*(PLEASE NOTE: Transcribed automatically by Vimeo, mistakes are possible/likely. Our apologies.)*

**TRANSCRIPT - GR 11 11 22** "Objective Measurements of Pain" **Alexander Niculescu, MD, from Indiana University Health**

- 00:16:28Okay. Very good.
- 00:16:30So uh thank you all for joining us. Uh, welcome to Medicine grand rounds for you, those of you in the audience, Sam. I'm. Excited to introduce Dr. Alexander Nicolasu, the third Dr. Nicolas, is joining us today from Indiana, where he works as a professor of psychiatry and medical neuroscience at the Richard Roadbush Va. Medical Center in Indianapolis.
- 00:16:51Dr. Nicholas, who completed his Medical school training at the University of Bucharest in Romania, followed by a masters in medical biology at the University of Geneva Medical School, and then a Phd. At Scripts Research Institute in Lahore, California.
- 00:17:05Fascinated by the intersection of neurobiology and psychiatric illness, he Then decided to pursue a residency in psychiatry and fellowship and biological psychiatry at the University of California in San Diego.
- 00:17:19Armed with this rigorous and extensive training as a researcher and a genuine desire to improve the lives of people suffering persistent, serious mental illness. Dr. Nicolas is passionate about the use of broad empirical discovery based science In the classification, risk, assessment and treatment of psychiatric disorders.
- 00:17:41He currently runs two research labs, a human subjects lab and a basic science, lab focusing on genetics with support, both from the Va Medical Center as well as Indiana University.
- 00:17:51Um. Where he holds a tenured academic appointment as well. His labs have higher engineered the use of functional uh genomics and blood biomarkers to enter uh, to interrogate our understanding of psychiatric illness with the recent focus on suicide risk assessment, and then a further extension of his work into fields of Alzheimer's and pain.
- 00:18:12So I invited him today to speak on the fascinating topic of objective biomarkers in pain. There may be few psychiatrists in the audience today, but as primary care doctors and and internists, You're all acutely aware of the destructive power of physical pain in the lives of our patients. My hope is that through
- 00:18:29my hope i'm sure, Dr. Nicholas, he's hope, too, is that through rigorous scientific approach um. We can learn more about pain in its pathways. It's effects on the body, and our ability to treat it So please give a warm welcome to Dr. Nicolowski.

**Alexander Niculescu**

00:18:46Thank you very much. Good to be with you. Can you all hear me? Okay,

- 00:18:50Yeah, we can hear you just fine, alright, great. So let's dive into it. So um i'm glad to have a, you know, actually an audience of non psychiatrists, because um this you know this area of pain and and even mental health is is broader than what we do in psychiatry. So
- 00:19:09you know i'm a professor of psychiatry. I still see patients um have a clinic at the University of day a week. I have a clinic at the Va. A day a week. I have the these research uh labs and um, you know,
- 00:19:24in psychiatry right now. Uh, there are more patients than can be seen by psychiatrists, and, in fact, eighty percent of uh mental health

- 00:19:35uh issues are first seen and seen for a number of years by primary care doctors, internists, and so on. So I think it's uh it's great if people um, you know. Uh get involved um get comfortable with how things are being done.
- 00:19:52Um! And um apply some of the insights that we have in psychiatry that are coming down the bike from research into their daily practice. So that's what I'm going to try to do today, and I'll focus specifically on paying. And
- 00:20:07you know the question is, you know why pain? You know why somebody who's a primarily a psych psychiatrist of psychiatric researcher. Um
- 00:20:18went into this area, and it was
- 00:20:21for three reasons. So first of all, um a lot of um Psychiatric patients have pain issues, acute pain, chronic pain issues, in fact, having depression and other psychiatric disorders, magnifies your perception of pain makes pain um more chronic the more difficult to treat. Um.
- 00:20:40Second reason is a lot of the medications that are being used for pain. Disorders are actually medications that are being used also in psychiatry. So, starting way back with tricyclic antidepressants,
- 00:20:53I'm. More recently with uh serotonin or ipine for you up. Take inhibitors um like symbol time. So on um, and I convolve since Gaba bentin um,
- 00:21:05and so on. So there is this probably biological and pharmacological overlap between
- 00:21:14psychiatric disorder, specific going with disorders and pain. When people are depressed, I feel paying more when they are,
- 00:21:22and with this elevate that they feel no pain.
- 00:21:26And then the third reason is, um,
- 00:21:28we've developed this platform that I'll show you where we can actually
- 00:21:33through careful um
- 00:21:36uh work uh identify um rnas in the blood that track
- 00:21:43signs and symptoms that track things that are happening in the brain. And we did it for
- 00:21:50mental health disorders for psychiatric disorders. But we, you know, we just realized, You know pain is very similar to what we're dealing with in psychiatry. Somebody sort of endorses something
- 00:22:01uh it's their subjective perception. Uh you have to rely on that to make your diagnosis to a guide treatment. Uh and uh,
- 00:22:11wouldn't it be great if you also had a blood that something objective to
- 00:22:17um give you some indication about severity to help
- 00:22:20match people to medications, to monitor response to treatment, and so on. So that's why we sort of delve into doing this work for paying.
- 00:22:30So. Um,
- 00:22:38do you see my second slide here uh disclosures.
- 00:22:42Uh, no, it right now. It's just still on the first slide.
- 00:22:47Um,
- 00:22:49there you go. It popped over to disclosures
- 00:23:03an inventor on patents related to precision medicine that were filed by Indiana University, and i'm a co-founder of mind that sciences which is a start up that uh
- 00:23:14uh develops and commercializes uh um precision medicine tools for mental health.
- 00:23:25So um the work that we've done over the years have has been highly translational, and we've um
- 00:23:34um include that um animal model studies in our approaches. Human studies. Um, there are things that you can do in animal models that you cannot do in humans.
- 00:23:44Um, they are um
- 00:23:47um things that obviously um

