Best-Practices to Achieve Quality PV Loop Data:
Webinar Q&A Report

Q: Can't someone just start with a larger piece of nylon fiber, make the loop, and then cut the ends to the desired length? I feel that would make the process easier.

(FKonecny): Yes it might make a tool to perform pre-load reduction more standardized. Unfortunately the procedure is still not enough standardized to allow comparison in the current published literature.

Q: what anesthesia is most widely used by PIs doing PV measurements?

(FKonecny): Majority 1) the isoflurane gaseous anesthesia using pure oxygen as a driving gas and 2) ketamine +xylazine sometimes still with isoflurane that is driven by air (some PI's) using pure oxygen at that set up.

Some still using Pentobarbital monoanesthesia but this is minority. Pentobarbital has some of quite negative hemodynamic side effects please if interested see (page 41-42 of the PV workbook)

Q: I often check the pressure catheter balance with a manometer. It often doesn't match the output of the ADV500 box… why?
(PPlouf) Assuming we are speaking of a catheter in the aorta being compared to a fluid filled manometer in the same location, the most common reason is because the catheter was not soaked in saline for 20 minutes or it was balanced either in a column of water or in air.
(FKonecny): best to contact Transonic office for further discussion

Q: Where, how and with what tools and technique do you get good, repeatable IVC occlusions?

(FKonecny): Very difficult question. As there is no published SOP (standard operation procedure) for this method, almost every researcher designed and in my opinion should have (validated>??) this technique before publishing it. There is scarcity of good IVC occlusion technique that has been published. In recently published PV workbook we have come up with e.g. one on page 73 for mouse. It is far from perfect. Steps to get good occlusion has to be validated against gold standard that is most likely some type of pneumatic occluder placed around the vessel with ability to have instantaneous read out of force /area placed on the segment. This way you are able to validate your preload reduction.

Q: is it possible to conduct PV Loop research in GLP environments?

(Fkonecny) Unquestionably yes. I do strongly believe that diligently performed PV has an enormous value in hemodynamic research. Also e.g. when testing of unloading with variety of VADs, PV hemodynamic read outs are the only one valid as you cannot continuously ensure monitoring using e.g. cardiac Magnetic Resonance Imaging, moreover you will not be capturing instantaneous changes and not be able to measure e.g. pressure in the system.

Q: how would the use of barbiturates affect hemodynamics?

(Fkonecny): I have included some of the effects of barbiturates (page 41-42 of the PV workbook). Also when using this older anesthetisia concept, you need to confirm that you standardize your protocol well, as in hemodynamics especially in rodents the very critical parameter of HR is necessary to be aware of. Slight change of HR has direct impact on dpdt. If HR is low (mouse under e.g. 450 bpm), comparison is very difficult within the published domain, as many PI's are switching to non-injectables. Also one other footnote in injectable anesthesia you need to ensure similar plain of anesthesia throughout the PV procedure and for (data recordings) that is w/o frequent re-injections as they have effect(s) on the captured hemodynamics.

Q: We are struggling with positioning the catheter in the center of rodent right ventricle, which has a crescent shape. What should we do?

(Fkonecny): New PV workbook available at this year's EB will have RV PV note using closed chest. It also has PA measurement using mouse open chest RV pressure catheter insertion. Please contact me for more directions and difference of the shape of RV PV loops when O/c vs. closed chest in mouse.

Q: when doing RV pressure-volume loops, are there any parameters for volume calibration that need to be adjusted compared to those typically used for LV studies?
Q: Do you have information on expected parameters for rabbits (both admittance data ranges and expected PV outcomes)?

(Fkonecny): Great question. We are still evaluating RV PV. For mouse new PV workbook is available at this year's EB will have RV PV note using closed chest. It also has PA measurement using mouse open chest RV pressure catheter insertion. Please contact us for more directions and differences for RV PV loops when O/c vs. closed chest in mouse.

No at this time I do not have any collaborators working on RABBIT in-vivo RV PV. Would you be interested to produce a RV PV rabbit specific Application note for our 2016 PV workbook?

Q: Which gas should be used during ventilation? 100% O2 or room air?

(Fkonecny): It is based on your animal protocol. I would use (establish) baselines using both driving gases. As many collaborators have different opinions, I did my research using pure oxygen. All my PV baselines were using 100% O2.

Q: how does disynchrony of the heart influence the loops? Does disynchronuous contraction, due to pacing for example, influence the PV catheter measurements?

(PPlouf) It would be difficult to predict in a general sense what the resulting loops look like. If pacing to induce a dysynchronous condition is performed AFTER a proper baseline scan, I would expect that the loops will reflect an observabke reaction in the Magnitude, Phase and Volume traces.

