Ralph DePalma: It's a pleasure today to have James Stone who's MD, PhD, Vice Chair of Research, and Associate Professor of Radiology at the University of Virginia. He is joined by Brian Avants, who is PhD, Visiting Associate Professor at MVA and Radiology. He's Director of Machine learning at a firm called Invicro. So, we're looking forward to their presentation, outlining the changes that are seen and Neurological Correlates of Repeated Low-intensity Blast Exposure in Operational Personnel. Dr. James?

James Stone: Thank you, Dr. DePalma. I appreciate that introduction. So, I am grateful to be able to spend some time with you all today, along with Dr. Avants, talking about the Neurological Correlates of Repeated Low-intensity Blast Exposure in Operational Personnel.

Now, when we think about the exposure of the brain to blast, oftentimes, the association that may come up is an individual blast injury event where you may have a military service member who is exposed to a large single explosive, or may find themselves in a vehicle that's subjected to, perhaps, a roadside bomb. And that individual may be exposed to the not only the shockwave event but also, perhaps, penetrating shrapnel to the brain or maybe thrown some distance by the blast, or the vehicle that they may have been in may have experienced, perhaps, an accident as well resulting in an impact and perhaps acceleration, deceleration as well.

And so, that's obviously an important entity to be able to understand to be able to appropriately manage. But what we're going to spend our time talking about today really is repeated low-intensity blast exposures; and so in contrast to a traumatic brain injury blast-associated event, this really relates to repeated exposures that operational personnel may experience such as breachers who work with small charges in order to be able to make it through hardened structures or, perhaps, artillery personnel as well who may be exposed to many individual blast events during training and operations as a result of just their proximity to these heavy weapons that generate over pressure. And again, in contrast to an actual diagnosed traumatic brain injury such as mild, moderate, or severe TBI, what we're talking about really lies more within the subclinical realm where you may be looking at multiple events with the gradual emergence of neurological changes after a career of exposures.

And so, just to talk a little bit about the history of what led to some of the work in this field, back in 2005 and 2006, military and law enforcement breaching communities began to express some concern about their risk for neurological changes over time as a function of their overall exposure to blast. And some of the anecdotal reports that were coming from the communities at that time, including changes in sleep pattern, short-term memory loss, headaches, and mood changes as well, and that really led to questions being generally raised around whether breachers demonstrated evidence of injury associated with their training; and if so, what blast exposure levels were associated with any of these abnormalities.

And just to speak further to some of the anecdotal reports that were being self-reported by these communities, this was a study that was done by Charmaine Tate, Dr. Walter Carr, and others that looked at a community of breachers over their training exercises and showed that there was an increase in symptoms--self-reported symptoms--such as headaches, slowed thinking, sleep disturbance, and balance changes as well that seemed to correlate with their training activities.

So, back in 2005, 2006 when some of these questions were starting to come up, there was a multi-institutional collaborative group that came together to look at a breacher training course. This involved collaborations with Dr. Steve Ahlers at the Naval Medical Research Center; Dr. Walter Carr as well, both of whom have been involved in many of the studies looking at these communities over the years. And so, the goal of the Quantico Breacher Study really was to look at a two-week training course, to evaluate the students that were participating in that course before and after their participation in the two-week training course where they were exposed to some of these breaching charges, and to also look at them throughout their training activities as well. And the primary objective of that study was really to try and get a better understanding as to whether the students that were participating in this course were at risk of injury during their standard training exercises. So, it was very sort of laser-focused, at least initially, on trying to basically assess the safety of the two-week training course itself.

And as we moved along with the overall experimental design and execution for this effort, the instructors expressed an interest in being involved with the course as well; and they were, of course, included; and frankly, it made sense to include them given that it was really more of sort of the senior breachers that had expressed some of these anecdotal reports that led to the study being done in the first place.

And so, one of the key observations that came from the study came from a set of neurocognitive tests--computer-based neurocognitive tests--and what was noted was that although the students that were participating in this two-week training course really didn't show any significant differences from the start of the course to the end of the course, when we looked at the comparison between students and instructors--which, again, the instructor cohort was pretty small--we saw that there were significant differences particularly on those computer-based tasks where there was a significant memory demand.

So, that work was reported back in 2016 by Carr and colleagues and it really led to a series of follow-on studies that were more focused upon the questions that emerged from the Quantico Breacher Study which is that, although it was clear, based upon the tools that we had available at that time, that a two-week training course seemed to be safe, there were questions that emerged around whether there was a cumulative risk across a career to individuals that were engaging in breaching activities. And so, again, as a result of that, there were a number of follow-on studies that emerged and there was the Blast Exposure Standards Program that also was established as well with support from JPC-5/ Military Operational Medicine, and under the leadership of Dr. Steve Ahlers up at the Naval Medical Research Center.