- 00:23:51can only doing humans in terms of uh specificity and uh relevance to the disorder um By combining those studies by combining those data sets at the primarily at the genomic level, but also at other levels, we were able to um make progress.
- 00:24:09And um,
- 00:24:10this is, you know, psychiatry for dummies, and I'm not referring to you. This is how I like to look up psychiatry myself. I like to have sort of things simple. And, uh, you know, boil down to sort of the Ds and send
- 00:24:24uh, Basically, enough. Psychiatry has three broad domains. The anxiety disorders domain, the move to Service domain, the cognitive disorders domain. There's a lot of overlap between them. There's a lot of heterogeneity inside them, and stress is a major trigger of psychiatric.
- 00:24:42So that was the you know the landscape that we were trying to a map. Um!
- 00:24:49About two decades ago, when I started on this journey. Uh, we initially started our work at Uc. San Diego, when I had my first lab, You know, when I was a
- 00:24:59chief resident and fellow there. Uh, I had some funding, started the lab and junior faculty, and then I came to Indiana in two thousand and four, specifically to do this type of work. Large scale genomics in a stable population followed over time.
- 00:25:16Um! So that was the you know, the landscape that we were trying to map, and over the years
- 00:25:25uh we've um done studies that have uh identified genes and gene expression patterns in the blood for uh those disorders. And uh, these are. These are all studies from my group over the years. Um,
- 00:25:41and um! There's a lot of uh,
- 00:25:43you know, a lot a lot of overlap at the genetic level between these disorders. Um, it's not one gene for one disorder. There are, you know, panels of genes that can um track a disorder. Um. In genetics we call that polygenic risk course. You might have heard that
- 00:26:02for gene expression, which is our area of focus. Those are panels of Rnas in the blood that we measure
- 00:26:11tracking people
- 00:26:12and um.
- 00:26:14So what we started to do um eighteen years ago, and I was recruited to Indiana was to
- 00:26:21have like a Mini Framingham project. You might have heard of that. The framing ham project that was done over decades where they follow people to identify cardiovascular risk factors to understand cardiovascular disorders. Primarily we wanted to do the same for um mental health, and we started that um initially. We wanted to sort of develop a
- 00:26:46cohort of about five hundred people that we followed over many years.
- 00:26:51Uh, we were calling it the Indy Five hundred uh cohort. It has sort of a catchy ring to it for um,
- 00:26:58you know. Car race uh fisher now does like me. Um right Now we're
- 00:27:03we're way. Beyond that We have about six hundred people over the last uh
- 00:27:10fifteen or so years. Um! They are tested every three to six months, either in the lab or on the inpatient unit. When they get hospitalized.
- 00:27:20We do comprehensive uh narrow psychological testing at each visit. So for the phenotype, we collect uh blood and saliva samples. Um
- 00:27:30for um,
- 00:27:31you know all sorts of studies. Multi-level approaches. All of them have um electronic medical record. Follow up um.
- 00:27:40Is that primarily V. A patients uh. So it's a stable population, or with uh electronic medical records,
- 00:27:48health system which makes it for a studies where you look at outcomes between the markers years ago, and what happened to patients subsequently, and we've developed some new instruments

for tracking things that now are sort of uh apps and are used for um in conjunction with the blood studies

