WEBINAR: Using EKG to Confirm MI in Mouse Model of Permanent LAD Occlusion

Questions and answers from the October 8, 2020 webinar titled "Using EKG to Confirm MI in Mouse Model of Permanent LAD Occlusion"

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.

You mention cd8 T cells in the beginning of your presentation. Would you say that they are more beneficial than detrimental for post MI wound healing or, in your opinion, is it the other way around?

Based on our echo data, I think that having them is probably more detrimental. It seems that they are playing multiple roles and that may be because the cd8 T cells are heterogeneous. It is very hard to classify cd8 T cells as just one thing. There are so many different variabilities: memory, effector of memory. So, we really want to tease that down a little bit more. Is it that the memory are good and the effectors are bad or and it is that it is temporally different? For example, are CD8+ T-cells good acutely and bad chronically? I do think that if you take it at a temporal time frame and look at every single day post MI, their jobs are going to change. This is something we are looking at now.

Do you think the sex of the animal model makes any difference in post MI healing?

Definitely, in fact we had a study that was published in Basic Research in Cardiology where we looked at immune cells in the post MI healing process and we found that neutrophils specifically, are what we like to quote "more efficient in women (or on the female animals) at cleaning up necrotic debris" so they were able to initiate resolution of inflammation a lot.

How long after the ligation are these measurements taken?

For our EKG's we looked within the first minute. We don't normally record for very long, but we have discussed recording for longer, say 5 minutes, to see if the first minute looks different than at the five-minute mark.

Do the EKG changes you measured correlate with infarct size by TTC staining or is the EKG a binary yes/no infarction?

We did try to do that with our data and with our current sample size of 8, there is no correlation with our TTC staining. The changes in RS intervals, for example, were quite variable so I'm not sure that we can actually correlate with infarct size right now.

Is the immediate time point most sensitive or can you use a 24-48 hour window as well?

Based on what we know clinically, we know that as a wound initiates and you start having that change in the structure of the left ventricle. But I'm not positive if the changes in the EKG at the later time point will better represent infarct size or not. We haven't looked at the 24-hour and 48-hour time points. We do see them on our echocardiograms (these include visualization and capture of EKG waveforms), but we haven't quantified anything at these longer time points. Our focus is usually on those first couple of minutes.

What's your opinion on the best timing to get the best MI? (morning vs afternoon)

There have been lots of studies that look at survival if you give an MI in the early morning vs. later in the afternoon. We have consistently seen it to be better in the early morning if you want improved survival. We tend to do all of our surgeries within the first couple hours in the morning, and try not to do any more after lunch.

How do you analyze the EKG's for MI? Is this automated?

We are using iWorx software. We import the data into their LabScribe software and perform the analysis, but we know it is possible to acquire the signals in real-time from the Indus Instruments Rodent Surgical Monitor system. We have yet to try that but plan to in the future.

Do you know if it's possible to analyze distal and proximal infarctions in the ECG?

That is a great question, but uncertain of the answer.

Can this same system be used to confirm MI in a rat model by analyzing EKG?

Yes, absolutely. In my slide presentation I showed a photograph of a mouse connected to the EKG platform and you likely noticed the green tape distal to its paws. The green tape is covering the rat EKG contact electrodes so that we don't accidently touch them during surgery. The Indus Instruments Rodent Surgical Monitor system is designed for both mice and rats.

Are there any changes in the ECG after occlusion when the thorax is closed?

I would assume that there would not be a change. I don't think that closing the chest cavity would change the EKG, but we haven't really looked at that.

During my surgeries and after ligation, we see an obvious ST elevation, however, after one minute or so the ST elevation turns negative. Is it still an infarction?

As we are doing our temporal changes, we have found that with the 15 seconds mark and the one-minute mark, 15 seconds seems to be more drastic as far as the change of the ST elevation. However,

by 1 minute it has gone down a little bit and we're not really quite sure why that is. It is still not a normal EKG so it's hard to say for sure without doing our timing studies, which we are working on currently. As long as you still see an increase in ST elevation, you can feel confident that it is a good MI.