And the Blast Exposure Standards Program encompasses a number of different ongoing studies, both preclinical, translational, and human-based as well; but one of the key focuses of the Blast Exposure Standards Program is really to try and get an understanding of the potential cumulative effects of blast in human populations. And so, the Experienced Breacher Study was really the first study that was established in order to try and compare and contrast a group of individuals that had been engaging in breaching activities across a career to a set of well-matched controls. This work was done at the NIH Clinical Center under the PI leadership of Dr. Eric Wasserman; it involved experienced breachers going to the NIH Clinical Center for several days to undergo an extensive set of assessments.

Those assessments included an extensive set of neuropsychological analyses and questionnaires; it also included a set of balance assessments as well; included audiological assessments which, of course, are important in blast-exposed personnel, and also included subtests in order to be able to differentiate any changes in peripheral auditory function from central auditory function; it included a collaboration with Dr. Jessica Gill up at the National Institute of Nursing Research in order to look at fluid-based biomarkers using this highly-sensitive Simoa approach that allows for single-molecule sensitivity, and also utilizing approaches that exist in her laboratory around being able to look at the concentration of these molecules not only within serum, but also to look within brain-derived exosomes to get a direct sampling of the protein content, the molecular content of specific molecules that may be directly related to the brain.

And neuroimaging assessments were included as well, including a whole series of advanced approaches utilizing MRI, that included functional connectivity, looked at cortical morphometry as well, looked at the white matter, and also looked at brain perfusion.

So, this is basically the cohort that was recruited for the Experienced Breacher Study. So, there were a total of 20 experienced breachers that were recruited and 14 well-matched controls. These individuals were matched across a number of different key areas including age, ethnicity, handedness, service, duration of service, any prior concussion with the primary differentiator between these populations being the number of explosive breaches over a career as well as the number of breaches within the last year.

Now, when we looked at the neuropsychological results, although a total of 69 individual assessments were performed, only a handful of those assessments were found to be significant; and really, one of the most significant measures that showed up was basically a self-report of tinnitus. With respect to the balance results, there were significant changes that were seen between experienced breachers and controls related to both movement velocity and also reaction time. And so, the movement velocity was seen to be slower within breachers and the reaction time was seen to be longer within experienced breachers as compared to controls.

Looking at the audiological data, there was a significant decrement in auditory function that was seen within experienced breachers compared to controls; and again, there was this significant signal related to tinnitus as well, and these findings were published just recently in Frontiers in Neurology by Modica and colleagues.

Now, in addition to the balance, the auditory, and the neuropsychological findings, there's also this series of fluid biomarker assessments that were performed. And for the fluid biomarker assessments, this essentially involved using the sensitive Simoa technique and then looking specifically at brain-derived exosomes versus serum. And there are a number of different molecules that were looked at, including structural proteins, tau and NfL, and Aβ42 as a marker--a potential marker of neurodegenerative processes.

And when we looked specifically at the structural protein, tau, within the brain-derived exosomes, comparing breachers versus controls, it was seen that there was an increase in the brain-derived exosome-related tau within the experienced breacher cohort compared to controls. However, when we looked at serum tau, there was no significant difference between the two. And when we looked at NfL and Aβ42 as well, there was no difference between breachers and controls within either the brain-derived exosomes or within the serum.

Now, moving to some of the inflammatory markers that were looked at in this population, the TNF-α, IL-6, and IL-10 were all looked at both looking in whole serum and also looking within brain-derived exosomes. And what was seen specifically looking in the whole serum is that there was a slight reduction in TNF-α within the whole serum within breachers compared to controls. However, when we shift to the brain-derived exosomes which, again, is a direct sampling of what's occurring within the brain versus looking at sort of all inflammation within the circulating serum, we see that within the experienced breacher cohort, there was a significant elevation in TNF-α, there's a significant elevation in IL-6, both of which are pro-inflammatory; however, there was a reduction in IL-10 which is considered more of an anti-inflammatory molecule as compared to TNF-α and IL-6.

And then looking at the IL-6 to IL-10 ratio which is a general index of sort of the balance between pro-inflammatory and anti-inflammatory factors, there is a marked increase in the IL-6 to IL-10 ratios looking within the experienced breacher cohort compared to controls. And so, this signal that was seen within brain-derived exosomes with some of these inflammatory markers was highly suggestive of there being some kind of a process occurring within experienced breachers related to controls that may involve inflammation.