- 00:28:12so as as mentioned, you know, we had um. You know animal studies that weren't done in conjunction with this um large scale human studies I won't go into these today.
- 00:28:23Um.
- 00:28:25So
- 00:28:26why go for blood biomarkers? Uh: Well, in psychiatry we can't bi off see our target organ, the brain, and not in live individuals. So um, you know the blood is easily accessible.
- 00:28:39Um, In almost any clinical setting um as opposed to, you know, trying to find brain imaging biomarkers. Those are more cumbersome, more expensive, might require specialized settings.
- 00:28:52Um, Even Csf, which might have some informative uh molecular markers is not. Um, you know It's not something that's very practical. Most patients are not super enthusiastic about getting spinal taps and so on.
- 00:29:06Um! The blood is re, you know, readily accessible. So the question was, Can we actually find any signal there that would track uh what's happening in the brain? And that could be used clinically. So when we started on this journey wasn't obvious that we could. But there were some reasons for
- 00:29:23to think that we might. One was that you know the brain, The nervous system in the immune system have a common developmental roots. There's a lot of um shared biology.
- 00:29:35Um, and um. They're, you know, after birth and throughout life their bi-directional brain, immune system, interactions, the brain affects the immune system, the immune system side the kinds and so on, affect the brain. So there is sort of this close relationship, and you could potentially find signal and
- 00:29:54the easily accessible one and the blood that tracks what's happening in the brain.
- 00:29:58And you know, from a genomic standpoint there are similar gene expression changes in brain and blood that are also due to them, having sort of some of the same genes, the same promoters, and the blood cells in the immune cells and in their brain cells
- 00:30:14that respond in a similar way to the internal familiar to external environmental changes, to stress, to medications. So that's probably the underlying basis. Why we are able to find these biomarkers and um
- 00:30:30um! Why, um! The signal is there?
- 00:30:34Uh? The signal is, uh,
- 00:30:36you know, like a needle in the haystacks that do you know, there are many things that change in the blood. Uh, in terms of gene expression. How do you find the relevant one so to do that we did this sort of careful
- 00:30:49erez agmoni four-step approach, where we have a a discovery step, a prioritization step of validation step, and a testing an independent cohorts step and one
- 00:31:00for discovery. We use this um powerful within subject design where you follow people over time. You catch them when they are in this case for pain when they are not in pain, then it some other visit, when they are in high pain, some other visits. There are no pain. So you have these trajectories, clinical trajectories in people, and at each
- 00:31:20of those extreme points you have blood sample, so you can see what's different within each person between those States, and that's very powerful, because,
- 00:31:29uh, you're subtract away all the genetic variability between people by doing that. Um,
- 00:31:35uh, we also, you know, have built large literature and experimental databases. So once we find something that
- 00:31:43a candidate or a list of candidate biomarkers, we can look what other converging evidence supports them, we

- 00:31:50and then we can then take that forward into validation and independent cohorts, testing for clinical utility and independent cohorts. And
- 00:31:59this reproducibility and testing and independent cohorts is key. Otherwise, you know, if you do something in a cohort to discover something has a good P. Value or sort of deluding yourself
- 00:32:12and polluting the field by publishing that without trying first to replicate it. So rep, you know replicating things in a dependent call parts is the goal standard,
- 00:32:21and that's how we've done our studies, and when I'm a reviewer on Grants or
- 00:32:26uh, you know, at the top journals uh insist on that, you know people need to replicate their findings and independent cohorts before publishing it;
- 00:32:36that in their paper not
- 00:32:39communicate something and wait for the rest of the field to try to reproduce

**UVA Medicine Grand Rounds**

00:32:43uh Dr. Nicholas, I'm not sure. If your slides are advancing, you may want to try. Just re-sharing. Them

**Alexander Niculescu**

00:32:59Let me see if because I have two screens over here. Maybe I'm being overly technological

- 00:33:06uh which slide. Do you see over there, guys
- 00:33:10the one with the

**UVA Medicine Grand Rounds**

00:33:16And now you're moving through a couple of them.

**Unknown Speaker**

00:33:19Okay,

**UVA Medicine Grand Rounds**

00:33:20Do you see the one with four steps now? So now we just see a blank screen, but it still looks like a Powerpoint slide

- 00:33:33there. Okay, mapping the genomic landscape. That's where we're on now.

**Alexander Niculescu**

00:33:39Okay, I got it

**UVA Medicine Grand Rounds**

00:33:42perfect,

**Alexander Niculescu**

00:33:46all right. So that's what I was telling you about. The steps that we take to discover that and validate the biomarkers. And so we We've done this Um. This studies and publish papers on mood disorders suicide on pain on

- 00:33:59um, stress, on memory, on longevity. And really, you know, our blood tests are like liquid biases. Essentially what's being done now in cancer. You might have heard how Grail Foundation medicine, you know other one hundred and fifty
- 00:34:14companies like that uh try to develop liquid biopsy. So you don't actually have to biopsy the tumor, or you can sort of look in the blood early on before things um for full-blown tumors in that case, or full-blown clinical illness um
- 00:34:28in our cases.
- 00:34:30So we've developed these liquid vibes for these indications, and we also have now studies under review for anxiety disorders and for schizophrenia.
- 00:34:39And these biomarkers really help you with um
- 00:34:43having some something, for you know, an objective assessment. Um, you can
- 00:34:50calculate the score based on those panels uh something to um the treatment uh you can match people to actual existing medications based on the
- 00:35:01markers in the panel. You can monitor response to treatment, and they also from a window into the biology of the disorder. So those are the three main um, you know, uh utilities of uh buyer martyrs
- 00:35:16and um, you know this is sort of a very simplified uh, again, part of calculations for dummies, and in that category I include myself, I mean, we have very sophisticated um biased statisticians, and so on in on our teams, on our grants and our projects. But I always sort of
- 00:35:34like to ask them. You know what's the bottom line. Not maybe this may be that, and so on. So um for genetic studies like you've seen, you know, published, and in the headlines all the time, or does those large genome, wide associations? That is you?
- 00:35:48Um, You need very large cohorts, because, you know, if you do case control type design, so hundreds of thousands of people for gene expression studies where you look at Rna, you know the level of activity of our gene as we do in the blood for the markers that we've identified.
- 00:36:05You need the much fewer subjects, and when you have these within subject design where you follow people over time you need the
- 00:36:13these are the most powerful. And so there you need dozens of subjects to discover things.
- 00:36:18Um, those things are very hard to do, because you need to follow people over many years and catch them when they're severely symptomatic when they're not, and so on, and build cohorts like that. Nobody wants to put that time. They all want to have large data sets from other people and do the studies. So
- 00:36:36um
- 00:36:40and um, one of the reasons. Um, we were interested in paying was um, you know we're on this mission of
- 00:36:49identifying who's at risk for suicide preventing um it
- 00:36:55intervening early to do that, and um one of the three main upstream drivers of suicide. Besides, you know, mood disorders. Besides, stress disorders is pain, untreated, pain unmanaged thing.
- 00:37:11And um.
- 00:37:12We lose a lot of people to suicide daily. Today's veterans Day we lose over twenty veterans every day to suicide.
- 00:37:21Um!

