Comparison of Blunt Versus Surgical Dissection for Aortic Flow Probe Placement in the Dog

by Z.Y. Peng³, L.A.H. Critchley¹, * & A.E. James²

¹Department of Anaesthesia & Intensive Care and ²The Laboratory Animal Services Centre, The Chinese University of Hong Kong, Shatin, Hong Kong, China ³Department of Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, USA

Summary

In animal research placement, of a flow probe on the aorta provides more accurate cardiac output measurements than alternative clinical methods, such as thermodilution. However, good advice on how to place such a probe in the laboratory setting is lacking. In twenty anesthetized dogs midline sternotomy and left thoracotomy approaches to the ascending aorta, using surgical (forceps with scissors) or blunt finger dissection to separate the aorta from the adjacent pulmonary artery (four groups of five dogs), were compared. A Transonic A-probe was placed around the aorta. Hematocrit was compared before and after surgery. The operative site was inspected for bleeding at post mortem. Two dogs died from massive pulmonary artery hemorrhage in the surgical dissection groupings. At post mortem, bleeding around the probe had occurred in five dogs in the surgical dissection groupings (P<0.05). Thoracotomy with blunt finger dissection to mobilize the aorta was associated with minimal operative blood loss. It was subsequently used successfully for flow probe placement in over fifty dog experiments each lasting 8-12h.

Introduction

Animal experimentation using flow probes allows researchers to measure cardiac output to a greater degree of precision. Furthermore, it permits a wide range of conditions such as hemorrhagic shock and acute sepsis to be simulated, drugs such as vaso-pressor agents to be tested over a range of doses, and equipment such as bioimpedance monitors to be validated (*Patterson & Witsoe, 1999; Treggiari et al., 2002; York et al., 2003*) that could not ethically be produced or performed in humans.

Placement of a flow probe on the aorta requires the heart and aorta to be exposed. This can be done either by a midline sternotomy or lateral thoracoto-

*Correspondence: Lester AH Critchley, MD, FFARCSI Department of Anesthesia and Intensive Care, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China Tel: +852-2632-2735 Fax: +852-2637-2422 E-mail: hcritchley@cuhk.edu.hk my. The aorta also has to be prepared by separating it from the pulmonary artery and cleaning the vessel surface of fatty tissue. Bleeding and heavy blood loss is a distinct possibility. In particular, rupture of the pulmonary artery may occur (*Folts & Rowe,* 1973). A simple method of applying the aortic flow probe that minimizes the risk of surgical bleeding is desirable. However, there is limited guidance in the literature on how to perform such a procedure, despite numerous publications citing the use of aortic flow probes.

The key issues in placing a flow probe are how best to approach the aorta and how best to mobilize the aorta from the adjacent pulmonary artery. To address these issues, we performed a pilot study using twenty anesthetized dogs. We compared blood loss and local bleeding using the sternotomy and left thoractomy approaches. We also compared two methods of separating the aorta from the pulmonary artery, surgical dissection using forceps with scissors and blunt separation of the vessels using a gloved finger. A detailed account is provided of our recommended method as guidance for future researchers.

Materials and Methods

Ethical approval

All experiments were performed under license from the Government of the Hong Kong SAR and endorsed by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong.

Supply of animals

Male domestic dogs (*Canis familiaris*), aged 2-3 years and between 11-25 kg in weight, were supplied by the Animal Management Centre, Shatin, New Territories, Hong Kong. This is an animal pound maintained by the Department of Agriculture, Fisheries and Conservation of the Hong Kong Special Administrative Region Government. The dogs were supplied with the written approval of the Senior Veterinary Officer in charge of all Animal Management Centres throughout Hong Kong.

The dogs' microbiological and parasitological status were unknown as these were animals that had been impounded by the Department of Agriculture, Fisheries and Conservation after being found wandering within various human communities in Hong Kong. All dogs were held for over 30 days in wired pens, open to the environment but sheltered from adverse weather conditions due to the pens being roofed and having kennels at the rear of the areas. The pens were cleaned daily. The dogs were fed once a day with commercial dog food, bought from a local distributor (Star Pro® Premium PT153), and supplied ad lib with potable town water supply.

The dogs were delivered to the Laboratory Animal Services Centre of the Chinese University of Hong Kong in a delivery van. They were sedated for transport using intramuscular Xylazine 2 mg/kg and Atropine 0.05 mg/kg. The transfer took around 30 minutes.