Now, when we shift over to talk about some of the neuroimaging findings related to experienced breachers, we really start to run into an issue of dimensionality. Now, what do I mean by dimensionality? So, every factor, every variable that is acquired as a part of a study for statistical purposes is considered a dimension; and when we look at the brain, the brain imaging is basically comprised of a number of three-dimensional pixels which are also referred to as voxels; and with the advanced imaging that we have available to us these days, a single brain volume may be constituted of over a million brain voxels--and again, each of these are our individual variables. When we bring together all of our advanced imaging, which includes everything from connectivity, to perfusion, to white matter, to volumetrics and such, we're dealing with many millions of dimensions, many millions of samples for each individual subject across the population that we're studying in addition to all of the individual variables that we have associated with the non-imaging measures.

And so, for a study like this where we have a very broad sampling of individual ways that we've looked at these subjects--and we'd like to be able to understand this information in the context of each other rather than looking just specifically at each measure and then trying to basically reconcile those measures at the end of the analysis--we really run into a problem related to dimensionality.

And so, at this point, what I’d like to do is to turn over the presentation to Dr. Avants to talk a little bit about a unique statistical framework that evolved alongside some of this work that we were doing with experienced breacher populations; and the statistical framework became very a very powerful tool for us in looking at this very rich data set in order to be able to identify the latent signal that was found within this data.

And so, at this point, I’ll turn it over to Dr. Avants.

Brian Avants: Great. Thank you very much, James, for the wonderful motivation for the solution that we've been using to resolve these issues of very high dimensional data that's also diverse in terms of not just types of images, but also types of measurements such as psychometrics, or inflammatory markers, et cetera.

The method that we've been using is similarity-driven multi-view linear reconstruction; it is an efficient statistical integration method now that can identify hidden signals embedded within multiple modality data sets. In the context that we're discussing here, these data sets are through imaging and also fluid biomarkers capturing measurements of neuronal loss, inflammation, demyelination, and brain metabolism as well as self-reported clinical symptoms. And the goal of the tool is to be able to look at each of these measurements as a complementary and informative viewpoint on the underlying patient. This method was published recently in Nature Computational Science last month.

So, a shorthand description of this methodology would be that it's a statistical hub that links these disparate measurement modalities together. This picture is hoping to capture the scientific intuition about what SiMLR was trying to achieve using the fable of the blind men and the elephant. And in this fable, five blind men are walking through the jungle and they encounter an object that they know is alive, but they don't know what it is; and each of them is walking around the object and touching a different part of it, and declaring what they decide that object is. One blind man is feeling the tusks and declaring it as spear; another feels the trunk and declares that it's a snake, et cetera.

In the analogy that we're using here, the elephant is itself, the hidden ideology of our disease of interest or other characteristics of interest. Each blind person here would represent a type of data or measurement; so, a measurement device, a type of imaging, a fluid biomarker, which is capturing something that's real about the underlying subject, but is not the whole picture. And SiMLR, at least, at the conceptual level, is trying to put these pieces together in a statistical framework allowing us to get a more complete picture.

And so, making that analogy a little bit more explicit, if we imagine that we have, where each blind man is feeling, say, the ear of several elephants and reporting back on each of them, that's what we have in our patient populations and cohorts; we have multiple measurements for each type. And we collect all of these in our cohorts and put them together, and feed them into the method--and the method is looking at the covariation, larger ear correlates with larger, with bigger legs with longer tusks, et cetera, and using that to reduce down the space in a more interpretable manner.

And the other, I think, the key point about this technology that makes it a little different from things like some of the tensor decomposition methods that are out there these days, is that it very naturally incorporates even different types of modalities such as in the case of with the elephant analogy, acoustic measurements of the noises that the elephant might make. And in the end, by filtering all of this data through each of the other types of measurements, the techniques that are able to identify the right parts of each of those measurements.

So, for instance, in the case of these measurements of the leg, you see some background where there looks to be trampled grass or something like that, so it'll throw that stuff away because it doesn't relate at all to the ear or to the tusk measurement, and keep and highlight the parts that are related. And beyond that, it tells us also how to combine these measurements to link together a more complete picture of the whole cohort and/or individual subject and that's what we're trying to output as the whole picture here represented by Rembrandt drawing from 1637.

So, hopefully, that gives you a conceptual idea of what this tool is doing and I just thought I’d say a few words about the technical context. So, this is really based on--we've been working with principal component analysis and canonical correlation analysis for quite a long time in our group; and as a field, we've been using these tools, and this is sort of a natural extension of those methods. CCAs dates back to the 1930s, so it's been there--the key idea was there. CCA is for two measurement and SiMLR is for really an arbitrary number of measurements; in the papers that we refer to in this talk, we use up to eight different types of measurements; and each of those measurements is very high dimensional. Beyond that, we're taking advantage of both blind source separation and data similarity terms in the method, and the specific variations of SiMLR reduced to more classical forms like linear regression and then PCA.