- 00:37:22It's it's a it's a issue in which
- 00:37:25um in terms of clinical practice, we haven't made that dent, although we're making a dent um a lot in terms of research nowadays. So the next step is translating all these things. Um, making them easy for our clinicians to understand and apply.
- 00:37:42So a pain is, you know, something that can severely affect quality of life, as you know from your patients from your own lives. If you got, you know
- 00:37:53I hurt doing sports or other things. Um, It's a What if you suffer from a cute severe pain, or from a chronic pain, to sort of severely affect the quality of your life, Your ability to do things,
- 00:38:05and can drive some people to depression, um substance, abuse, and suicide.
- 00:38:11Um. So
- 00:38:14it took us about four years to actually, from conception to publication of the study to discover paying biomarkers. So in general for those of you who are doing research, or might do some research, you know, or see other people sort of get headlines.
- 00:38:30You only see the tip of the iceberg like a nice published study. There's some media uh university. Does the press release Everybody, you know is, uh, you know, happy, et cetera. But
- 00:38:41you know there are years of work. The actual iceberg is years of work, of trial and error of um, you know, putting in long hours of things that don't work, and you try different approaches, finding the right approach, then reproducing things, and so on.
- 00:38:59Um, it's a work well journey, and uh, you know no pain, no gain, no pun intended.
- 00:39:06All right. So our approach is, uh, So this is actual data from the pain biomarker study. Um that um received a lot of attention when it was published over two, three years ago. And um
- 00:39:20that. Um!
- 00:39:22It's probably the reason why I was invited to present here.
- 00:39:27We're nine. The process of doing a fall off Study on this, where the results from the past have held is not yet under review, and we've expanded. But at that time when we publish this, if you see these trajectories over here. These are actual patients, and blue are low-paying visits, red high pain visits, and we follow them
- 00:39:47over years. And sampled got blood samples when they were in different states, and so they sort of whole um Genome Rna, sequencing to find what was changed. Um! And in those different states, and conducted some sort of sophisticated
- 00:40:05by formatic analysis to discover candidate biomarkers, then prioritize them, using our literature databases,
- 00:40:14you know, kind of a Google page rank
- 00:40:17algorithm approach where the more evidence for a marker. The hired r is up on the list just like when you do a website. The more links to a page, the how it comes up in your search, and then uh, we validated them in people who had um uh pain disorders uh to have severe um
- 00:40:35self ratings for pain, and also had
- 00:40:38severe self ratings for impairment in terms of quality of life.
- 00:40:43And these are, uh
- 00:40:46examples of uh, two of the top biomarkers that we we've identified, and I'll mention a little bit about their biology. So here in this histogram, when you see uh blue, it's uh, you know it's levels of the market, and people who have low pain, red low levels of the market and people have high pain.
- 00:41:05And uh, lack is our Uh levels of democracy people have severe pain, so you can see this sort of nice step-wise decrease. This is a
- 00:41:15um decreased by a marker. Um!
- 00:41:19It's almost like a pain, suppressor gene. So when it's decreased in expression, then time can
- 00:41:25explode.
- 00:41:27Um.

