WEBINAR: Transverse Aortic Constriction – The Importance of Monitoring Surgical Outcomes

Questions and answers from the March 6, 2019 webinar titled "Transverse Aortic Constriction – The Importance of Monitoring Surgical Outcomes"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address. Questions have been grouped into relevant categories.

Considerations during Surgery

1. **What affect can anesthesia have on surgical outcomes and on measurements of surgical success and cardia function?**

   As always, please consult with your animal care committee when making any decisions regarding anesthesia and follow their directives.

   When considering anesthesia, it is important to think about the safety and flexibility of using inhaled anesthesia, such as isoflurane, as the levels can be more tightly controlled and adjusted when compared to an injectable anesthetic agent. In this way, you may be better able to control the plane of anesthesia, and you can minimize the effect on cardiac function when doing any measurements. When using inhaled agents please also consider the safety of the user, and consider active scavenging of the waste gas to reduce exposure and possible side effects.

   For both improved surgical outcomes and all measurements it is imperative to maintain the temperature of your animal. During surgery, this will help improve the survival and improve the outcomes. While making measurements, this will ensure that cardiac function is not supressed due to a drop in the animal's core temperature.

   Having the ability to monitor the animal's core temperature, respiration rate, heart rate, and ECG signal, allow the surgeon to monitor the plane of anesthesia, and also to maintain specific safety zones within each of these to perform their surgeries. For measurements these physiological parameters can be used to ensure consistency between animals across the longitudinal study when measurements are made such that

the data is reproducible and not artificially influenced by changes in heart rate or temperature.

2. **To improve reproducibility, would it be best to pre-image the transverse aorta to measure the diameter and therefore select the appropriate needles size to use for that specific animal?**

   Imaging the transverse aorta, using ultrasound for example, is one way to pre-determine the size of the aorta and select the appropriate size of needle to use to create the constriction. However, you may also visualize and assess the transverse aorta once the surgery begins to determine its size. You would need to have a selection of needle sizes available, and would simply select the appropriate one just prior to placing it during surgery. This may save some time in preparing for surgery.

   If you are planning to measure the carotid artery flow velocities as a means of confirming surgical success, or to determine the tightness of the band we would recommend measuring the flow velocity in the vessels prior to surgery to ensure that there isn't any difference pre-surgery, this will give you confidence that post surgery differences are in fact due to the constriction placed. You may choose to measure the aortic diameter at the same time using an ultrasound system.

3. **Does animal weight correlate with aorta diameter?**

   Some studies have shown that aortic diameter does increase with increasing animal weight and age, however two animals of the same weight but different strain, transgenic knock-out, sex etc. may not have the same size aorta. It is best to confirm the diameter of the aorta to reduce variability in your surgical outcomes.

Confirming Surgical Success

4. **How long after surgery can one confirm the successful placement of the constriction?**

   The Doppler Flow Velocity System (DFVS) discussed in the webinar can be used to confirm surgical success immediately after flow returns through the site of stenosis, prior to closing the animal. This is possible as the probes are very small, and measurements can be made by placing just a small drop of sterile water/saline over the carotid arteries and using the sterilized probe to perform the measurements. The location of the probe is far enough away from the incision that it should not interfere with the surgical procedure.

Alternatively, you can close the animal and perform the measurements on the carotid arteries at any subsequent time point, as the changes in flow velocity are immediate and long standing, that is they don't resolve over time.

5. **How do you calculate the pressure gradient across the site of stenosis; what equation did you use to calculate ΔP?**

   The simplified Bernoulli's equation can be used to approximate the pressure drop across the band, as sited in Hartley et al. Ultrasound Med Biol 34, 2008.

   $$\Delta P = 4V^2$$

   Where P is reported in mmHg, if V is in m/s.

6. **When discussing the ratio between left and right carotid arteries, as a means of estimating the tightness of the band, what is the best ratio for the TAC model and what ratio could be used to cause hypertrophy alone without progression to heart failure?**

   The ratio will indicate the tightness of the band placed around the transverse aorta. The ideal ratio is entirely dependent on the purpose of your study and must be considered when choosing how to develop your model.

   The higher the ratio, the tighter the band, and this will affect the outcome of your surgeries in terms of development of cardiac hypertrophy or heart failure. The variability within the TAC model is dependent on many other factors including strain, sex, surgical technique, etc. that you must consider along with the goal of your study to select the most appropriate approach to your surgeries.

