**Aged Mortality Minutes**

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| Meeting: | SPAN Stage 2 Aged Mortality |
| Location: | Zoom Meeting |
| Date: | July 21,2021 11-12:00pm PT |
| Present: | Patrick Lyden, Jessica Lamb, Yanrong, Andrew Goh, MB Khan, Kris Wood, Ligia Boisserand, Taylan Erdogan, Basav Sanganahalli, francesca Bosetti, Nirav Dhanesha, Senthil Gounder, David Hess, Ray Koehler, Pradip Kamat, Dan Thedens, Rakesh Patel, Ali Herman, Conor Johnson, Lee, Kris Dhandapani, Anil Chauhan, Cenk Ayata, Lauren Sansing, Suyi Cao, Enrique Leira, Andreia Morais, Louise McCullough, Karisma Nagarkatti |

**Agenda**

1. Mortality data review provided by Jessica as of this morning:
   1. SHR: 2/36 (before D28); 2 females
   2. OB: 6/34 (before D28); 4 females, 2 males
   3. Aged : 21/44 (before D28); 8 females, 13 males
2. Site Feedback on Aged Mice Mortality
   1. JH: Dr. Koehler reported on 20 surgeries, out of 10 deaths, 5 occurred during surgery. Site switched surgeons and settings and so there may be a learning curve. 4 of 1st 5 mouse stopped breathing at various points during surgery. Out of 15 mice that were done subsequently only 1 died during surgery. Anesthesia control and accuracy is an issue. Other issue site had is in the first and second week of doing the 48 h MRI, 4 of them died in the MRI even before the animal was in the scanning block. Some died at the end of MRI. Some during the MRI. Today, 3 more MRIs were done, they seem to have done better. Does not believe reducing MCAo duration will help much. The animal that had the biggest infarct is still alive. A few animals had tremors on D2, they are certainly stressed. Can we improve survival by getting rid of D2 MRI?
   2. AG: Dr. Pradeep reported that the site lost a lot of animals in the first week during MRI. This week site has not lost any animals in MRI. After adjusting, AG may have partially resolved the issue. Bodyweight of females was very low for first group. Strokes are occurring in striatal region. Suggestion to reduce occlusion time from 60 to 45 min. During pilot they did 7 mice and all survived to 30 days.
   3. Dr. McCullough: Duration of ischemia, 60 min gives more consistent outcomes. At 45 min, some subjects have strokes, and some have nothing. A lot of this early has to do with getting used to handling aged mice, even injecting them. Aged males are fatter and retain a lot more anesthesia. Recommends that sites be very careful when the mice are under anesthesia. If breathing rate is decreasing need to reduce anesthesia. Keep a close eye on the anesthesia. Make sure to put food mash (water plus chow) at the bottom of the cage (wet mash), this reduces the amount of stress. We see greater LPS, sepsis after 48 hr. Notes that it is important to keep up with the fluid resuscitation. They do get cold, make sure they are housed in groups to avoid shivering due to hypothermia. Do not touch the mice. If you can avoid stressing them out in any way this will increase survival. Peak inflammation is 48 hr. If they survive 5 days they will survive long term. Survival is improved if the mice are on the plumper side, so there is leeway if they are not eating well. If you can do the weight and do the neurodeficit score, then put them back in the cage with other mice. Females will lose more weight than males. If you are getting mice that are under weight, they need to be housed for at least a month to reduce stress.
   4. CC priority: First priority, is having all animals in the same age range. If mice arrive under weight are there any trick to increase weight?
      1. Dr. McCullough: You will need to just wait. We see high mortality in 24-26 mths. So weight might be the more important variable for survivability. If mice are 1 month or a few weeks older it should not be an issue.
      2. Dr. Koehler: We have not seen a weight issue in our Aged mice
3. Questions
   1. Are we powered for 50% mortality?
      1. CC: We could statistically tolerate a 50% mortality at the end of Stage 3.
      2. Dr. McCullough: It will not be that high, once sites get used to it, mortality will go down. Another thing that could help is not doing the D2 MRI
   2. Can we get rid of D2 MRI in Aged mice or do a D7 MRI?
      1. Dr. Ayata: I agree with Louise on handling the mice, but we are not slated to do Aged mice now—we have done them in the past. There is a true learning curve. I am reluctant to change the MRI protocol because it will be difficult to compare the outcomes. Changing MCAo Duration would add more variability especially because some sites have smaller strokes. We are not relying on D2 as much as we are relying on tissue loss on D30. This would be one less readout that is comparable to what is in the literature. With that in mind, I am hoping mortality will improve over time and we will not need to change the protocol
      2. Dr. Dhanesha: I am in favor of continuing D2 MRI. There is only one readout we have from that treatment/group. If mortality is reduced in the coming weeks, then we should continue with D2 MRI.
      3. Dr. McCullough: I am concerned that MRI is being done at the peak of the post stroke inflammatory response when the animals are most susceptible to stress induced mortality.
      4. Dr. Leira: I think it is important to stay the course. Moving the MRI time would not help the experiment.
      5. Dr. Sansing: I tend to agree with staying the course for now. HOwever, would like to ask whether it make sense to switch to 2 new labs that haven’t done Aged yet and go through the learning curve again. Maybe this would balance out the mortality. CC: Stats had planned this round robin because of seasonality and to balance out other sources of variation across sites.
      6. Anjali Chauhan: There is an anesthesia issue. All surgeries should be done within 10 min. UTH has not done Aged mice yet. This is something that could be playing a major role. Only 1 mouse that underwent MRI died. All the mortalities that are occurring now are before the MRI itself. Anjali supports staying the course.
      7. Jessica Lamb requests AG and JH to come up with suggestions for what made the biggest difference in the MRI survival—tips and tricks that may benefit the other sites.
         1. Dr. Koehler: JH usually induces isoflurane between 2-4% however for MRI, JH induces with 1.5%. There is still twitching and moving around. JH keeps them on 1.5%, then drops 1% when mice settle down or 0.4% if mice are weaker. Site voices concern of awake protocol is neck movement; this could be why JH is getting smaller infarcts. They are using a longer filament from Stage 1. Agree to continue to do D2 MRI and stick with 60 min. The hope is that there is a learning curve.
   3. How many sites reactivate LDF before deocclusion?
      1. MGH: we sometimes do it.
      2. JH: Suyi has seen some circling at 15 min MCAo that then disappears by the time of de-occlusion. This suggests movement of the filament and premature de-occlusion.
4. Consensus Decision on Aged mortality : Sites agree to stay the course for another 2 weeks.
5. Stage 1 Control Results (IV control/IP control/ RIC Sham)
   1. All though there is inter site variability which we know about there is no intra site variability in volume lesion site. Group sizes are on the small side so there is a potential for type II error. Nevertheless, it appears that the IV and IP control groups are comparable.
6. Dr. McCullough posted paper. Original papers done regarding edema in aged mice. There is much less edema, inflammatory response is different.
   1. Liu F, Akella P, Benashski SE, Xu Y, McCullough LD. Expression of NA-K-Cl cotransporter and edema formation are age dependent after ischemic stroke. Exp Neurol. 2010 Aug; 224(2) :356-61. PMID: 20406636; PMCID: PMC2906683.