- 00:41:28Both of these are sort of decreased in expression in pain. So these are molecules that probably when they are expressed higher, or if you increase them with treatments uh
- 00:41:39keep being so suppressed, and when
- 00:41:42um they are decreased in expression, you have high levels of pain,
- 00:41:47like in cancer where you have, you know, oncogenes tumor suppressaging. So this would be that you want to of a tumor, suppressor gene or a pain suppressor team. And, in fact, we use a lot of the tools and um
- 00:42:00mindset from cancer. And this types of study, not only liquid by. But how we do the genomics, how we try to be precise about phenotype, how we
- 00:42:09approach things. And my um! That work that's Chris was actually in cancer, molecular genetic. So we've when we move to the brain and so on. We brought a lot of those um understandings and approaches into this field.
- 00:42:25So um. So at the end of you know, doing those four steps, discovery, prioritization, validation, testing you identify a number of biomarkers, and then you see an independent cohort of of patients. How good are they at predicting who's in pain, self-reported pain
- 00:42:43and these are patients that were not used as part of the studies.
- 00:42:47So
- 00:42:56um, so on the y-axis you can see area under the curve. That's a measure of um predictive ability of a marker to hire the
- 00:43:05value, the higher the predictive ability uh point five is, you know, uh flip of the coin. Uh and uh, you know above that point six point seven point eight point nine. It's a hypothetical ability.
- 00:43:19So when we look in in all the patients in this independent call Court, there are some markers that, like contacting one depicted here, and so on. They can predict uh pain, state of the individual, but the with modest uh, you know,
- 00:43:35point six point seven A. You see. So sixty, roughly speaking, sixty, seventy percent. The accuracy.
- 00:43:41When you go by gender, uh, you start having better results, particularly in females. So, consistently not just in the pain by mark or but in other studies and in depression and stress and suicide
- 00:43:55we always get stronger results in females. Um. We're not sure. Why that is, It could be that, uh, in the initial discovery work uh uh females are more
- 00:44:06um accurate that self-reporting how they feel. So then their initial discovery is that they' to that more accurate self-report. They are more in touch with how they feel with their feelings, et cetera. Also Um,
- 00:44:19the uh brain. Immune interaction is much stronger, and uh and females, females have two X chromosomes, and that the a lot of the immune um genes are on the X chromosome.
- 00:44:32The brain immune interactions are stronger. So that's probably why uh females have more um autoimmune disorders. Immunological uh reactions are stronger, and so on. And we see this on in our biomarker work. The bomb marker and uh females are stronger. Um! And
- 00:44:51when you go personalized by gender and diagnosis, and these are people with different uh males, and you know, with different mood disorders. You see um
- 00:45:02very strong predictive ability. So here, for example, These are females with a bipolar disorder which is a severe mood. Disorder, Manic depression used to be called, and you see strong markers for pain.
- 00:45:15So, with the personalized approach by gender, gender and diagnosis, you get stronger results.
- 00:45:22Uh, we also looked at our ability to predict future um er visits for pain. And uh
- 00:45:29here on the y-axis our odds ratio. You know
- 00:45:33how much more likely are you to come to the er for pain related reasons. And

- 00:45:40Um! You know you have, you know, odds ratios of two, four, six, you know, sometimes very high ratio, much harder than in genetics, so you know. Now it's ratio of two is
- 00:45:52now, roughly speaking, some of these twice as likely to come to the er for a pain visit in the year ahead compared to somebody who
- 00:46:01doesn't have that mark or elevated.
- 00:46:04So again, you know, there are some markers that are show increased uh risk of somebody coming to the they are, and probably developing sort of a more chronic pain Condition and um
- 00:46:15uh, when you personalize by
- 00:46:18gender and by diagnosis, you have very strong results, particularly, for example here, female Mdb. Females with depression. You have very high odds ratio for some of these markers,
- 00:46:29and so on.
- 00:46:31Um, and that's sort of a blow up of the previous slide. I want to spend time on this. Okay? So what what about some? What's the biology of some of these markers? And we started, you know, agnostic, you know we started with the whole genome. We sequence the Rna expression from
- 00:46:48all of the genes in the body, in the body, in the blood, and then we sort of narrow down the list to our studies.
- 00:46:55We didn't have a hypothesis as well. We think that maybe this or that um gene is involved in pain so agnostic no
- 00:47:04um prior hypothesis discovery based.
- 00:47:08So one of the top biomarkers we identified as this gene uh mfap three micro fibril associate protein three. It's a
- 00:47:17new, not implicated in pain before our work. Um! It's a decreased in expression and blood in high pain States. So it's a gene that we think is protective for pain when the levels are decreased and can manifest more.
- 00:47:31Um. Very interestingly, it's also Gen. That's decreasing expression in blood in our suicide studies. So that shows the sort of
- 00:47:40comorbid that the same gene, protective gene is decreased in expression and pain decreased in expression and suicide, and then bad things happen.
- 00:47:51Um,
- 00:47:52there's other evidence for it. It's a very interesting Um,
- 00:47:56you know. Um gene in animal models of chronic stress. It is decreased in expression,
- 00:48:02just like we seen our patients in pain decreased in expression and suicide, the Christian expression and animal models of chronic stress. It's decreasing expression in the brain. There we can look in the brain
- 00:48:13in Amygdala, which is a region of the brain that's involved in the
- 00:48:18sort of um
- 00:48:19Anxiety Emotional response to bad things happening
- 00:48:25um in human post-mortem brain studies, colleagues of us have found this stream to be decreasing expression in the prefrontal cortex in alcoholics
- 00:48:35and um another independent study, somebody. Some other group reported that Um,
- 00:48:43if this gene is increased in expression or normalized in expression,
- 00:48:49uh it improves sleep.
- 00:48:51So you have all these sort of phenotypes that where the gene sort of does the same thing, the expression of the gen is decreased in pain.
- 00:49:01Um, it's decreased in stress. Increase in alcoholism. Um
- 00:49:09um! The decrease is associated with poor sleep

- 00:49:13erez agmoni very very similar to sort of the comorbidities we see in our patients or people present with, You know, chronic pain issues. They might have some so subtle aviation. They drink one hundred and fifty.
- 00:49:24They don't sleep well. They are stressed out.
- 00:49:27All these phenotypes are underlined by this team.
- 00:49:32We've looked at some of the networks involved. I won't bore you with that. This is sort of like
- 00:49:39there are these stuff. But um,
- 00:49:42they're very interesting uh pathways. They can be targeted um for um,