   When aiming to induce only hypertrophy and not heart failure, again there are many considerations to take into account, the tightness of the band is only one. The strain, sex, and surgical technique must also be considered when designing the study with a specific goal in mind.

Monitoring Cardiac Function

7. **What is the difference between cardiac function measured with the Doppler Flow Velocity System (DFVS) versus that measured with the ultrasound system (Prospect T1)?**

The measurements made with the Doppler Flow Velocity System (DFVS) are based solely on the blood flow velocity measurements in the heart. Specifically systolic function is measured as the peak velocity of flow through the aortic valve. While the diastolic function is measured on the flow spectrogram through the mitral valve. These measurements will provide a means of assessing cardiac function, however they will not provide measures of wall thickness which are important in assessing cardiac hypertrophy and progression to heart failure.

Ultrasound measurements of cardiac function are based on structural movements of the heart as it moves through systole and diastole. Specifically in the case of measures of wall thickness, these can be obtained, and are used to differentiate between hypertrophy in which cardiac function appears normal but the walls are thickened, and heart failure where cardiac function decreases and the walls begin to thin.

8. **In your opinion, which is the best system to monitor surgical outcomes (Doppler Flow Velocity System, Prospect T1 Ultrasound, M-series MRI), both surgical success, tightness of the band and cardiac function?**

   This is a challenging question to answer as for surgical outcomes the Doppler Flow Velocity System (DFVS) is the quickest and most accurate way to assess flow velocity spectrograms from the carotid arteries. The DFVS is also the best system to assess the tightness of the band to estimate the pressure gradient across the stenosis. However the DFVS does not allow for measurement of structural changes in the heart as hypertrophy and cardiac failure progress, the best systems for this are either ultrasound (Prospect T1) or MRI (M-Series).

   When deciding on a piece of equipment one must take into account the goals of the current study, long term plans with the lab, as well as others who may benefit from using the system.

   We do offer a system bundle in which includes both the DFVS and the Prospect T1 so that you may use the most appropriate tool to answer the relevant questions pertaining to your study.

The TAC Model – Variability and Disease Progression

The questions in this section are intriguing questions that we feel would actually be a great focus of an interest group to discuss.

There are many researchers working with TAC models on a wide variety of animal species, strains, and with a variety of surgical techniques. Your peers would be better able to help address these questions.

We hope to initiate an interest group on LinkedIn over the next couple of weeks and will invite you all to participate in this discussion. We hope that you will join the group and actively participate in the discussion. Please keep an eye open for an invitation to join the discussion.

9. **What is the percentage of mortality due to TAC surgery, are differences noticed between male or female animals? What are some suggestions to help improve the survival rate?**

   Mortality is one of the surgical outcomes which varies greatly amongst research groups, surgical techniques, and various animal models.

   Important things to consider to help improve survival include the following:

   - Maintaining core body temperature throughout the surgical procedure, while monitoring ECG and respiration rate to ensure the plane of anesthesia is sufficient for the surgery but not overly deep
   - Proper intubation and ventilation when using the open chest procedures
   - Aseptic techniques
   - Working quickly and precisely to minimize surgery time, while being cognisant of performing the surgery accurately and minimizing tissue damage
   - Providing support during the recovery period to ensure the animal is warm and stable while the effects of the anesthetic wear off
   - Ongoing monitoring to ensure the incision heals well and the animal fully recovers from the procedure

10. **What level of difficulty do you consider the rat and mouse TAC model?**

    As with any microsurgery technique on small animals they can be difficult to learn, however with practice, appropriate tools, and proper instruction the surgery can be learnt.

    The best way to learn these techniques is to learn from an experienced surgeon. There is a very good microsurgery workshop hosted by InsideScientific lead by Dr. Timothy Hacker at the University of Wisconsin. More information can be found here:

https://insidescientific.com/workshop/rodent-microsurgery-hemodynamic-measurements-training-program/.

11. **Do you have references for strain differences in response to TAC?**

    There are numerous publications on this topic, and also a lot of anecdotal experience from researchers around the world.

    Here is one such reference looking at differences in sub-strains of C57Bl/6 mice from Garcia-Menendez et al. Am J Physiol Heart Circ Physiol 2013.

12. **At what time point do you typically see TAC progress to heart failure?**

    As mentioned the variability in the TAC model, and with that the time point of progression to heart failure depends on a number of factors. The tightness of the band, surgical technique, as well as strain and sex of the animal will play a role in the timing of progression to heart failure.

