3 Target site for Autologous clot retrieval

Figure 4: Necropsy Image of animals P1, P2, and P3

Histopathology

In this study, branches of the carotid artery were harvested to assess clot deposition and retrieval activities, following the sponsor's guidelines. The arteries were collected from 2-5 cm above the proximal end and 2-5 cm below the distal end of the clot retrieval region. After collection, the arteries were flushed with normal saline and preserved in 10% formalin. Comprehensive evaluations, including photography, gross examination, and histopathological analysis, were conducted on the harvested arteries and standard organs. Histological evaluations involved staining the specimens with hematoxylin and eosin (H&E) and performing histomorphometry to analyze various parameters. Histopathology Scores are mentioned in table 11.

Table 11: Histopathology Score for Carotid Artery branches of Clot Retrieval Device

Device Location	Animal no.	Inflammation	Vascular Injury	Smooth muscle cell loss	Fibrin deposition	Endothelial loss	Total Score
Carotid artery branches (Clot Retrieval Device)	P1	Right	0	0	0	0	0
		Left	1	0	0	3	4
	P2	Right	1	0	1	1	3
		Left	1	0	1	1	3
	P3	Right	0	0	0	0	0
		Left	0	0	0	0	0

Histological Analysis

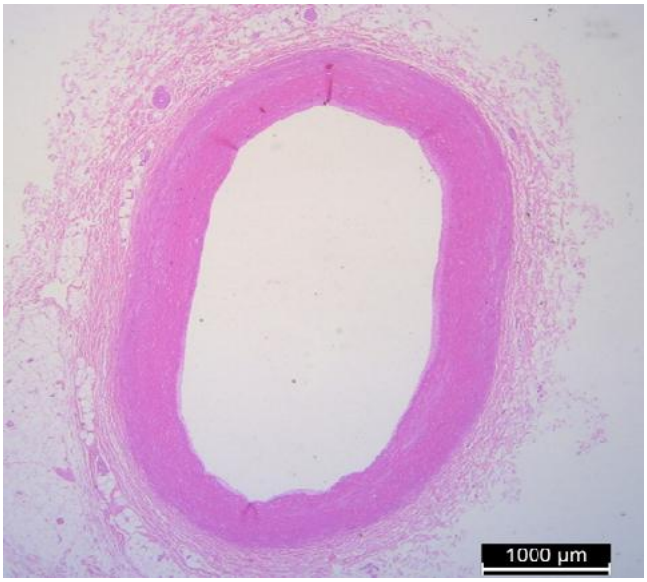
Carotid artery branches were harvested from the region associated with clot deposition and retrieval activities, specifically 2-5 cm above the proximal end and 2-5 cm below the distal end of the clot retrieval site. After collection, the arteries were flushed with normal saline and preserved in 10% formalin. They underwent gross and histopathological evaluation, including staining with hematoxylin and eosin (H&E), to assess any pathological changes related to the thrombectomy procedure. This analysis aimed to provide insights into the effects of the clot retrieval process on the arterial tissue.

Microscopic examination

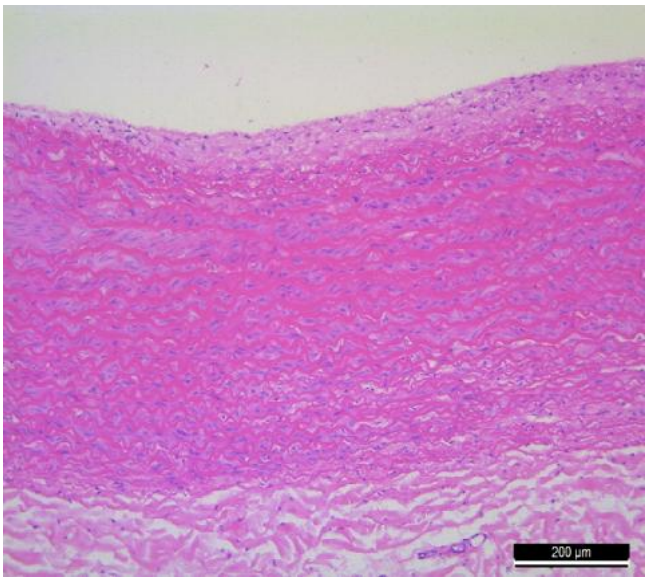
The animals were observed for 30 days and exhibited normal behaviors, with a pain score of 1 for the first 3 days following a minimally invasive procedure, leading to the administration of tramadol (2 mg/kg IM) for 3 days to the P3 animal. Microscopic examination revealed few inflammatory cells in the left carotid artery, moderate endothelial loss, and a histopathology score of 4, while the right carotid artery showed no inflammatory cells, no endothelial loss, and a score of 0. Histo-morphometric measurements showed no remarkable changes in the medial or neointimal areas, and no stenosis was observed in the carotid arteries of animal number 1 after clot removal.