**Unknown Speaker**

00:49:48you know

**Alexander Niculescu**

00:49:49for treatments, and also in terms of understanding. So one of the main um gene networks involved in time has to do with immune response,

- 00:49:57and we have some biomarkers, some of our top biomarkers map to that. Another one has to do with the growth and trafficity uh, and another one has to do with um
- 00:50:10connectivity and signaling.
- 00:50:12So it could be that you have, you know, different types of pain, not only sort of um in terms of clinical presentation, but biologically, and um that um
- 00:50:25one size does not fit all you need to stratify people based on their clinical symptoms and on their molecular markers, and treat them appropriately.
- 00:50:36So this issue of comorbidity with suicide that I mentioned, you know. Uh again, you know, when we looked at our top biomarkers, the top sixty
- 00:50:45um genes that we identified as being involved in pain.
- 00:50:49Um! Over eighty of them also had popped up in our suicide studies suggesting this very extensive comorbidity. So
- 00:50:59be careful with your pain, patients. If somebody comes to you for treatment of pain, always ask about to sidel aviation. Do they have any family history of suicide, attempts, or suicide completion, always ask for that. Offer them resources in case they go through a crisis like
- 00:51:17nowadays the nine, eight, eight, number, and so on.
- 00:51:20Um, it's a very high comorbidity between pain and suicide.
- 00:51:27It could be that there's sort of this almost like psychic pain component that occurs in a depression and in other things that goes along with the physical thing.
- 00:51:38So one of the useful things for clinicians that you can do with these tests are that based on which markers are changing your patient. You can match them to different medications.
- 00:51:51Um. For pharma companies. You can use this uh markers to identify which drugs off the shelf could be repurposed for
- 00:52:02treating pain disorder. So we did this exercise in our paper,
- 00:52:07and we asked, You know, out of our top markers uh what
- 00:52:12drugs that they match that are currently
- 00:52:15drugs are an adjusted disc that are currently approved in use for other indications,
- 00:52:21and um.
- 00:52:23Our top hit was actually an unsaid non-steroid al anti inflammatory that's not uh um
- 00:52:31in using the us it wasn't used in Europe. Um, and um

- 00:52:36which is sort of interesting, like a positive control. Your top it wasn't and say Then again, you know, I started with that whole genome end up with the small list of markers, and your top hit is on, and so you must be doing something right.
- 00:52:49Uh, there were a lot of nutritional, so
- 00:52:54which is a form of vitamin. B. Six came as a top. Hit um
- 00:53:00um
- 00:53:02vitamin b Twelve came as a top hit, so uh it might behoove you to supplement your patients with V. Six P. Twelve Uh. If they have ner up at your other chronic pain. Conditions um
- 00:53:14and um a mocks have been a tricyclic anti-depress, and was a hit. This is sort of an older one that started that's been neglected surprising hit for Hal, though for Haloperidal, um you know.
- 00:53:28Uh, but it's very interesting that patients with schizophrenia have very little pain. They They have an abnormally low perception of pain. So how much of that is them being actually treated with and type of psychotics, we don't know, but it's an interesting question.
- 00:53:43Um!
- 00:53:47So you know, these markers can be used in clinical trials as opposed to sort of trying to develop drugs for pain, using things that are using sort of rating skills and patients might or might not be accurate in terms of reporting things. You can use these markers for clinical trials.
- 00:54:05And uh, this is how a report would look for a doctor uh you would get sort of a pain Score Um! Looks like your credit. Report that you pull from your bank right? You get that
- 00:54:18uh a score for severity of paying at that moment in time, and then I can get uh sort of subscribers for sure dumb risk in the year ahead long-term risk of chronic pain, matching with nutriticals uh matching with pharmaceuticals. And this is sort of a
- 00:54:37an actual patient done by the company that I call found in mind that signs. This is the identified here, but this is a report that the company provided to a doctor and um
- 00:54:48What was very interesting to me as a researcher, and to that doctor we got feedback from him was that some of the top hits were non opioids. If you could see here morphine and so on, are at the bottom, so he could use the report as a
- 00:55:02um, you know, as a starting point for a conversation with the patient, how other options like uh
- 00:55:09clocks inhibit their like, you know, motoring uh steroid, and so on, would work better, better, better fit for that patient than going down the opioid route which creates a lot of problem.
- 00:55:21Then there are a lot of neutral vehicles that were, you know, even higher matches than the pharmaceuticals. So Omega three fat, the assets we just anti inflammatory, and so on, should be used more. They have very little downside, unless you have a leading the Z, that they can be used uh
- 00:55:37for allergies to fish there, you know. Great supplements to have your patients on vitamin d was a hit here, and so on.
- 00:55:45So that's the story i'd like to acknowledge. Uh, You know my teams at the High School of Medicine at the Va.
- 00:55:54At Scripts, and I agenda va for funding us over the years.
- 00:55:58And um
- 00:56:00these are my current um
- 00:56:02lab members. Um,
- 00:56:04We have terrific people doing the neurop psychological testing and bio-banking primarily at the Va. Where we follow people all these years, and then at the University of the medical school, we have a strong genomics and buying Formatics group. So thank you very much for your attention, and i'm happy to take a few questions.

00:56:24Thank you.