Anesthesia

On arrival at the Laboratory Animal Services Centre, the sedated dog was anaesthetized with intramuscular ketamine (5mg/kg). The tracheal of the dog was then intubated with an endotracheal tube (ID 9mm), which was tied to the jaw using a hollow (20 cm in length and 2.5 cm in diameter) plastic pipe through which a perpendicular hole had been drilled to accommodate the endotracheal tube. The pipe and tube were tied such that the pipe lay across the bit of the jaw. This method of fixation overcame movement and slipping of the endotracheal tube due to secretions.

Anesthesia was subsequently maintained with inhaled halothane (0.5-1.5%) in oxygen, the inhaled dose being adjusted to prevent spontaneous movement. The lungs were mechanically ventilated using a Manley 4 Ventilator (Blease Medical Equip Ltd, Chesham, Bucks, England) minute volume divider, ventilator at a tidal volume of 10-15ml/kg and fresh gas flow of 1-2 litre/min. Blood gas analysis was not available to facilitate normalization of the PaC0₂. The anesthetized dog was placed on its back on the operating table. Intravenous access was then secured via the forelimb. The fur over the vein was shaved, the skin cleaned with alcohol, a 20 gauge catheter was inserted and secured with tape. The catheter was connected to an intravenous infusion of normal saline (2 ml/kg/h) that was given to compensate for the lost body fluid. During the procedure the infusion rate was adjusted to maintain the mean blood pressure at $\pm 5\%$ baseline levels (about 75-85 mmHg). Significant blood loss was replaced with colloid solution (10 ml/kg boluses). The dog was covered with a blanket to prevent heat loss. Beneath the operating table was a water-bath and pump system made by the Multidisciplinary Laboratory of the Chinese University of Hong Kong that circulated warm water through the operating table.

Monitoring lines

Blood pressure was measured via a 20 gauge catheter inserted into the femoral artery. The fur

over the groin was shaved and the artery surgically exposed via a 2-3 cm skin incision. The catheter was kept running using a saline infusion (1 ml/h). Central venous pressure was measured via a 16 gauge catheter inserted into the left internal jugular vein to a depth of 10 cm. The fur over the left neck was shaved, the vein surgically exposed, bleeding controlled by two sutures looped under the vein and the catheter inserted using a guide-wire-throughneedle technique.

Placement of the flow probe

Two different surgical approaches to expose the aorta and separate it from the pulmonary artery were used: A mid-line sternotomy and a left lateral thoracotomy. Mobilization of the aorta was performed by either surgical dissection using forceps with scissors or blunt dissection using a gloved finger.

The flowmeter and probe

Ultrasonic transit-time technology (Transonic System, Ithaca, NY, USA) was used to measure aortic blood flow in our animal model. A number of recent publications had shown the Transonic system to be more reliable than the older electromagnetic flow probes (Koenig et al., 1996). The Transonic system included a bench-top electronic meter that was connected to a volume flow-sensing probe. The meter automatically identified the calibration factor of the probe. It provided a digital readout, an analog meter display, and an analog signal output to a computer that processed the data using the data acquisition program WinDag (DataQ Instruments, OH, USA). The probe was connected to the meter via a flexible cable. A size 12, 16 or 20 mm A-series probe was used (Figure 1). This probe was designed for large blood vessels such as the aorta and had a four transducer array, which utilizes a dual beam Xpattern of ultrasound illumination. The transit-time of the beam passing perpendicular to the blood flow is measured rather like the Doppler shift signal used in older ultrasound devices. The A-series probe is designed to accommodate ultrasonic gel. It has a

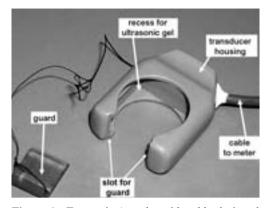


Figure 1. Transonic A-probe with cable designed for use on large blood vessels, such as the aorta or pulmonary artery. It contains a four-ultrasonictransducers array. The probe housing contains a recess to accommodate the ultrasonic acoustic gel needed when using the probe. There is a guard that clips into the housing to complete the ring.

guard which slots into the body of the probe and allows the probe to be fitted around a large blood vessel with the probe fully encircling the vessel.

Midline sternotomy approach

With the dog lying on its back, the fur over the sternum was shaved. A longitudinal skin incision was made in the midline extending from the sternal notch to the ziphoid process using cutting diathermy. The bony sternum was then cut in the midline using a pair of sternal scissors. Bleeding from the cut bone edges was stopped using diathermy and bone wax. The bone thickness was approximately 1 cm. A rib separator was inserted and used to expose the contents of the pericardial cavity. The heart and root of the aorta were easily identified.