Microscopic analysis of both the left and right carotid arteries revealed a few inflammatory cells, with no signs of vascular injury or smooth muscle cell loss; however, minimal fibrin deposition and endothelial loss were noted in both arteries, resulting in a total histopathology score of 3 for each. Histo-morphometric measurements, including lumen diameter and the diameters of the internal and external elastic lamina, showed no significant changes in the medial or neointimal areas. Additionally, no stenosis was detected in the lumen of the carotid arteries of animal P2, as any clots were removed prior to gross pathological examination, with negative stenosis values recorded as zero.

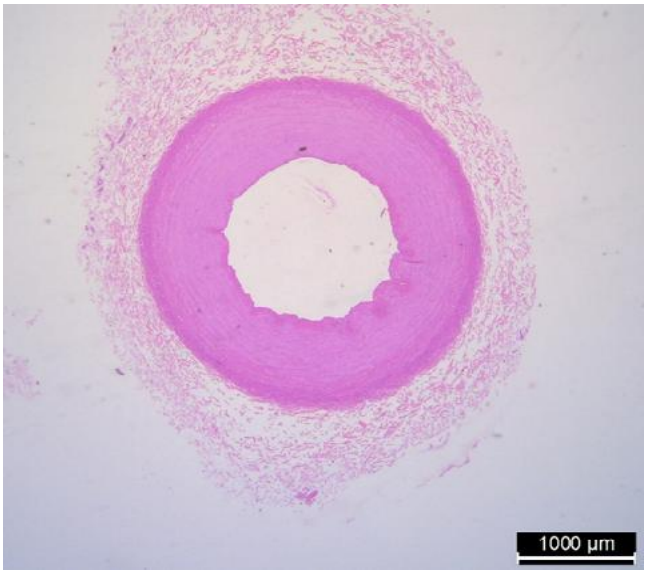
P3 - Microscopic examination of both the left and right portions of the carotid artery revealed no inflammatory cells, vascular injury, smooth muscle cell loss, fibrin deposition, or endothelial loss, resulting in a total histopathology score of 0 for both arteries; histo-morphometric measurements, including lumen diameter and the diameters of the internal and external elastic lamina, showed no remarkable changes in the medial or neointimal areas, and no stenosis was detected in the carotid arteries of animal P3, as any clots were successfully retrieved using the Clot Retrieval Device, with negative stenosis values recorded as zero. Histopathology images of animals P2 and P3 were mentioned in Figure 5.



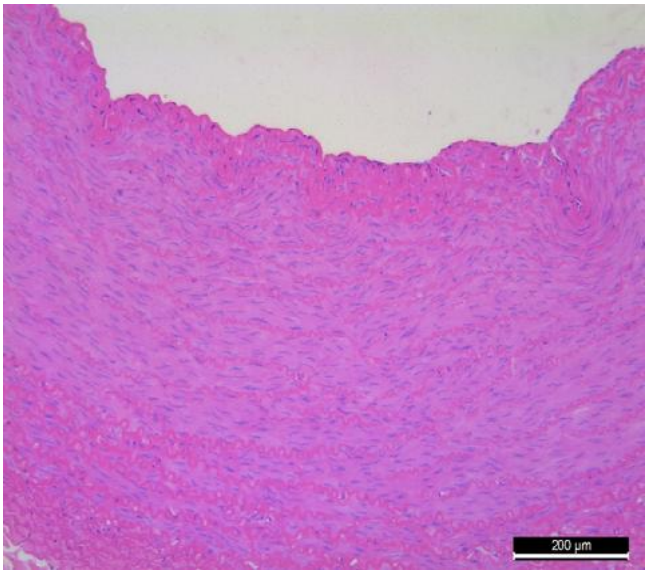
Animal No. P1: Carotid artery (Clot Retrieval stent), left, H&E 1.25X