- 00:56:30Thank you. Dr. Nikolowski. Uh one of the big questions I had you mentioned in uh, on one of your slides, briefly, was just how do you get over this golf between correlation and causation, and it is interesting to see kind of the idea of repurposing

**Alexander Niculescu**

00:56:46um different pharmaceuticals, and maybe that golf has been crossed. Given that, and says, are our top hit in that kind of paradigm. But a lot of this also. Just yeah, that's a great question. So uh short answer is, we don't know I mean. What we do know is that these markers track pain Well, and um, we've established that. That's why we've uh, you know

- 00:57:10we're using them moving forward
- 00:57:13um! But whether that's just correlation, or there's some causation for some of them. Uh we don't know for sure and um
- 00:57:22correlation doesn't mean causation, uh, and that's sort of a common cognitive fallacy. Right? You see it all the time. And in studies published in medicine, where people discover something, you know, taking, you know,
- 00:57:35having blue eyes uh might makes you at higher risk, for I don't know uh something, you know. All sorts of correlations uh establishing causation is very, very hard. Um! In our case it doesn't really matter like as a clinician. I'd like to have something that tracks
- 00:57:53um the disorder that helps me a little bit with the diagnosis, so I don't have to rely just on what the patient is telling me or my clinical impression they can monitor whether my treatments are working that can give some indications what medications I should be using.
- 00:58:07I mean, if you look at Psa. Um, you know um! Is it correlation or causation. If you look at a lot of the markers that we're, we're um having in medicine, you know.
- 00:58:23Is it correlation or causation? These are markers. They help you track the disorder. They're not necessarily all of them, or most of them involved in the biology. But, as you point out, some of them might be, especially when we look at what they do and the brain what they do in the nervous system, how they respond to medication. We think that at least the subset of the markers will be. But
- 00:58:44you know um,
- 00:58:46that's sort of beyond the the point of having something that's useful. It's sort of more understanding biology, and maybe using them as a inroads and targets for new drug development. And there, you know, you could
- 00:59:00in that, you know.
- 00:59:02That's why we do animal studies in animal studies. You can actually distinguish between correlation and causation, because you can knock out the gene or over express it. And then you know, it's more direct, Right? The change in that specific gene led to a changing the biology and the phenotype in humans, it's harder.
- 00:59:19Yeah, very good question. Yeah. Uh. We have a question here from Susannah Keller. Do you know the source that we obviously you do? But what is the source of the biomarkers and the black sample. So these are all from white blood cells, from immune cells. You know red blood cells. Don't have our um
- 00:59:36no close they don't have uh,

**Unknown Speaker**

00:59:39uh.

**Alexander Niculescu**

00:59:41So these are from

- 00:59:43white blood cells, from immune cells, and these are genes from those cells that get change in expression.
- 00:59:50Um,
- 00:59:51we've from the beginning. We, instead of sort of uh doing, you know. Uh phi call gradient, separating the immune cells, centrifuging them, and just having, you know the immune cells that we looked at. We want that from the beginning to see. Can we detect signal that's
- 01:00:06reliable, reproducible, and whole blood, so that we make it easy in the clinic, you know, in any clinic anywhere, you know, in Indianapolis.
- 01:00:14Um, you know, at your school. Um,
- 01:00:18my will be anywhere you could do it. You don't need sort of to go to all those steps of uh, you know, isolating by blood sales, extract and things just whole blood. So we've used from the beginning that tube
- 01:00:31to this day. We use it. That's you know It's called a packaging to you, because from then into the tube. It's immediately stabilized the Rna. You don't have to do any centrifugation. It's stable at room temperature for up to a couple of days,
- 01:00:44and uh we. We've looked at sort of gene expression in that Rna from the whole blood, but the you know, in all likelihood the sources from certain wildlife cells, and you know, in the early days we got questions from um
- 01:00:58from colleagues when we submitted our studies, and so on. Well, which one black cell it comes from, and so on. And now tell them, Look, we don't really care, you know i'm not the hematologist. That's sort of an academic question. It doesn't matter to us where they come from, as long as they are markets that reliably track,
- 01:01:15and the phenotype that are predictive, and so on. Um! So
- 01:01:20uh, now they we don't think they come from their brain. I don't think they leak from the brain into the blood. There are some disorders where you can have leakage from the brain of certain things into the blood. You can have exos on some feed vesicles with Rna can have, like leakage of traumatic brain injuries of certain proteins and Rnas. But, uh, in the majority of disorders that we studied exactly these changes in gene expression in the blood that we're measuring. And uh,
- 01:01:49um, these are not things that lead from the brain, and
- 01:01:53that's that's our understanding of where we're at with them.

**UVA Medicine Grand Rounds**

01:01:57Okay, that answers your question,

**Alexander Niculescu**

01:02:02What about so Swati? Ralph wanted to know kind of about implementation of some of this, so asking a question about costs of some of these tests, and maybe what your vision for getting this into the hands of physicians, and how they work. Yeah. So you know the company that I disclose that I’m a

co-founder of mindex sciences. Um has done this year an early access program, where certain physicians early adopters can order the desk for their patients, and so on.