Left thoracotomy approach

With the dog lying on its back, the left 4th intercostal space was identified and the overlying fur shaved. A left thoracotomy incision from the left sternal edge to the mid-axillary line was made using cutting diathermy and the pleural cavity entered (*White & Lang, 1982*). A rib separator was used to hold the cut edges apart (Figure 2). The left lung collapsed rostally to reveal the pericardial sac. The collapsed lung did not have to be actively retracted.

The left phrenic nerve was easily identified crossing the pericardial sac longitudinally (Figure 2) with the more anterior pericardiacophrenic blood vessels (Figure 3). Within the pericardial sac the auricular appendage of the left atria was also identified, as it moved with heart contraction (Figure 2). The superior limit of the auricular appendage marked the anatomical position where roots of both the aorta and pulmonary artery exited the heart. It was at this level that the flow probe needed to be

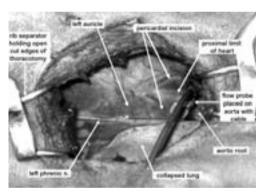


Figure 2. Photograph showing the structures within the pleural cavity following left thoracotomy. The anterior chest wall lies above and the back lies below. The ribs are separated by a surgical retractor, or rib separator. The underlying pericardial sac is seen with the overlying lobe of the left lung collapsed posteriorly. The left phrenic nerve is seen crossing the sac. The pericardium has been incised anterior to the nerve and superior to the left auricle. The auricle is identified by its movement within the sac as the heart beats. The opening within the pericardial sac reveals the proximal part of the heart and the roots of the aorta and pulmonary artery. The flow probe has been placed around the root of the ascending aorta, obscuring its view. placed on the aorta. The pericardial sac was opened at a point 1 cm anterior to the nerve and blood vessel to the diaphragm and at the superior limit of the auricular appendage (Figure 2). The pericardial sac was grasped with dissecting forceps and carefully cut parallel to the nerve and vessels using a pair of fine scissors. A longitudinal incision that extended 1 cm distally over the heart and auricle and 2-3 cm superiorly over the great vessels was made. The aorta and pulmonary artery were thus exposed (Figure 3). The pulmonary artery lay in front of the aorta with respect to the pericardial opening.

Surgical preparation of the aorta

The pulmonary artery and aorta were identified as they emerged from the heart. They were joined by adventitial tissue that needed to be carefully separated by 2-3 cm to allow placement of the probe around the aorta. The pulmonary artery could be easily ruptured during this procedure causing massive hemorrhage, which usually resulted in death of the dog (*Koenig et al., 1996*). The two vessels were separated by either surgical dissection using forceps with scissors or blunt dissection using a gloved finger.

The first step of separation was to pass a finger of the left hand through the pericardial incision and hook it over the aorta, so that it lay behind both the aorta and more lateral pulmonary artery (Figure 3). This enabled the operator to stabilize both the aorta and pulmonary artery and facilitated separation. The gap between the two vessels was identified by digital palpation.

Surgical dissection involved inserting forceps into the adventitial tissue between the two blood vessels and carefully dissecting down on to the supporting finger that lay behind the two blood vessels. The gap was then enlarged using forceps and scissors.

Blunt dissection involved supporting the two blood vessels with the finger and using the gloved little finger of the right hand to gently separate the adventitial tissue and open up a space between the two blood vessels. This was done by gently rotating the gloved finger. Once a hole had been created, the

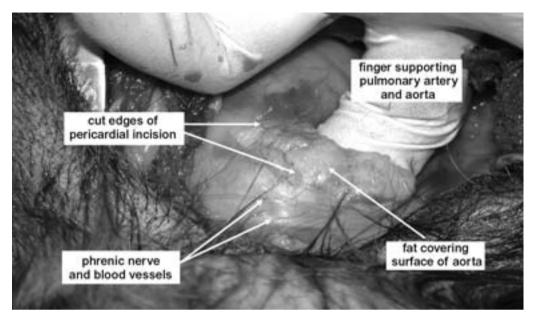


Figure 3. Photograph showing the aorta exposed by the incision in the pericardial sac. The aorta is supported by a gloved finger. Note the fat covering the surface of the aorta, which needs to be removed surgically before the flow probe can be placed.

little finger was changed to the index finger and the hole gently expanded until it was large enough to accommodate the flow probe.

Once the two blood vessels had been separated, and a hole large enough to accommodate the flow probe produced, any fatty tissue surrounding the aorta was removed (Figure 3). This was an important stage in the procedure, as it assured good aorta-probe contact. Careful cleaning and tidying of the surface of the aorta was performed using forceps and scissors, whilst supporting the vessel with a finger. Hemostasis was then assured by placing damp cotton wool balls around the aorta for 10 minutes to stop any oozing from small adventitial blood vessels. Surface fat and blood clot formation from local bleeding would adversely affect the performance of the probe.