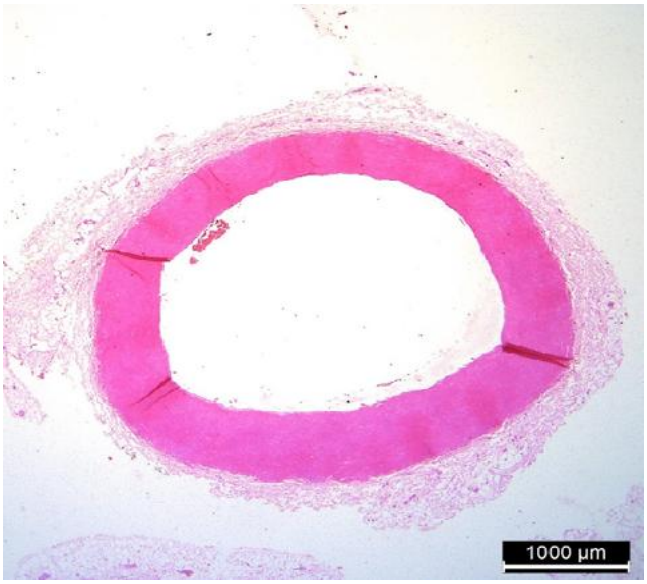
Animal No. P1 Carotid artery (Clot Retrieval stent), left, H&E 10X



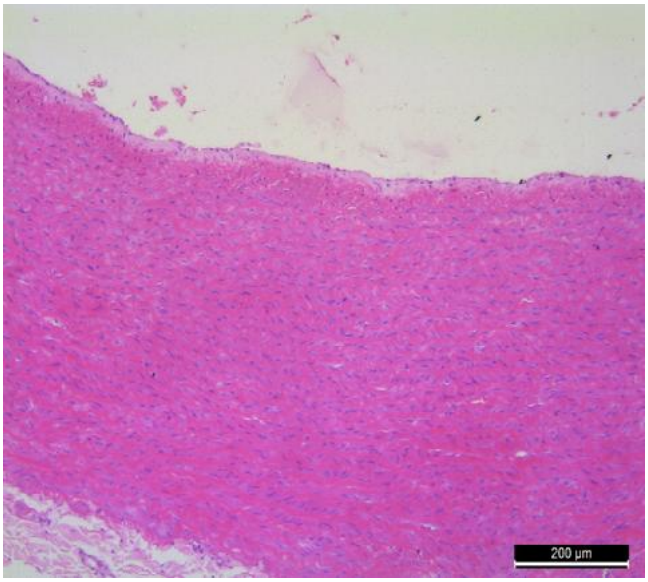
Animal No. P1: Carotid artery (Clot Retrieval stent), Right, H&E 1.25X



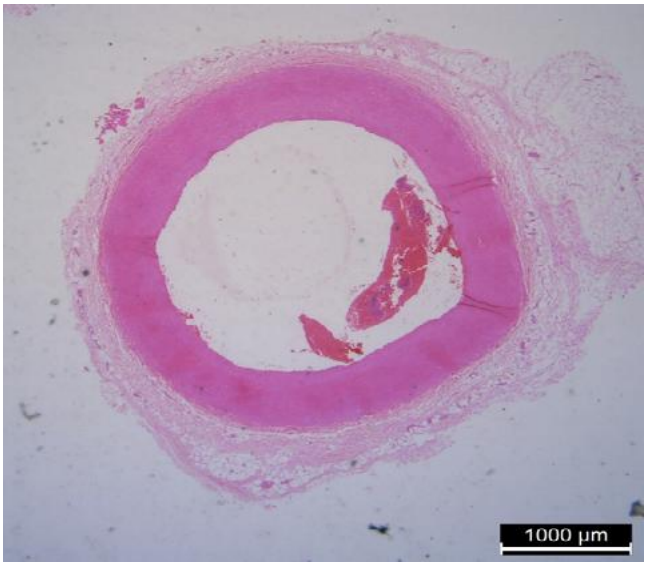
Animal No. P1: Carotid artery (Clot Retrieval stent), Right, H&E 10X



