Q: What are landmarks for you to target the probe to different locations of the aorta?

Anilkumar Reddy: Typically, flow in the aorta is measured at the aortic root, transverse aortic arch, and the abdominal aorta. Each location requires a different orientation of the probe. For example, the Doppler flow velocity at aortic root is measured by placing the probe tip just under the xiphoid process at the base of the ribcage, oriented towards the head of the animal, with the long axis of the probe almost parallel to the direction of the blood flow, which will have the probe almost horizontal to the heated platform (please refer to Reddy et al., Ultrasound Med Biol 35:2042-2054, 2009 for information on probe position and considerations of errors introduced when the probe is held at an angle to blood flow). With this the sound wave will travel from the probe tip through the apex of the heart straight to the aortic root. By listening to and observing the Doppler waveform and adjusting the probe and range depth, the user is able to identify the location of the strongest signal. Similar approaches are used to acquire the Doppler waveforms at other aortic locations.

Q: How do you adjust angle and position of the probe without an anatomical image of the heart? Do you determine only from shape of waves?

Anilkumar Reddy: Yes, the ideal position of the probe, in terms of location and angle, is determined by the shape and sound of the waveform along with the waveform timing with respect to the accompanying ECG signal. The strongest most consistent signal is the goal, and this can easily be gauged by either the sound or visual appearance of the waveform which is displayed in real-time. Knowledge of the cardiovascular anatomy is also important.

Q: How do you control the angle of probe-vessel incidence in order to minimize variation in velocity measurements?

Anilkumar Reddy: Our Doppler probes are 2.5mm or less in diameter and therefore allow for easy manoeuvring and achieving an optimal angle. This will ensure that the error from angle between the probe and flow within the vessel is measured consistently between animals and time-points, thereby minimizing the variation in measurements. The key point to remember is that the angle of the probe and its sound beam should be kept within 15° of the axial direction of the flow. That is, as long as the sound beam intersects the blood flow at a 15° or less, the error in peak velocity measurement in very small (refer to Reddy et al., Ultrasound Med Biol 35:2042-2054, 2009 for information on angle errors).
Q: How deep can the Indus Doppler Flow signal penetrate?

Anilkumar Reddy: Flow velocity signals can be measured from depths up to 10 mm.

Q: How would the signal be affected with a plastic cuff inserted in the vessel?

Anilkumar Reddy: We presume the plastic cuff you mention is for your own research purposes, and not in reference to a cuff which can be placed around the vessel to measure Doppler flow directly.

The ability to measure the Doppler signal in the area covered by the cuff would depend on the type of material used, its shape, and its thickness. The Doppler signal may be attenuated by this material, without testing it is hard to answer this question definitively. However, the signal would not be effected on either side of the cuff, and signals could be measured in these areas.

Q: What are the rates of sampling the waveforms -- how much memory is required to analyze variability over time - for example, 10 minutes, etc.?

Anilkumar Reddy: Data is sampled at the maximum rate of 125 kilo samples per second and each saved file containing 2 seconds of data is typically 1 MB in size. Sampling rates can also be set below 125 kilo samples.

For interventions, it is suggested that 2-4 second segments are collected starting with before intervention and at regular intervals after physiological or pharmacological intervention (for example, 30 seconds, 1 min, 3 min, 5 min, 7 min, 10 min, and so on). The selection of the intervals to collect data depends on the type of intervention used and should be decide by the investigator.

Q: How do you measure the distance between the two sites for Pulse Wave Velocity?

Anilkumar Reddy: The distance between the two sites used to measure Pulse Wave Velocity (pulse transit time) can be measured using a set of calipers or a simple ruler. The measurement is made from the tip of one probe/placement to the tip of the second probe/placement. We understand that there may be error in estimating the distance due to curvature at the arch (if measured from Arch-Abdominal aorta), but this error is factored in as the same procedure is followed in every mouse.

Q: Regarding Pulse Wave Velocity, how much error is there in placement of the abdominal probe? E.G. is it necessary to have a fiducial marker so that the probe is placed at the same position for each subsequent PWV assessment?