So, saying a little bit more about the history of the tool--I don't want to go into the details of the mathematics here, but I would just point out to you that even if we can relate SiMLR all the way back to linear regression--in the highlighted colors here, the blue highlighted predictor is more like an outcome and the orange is more like predictor--and you can see that the structure of each of these methods kind of builds on the prior ones. So, Principal Component Analysis builds on the regressions; Canonical Correlation Analysis builds on Principal Component Analysis; and SiMLR builds on all three. And please, refer to the original publication for more details on this.

I would also like to just highlight that it's a--it's not just one method, it's sort of a framework. So, depending on how you want to use it, you can sort of manipulate the different pieces and different assumptions that are underlying the framework or the piece of the framework to help not just analyze data in an unbiased way, but also to ask very specific multi-view hypotheses, so hypotheses that span modalities in a natural way.

So in the paper, we evaluated this framework fairly extensively on five different data sets; we showed that we can simulate data with known hidden signal and recover it very successfully relative to related methods; we showed that in a survival prediction machine learning paradigm, we were able to use a combination of gene expression transcriptomics and methylomics to predict survival in a data-driven manner from glioblastoma data--that's public data; we looked at a number of different brain-related data sets including the PING data and ADNI, which is Alzheimer's disease data. And most of these experiments, except for those that we were not allowed to share, are shared in a public cloud computing platform where you could play around with these tools and look at these results interactively if you so choose.

I just wanted to highlight, just make something clear here to, perhaps, help out the remainder of James' presentation. I’m just showing a picture here that's indicating, in the case of a neuroimaging study like we're looking at here, that while we're doing a bunch of things that look like PCA, Principal Component Analysis, in the end, we do get predictors that are very focal and sparse, and so we can take pictures; if we look at the lower right portion of this figure, we can make pictures that are kind of what we think of as classic pictures and brain mapping where we show the networks that are involved and that are key to driving the relationships to the other variables of interest, for example, blast exposure, or years of career breaching, or things of that nature.

So, in the end, we get something that can be interpreted in a natural way and in a way that's very relatable through linear regression. So, there's a lot of machine learning magic underneath, it ends up--we can look at these high dimensional data sets in a very, very low dimensional space--at least, low dimensional compared to the original data--and interpret the results with linear regression with a picture attached telling us where in the brain and what parts of the brain are driving the results that we're seeing.

And with that, I think I can hand this back to James to discuss further. Dr. Stone?

James Stone: Thank you, Dr. Avants for providing that wonderful overview of SiMLR and providing just insight into this tool that really has been quite transformative in the way that we've looked at some of our data sets.

And so, coming back to the experience breacher cohort that we've looked at and considering the neuroimaging data, what our goal really was with the neuroimaging analysis is to be able to look at the multiple modalities of imaging that we were acquiring in a true multimodal fashion. So, those of you who are familiar with brain imaging research studies, oftentimes, the approach that's taken is to basically look at each individual modality on their own, analyze them between groups or analyze them in relation to a variable or a set of variables of interest, and then try to reconcile those individual assessments in more of sort of a post-hoc kind of way. And our goal utilizing SiMLR was to basically utilize that unique statistical framework to be able to bring all of this data together into the same analytical space, and to be able to consider it against some of the non-imaging information that was acquired, including the fluid biomarkers, the neuropsychological testing, and other measures that were required.

And so, the imaging that, again, was acquired for this ranged from connectivity through structural and perfusion measures; and when we used the SiMLR-based approach to look at this data, we were able to see that there were changes in relative perfusion that were seen within experienced breachers compared to controls; and so, there was a reduction in perfusion that was seen within experienced breachers across a number of different brain regions compared to controls.

Looking at cortical measures, interestingly, there seemed to be an enhancement of cortical measures, particularly thickness in experienced breachers related to controls, and we'll talk a little bit later about some neuropathological observations in these populations that might help to explain that finding. Looking at white matter, specifically looking at fractional, and isotropy, and radial diffusivity which are both the fusion tensor imaging metrics and basically, diffusion tensor imaging is essentially an approach that allows us to be able to look at water diffusion within white matter and to be able to look at the changing shape of that water diffusion to determine whether there are changes in the overall integrity of the white matter; fractional and isotropy, basically the closer that number gets to zero, the more sort of spherical or unconstrained the overall diffusion is with radial diffusivity, which basically looks at the overall diffusion of water across or basically orthogonal to axonal tracts; the radial diffusivity is, again, the more that's a reflection of changes in white matter integrity.

And so, looking at the experience breacher population, basically, we saw a drop in fractional and isotropy compared to controls, and we saw an increase in radial diffusivity compared to controls, both of which suggest that there is a reduction in white matter integrity within the experienced breacher cohort compared to controls.