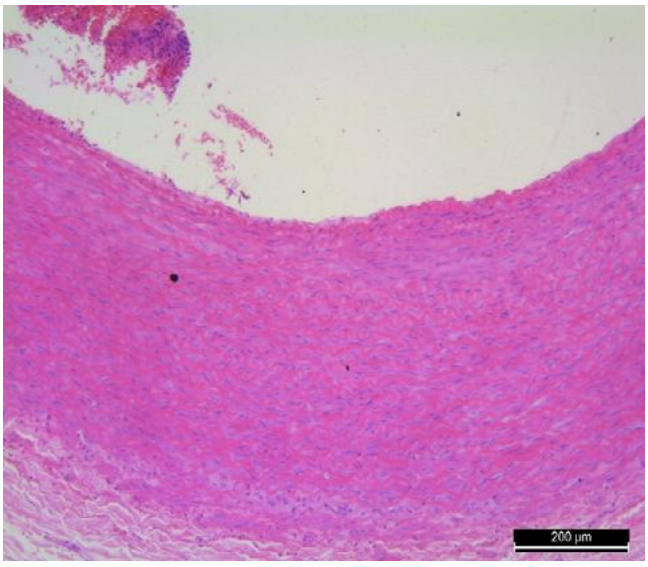
Animal No. P1: Carotid artery (Clot Retrieval stent), Right, H&E 1.25X



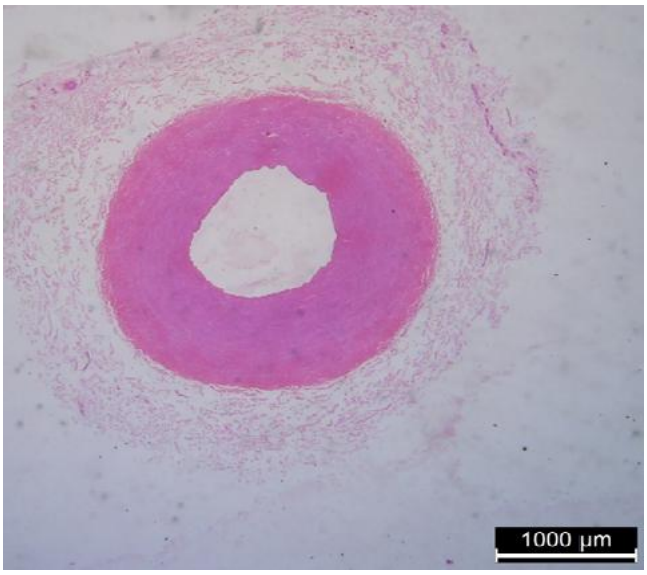
Animal No. P1: Carotid artery (Clot Retrieval stent), Right, H&E 10X



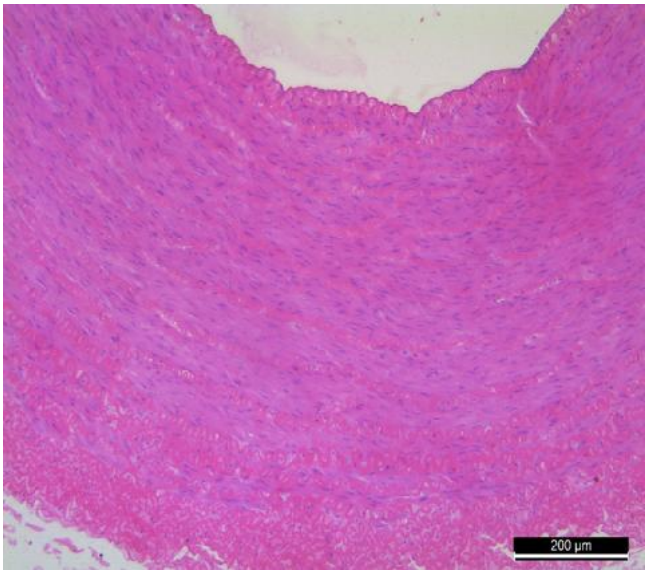
Animal No. P2: Carotid artery (Clot Retrieval stent), Right, H&E 1.25X