Anilkumar Reddy: It is not necessary to place a fiducial mark per se. If the probe tip at the abdominal site is placed at distance either smaller or larger than the previously measured location, the time of travel of the pulse is also going to either decrease or increase accordingly in the subsequent measurement. Nevertheless, marking the two locations will make it easy to locate the site of measurement. Using a tattoo instead of a permanent marker is a better option.
Q: Do you have any experience using this platform to evaluate changes in flow/velocity with drugs/compounds which have been shown to cause vascular injury (e.g. in animal toxicity studies)?

Anilkumar Reddy: I do not have specific experience with vascular injury models. The closest experience I have is the use of 10% FeCl₃ solution on the carotid artery to elicit thrombus formation and the downstream flow was monitored with the Doppler system to determine the time it took for the flow to go from maximum to zero (Zhou et al., J Immunol 197:288-295, 2016). Other studies include the administration of methoxamine to α-smooth muscle actin null mice to determine their response to a vasoconstrictor. As I mentioned in the webinar, we also used phenylephrine to show its effect on PWV in normal mice.

Q: How would this system be used in hindlimb ischemia models of PAD? Would you measure one vessel repeatedly or is there a way to get a larger picture of the muscle vasculature?

Anilkumar Reddy: We have not done such studies. However, the system was used by Zhang et al. (Human molecular Genetics 22:3720-3729, 2013) for peripheral vascular flow measurements. In this study the measurements were made before and after interventions during a single experiment. However, longitudinal studies would entail repeated measurement at a give site on the vessel and calculate pulsatility index from the velocity waveforms to determine the characteristics of the distal vasculature.

Q: What is the limitation of the vessel size that the velocity can be reliably measured?

Anilkumar Reddy: The small vessel we were able to measure from is the coronary (about 200 μm). We have measured signals from vessels in the brain but are not sure which ones (cerebral arteries diameters can range from 120-160 μm).

Q: Can you measure venous flow, such as the IVC or portal vein?

Anilkumar Reddy: Veins by nature do not have pulsatile flow. However, we can measure venous blood flow with Doppler provided we know the location of the vessel we are measuring from. Specific abdominal vessels are harder to locate despite the ability to measure their flow velocity with the Doppler system. To measure from a specific small vessel such as the portal vein one may need to use an implantable Doppler cuff probe or make the measurement with a regular Doppler probe during an acute invasive study.

Q: Is it possible to measure Pulse Wave Velocity (PWV) when the disease mice have (are undergoing) aortic regurgitation?

Anilkumar Reddy: We have not worked with mouse models of aortic regurgitation (AR). In clinical studies AR was shown to increase pulse pressure which leads to increase in aortic dispensability causing it to be stiffer. Thus one would expect PWV to be higher in AR. We think that it is possible to measure PWV in mice with AR.
Q: Can you measure RCA, LCA, LCX flow in the same mouse?

Anilkumar Reddy: At present we can only measure from LCA. We have not tried measuring from RCA or LCX. We hope to develop methods to measure from these locations and validate them in the future. Once the methods are established it is possible to measure from each of these locations in the same mouse.

Q: Is it possible to use this device for larger animals like pigs? How can we proceed? Is it similar to the mice?

Anilkumar Reddy: When thinking about using this device on larger animals the consideration of depth of penetration for the 10MHz probe must be considered. This probe can typically penetrate 10 mm. So in larger animals, one can place 15-20 mm diameter cuff probes around the vessels of similar diameters and measure flow velocity. However, cuff probes have to be chosen carefully to avoid measurement errors.

Q: I should be able to combine the use of this technique with high resolution Echo, but is it well justified?

Anilkumar Reddy: You need a Doppler system to measure blood flow velocities. You need an Echocardiography system to measure structures and dimensions. Blood flow velocities can also be measured with echo system but the large foot print of the echo probe requires angle correction and angle correction at angles larger than 15° results in larger errors in velocity measurements. Additionally, it is not easy to measure pulse wave velocity with an echo system/probe. Blood velocity measurement in peripheral vessels of mice or other small animals can also be challenging with echo system/probe. Echo and Doppler systems, each provides a different set of parameters which can be used to assess cardiac function. However, it is important to note that it is not necessary to use echocardiography to acquire the Doppler blood velocity signals.