Placement of the flow probe

A probe size was chosen that would neither compress the vessel nor allowed too much room to cause kinking of the aorta and poor contact. The guard was removed from the probe and the housing was filled with ultrasonic gel (Figure 1). The aorta was supported from behind using a finger. The probe was held in the right hand with the cable extending downwards. The probe was then slotted over the aorta. This maneuver was technically difficult and often required several attempts. Once the probe was positioned around the aorta (Figure 2), it was rotated through 180 degrees towards the midline, such that slot for the guard became visible and accessible via the pericardial incision. The guard could then be easily slotted into position. The probe was then repositioned to allow easy exit of the cable (Figure 2) and additional gel was syringed around the aorta-probe interface to assure a good acoustic contact.

Wound closure

When using the mid-sternal approach the probe cable exited the pericardium via the sternotomy wound. The sternum and skin were closed. When using the thoracotomy approach the probe was positioned so that the cable ran posterior through the pleural cavity. The pericardium was closed with interrupted sutures. The rib retractor was removed and chest drain to underwater seal inserted. The lung was expanded. The rib cage was closed by interrupted sutures around the separated ribs, the muscle layers sutured to cover the ribs and skin incision closed.

Study plan

Twenty anesthetized male dogs (age 2 to 3 years) were randomly used to evaluate the four different surgical techniques: (i) Midline sternotomy and (ii) left thoracotomy approaches using (a) surgical or (b) blunt dissection; four groups of five dogs. The extent of blood loss due to the surgery was assessed by measuring the hemoglobin and hematocrit before and 60 minutes after probe placement. The performance of the probe was assessed by measuring the coefficient of variation (SD/mean %) of cardiac output measurement over a 10 minute period following completion of probe placement. All the dogs were later killed by injecting air into the heart and at post mortem the aorta and the adjacent tissues including the probe were carefully

examined to identify any bleeding or coagulation mass.

Statistical analysis

The Mann-Whitney test was used to make statistical comparisons. Results were presented as mean and range. P<0.05 was considered statistically significant.

Results

Data from eighteen dogs are presented. The surgical groups were similar with respect to weight (Table 1). Two dogs died from massive hemorrhage following rupture of the pulmonary artery when using the surgical method of mobilizing the aorta and were excluded from our final analysis (Table 1). At post mortem, bleeding from the aorta and the surrounding tissues had occurred in five dogs in the surgical dissection group but none from the blunt dissection group. There were also significant decreases in the hemoglobin content and hematocrit following placement of the probe in the midline sternotomy group and those dogs where surgical mobilization of the aorta was used (Table 1; P < 0.05). The coefficients of variation (4.7 to 6.2%) of the flow probe measurements were similar in all four groups (Table 1).

Table 1. Data comparing the four surgical methods before and after placement of the flow probe.

	Sternotomy		Thoracotomy	
	Surgical [#]	Blunt	Surgical [#]	Blunt
	(n=4)	(n=5)	(n=4)	(n=5)
Weight (kg)	17.2(13-24)	17.8(15-23)	17.4(11-23)	18.2(14-25)
PM-blood clots	2	0	3	0
Hb-before (g/100ml)	13.6(10.7-15.9)	13.8(10.0-15.2)	13.2(10.9-14.2)	13.3(10.3-16.3)
Hb-after (g/100ml)	9.7(6.2-11.0)*	10.2(7.3-11.7)*	10.0(8.5-11.5)*	13.1(10.1-16.1)
Hct-before (%)	43(40-45)	43(39-44)	43(40-45)	43(39-46)
Hct-after (%)	32(29-36)*	35(30-37)*	34(31-37)*	42(37-44)
CV (%)	6.2(5.3-6.9)	5.3(4.8-5.9)	5.3(4.6-6.0)	4.7(4.3-5.2)

Data presented as mean (range). PM = post mortem, Hb = hemoglobin, Hct = hemotocrit and CV = coefficient of variation of flow probe readings. *Significant difference compared to pre-surgery value P < 0.05. #Results for only 4 dogs as one died from hemorrhage.

Subsequent experience

We have since used the thoracotomy with blunt dissection method in over fifty dog experiments (*Peng et al., 2004, 2005; Critchley et al., 2005a, 2005b*). Using this method we have lost two dogs during preparation from pulmonary artery hemorrhage. It took about 1-2 hours to prepare the dog. The most time consuming and delicate part of the procedure was separating the aorta from the pulmonary artery though positioning the probe could also be tricky. The probe performed without fault for the duration of the experiment (8-12 h) in over 90% of cases. When the probe signal was faulty and the probe had to be re-explored, the problem could usually be resolved by clearing any blood clots, assuring hemostasis and applying more gel.