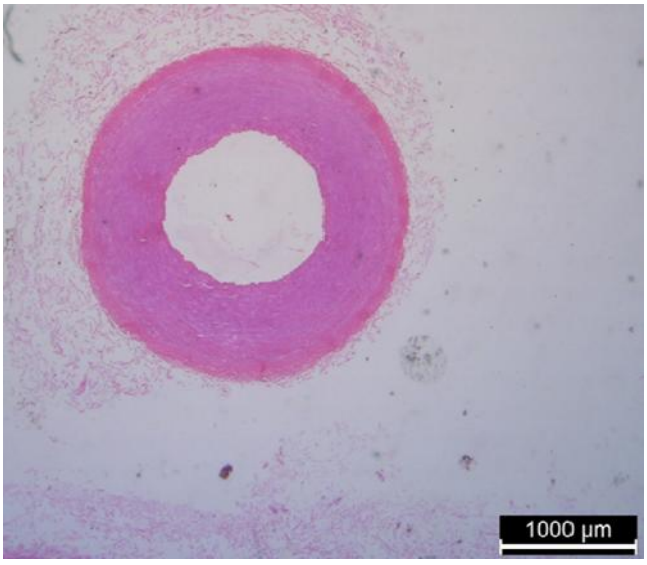
Animal No. P2: Carotid artery (Clot Retrieval stent), Right, H&E 10X



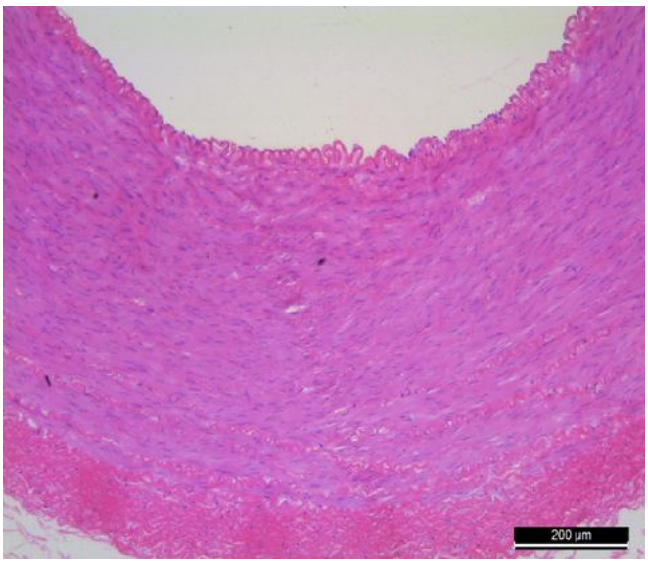
Animal No. P3: Carotid artery (Clot Retrieval stent), left, H&E 10X



Animal No. P3: Carotid artery (Clot Retrieval stent), left, H&E 1.25X



Animal No. P3: Carotid artery (Clot Retrieval stent), Right, H&E 1.25XAnimal No. P3: Carotid artery (Clot Retrieval



Animal No. P3: Carotid artery (Clot Retrieval stent), Right, H&E 10X

Figure 5: Histopathology images of animals P1, P2 and P3

Result

During the study, the health and well-being of the animals were meticulously monitored from Day 1 to Day 30. Observations indicated that all animals were eating, drinking, defecating, and urinating normally, and they consistently appeared bright, alert, and responsive throughout the observation period. Following the minimally invasive procedure, the pain score was assessed at 1 for three days, indicating minimal discomfort. To manage this, animal P3 was administered tramadol at a dose of 2 mg/kg IM for three days to ensure its comfort during recovery.