Q: Is there a similar system to the pulse Doppler to measure all these velocities and pressures non-invasively in newborn humans in order to identify early cardiac lesions?

Anilkumar Reddy: The size of newborn humans would require the use of lower frequency ultrasound signals. The small animal Doppler system uses 10 and 20 MHz. However, newborn humans will need 2 to 1 MHz systems. These frequency probes are usually available with pediatric echocardiography systems. There are portable/handheld Doppler systems with single frequency probes that are used clinically on radial or brachial arteries in adults that may be used in newborns. In order to locate specific structural cardiac lesions, one may need to use an imaging system.
Q: Do you think it is possible to detect changes in viscosity or cell volume inside vessels using the Doppler technology?

Anilkumar Reddy: We have not done studies using Doppler only but measured pressure - Doppler velocity relationship studies in polycythemic mice (Reddy et al., Effect of cellular elements on the pressure-velocity relationship in mice. Proceedings of IEEE-EMBS, 26(2):3720-3722, 2004). Here we measured hyperemic responses after we occluded the abdominal aorta distal to renal branches but proximal to iliac branching (an invasive procedure). Noninvasively, one may try to do this on the tail using a tail-pressure cuff to stop blood flow and measure Doppler velocity in the tail before and after the occlusion. However, one should keep in mind that tail blood flow mechanism is dependent on body temperature as well.

Q: When mice are under isoflurane, I actually notice that PWV tend to be lower when HR is lower. So I am surprised to see the slide showing irrelevance between PWV and HR. Could you talk more about this?

Anilkumar Reddy: We recently did a study (unpublished) to determine PWV in mice anesthetized at 1.0%, 1.5%, 2.0%, and 2.5% isoflurane, resulting in heart rates of (mean±SE, n= 7) 468±12, 491±17, 524±12, and 537±15, respectively and PWV of 312±8, 314±6, 313±7, and 308±8, respectively. While heart rate changed significantly from 1.0% to 2.5%, PWV did not change significantly. I need to see the data and learn of the conditions this data was obtained to find out what caused PWV to be lower with HR in your studies (please send me the set of files that you saw these changes so that I can comment further).

Q: With hyperthyroid young mice, will you see hyper-contractility or increase of LV ejection based on measurement of pulse wave Doppler aortic outflow velocity?

Anilkumar Reddy: In hyperthyroid mice we observe an increase in peak aortic velocity compared to control mice indicating that hyperthyroid mice have increased contractility and increased LV ejection. We haven't made comparative measurements of contractility or LV ejection.

Q: Is there a hands-on course to attend to learn this technique?

Anilkumar Reddy: Onsite training is available for a nominal fee to all of our valued customers. Benefits to doing training on-site, are that you get to use your specific animal models, and get used to using the equipment in the environment where you will be conducting your studies.

We do also, on occasion, offer a free course in Dr. Reddy's lab in the Houston, TX area.
Q: How difficult is it for a sonographer skilled with human subjects to learn these techniques with small animals?

Anilkumar Reddy: The principles of ultrasound remain the same no matter the size of the imaging subject. With small animals, you can imagine, the structures are smaller but the anatomical features are very similar. As we suggest for all our users, one can learn the technique quite quickly, and having a fundamental understanding on the technology is beneficial, but to become proficient and to get reproducible results, one must practice for about one hour per day for approximately 2 weeks. At this point muscle memory is achieved and your results should be consistent throughout your upcoming studies.

Q: What is the cost of this system?

Anilkumar Reddy: The cost of the system is dependent on the configuration of the system. The best approach is to discuss your specific applications with the team at Indus to ensure the appropriate configuration is established for your lab. A well configured system will range in price from $32,000-$39,000 USD.

If you have additional questions for Dr. Anil Reddy regarding content from his presentation or wish to receive additional information on the Indus Instruments Doppler Flow Velocity System, please contact Dr. Reddy by email:

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