(Fkonecny): That is very hot topic. Many researchers are currently working on this problem. It e.g. depends where you are pacing from. You can pace from multiple sites influencing not only ventricles. Dyssynchrony has influence on load dependent but also on load-independent indices of cardiac function. Is the second question more about electrical interference?

If yes please contact our office we can discuss further.

Q: in addition to my previous question: dyssynchrony of the contraction can influence the position of the PV loop catheter. How are measurements influenced by a changing position during the cardiac cycle?

(PPlouf) We need to differentiate between a catheter position change and a change in myocardial contractility as it relates to the catheter in a given position. If the catheter is ejected or otherwise forced into a position that is not ideal, then the baseline scan and transient maneuver will have to be repeated. If the "change in position" is due to the heart contracting either more of less aggressively around the catheter, then there is no problem; the phase angle tracks the muscle incursion and reports on the cardio dynamic situation as it is happening. The way to determine which of the two events might have occurred is to look at the phase angle before and after the intervention: if the phase angle returns near the pre-intervention baseline, the catheter has not moved out of position.

(Fkonecny): Again, currently many researchers are working on this problem. What I can only suggest is to establish baseline with known position of the catheter using e.g. echo or CT scan. You also know the phase angle at this position, and at this time. To validate the catheter position please use2D imagings like e.g. echo or CT to establish catheter position before e.g. the pacing train. Now you pace the location and try to run at the same time contrast into ventricle while looking on the catheter position live. Remember you are also able to collect Phase angle signal (range and amplitude) to guide you further. Measurements are influenced as e.g. the phase angle changes and observation of catheter whip can be seen on echo or CT.

All is depending on your SOP for the procedure. I have seen diligent control of these conditions when pacing using CT as another "eye" if you will. Also catheter is usually (post-pacing) "searching" for similar location within the cavity as before pacing. Phase angle and CT recordings is frequently confirming this.

Q: Regarding SV calibration factor, let's say you have two mice groups (one control and the other myocardial infarction), do you need to use one SV calibration value for the control group and another (lower value in this example) for the myocardial infarction group? If Yes, How does this volume calculation by admittance change in the event you do not use unique SV calibration for each group?

(PPlouf) Regardless of the system being used, the need for a SV calibration factor is required for all PV studies unless the study is limited to relative values.

(FKonecny): You need to establish SV from control group and for MI group this SV factor should be also ascertained. Volume calculation(s) is partially influenced by not using good (correct calibration) factor. Analogous example of non-calibrating one of the signals might be during ECG if e.g. not using proper voltages (not calibrated) but only calibrate time of signal propagation. Here you would observe similar propagation timing but signals in some cases would be low or high or in some situation not-collected at all.

Q: What are you gaining from the PV loops measurement if you need to measure SV or CO using another technique?

(PPlouf): This is a very common question. The answer is "Quite a lot of information about how the heart is working" What you are gaining if the ability to track hemodynamic changes and/or reactions, in real time on a beat-by-beat basis. The ADV500 uses the term "Baseline Scan" to refer to the point where the system is calibrated and ready to observe any changes that are the result of the experiment. It is also well accepted that PV loops, when properly calibrated, are also the only technology that will provide load free index of myocardial contractility

(FKonecny): you gaining exact, precise, unique, unbelievably correct trace of SV (CO) at every beat (as at every heart beat the SV is not the same). Also when using variety of compounds (studying pharmacokinetics particularly with hemodynamic interests) you can directly trace the change "on the fly" of SV as it is changing based on the instant hemodynamic conditions you have created. Moreover, any imaging system AT THIS age of technological progress does not allow you to collect the longitudinal data (in time) of this magnitude. With Echo you are not performing continuous scan of every heart beat, same applies for CT scans. Please see our PV book page 2 and 3 for cardiac volume measurement methods comparison. I have listed major cardiac imaging volume tracing methods.

As a footnote : With Admittance you are using 3D catheter positioning-live rather than using 2D echo, 2D Fluoroscopy etc. for collection of hemodynamic parameters. Moreover you are not collecting pressure in direct relationship with volume using any of the selected hemodynamic technologies.

Q: Can you expand on larger animal catheters? Can your system accommodate sheep hearts (roughly 250g), and how would one select the correct VSL style catheter?

(PPlouf) Some due diligence on the part of the researcher is required to establish a long axis length for the animal. Mitigating the need for absolute accuracy in this LV long axis measurement is the fact that all Scisense large animal catheters offer a selection of 4 different lengths from a single catheter. The different segment lengths are selectable at any point during the study.

(FKonecny): yes we have collaborators and sheep is one of the models we use. Please call our office for VSL info.

Q: If I am correcting my volume signal with a reference Stroke Volume (measured by a flow probe), do I need to measure the blood resistivity or do the standard cuvette well measurements as well? Does the reference SV not eliminate the need for this?