13. **Out of the 3 surgical models discussed (Rockman, Hu, Melleby) which provides the best outcomes?**

    I'm hoping that through the LinkedIn focus group we hope to begin that your colleagues will be able to comment on this question. Primarily because it is not just the surgical technique that influences the survival or outcomes, but a whole host of factors including surgical technique, strain, sex, and of course the experience of the surgeon in doing successful TAC banding.

14. **We use a 26G needle for a guide, is this still the standard?**

    As discussed in the webinar, the techniques from Rockman and Hu initially worked with a 27G needles, however Melleby et al used a variety of sized O-rings. More important than the size of the needle is the percent stenosis caused, and that has to do with both the size of the transverse aorta before surgery and the size of the needle used. When working with different strain, sex, weight, or transgenic animals it is important to initially assess the diameter of the aorta and choose the appropriately sized needle to maintain consistent percent stenosis across all of your animals within a study.

15. **We know that FDG PET indicates that an animal which has undergone TAC surgery have increase FDG uptake in the left ventricle. Could we use FDG PET to monitor hypertrophy and cardiac failure?**

    I was able to find some papers that used FDG PET to monitor disease progression in a TAC model, however they also used other forms of *in vivo* imaging and *ex vivo* histology

to monitor cardiac function and to assess scar formation. Here is one example from Todica et al. Mol Imaging Biol 2018.

As was mentioned in the webinar, the M7 compact self-shielded MRI system has the capability of doing simultaneous PET/MRI and would allow for both structural assessment of cardiac function as well as PET imaging on the same model.

16. **Would the disease progression be the same if the cardiac load is the same in all of the animals?**

    Many researchers have noted that despite conducting their experiments in a very controlled manner, i.e. using an inbred strain of animals and controlling as many aspects of the surgical technique as possible, that variability in disease progression still exists within the model.

    This is where stratification of the animals based on disease progression or a specific functional marker becomes very important as it may not be appropriate to assume that the disease will progress in the same way in all animals despite our best efforts to control the resulting cardiac load from the TAC surgery.

17. **Does every TAC show hypertrophy?**

    This question may be best answered by your peers, however to the best of my knowledge as the heart experiences a pressure overload as a result of the TAC surgery it will initially enter a phase of compensatory cardiac hypertrophy in an effort to maintain cardiac function. However, the variability exists when talking about progression to heart failure; there are some animals that no matter how long you wait will not progress towards heart failure. There are other animals that quickly progress from hypertrophy into heart failure, so if monitoring is not performed frequently the hypertrophy stage may be missed.

18. **Does collateral circulation play a role after TAC?**

    To the best of my knowledge I have not seen any publications on this question. However, I am hopeful that your colleagues will be able to help answer this from their own experience through the LinkedIn interest group we hope to create over the coming week or so.

Doppler Flow Velocity vs. Ultrasound Measurements & Systems?

Scintica: INSTRUMENTATION

19. **Before surgery we have measured the left carotid artery with a conventional ultrasound system (PW Doppler) and the Doppler Flow Velocity System (DFVS); the ultrasound provided a velocity of 280mm/s while the DFVS gave 400-450mm/s. Can you help me to understand this difference?**

    Most often the differences measured on with a conventional ultrasound system versus the DFVS have to do with the angle at which that measurement was made, and the correction that may or may not have been applied to the measurement to get the final velocity output.

    It would be important to see in a bit more detail the procedure used to perform both measurements to be able to know for sure.

    However, with the small single element probes of the DFVS, as we saw in the webinar, a small angle between the probe and the blood flowing through the carotid artery can be achieved. With the conventional ultrasound system the probes are quite a bit larger, as they are designed to acquire images, making the angle that can be achieved between the probe and the flow in the vessel quite large. As the angle increases the error in the measurement also increases; if the angle correction applied to the measurement is also incorrect or off by a small amount the error is magnified.

    Most important when making these measurements is to try to achieve the smallest angle possible, and apply an appropriate angle correction. Ideally, the same angle and correction is used to measure both the left and right carotid arteries. In this way, if an error is introduced, it would be negligible when calculating the ratio.

