Webinar Q&A Report:

A Noninvasive Alternative to +dP/dtmax: Peak Aortic Blood Acceleration

Q: You mentioned several times that the Doppler flow velocity system can measure very small angles. Would you please elaborate on what that means and why it is important?

Anilkumar Reddy: When measuring Doppler flow velocities, the angle between the sound beam and the blood flow is important. The Doppler frequency shift due to blood flow is expressed as $\triangle f = (2vfo \cos\theta)/c$, where θ is the angle between the sound beam and the blood flow, v is velocity of blood flow, fo is the Doppler transducer frequency, and c is the velocity of sound in blood/soft tissue. As θ increases from 0 to 90 degrees, the value of $\triangle f$ decreases from a maximum (at 0°) to zero (at 90°). Since we calculate the velocity values from $\triangle f$, blood velocity v will follow $\triangle f$. Thus, one needs to keep the sound beam angle to a minimum ($\leq 15°$). At angles closer to 0° the effect of slight changes in angle due to operator handling results in negligible changes (or errors) in the measurement of blood velocity. This is important especially in serial measurements because when probe is held at angles higher than 15° even the slight changes in sound beam angle due to operator handling will generate larger errors in measurement to measurement and cannot be fixed by using angle correction. If the angle is consistently within 15^{o} for every measurement then the generated error is negligible. Also, the small DFVS probes (with probe head/tip and body 2.5-3mm in diameter) allow for easy maneuverability and positioning to acquire Doppler flow velocity spectrograms with smaller angles, traditionally below 15°.

Q: During the talk, the images of the probe location looked like it was being placed over the abdominal aorta (AO), but it was mentioned that the probe is placed on the ascending aorta. Will placing the probe below the xiphoid process pick up the ascending aorta?

Anilkumar Reddy: On a mouse, to acquire a strong Doppler signal from the ascending aorta the probe is placed below the xiphoid process and aimed towards the outflow tract of the left ventricle at an angle within 15° of the flow velocity vector. The depth range may be adjusted to place the sample volume within the ascending aorta (typically between 6-8mm in a mouse). The spectrogram will have small yet clear reversal in flow when the signal is being acquired right above the aortic valve, as the closure of the leaflets pulls a small amount of blood back towards the heart. This backwards flow signal in the spectrograph is used to help ensure the sample volume is placed accurately. If the probe is angled steeply (>45°) at the xiphoid, it can pick up the upper abdominal signal which we normally use for the measurement of pulse transit time with respect to the signal measured at aortic arch location for the calculation of pulse wave velocity (PWV). Higher angle here does not matter because we are only measuring time when the blood pulse passes through the measured location, mainly at the foot of the waveform (thus known as foot-to-foot aortic PWV) and not the peak velocity.

Q: Regarding E/A ratio, E and A are often fused due to the high heart rate of the mouse. How do you separate E and A waves?

Anilkumar Reddy: E and A waves tend to fuse for heart rates above 500 beats/minute. Under isoflurane anesthesia heart rates vary from about 400 to 550 beats/min. The high pulse repetition frequencies and high sampling rates of the DFVS (PRFs -62.5 & 125 kHz; sampling rates up to 125 kilo samples/second) allow for high temporal resolutions that facilitate separation of E and A wave peaks in most cases for heart rates up to 500 beats/min. Please note that in some animals the E and A waves may not separate due to the physiology of the animal.

Q: Do you have any suggestions on how to control for the effect of afterload when studying ejection indices?

Anilkumar Reddy: The ejection indices with the DFVS are aortic flow velocity and its acceleration. In patients, it has been shown that both aortic velocity and acceleration are dependent on loading conditions and the LV ejection indexes of ejection fraction, stroke work, fractional shortening also depend on loading conditions [Bedotto et al. Am J Cardiol v64:856-9, 1989]. We expect the same to happen in mice. In mice, these measurements are made under anesthesia which may mildly lower afterload in both normal and affected animals at baseline. Therefore, it is important to keep the anesthesia levels and also the core body temperature tightly controlled during experimentation. If the baseline measurements between groups do not show expected significant differences, then interventions may be needed to bring out the phenotypes and this may include changing loading conditions. We are currently conducting carefully controlled studies to determine the effects of altered loading conditions on aortic flow velocity and acceleration in mice and hope to present these results in a future webinar and publication.