What is the effect of strain of mice on the EKG changes with MI surgery?

There have been some studies that look at changes in EKG with different strains in the animals. I am not well versed on that, so I would encourage you to look at the literature to see what has been published.

Is it possible to see a difference in the ECG if other coronary arteries are occluded? For example, the circumflex arteries?

I would assume the answer to this question is yes, however, we only occlude the LAD and we have not really looked at other vessels in details so cannot confidently answer this question.

Do you see a difference in EKG with MI reperfusion vs. MI?

The study that I had cited in my presentation was looking at changes in EKG after ischemia reperfusion. That study looked at 30 minutes, 1 hour of ischemia, and then the reperfusion process and they did find changes. During ischemia you see ST elevation and they did see changes after reperfusion.

Do you see a change in polarity of the ECG? For example, the QR waves flipped from more upright to upside down? What does this mean in terms of the MI?

We do see this and it tends to be on Lead III when it happens. I have not looked as far as what exactly that is telling us based on the MI surgery itself. We find it interesting that this seems to happen specifically on Lead III.

Is it normal to have a 50-60% survival rate at day 7 in C57 background mice? I've tried decreasing the size of my infarcts and it does seem to help a bit (still getting consistent decent sized infarcts). The females definitely have higher survival rates compared to the males.

A lot of studies show closer to 60% survival rate and in our study we did give fairly large infarcts. Obviously, we want to improve that survival. This is one of the main reasons why we started looking at EKG changes in order to quantify them and essentially make our infarcts consistent. Decreasing the infarct size would definitely improve our survival, but most publications in C57 animals suggest their survival is going to be in the 60-65% range at day 7 post MI. That is in males specifically.

Is there any sex or age variabilities in terms of the EKG phenotypes post MI?

Not that I have noticed and not that we have actually evaluated ourselves.

How long does it take to see dilation after ligation surgery?

It really depends on how large you give the infarct. If you give a fairly substantial infarct you can start seeing a little bit of dilation as early as day 1 (if a very large infarct). Usually by day 3 you start to see the

actual dilation. The biggest thing we look for is lack of movement in the apex which you should be able to see by echocardiography within a few hours after ligation.

What do you think about the clinical translation of the transient LAD ligation model?

Clinically, about 80% of the patients get reperfused. There has been some concern that the permanent occlusion model is not going to be as clinically relevant. However, there have been studies, and a statement by the American Heart Association, stating that the permanent occlusion model is a really good model for heart failure which is really what we are looking at. In addition, because we look at the immune system, we want as robust of a flare up, or inflammatory response, that we can get. This is another reason why we tend to focus on the permanent occlusion. We would like to start going into the ischemia reperfusion model just to confirm if what we are seeing is also true in that model.

Can you see arrhythmias with the EKG during the initial Reperfusion (in mice)?

Sometimes we can see them. It's not something that we look at in a lot of detail.

Do you have an ideal age range for surgery survival in mice? (For permanent occlusion)

We tend to focus our studies on younger animals in the 3 to 6 months of age. however, I have been part of other studies where we looked at very old mice (as long as old as 36 months) for aging studies.

Did you have the chance to try some post MI care such as heating and oxygen delivery to increase survival rate or any other methods to increase your survival rate?

The Indus Rodent Surgical Monitor platform is heated, so adequate heating is maintained during surgery and throughout the recovery. If not on the heated surgical platform, we sometimes place them in a heating area. Before we take them off the oxygen, we assess if they are breathing on her own and if they are moving and walking around, etc. Obviously, we will move them to a clean cage that is on a heating pad so that they can recover. All of these things will affect survival of your animals. As long as you take very good care of them during recovery, and obviously keep an eye on them for things like signs of pain, etc. they will be fine. We also tend to give saline to our animals after surgery and that has also significantly improved our survival.