20. **Beyond the smaller size of the probes on the Doppler Flow Velocity System (DFVS), what are the advantages over using PW Doppler Mode on a conventional ultrasound system for these measures?**

    The DFVS and the PW Doppler mode on a conventional ultrasound use the same principles of ultrasound, that is sound is transmitted at a specific frequency and the systems are listening for sound coming back at a slightly different frequency due to the reflection from a moving object, i.e. red blood cells. The systems then convert this Doppler Shift into the blood flow velocity.

    The equation to calculate velocity is:

    $$V = (c\,\Delta f) / (2fo \cos \theta)$$

    Where:

V = flow velocity (cm/sec)

c = velocity of sound (cm/sec)

Δf = Doppler shift (Hz)

f_o = transmission frequency (Hz)

θ = angle between velocity vector & beam vector

From this equation we can see that θ is very important to the velocity measurements made with either of these systems.

The smaller probes (2-3mm in diameter) of the DFVS allow the user to achieve a much smaller angle between the probe and blood flowing through the carotid artery. This is not possible with a conventional ultrasound probe due to the size – these probes have been designed for imaging and achieving a reasonable angle on the carotid artery often involves compression of either the chest or head, neither of which is advisable.

Outside of measurement angle is the ease of performing the measurements. It has been my experience that getting a nice Doppler spectrogram is easier using the DFVS than a conventional ultrasound system.

21. How do you account for angles between the probe and flow within the vessel for both a conventional ultrasound system and the Doppler Flow Velocity System (DFVS)?

In the previous question the equation for calculated flow velocity is shown, and the importance of angle is discussed.

When performing these measurements the user must estimate the angle between the flow probe and the blood flowing through the carotid artery. This value can be entered into either software to apply the appropriate correction.

Specific Systems Discussed During Webinar – Doppler Flow Velocity System, Prospect T1 Ultrasound, and M-Series MRI

22. How long does it usually take to get trained to use the Doppler Flow Velocity System (DFVS) to ensure accurate placement of the probe, and appropriate angle, to get the signals discussed?

When working with new customers who have not used the Doppler Flow Velocity System (DFVS) previously we recommend a 1.5 day on-site training. During this time you

will have the opportunity to learn how to acquire the signals you would like, practice, and review the possible measurements with the system. In 1.5 days most people with an understanding of basic cardiovascular anatomy have no problems learning the techniques. Of course practice is imperative, and we do recommend that people spend some time each day following the training (1-3 mice) to practice on a variety of animals so that they see the possible variations that exist within their models. Once confident in acquiring strong signals it would be appropriate to start a study.

23. Does the ultrasound system (Prospect T1) you carry have Doppler Mode?

Yes, the Prospect T1 system does have a collection of Doppler modes, including Color, Power, Pulsed Wave, and Tissue Doppler. Please see previous questions (7, 8, and 20) for more details on how the Prospect T1, which is analogous to a conventional ultrasound system, and the Doppler Flow Velocity System compare and when and why you would use one over the other.

24. Is there a way to arrange for a demonstration of these systems so that we can see how they work in person?

Yes. For the Doppler Flow Velocity System (DFVS) and the Prospect T1 ultrasound system we can arrange an on-site demonstration in your laboratory so you may be able to see them in action on your specific animal models, and if time allows actually get a chance to try the systems for yourself.

For the M-Series MRI systems we can work together to find an appropriate demo site at an existing customer based on location and available models. Although these systems are compact, shielded, and don't require additional infrastructure, there are complexities in moving them around for a demonstration which are not easily overcome.

Please reach out to us directly and we can continue this discussion.

25. Could you please repeat the Model#/manufacturer of the systems discussed, and prices?

Scintica Instrumentation distributes all of the systems discussed during the webinar. We work closely with the manufacturers to ensure our customers get the best products and support to meet their research needs.

More information on the Doppler Flow Velocity System (DFVS) can be found here: https://www.scintica.com/products/indus-instruments/doppler-flow-velocity-system/.

More information on the Prospect T1 high-frequency ultrasound system can be found here: https://www.scintica.com/products/s-sharp-prospect-t1/.

More information on the M-Series MRI systems can be found here: https://www.scintica.com/products/aspect-imaging/.

While visiting our website please feel free to navigate around to see the other products that we distribute which may be relevant to the work that you are doing, including tissue vitality systems, surgical monitoring, anesthesia, as well as a host of products for studying hypoxia and a number of other research models.

Feel free to reach out with any questions you may have regarding the systems we discussed during the webinar or others that you may be interested in learning more about.