Body weights were recorded at the beginning of the study (Day 0) and again on Day 30, just prior to euthanasia. The weights were as follows: P1 was 42.4 kg, P2 was 43.1 kg, and P3 was 40.2 kg on Day 0. By Day 30, P3's weight was recorded at 42.2 kg, demonstrating that there was no significant reduction in body weight for any of the animals throughout the study period. This stability in body weight is indicative of the overall health of the subjects during the study.

Importantly, there were no incidences of morbidity or mortality among the animals subjected to the test item, which further underscores the safety of the procedures performed. Clinical pathology assessments were conducted through blood collections on Day 0, prior to the procedure, and on the day of euthanasia. These assessments included a comprehensive analysis of hematological parameters and biochemical markers, such as LDH, AST, creatinine, and electrolytes. The results indicated that all hematology and clinical biochemistry parameters remained within normal ranges at both baseline and follow-up time points, with no abnormal findings reported.

At the scheduled sacrifice dates, animals P1 and P2 were euthanized on the same day, while P3 was euthanized on Day 30. The euthanasia was performed using an overdose of thiopental sodium, followed by exsanguination. Pathological examinations were conducted by a qualified pathologist, who assessed both external and internal gross pathological changes. The examination revealed no significant external lesions. However, internal examinations identified a blood clot in the left carotid artery of animal P1 and in both carotid arteries of animal P2. In contrast, no clots were observed in the carotid arteries of animal P3.

Microscopic evaluations of the carotid arteries revealed few inflammatory cells in the left carotid artery of animal P1, along with moderate endothelial loss, resulting in a histopathology score of 4 for the left artery and 0 for the right artery (Safer limit of a histopathological score ranges from 1 to 5), where 1 indicates minimal changes and 5 represents the most severe. Animal P2 showed similar findings, with few inflammatory cells and minimal fibrin deposition, yielding a total histopathology score of 3 for both arteries. Notably, animal P3 exhibited no inflammatory cells, vascular injury, or endothelial loss, resulting in a total score of 0 for both carotid arteries.

Histo-morphometric measurements were taken to assess the lumen diameter, internal elastic lamina diameter, and external elastic lamina diameter. The analysis indicated no remarkable changes in the medial area or neointimal area across all animals. Importantly, no stenosis was observed in any of the carotid arteries, as the clots had been effectively removed during gross examinations.

In conclusion, the study demonstrated that the test item was safe and effective for the retrieval of clots without causing significant injury to the target arteries. The overall health of the animals remained stable throughout the study, and the absence of adverse pathological findings supports the efficacy of the Clot Retrieval Device in this porcine model.

Discussion

The performance evaluation of the stent delivery systems revealed efficient trackability, handling, and haemostasis, with no instances of leakage observed. Deployment accuracy and efficacy were confirmed by the rapid self-expansion speed of the stents and normal flow in all deployed stents, as seen in angiographic evaluations. Radiography and angiographic findings further supported the stent system's satisfactory performance across all parameters. Clinical pathology results demonstrated normal hematology and clinical biochemistry parameters, indicating no adverse effects on blood parameters. Necropsy and histopathology examinations revealed no significant pathological lesions, internally or externally. Histopathological evaluation of stented arteries confirmed the biocompatibility of the Clot retrieval device material, Overall, these findings suggested that the Clot Retrieval Device was a safe and effective option for venous applications. The consistent performance and minimal adverse effects observed in animal models provided valuable insights for potential clinical use in treating venous disorders in humans. Three female swine's were selected and fasted overnight without access to water. All animals enrolled in the study passed the study acceptance physical examination criteria, with no major clinical observations noted. The animals were prepared under appropriate analgesia and anaesthesia for each procedure on Day 0. P1 and P2 were deployed with clots, which were retrieved using the test item, while P3 was also deployed with a clot, but the retrieval occurred on the procedural day. On Day 0, each animal was placed with autologous clots and synthetic occluders (Menox). The test item was utilized to retrieve the clots under proper heparinization, and deployment was evaluated. Angiography and measurements were conducted pre- and post-implantation. The stented veins were harvested on Day 0 from Animal P1 and P2 & day 30 for animal P3 for histopathological evaluation. Results showed the autologous clot was easily and completely retrieved, while the synthetic clot could not be retrieved but showed no clinical signs. Additionally, there was no decrease in body weight for P3 on Day 30.