Q: For LV systolic function, would it not be useful to use a micromanipulator to stabilize/standardize the angle of the Doppler Flow Velocity probe?

Anilkumar Reddy: We have not found this to be necessary, as small adjustments to the angle and position of the probe on each animal is best practice to optimize the Doppler spectrogram. With the approach from the xiphoid process the angle between the sound beam and the blood flow is minimized, so errors in angle measurement are greatly reduced. However, if you are making before and after acute interventions it is recommended that you use the micromanipulator to hold the probe in place.

Q: When measuring diastolic function, do you evaluate it from Apical 4 chamber (mitral annulus), or from the parasternal short axis with sample volume positioned at Posterior wall?

Anilkumar Reddy: The approach taken for mitral valve flow is from the xiphoid process, so slightly more midline than an apical four chamber view. The sample volume is placed mid-ventricle near the valve opening.

Q: Is this Doppler probe able to perform the same measurements in Rats?

Anilkumar Reddy: We have two probes available for the DFVS, 10MHz and 20MHz. Traditionally, the 20MHz probe is used on mice, while the 10MHz probe is used on rats. The positioning of the probe on the rat may vary slightly from that of the mouse depending on the size of the rat. Signals are obtained within 10mm of the probe tip/head's surface, so adjustments to the probe position may be necessary for larger rats to obtain the same measurements. While peripheral signal measurement techniques on the rat are similar to the respective measurements in mice, the cardiac signals are not measured from the xiphoid because of depth limitation. So, aortic outflow is measured from the sternal border similar to the right carotid artery and the mitral inflow is measured from a diagonally opposite site located between the ribs on the left side of rat chest.

Q: Can Anil talk about various stages of E/A (pseudonormal etc.), that can impact how you interpret the data?

Anilkumar Reddy: The diastolic flow velocity (aka mitral flow velocity) is biphasic with a passive early (E) filling flow velocity which occurs when the left ventricle (LV) pressure during isovolumic relaxation drops below left atrial pressure and then a second atrial (A) flow velocity that results with atrial contraction (after the occurrence of p-wave). The following are the four stages of E/A that can be used to interpret the data.

- In normal young mice, you will see that E-peak velocity is 2 to 3 times higher than the A-peak velocity (2 ? E/A ratio ?3). This means that the majority of LV filling is occurring during the passive early phase meaning the heart is relaxing efficiently and very little flow occurs during atrial contraction. Also, IsoVolumic Relaxation Time (IVRT) and E-wave Deceleration time (DT) is normal.

- If LV relaxation is impaired as with aging or other diastolic dysfunction the passive early filling is reduced and greater flow occurs during atrial contraction (E/A ratio <1). Both IVRT and DT are prolonged.

- During the initial phases of diastolic dysfunction physiological compensation occurs by increasing preload thereby increasing atrial pressure, leading to the appearance of normalization E/A ratio and is know as pseudo-normalization of the E & A velocity waveforms (1< E/A ratio <2). Both IVRT and DT are shortened. Since pseudonormal E/A looks like normal E/A, the status can be unmasked by reducing preload which results in the E & A waveforms pattern reversal looking like those in impaired relaxation and E/A will become <1.

- As preload continues to increase resulting in significant elevation of atrial pressure. This condition is called restrictive pattern. Here almost all the blood flows into the LV during early phase and little to no blood flows during atrial contraction (E/A ratio >4). Both IVRT and DT are then very short. Reducing preload may shift the restrictive pattern into pseudonormal pattern. Both normal and restrictive E/A ratio can be in the range of 3-4 and can be distinguished by looking at IVRT, DT, and E-duration.

Q: Have pulmonary flow velocity profiles ever been used to attempt to quantify systolic function of the right ventricle?