Now, looking at the functional connectivity measures, we looked at this from a couple of different perspectives. One of which is looking at resting activity within the default mode network; and the other of which is to look at inter-network connectivity, so the amount of connectivity out of the default mode network into other regions. And what we saw within experienced breachers is that there is a reduction in resting activity across a number of brain regions within the experienced breacher cohort, while there is an enhancement of this inter-network connectivity within the experienced breacher cohort compared to controls. Now, this enhanced inter-network connectivity has been seen in other neurological disease processes as basically potentially a reflection of an adaptive or, perhaps, a maladaptive response to disruption of the default mode network.

So, just to summarize the findings of the Experienced Breacher Study using the SiMLR framework, we were able to see alterations in structural imaging both white matter as well as cortical measures; we were able to see changes in perfusion as well as changes in a couple of different measures of functional imaging looking at changes in connectivity; we also saw alterations in the fluid-based biomarkers, alterations in the audiological measures, and alterations in balance as well. The neuroimaging components of this work was published in the Journal of Neurotrauma late in 2020, and there are a series of other publications that have either already been published or are in the process of undergoing review related to the findings of the study.

So, just to summarize some of the breacher-related work. Again, the Quantico Breacher Study, which is really sort of the start of a lot of this work, showed that there were significant findings that were specific to some of the more experienced instructors. The Experienced Breacher Study was basically a follow-on to the Quantico Breacher Study that really sort of extended the overall question towards looking at cumulative change over a career of breaching activities; and that experienced breacher effort basically showed that there were significant changes with imaging, both structural and functional imaging, there were significant changes with the brain-derived exosomes looking at the fluid biomarkers, particularly as it relates to some of the inflammatory markers. And I just want to underscore that this study was really the first to basically perform a comprehensive analysis looking at differences between populations that are exposed to blast over a career--repetitive low-intensity blast over a career--and compare them to well-matched controls.

So, as this work related to breachers have evolved, there's also been Congressional interest that has emerged around getting a better handle on the overall effects of blast. The 2018 National Defense Authorization Act within Section 734 basically established a requirement to get a better handle on the overall biological effects of blast; and the Section 734 Working Group was established as a response to that requirement. This working group has basically established five key lines of inquiry that range from surveillance, to weapon systems, exposure environment, blast characterization, health and performance, and across all of these areas, there's been a lot of activity around understanding what the overall state of the science is within each of these areas; and also identifying the gaps, areas where there is a need for facilitating ongoing activities around filling in key knowledge areas that are needed to progress our understanding of the biological effects of blast.

Now, some of these gaps include understanding what the limits of safe exposure are to repetitive low-intensity blast--that's one of the major gaps in this area and it's a key piece of information that's necessary in order to be able to establish practices and policy around mitigating some of these effects as they're observed. Other questions relate to understanding what the overall chronic neurological changes may be that might be seen in other non-breaching groups, such as those that are exposed to heavy weapons, artillery, or perhaps, the EOD, the Explosive Ordnance Disposal community as well. And then developing a better understanding of the overall molecular mechanisms of the brain in response to repetitive low-intensity blast exposure.

Now, when we consider this first gap, one approach would be potentially to heavily-instrument a cohort of blast-exposed individuals across a number of different populations, and to perform an extensive longitudinal evaluation over years or, perhaps, decades in order to be able to understand what the neurological manifestation may be, and how it relates to measured blast exposure over time. That would obviously be a very difficult study to do and it would be quite some time before we were able to get the information that we need in order to be able to feed data back to the operational communities to help to mitigate any of these observed changes.

An alternate approach is to look at the populations that are available now that have had some level of historic blast exposure and to perform a very sort of precise and calibrated structured survey or interview around trying to understand what an individual's context for exposure has been. So, when did their symptoms emerge; what are the blast-exposed communities that are most at risk, and to try and understand what the overall weapon systems have been that they've worked with, what explosives and types of explosives have been that they worked with as well? And this was the approach that was taken under the Blast Exposure Standards Program under the leadership of Dr. Ahlers with this particular activity led by Lieutenant Claire Modica.

And so, essentially, what this activity did was to construct a survey known as the Blast Exposure Threshold Survey that contained many of the elements that are needed in order to be able to understand the context of an individual's exposure. This survey was administered across all of the DoD blast communities and it was its goal really was to determine when, during a career, do some of these self-reported symptoms emerge; what are the symptom differences as a function of weapon systems; what are the relationship of the number of cumulative blasts and symptoms; and to allow the survey really to serve as an online event-counting tool.

Now, the survey was administered to a total of 984 respondents across a wide array of blast-exposed and non-blast-exposed communities; importantly, there were significant age diversity and some of the key signals that emerged from the survey were reported changes in hearing, tinnitus, forgetfulness, changes in taste and smell; another pattern that emerged was that the symptoms were evident in groups that had exposure to higher-intensity weapon systems, and they tended to be reported in individuals that are a little bit older, so 32 years of age or higher.