(PPlouf) The admittance technology, unlike traditional Conductance, does not rely on "Correcting" the signal after the fact. The admittance system uses calibration values to calculate an accurate volume signal. Blood resistivity is one of the calibration factors. The ADV500 has species dependent default values that will appropriate for most studies. Measuring this parameter from a very small sample of blood is easy to do with the provided probe. There is no need for cuvette calibration of any sort with the ADV500.

(FKonecny): There are two separate answers. SV collected by flowprobe is cal factor for SV. System has to start functioning at certain point, you giving the system starting point by using this SV (outside validated number). Blood resistivity (as a property of tissue called blood) with measurement of Muscle resistivity and phase angle are used for determination of 1) live subtraction of muscle from instantaneous blood pool in the LV/RV or other cavity 2) Phase will help you to determine (live at 3D) where is your catheter in this cavity to make eventual adjustment(s). When using cuvette you tend to position your catheter to the center of the well. You are instructed to position catheter in the cuvette so it see all blood (you are not try to position your catheter to the side of the well) to lessen the voltage coming back and use this to correlate for volumes. I would encourage to use this static plastic well (not beat to beat-live strategy) technique to prove yourself that reading of voltages from the non-centered catheter position might be very important and starting making charts for later recalibrations of bad positioned catheter in cases when you have observed very low amount of conductance coming from heart (well….. the question would be what is enough live conductance measured in the cavity ??), at this stage you would still benefitted using Phase angle(s).

Q: If I believe the catheter (electrode spacing) may be too long for the subjects heart, are there "signs" that will indicate this when acquiring data?

(PPlouf) If the catheter is too long, you will be unable to get proper shaped loops. PVCs on the LVP trace and artefacts on the lower and upper right-side of the loop indicate the catheter being ejected.

(FKonecny): yes we have PV workbook tech note about it. Please contact me for copy. It is on page 30, referring to position #3.

Q: Is there a reason to NOT use a VSL catheters for rats or larger animals? These catheters are typically more expensive, so how does one understand the need and value versus buying single segment catheters?

(PPlouf) VSL catheters are only needed in cases where the animals studied are going to vary in LV long axis or in cases where the animals are being studied after a time period where they might have grown and increased long axis length.

(FKonecny): Thank you that is one of major reasons why VSL catheter has been developed.

I would not use VSL however in cases (especially in rodents) where the amount of rings make this catheter more rigid and turns in vasculature(s) might less easier as compared with non-VSL type.

Q: We have an older SciSense PV loop system and I don't recall a "heart type" input during calibration for our large animal studies. Is this a new setting for the ADV500, and if so, how might heart type affect our studies?

(PPlouf) In order to answer this question, I would like to know what system you do have. If it is an older conductance system, then it does not apply. The original admittance system used the electrical engineering term "Sigma/Epsilon Ratio" to describe the muscle electrical properties. The term, but not the function was changed to make it more relevant to physiology.

Q: Once you are sure that your catheter is correctly calibrated and in the right place, how many cycles would you select for your baseline measurements?

(FKonecny): You are driving this comparison as independent researcher; you should be able to compare baseline data (similar cardiac beats with your data of interest ..similar cardiac beats). I would suggest making SOP-like document. I have created one in excel sheet that you can download from the website. All can be changed based on your specific need.

Q: Are there any particular precautions for those doing open chest experiments?

(FKonecny): In certain research cases there are no alternatives as in e.g. of severe TAC (TAB) studies. In case of e.g. ECG collection during o/c vs. closed chest the ST segment was found to be stat. sign. Depressed post thoracotomy. Attached link does not however explains the mechanism http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2994054/

On the chest opening e.g.(tachycardia, tachypnea, hypoxemia, hypercapnia) is occurring during the initial stages. Moreover, abnormal collection of air or gas in the cavity interferes with normal breathing. It is often called collapsed lung, although that term may also refer to atelectasis. On chest opening you are preventing all these path-physiological changes by controlling and constantly adjusting your ventilator set up to partly reverse all these path physiology conditions to provide ideal access for cardiac catheterization.

Q: Do you recommend muscle relaxants (paralyzer) be used to control gasping?

(FKonecny): I will answer this Q based on my experience that I have had with muscle paralytics. Currently have 2 collaborators using muscle paralytics in large animal studies during PV. Their IACUC committee have questioned but approved the protocols and currently observing almost every animal…as relaxants characteristically interfere with the transmission of impulses from motor nerves to skeletal muscle fibers. Characteristically, pain induced withdrawal reflex is missing. Animal should be under diligent pain control for this reason. IACUC was there all the time to ensure compliancy.

On the other note using different muscle relaxant has variety of influences on cardiac function that you have might need to account for …and IACUC usually does not like this one. Side, additive or other effects

on your tested compound you might experience when testing hemodynamics or pharmacodynamic of (novel compound) might hinder your research. For more info please see ref. http://ceaccp.oxfordjournals.org/content/4/1/2.full

Q: I get gasping in well ventilated mice. Could it be a response from going through the diaphragm? Can you use pancuronium in mice?