Conclusion

The results of the present study demonstrate that Clot Retrieval stent facilitates easy deployment and withdrawal from the test system. There are no clot-related lesions found in the internal carotid arteries supplying the brain for the autologous clot. Although the synthetic clot remains in place, it does not exhibit any clinical signs related to its presence, nor does it adhere to the artery. Overall, the test item proves effective for the complete and safe retrieval of autologous clots without causing injury to the target arteries. This conclusion is supported by findings from necropsy and histopathological evaluations conducted at all time points. These results suggest that Clot Retrieval stent is a viable option for clinical applications involving clot retrieval, ensuring patient safety and minimizing potential complications associated with clot presence in the cerebral circulation.

Ethics approval and consent to participate

Approved

List of abbreviations

NPO: (nil per os)
ECG: Electrocardiogram
H&E: Hematoxylin and Eosin
CRD: Clot Retrieval Device

ACT: Activated Clotting Time
QVA: Quantitative Vascular Analysis
RVD: Reference Vessel Diameter

Data Availability

Not Applicable

Conflicts of Interest

None

Funding Statement

None

Authors' contributions

The Author declares no conflict of work

References

[1] https://jnis.bmj.com/content/8/Suppl_1/A84.1
[2] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5939833/
[3] Kan, I., Yuki, I., Murayama, Y., Viñuela, F. A., Kim, R. H., Vinters, H. V., & Viñuela, F. (2010). A novel method of thrombus preparation for use in a swine model for evaluation of thrombectomy devices. American journal of neuroradiology, 31(9), 1741-1743.
[4] Gralla J, Schroth G, Remonda L, Fleischmann A, Fandino J, Slotboom J, Brekenfeld C. A dedicated animal model for mechanical thrombectomy in acute stroke. AJNR Am J Neuroradiol. 2006;27:1357-1361.
[5] Liebig T, Reinartz J, Hannes R, Miloslavski E, Henkes H. Comparative in vitro study of five mechanical embolectomy systems: effectiveness of clot removal and risk of distal embolization. Neuroradiology. 2008;50:43-52.
[6] Mordasini P, Brekenfeld C, Byrne JV, Fischer U, Arnold M, Jung S, Schroth G, Gralla J. Experimental evaluation of immediate recanalization effect and recanalization efficacy of a new thrombus retriever for acute stroke treatment in vivo. AJNR Am J Neuroradiol. 2013;34:153-158
[7] Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources. Commission on Life Sciences. National Research Council. National Academy Press. Washington, D.C. 1996.
[8] Organization for Economic Co-operation and Development - Principles of GLP and Compliance Monitoring (as revised in 1997).
[9] Compendium of CPCSEA 2018 Guidelines for laboratory animal facility 2015:7; Pg - Page 61-96.
[10] Biological Evaluation of Medical Devices-Part 2: Animal welfare requirements. ISO 10993-2:2006 (E).
[11] Hung, M. Y., Yang, C. K., Chen, J. H., Lin, L. H., & Hsiao, H. M. (2021). Novel Blood Clot Retriever for Ischemic Stroke. Micromachines, 12(8), 928.
[12] Yuki, I., Kan, I., Golshan, A., Sohn, J., Murayama, Y., Vinters, H. V., & Viñuela, F. (2013). A swine model to analyze arterial structural changes induced by mechanical thrombectomy. American Journal of Neuroradiology, 34(8), E87-E90.
[13] Spangler, T. A., & Katzman, S. A. (2019). Pathological Safety Assessment in Preclinical Neurothrombectomy Studies. Toxicologic Pathology, 47(3), 264-279.
[14] Ulm, A. J., Khachatryan, T., Grigorian, A., & Nogueira, R. G. (2018). Preclinical evaluation of the stent retriever: safety and efficacy in the swine thrombectomy model. Interventional Neurology, 7(5), 205-217.
[15] Pre-Clinical and Clinical Studies for Neurothrombectomy Devices, Guidance for Industry and FDA Staff, Document issued on: June 18, 2007.