Now, one really important deliverable that also came from this experience with the survey is the creation of this index known as the Generalized Blast Exposure Value, also known as the GBEV. Now, the GBEV is really sort of built on the foundations of the Cumulative Head Impact Index that was reported by Montenegro and colleagues, and basically was used to characterize populations of professional athletes and to identify their threshold for risk. So, building upon that foundation, the GBEV was formulated based upon looking at the overall results of the Blast Exposure Threshold Survey and it factors in important items such as years of experience with a weapon or explosive months of experience per year, days of experience per month, the number of exposures per day, the frequency of back-to-back days of exposures; and it also factors in the overall differing types of exposures that may occur from small arms to artillery, all the way up to smaller or large explosives.

And basically, this formula that's presented here that underlies the GBEV, essentially provides a single index that's reflective of those individual elements. And what was found within the survey activity is that a GBEV value with about 200,000 is basically a threshold for significant symptoms. That work has been accepted within the Journal of Neurotrauma and is in the process of--is basically in print.

So, as one can imagine, this GBEV value and this survey type of activity, as we move forward and continue to basically study these populations that have a historical exposure to blast, this type of tool is extremely important for us to understand the individual context of exposure within any of the subjects that may be participating in this activity as we work towards, again, sort of defining this all-important question around what the safe limits of blast exposure may be.

So, another gap here is getting a better understanding for chronic neurological changes within other populations, so non-breacher populations that may be exposed to repetitive low-intensity blast exposure. That may include artillery heavy weapons, EOD, and others. And this is an activity that is underway; and so, building upon the experience breacher study, we're in the process of launching the Experienced Artillery Study, and so this is basically similar to the Experienced Breacher Study going to look at a cohort of experienced service members that have been exposed to artillery over their career and look at matched controls.

And for this study, there will be a total of 50 experienced artillery service members compared to the same number of matched controls and we, thankfully, had the luxury of some power analyses from the Experienced Breacher Study to help to inform how we put together the overall sample size estimate for this particular study. We'll be looking at some of the same measures that we looked at for the Experienced Breacher Study ranging from the neuropsychological assessments, to audiology balance, advanced imaging, and looking at fluid biomarkers as well.

Another important gap is understanding what the molecular mechanisms are of the brain response to repetitive low-intensity blast exposure. Now, if you recall from the review that we did at the Experienced Breacher Study, there was that very interesting signal that emerged within the brain-derived exosomes from the fluid biomarkers that suggested that there may be potentially a significant central neuroinflammatory response; and so, it's very important for us to gain a better understanding for that.

One study that has helped to inform our overall understanding of, perhaps, the unique pathobiology and perhaps, the underlying molecular mechanisms of blast exposure was this study that was published by Pearl and colleagues that described this unique pathological signature within blast-exposed personnel of astroglial scarring; and basically, there is this pattern of astrogliosis that was seen within the gray and white matter interfaces, was seen on the peel surface, and was also seen in proximity to the walls of the third ventricle, and within the lateral ventricle as well, along with perivascular regions.

And so, the signal that we saw within the experienced breachers along with this neuropathology really moved us in the direction of trying to get a better understanding for what some of the molecular signatures may be of repetitive low-intensity blast exposure. And while neuropathology is extremely useful and very powerful for providing insight into the underlying pathobiology of a given disease process, we'd also like to be able to look at some of these changes in a living individual as well.

And so, that's really where molecular imaging and the power of molecular imaging comes into play. And so, with molecular imaging, we can basically look at very low concentration but highly physiologically-significant molecules within the brain, in order to be able to determine whether there is a process of interest that may be occurring. And inflammation based upon the neuropathology and based upon the fluid biomarker work is pretty high on our list at this point.

Now, one of the approaches for being able to look at neuroinflammation involves using a ligand that binds to the translocator protein; translocator protein is basically a molecule that is associated with mitochondria; it's dramatically increased by cells--inflammatory cells--during periods of inflammatory activity; and also, interestingly enough, it's increased in expression in reactive astrocytes. And so, these TSPO ligands, given the neuropathology and given some of the findings within the serum biomarkers, were of high interest to us.

So, by using what's known as a radiopharmaceutical, which is basically a targeting peptide that binds to the translocator protein linked to a radioisotope that allows for us to be able to see it with imaging, we can basically look directly for neuroinflammation using imaging. And once we administer this radiopharmaceutical and allow for it to circulate, we can basically look directly for the presence of that radioisotope that's linked to the targeting peptide, that's linked to the translocator protein using Positron Emission Tomography that gives us a very high signal of the noise allowing us to look for low concentration, but also has a very high spatial resolution as well, so it allows us to be able to localize it fairly well to brain structures.