(FKonecny): Pancuronium has direct vagolytic and sympathomimetic properties. It causes an increase in HR, blood pressure and cardiac output. Has long duration of its action, especially in conditions of renal impairment. During my research I sometimes observe gasping even mouse is ventilated well. I have been using predominantly ventilators with volume control. For PV researcher it is necessary to have set of stable PV loops. In order to have comparable results I have used in past maneuver of stopping ventilator in the inspiratory phase. I have always observed 10-15 non-gasping –cardiac cycles before put the ventilation back in action. These loops were my control (I have always made note in the file for later analysis). As I have done novel compound testing hemodynamic analysis while using PV, I have been particular to not complicate the matter by using other agent e.g. neuromuscular blocking agents. If I would select the neuromuscular agent I would first search the literature and would do more tryouts before the real experiment. PV as central measurement of hemodynamics might answer (or in future) help to compare neuromuscular agents and its effect on load independent data.

Q: The open chest method would have lost the haemodynamics that we are measuring wouldn't that?

(FKonecny): In certain research cases there are no alternatives as in e.g. of severe TAC (TAB) studies. All would be based on diligent control (i.e. baseline vs. experimental condition) that should answer the research question.

Q: I still get gasping in well ventilated mice. Could it be a response from going through the diaphragm (open-chest surgery)? Should one use pancuronium to reduce gasping?

(FKonecny): Pancuronium has direct vagolytic and sympathomimetic properties. It causes an increase in HR, blood pressure and cardiac output. Has long duration of its action, especially in conditions of renal impairment. During my research I sometimes observe gasping even mouse is ventilated well. I have been using predominantly ventilators with volume control. For PV researcher it is necessary to have set of stable PV loops. In order to have comparable results I have used in past maneuver of stopping ventilator in inspiratory phase. I have always observed 10-15 non-gasping –cardiac cycles before put the ventilation back in action. These loops were my control (I have always made note in the file for later analysis). As I have done novel compound testing hemodynamic analysis while using PV, I have been particular to not complicate the matter by using other agent e.g. neuromuscular blocking agents. If I would select the neuromuscular agent I would first search the literature and would do more tryouts before real experiment. PV as central measurement of hemodynamics might answer (or in future) help to compare neuromuscular agents and its effect e.g. on load independent data.

Q: do you recommend echo based calibration of the raw conductance or magnitude data collected from the PV catheter?

(FKonecny): For rodent PV research echo is most commonly used calibration system for parameters you have listed. I have always struggled in my research with 1 major issue as echo is 2D and also other 2D e.g. CT …as performing e.g. (ventriculography) method. Methods listed uses formulas that applies to 2D and using in many cases models e.g. (prolate ellipsoid). The calibrated values are as good as the operator. Echocardiography in mouse is gained skill, it needs training, diligent approach and perseverance….similar to PV at the end.

Q: Regarding heating pads, if we use a non-water based heating pad would we not be able to off-set/remove any noise generated? Why is this such a concern?

(FKonecny): Concern it is due to non-linearity of the noise. If the el. Noise is randomly occurring it introduces random PV artifact. The solution is not to turn it off/ or lower it…. the incoming el. Current.

Noise is however less concerning as to NON-ABILTY to set temperature of the surface to control it during the procedure. Available systems can be set to e.g. 38.5 degree of Celsius and have clear mind while concentrating on PV. Sometimes I have unintentionally overheated / caused burns to animals using less effective heating. I do strongly believe that we are moving towards improvement in animal research. Temperature control of anesthetized animal is critical during PV surgery.

Q: on the particular point of monitoring ecg, bp, temp, and hr, are you suggesting that researchers should have a separate monitoring system in addition to the PV system?

(PPlouf) The ADV500 is one component of a system used to study PV loops. The basis of good science is always proper control over variables and the ability to show that the variables were controlled during the experiment.

(FKonecny): there are currently systems with complete solution (parameters) you have listed are all included in this one –system solution.

I do strongly believe that we are moving towards improvement in animal research. ECG will enormously help to collect and also help with your observation of cardiac cycle stages. Yes, if you ask about ECG I would use ECG concurrently when collecting PV data. Blood pressure ..if the question was more about collecting peripheral BP. Yes, in every large animal I have been collecting peripheral BP. In some cases even pressure in the aorta…based on different research questions (applications). Have done with my collaborators measurements of peripheral BP in mouse and still used PV (there were some research questions to be answered). Temperature control of anesthetized animal is critical during PV surgery. HR can be collected out of ECG or LV e.g. pressure.

If you have additional questions for Transonic regarding content from their webinar or wish to receive additional information about solutions for PV Loop research and hemodynamic measurements please contact them by phone or email:

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