Discussion

Our group had previously used anesthetized dogs to study catecholamine secretion from the adrenal gland (Critchley et al., 2004). We had been using bioimpedance technology to measure cardiac output for a number of years and wished to validate the method against a gold standard such as an aortic flow probe (Critchley et al., 1993, 1994; Zhang et al., 2004). Hence, we had acquired a Transonic flow meter (single channel T106) with aortic probes (Aseries) using a research grant. The problem we faced was how to place the probe on the aorta without compromising cardiopulmonary function or causing massive hemorrhage. From the literature we had learnt that hemorrhage from the pulmonary artery and removing fatty tissue from around the aorta to improve probe contact were the main issues (Koenig et al., 1996). However, there was little guidance in the literature on how to surgically place the probe and whether the sternal or thoracotomy approach was better. Thus a pilot study was performed to determine the best method in our hands of placing the probe.

To place a probe on the aorta the chest needs to be opened and aorta mobilized, which are both procedures that may lead to unavoidable trauma and bleeding. When working with experimental animals such complications need to be minimized because facilities to resuscitate are often limited. Furthermore, local bleeding from the aorta may affect the performance of the probe. In the present report we found that the lateral thoracotomy approach with blunt dissection to mobilize the aorta was associated with the least blood loss and clot formation around the aorta and flow probe (Table 1). The blunt dissection method was developed by one of the authors (ZP) in response to losing a number of dogs from hemorrhage using the forcepswith-scissors method. It should also be noted that unlike the human sternum, which is flat, the canine sternum is more rounded and thicker, because of the dog's standing posture. Thus, it requires a greater effort to cut with a greater tendency to bleed. Taking all these points into consideration, we subsequently used the thoracotomy blunt dissection method successfully in over fifty dog experiments, with reliable functioning of the flow probe for up to 8-12 hours in over 90% of experiments. However, our method was not totally successful as fatal pulmonary artery rupture still occurred in two dogs.

Our dog experiments were terminal so we cannot comment on the utility of the technique for chronically implanted flow probes. In one report on longterm implanted A-probes in dogs, two of eleven dogs died from pulmonary rupture within two weeks (Picker et al., 2000). Limiting blood loss and operative pain are clearly important and thoracotomy would seem better in this respect because the bony sternum is not divided. Furthermore, many countries regulate the use of opiates in animal research so the choice of analgesia is limited. Thus, it is desirable to minimize the level of pain during the experiment. In the present investigation we were also concerned that the thoracotomy approach may influence our impedance measurements. However, we subsequently found that collapsing and reexpanding the lung had little effect on impedance cardiac output measurements as most of the impedance current presumably traversed the thorax via the highly conductive and blood filled mediastinal structures.

Several authors have reported difficulty when inserting the guard into the body of the flow probe and have describe special tools and modifications to the probe to facilitate the insertion of the guard (Folts & Rowe, 1973; Moskowitz et al., 1978). We overcame this problem by rotating the probe through 180⁰, once it was placed on the aorta. However, compared to previous probe designs, the guard is more easily positioned when using the Transonic A-probe. Koenig et al in chronically instrumented primates have shown the transit-time flow probe to be superior to older electromagnetic flow probes (Koenig et al., 1996). These authors found an error against thermodilution of 6% using the transit time probe compared to 15% using the electromagnetic flow probe. Electromagnetic probes are prone to baseline drift and require frequent zeroing. They are also sensitive to hemoglobin content, probe misalignment and flow turbulence (Dean et al., 1996). In comparison, the transit-time probe by using two ultrasound beams is independent of vessel cross-section and misalignment. It is also independent of hemoglobin content and temperature. Compared to the electromagnetic probe it is smaller in size, lighter in weight and does not require calibration prior to use. The transit-time probe has also been implanted for many months in chronic animal studies (Bednarik & May, 1995; Picker et al., 2000). Hence the decision to use a transit-time flow probe in our and others animal research.

In conclusion, the left thoracotomy with blunt finger dissection to mobilize the aorta was the most appropriate method of flow probe placement and was successfully used in over fifty dog experiments. The method was found to minimize operative bleeding and we recommend it in the animal laboratory setting.

Acknowledgements

The flow meter and probes used in this project were purchased using a Direct Grant for Research (2040805) from the Chinese University of Hong Kong.

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