So, at the University of Virginia, we have the capability for being able to make experimental radiopharmaceuticals that can be used in imaging in our FDA-compliant radiochemistry laboratory, and we have the ability to be able to make at least one of these TSPO ligands known as DPA-714; this is an F-18-based radiopharmaceutical and again, this binds directly to the translocator protein. Using this radiopharmaceutical, we've partnered with a Special Operations command in order to perform a pilot study looking at ten blast-exposed individuals versus ten matched controls, with the blast exposed individuals having extensive repetitive low-intensity blast exposure over their career.

We'll perform a series of neuropsychological assessments and administer a number of questionnaires; and then we use PET CT neuroimaging to look directly for inflammation using the DPA-714 radiopharmaceutical. We'll utilize many of the fluid biomarkers that we've used in some of the experienced breacher work before to look at the relationship between some of these biomarkers for inflammation and any observed central neural inflammation; and we're using advanced MRI measures as well, giving us insight into connectivity, perfusion in white matter and gray matter.

We'll also employ the Blast Exposure Threshold Survey as one of the questionnaires that will allow us to be able to look for the context of blasts for each of the participants, and to be able to calculate this GBEV value.

Now, while his work is still underway--we're wrapping up recruitment at this point, we have just a couple of individuals left to recruit for the study. Once we've finished with recruitment, we'll basically move on to the overall analysis phase of the work, at which time we'll have quantitative information that will come from that analysis. But at this point, I can share some of the qualitative images that we've acquired from an example blast-exposed individual versus a normal subject that shows some of the patterns of uptake within the brain; and these two studies have basically been baseline to one another in terms of the overall color metric representation and what some of the base values are for representing that information.

So, just to conclude this talk, in general, there's emerging building evidence that confirms that repetitive low-intensity blast exposure may be an occupational risk for operational personnel; there's ongoing work that's looking at whether some of the signal that we've seen within experienced breachers is also seen within other populations that are exposed to repetitive low-intensity blast over their career. There's initial work that we just talked about looking at some of the molecular mechanisms involved in repetitive low-intensity blast exposure, and special operators, and there are standardized assessments that are beginning to emerge that allow us to really have a very precise insight into what the overall context for a blast exposure may be within an individual.

And then, just in closing here, as sort of one of the final content with a consideration of the overall goals of the work. This really is, as we move forward, looking more and more like an occupational hazard, an occupational exposure issue; and there are a number of different other occupational issues that have been encountered in other fields over the years where there are frameworks that have emerged in order to be able to help to protect or mitigate individuals from these occupational hazards. And one of these that's near and dear to me, as an interventional radiologist, is radiation exposure. And this is basically an image that's pulled from a radiation exposure literature that's showing a pyramid that describes controls with increasing effectiveness; and what we can see at the bottom is personal protective equipment, we move up to administrative and work practice controls, engineering controls and, ultimately, at the top, which is the most effective, is some form of elimination or substitutions.

And as we move forward as a community in order to be able to better understand the nature of these cumulative neurological changes within repetitive low-level blast exposure, and understand what the thresholds of safe exposure and unsafe exposure may be, we'll start to be in a position where we can construct these kinds of models that will help to inform how we can put policies and practices in place that will help to address mitigating some of these chronic changes that may be observed in some of these populations.

And I just want to close here just acknowledging the phenomenal team that that's come together to perform a lot of this work under the Blast Exposures Standards Program again this is led by Dr. Steve Ahlers, a long time colleague up at the Naval Medical Research Center, and I’ve just presented just a handful of the studies that are occurring under this program today. So, there are a number of different activities related to pre-clinical studies and other field-related studies and such that are ongoing as a part of this program; but this really is a highly functional and very dynamic team.

I’d like to acknowledge Dr. Wasserman up at NINDA, that was really key for some of the experienced breacher-related work; Dr. Gill up at the National Institutes of Nursing Research that we collaborate with across a number of different studies looking at peripheral biomarkers; our West Coast colleagues at the Naval Health Research Center, Drs. Taylor and Hernandez are critical members of this team; Dr. Bhomia at USU. I’d like to acknowledge Dr. Lisa Wilde at the University of Utah who is really our key colleague related to some of the neuropsychological-related assessments for our artillery-related work and our partnership with SOCOM, Walter Reed Army Institute of Research, and the team there that's involved in a number of different studies including some of the pre-clinical work that's being performed.

And last, but not least--certainly not least--is our partnership with Special Operations Command the incredible team down there that we've been working with on this pilot study. I’d like to really make a special call out to Katryna Deary who's been critical to our recruitment and in screening for that study; and I’d also like to acknowledge Lieutenant Modica who started at the Naval Medical Research Center with the Blast Exposure Threshold Survey, and is really continuing to be just an exceptional partner down at SOCOM as well.

And I’ll finish here and would love to take any of your questions.