Anilkumar Reddy: At this time, we are exploring a pilot study to optimize a protocol for pulmonary artery flow velocity evaluation. We are hopeful that this will provide the necessary validation so that we are confident the waveform observed is solely from the pulmonary artery and not mixed with signal from the aorta and thus indeed quantify systolic function of the right ventricle.

Q: How do you know if you are in placing the probe and measuring from the correct location if you have no image guidance?

Anilkumar Reddy: 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 highest velocity and 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. However, knowledge of the cardiovascular anatomy and function is a must.

Q: How do you set the source of the signal? When using echo ultrasound systems, one sets an area for measuring. In your model is there an adjustable data acquisition depth?

Anilkumar Reddy: On the DFVS there is a depth control setting, both on the system and by a remote which may be placed very near the animal during acquisition. This depth is set to millimeters from the probe surface and may be adjusted from 2-10mm.

Q: How much can we benefit from this non-invasive Doppler system in case of cardiac patients who are in ICU and need a quick assessment to decide strategy to improve cardiac function (especially the ejection fraction).

Anilkumar Reddy: This system is only for use on small animals, not for use on humans. However, if a quick assessment of cardiac function is required on an animal appearing to be in distress, the aortic outflow from the heart may be measured.

Q: How can one standardize the heart rate (HR) for optimal measurement?

Anilkumar Reddy: The DFVS includes our Rodent Surgical Monitoring system where the animal is anaesthetized and secured to the heated platform. The heart rate and ECG are monitored throughout the Doppler signal acquisition. It is important to come up with a target heartrate range for your study to reduce variability in the results. The core body temperature should be monitored and maintained, while the anaesthesia levels should be adjusted, by standardizing these two variables the target heartrate range should be achieved within a study group.

Q: Continuing the angulation topic, may I ask you how do you assess the angle for the angulation correction, based on anatomy?

Anilkumar Reddy: The main purpose of the system is to reduce the need for the assessment of angle and applying a correction. In most small animals such as mice and rats, all the major arteries are oriented parallel to the horizontal plane with the exception of pulmonary artery, some parts of coronary artery, and some (or parts) of the cerebral arteries. If we always position the probe to keep the angle below 15^{o} then we do not need to assess and apply angle correction. Also, when we make measurements of blood flow velocities at a certain angle before and after acute interventions and calculate ratio of the before and after measurements then we do not have to worry about the angle correction. We highly recommend the use of a micromanipulator to maintain the probe angle and location to determine the effects of intervention on blood flow velocity.

Q: What is the best reason to have this system in addition to an echo ultrasound?

Anilkumar Reddy: The Doppler system is specifically designed 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 does not allow for the probe to be oriented at angles close to 15^{o}. This requires angle assessment and correction. However, even small errors in the angle assessment and correction at higher angled measurements can result in large errors in velocity estimates. Another reason is that it is not easy to measure pulse wave velocity with an echo system/probe, whilst with the Doppler system it is possible to measure blood flow velocity simultaneously in two different locations (aortic arch and abdominal aorta for instance) to more reliably determine PWV. Blood flow velocity measurement in peripheral vessels of mice or other small animals can also be challenging with echo systems/probes compared to the Doppler System. 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 flow velocity signals.

Q: With ECG measurements, often noise will affect the signal and make it difficult to analyze. Does this also happen with the Doppler system?

Anilkumar Reddy: We have found that our ECG signal is quite clean and does not display noise routinely. However, there are various software filter settings and grounding options available to help remove or minimize noise when it is present. This ECG signal is then fed into the Doppler system capturing software. The noise in the Doppler system can be controlled through a set of pre-acquisition low-pass filters and post-acquisition high-pass filters along with automatic and manual Gain, Contrast, and Noise controls.

Q: What is the cost of this system?

Anilkumar Reddy: The cost of the system is dependent on the configuration of the system. We suggest discussing 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:

Dr. Anil K. Reddy, PhD: areddy@indusinstruments.com

Indus Instruments
721 Tristar Drive
Webster, TX 77598
USA

Phone: +1 (281) 286-1130