Moderator: Thank you so much for that wonderful presentation. We have a couple of questions lined up so I’m just going to go in order. Our first one is, "What clinical measures, if any, were taken as a result of the breacher findings?"

James Stone: So, the clinical-related measures were a number of the neuropsychological assessments, the auditory assessments, and some of the routine neuroimaging that was acquired. So, the T1, T2 susceptibility-weighted imaging and flare imaging as well. And this wasn't necessarily to sort of asked as a part of this question, but I think what we saw across a number of these sort of "clinical measures" that were acquired, that they tended to be a little bit less sensitive overall than some of the advanced research methods.

And so, I think one of the challenges with this work is how to translate some of the advanced methods, particularly some of the advanced imaging methods to be useful in the clinical environment, and that's probably an entire other lecture around how we get there to do that.

Moderator: Thank you. So, this question is a bit long, so please bear with me. "How have the conclusions made from the breacher cohort been extrapolated to other at-risk military occupational specialties, particularly in direct fire?" Sorry if I butchered that.

James Stone: So, one of the key follow-on studies from the Experience Breacher Study, or one of really sort of the series of follow-on studies, is to do exactly that, which is to understand what does this mean for other communities that may be exposed to blast overpressure. And so, that's why the Experience Artillery Study is, we feel, a critical follow-on measure in order to be able to understand whether these changes are also seen within other operational populations. And there are certainly other groups, such as EOD and others, which we really require an assessment as well to understand whether cumulative changes are an issue for them.

Moderator: Thank you. Here's the second part: "Are there studies currently evaluating the cumulative effects of 81/120 millimeters mortar systems?"

James Stone: I can't speak specifically to the weapon systems themselves; I know that for the artillery study, we're certainly trying to have a range of experience with those studies, but I’d have to get back to you on the specific weapons system question.

Moderator: Thank you. "Are suicide rates higher in experienced breachers?"

James Stone: I don't know the answer to that question. I think that that is an extremely important question and it's certainly something that we need to get a handle on, particularly given what an issue suicide and suicidality is for our service members in general.

Ralph DePalma: Thank you. "Is there a higher risk of cardiovascular disease in the breachers and could it be linked to higher inflammatory status?"

James Stone: Cardiovascular changes was not something that was specifically looked at; one of the things that did seem to be clear from the Experience Breacher Group is that the circulating--there didn't seem to be an elevated level of circulating inflammatory factors within the periphery; and in fact, that one TNF-α signal that we saw within the periphery, within experienced breachers was actually a little bit lower than within the control population. It really wasn't until we went to those brain-derived exosomes, which are those small membranous vesicles that are secreted as a part of some sort of intracellular communication that gave us a direct insight into the brain when we started to see some of those signatures.

So, I’m not sure, necessarily, that the link is there directly to sort of systemic inflammation, but I also don't know that anyone's looked specifically at cardiovascular risk factors in blast-exposed populations. But I do think that this question raises a very important consideration, which is that blast is a whole-body exposure. We've talked a lot about the brain here, but it really is the whole body that experiences the blast; and so, at that point, I think that really is sort of an unanswered question.

Moderator: Thank you. We have time for just one more. The last one is, "Fantastic talk. Have you validated your GBEV index in a separate cohort?"

James Stone: The work with the Blast Exposure Threshold Survey sending it out to the thousand respondents--or close to a thousand respondents--looked across a number of different blast-exposed and non-blast-exposed populations; and so it was it was a pretty diverse group that one could argue was constituted a number of different types of cohorts in that initial survey administration; and there's active work to increase the BETS circulation by orders of magnitude out to broader populations in order to be able to increase the overall sample size for the BETS and to basically feed that information into the GBEV calculation.

I will say that the work that was done for the GBEV did use a machine learning-based approach where there was basically a portion of the data that was utilized to build the model, and then there was a left-out portion of the data to test the overall validity of the model; but a natural progression of this is to basically get this out more broadly to the DoD community and to continue to test the model. So, that's absolutely the next step with that work.

Moderator: Great. Thank you so much. So, we're just right after the end of the hour; we do apologize for not being able to answer all your questions at this time. I'd like to ask if Dr. Stone and Dr. Avants have any closing comments?

James Stone: I’d just like to thank the organizers and thank Dr. DePalma for the opportunity to be able to present this work, and would just like to relay that if there's anyone in the audience that would like to connect, has any additional questions about this or our work, feel free to reach out to me directly and I’d love to continue the conversation.

Moderator: Dr. DePalma, do you have any?

Ralph DePalma: Well, I’ll just add my thanks to Dr. Stone and Dr. Avants for this elegant presentation that has been shared widely within the VA-mainly audience of about 128 people; it will do a lot of good for our providers and researchers who are seeing these patients to understand that this type of science is available for objective evaluation. Thanks again.

James Stone: Thanks so much.