- 01:02:30This is very expensive technology, as you can imagine. So, um. The tests are not currently reimbursed by Medicare insurance companies. Patients pay out the pocket for them. That's not optimal. So what um
- 01:02:45we're doing over the next year is to provide the data um to Cms, so that Medicare can reimburse these tests and build those years for insurance companies to, you know, show the clinical
- 01:03:00validate, the clinical utility, and so on, so that the insurance can cover it. So, then more people can afford them. Uh, we're also looking at um developing uh
- 01:03:11cheaper version of the test where we use Pcr on a on a small panel of markers as opposed to doing this sort of whole genome Rna sequencing, which is very expensive.
- 01:03:22And, uh,
- 01:03:23I I think you know we have to be very mindful that
- 01:03:28in order for these tests to be available and used widely, people should be able to afford them. They should be covered by Medicare by insurance companies, and so on. So that's where the next step is. I mean they are available now, but they are sort of uh
- 01:03:42early access program,
- 01:03:45you know, uh self paid type of thing which is so expensive, and that's not optimal. So we're working very hard on
- 01:03:53um. My group is working hard on making them, you know, on a Pcr platform which is less expensive, and our colleagues that mindex are working on insurance coverage to get this uh set of more widely.

**UVA Medicine Grand Rounds**

01:04:06Yeah, Great

**Alexander Niculescu**

01:04:07I you. You certainly have a interested listening listener here in Susannah Keller. Uh? She asked another question. Do you consider looking at changes in uh metabolites, peptides, hormones, instead of that. That's a great question. So over all these years, and you see, you know those uh freezers there. We've banked uh um

- 01:04:28plasma, c. Room for metabolomics for predominance.
- 01:04:32We've never done those studies yet. It's much easier with our approach, our convergent functional genomics approach and you know that the technology to go for gene expression for Rna. That's kind of the sweet spot between genes and environment. Genes the N. I. The environment come together, and you have
- 01:04:50gene expression changes. So we went for that. It can be done whole genome precisely relatively, inexpensively, and so on. Uh, you know it's hard to the discovery on the whole protein. Uh, it's hard to do discovery on the home at tab alone.
- 01:05:05Now, now that we've narrowed down the list of markers to well validate the panel. Uh, I think you know we can, and we are active in the process of looking at some of those markers some of the very best markers at the protein level,
- 01:05:19because then you could potentially take, you know your top one or two markers and make them into an Eliza that can be done. Point of care in a doctor's office in a er setting, just like a stre

throat or a Covid test, or something, so that you know you have the result there. Uh, within, you know, fifteen minutes, thirty minutes an hour as opposed to this being set up like an on college. Your lab send out and you get the result.

- 01:05:44Yeah,

**UVA Medicine Grand Rounds**

01:05:46you got a question coming up from the audience,

- 01:05:49is it? We got to turn it out.

**Alexander Niculescu**

01:05:53Thank you. Dr. Nicolasco. Um. One question I had is, Is there any sense of the correlation on the timeframe between changes and symptomatology and changes in expression levels of these um rnas.

- 01:06:08We don't know exactly um how we know that gene expression changes within hours, certainly within that day or so on. But, um,
- 01:06:18please,
- 01:06:20you know
- 01:06:21the question is, are the markers still on when somebody is not feeling pain? And we've had examples of this in our studies, and when we did our clinical validation. For, uh, you know, for clear lab approval of those tests where there were patients that were on self report of pain that have very low pain.
- 01:06:41But the markers, the score from the markers was high, so we were intrigued. Why, that discrepancy, and those are instances where the patient, you know, took their Viking or something an hour to before the testing. They were not feeling the pain anymore, so they mark their pain as low, but the markers were still changed. Uh
- 01:07:01and um, we had to develop a clinical context core uh to try to sort of look at this discrepancy and the clinical context core, you know, for the you know, having a pain diagnosis being on pain, medications having er visits, and so on. That composite score
- 01:07:19uh correlate that very well with our tests. But the self report sometimes doesn't correlate with our test, because the patients might have taken a medication that kicked in, and they are not feeling stressed or anxious, or depressed, or in pain at that time. But the biomarkers are still turned on.
- 01:07:35So there's a lag between. You know how you feel at that moment in time, and the biomarkers, the biomarkers may have also more like a more chronic or trade like component um, for you know, having that disorder being at risk, they may change more slowly than your self perception

**UVA Medicine Grand Rounds**

01:07:56great. I think you kind of mentioned this towards the end. But Swati Row also was wondering about discordant results. Um, but I guess your clinical contact score might kind of approach that in that way.

**Alexander Niculescu**

01:08:11and you know, when we looked at the sensitivity of specificity of the test, it was significantly um better than we thought when we took look at the clinical context score into, you know, when we looked at that to that into account.

- 01:08:25So and that sort of uh fits some of the feedback early feedback that we're getting from some of the doctors that have started using this test. And again, it's very small scale early days early access program, but they feel it's it fits what the patient has. Um, you know more broadly, even in the instances where it doesn't exactly correlate with how that patient felt at that moment in time.
- 01:08:48So again it might be more like a sort of a hemoglobin, a one, c. Right, some more of a kind of a trade thing as opposed to a glucose That varies very fast.

**UVA Medicine Grand Rounds**

01:08:59Yeah, correct. Um. I don't think there's any more questions from the audience here. I'll just look at the chat one more time.

- 01:09:09But um no, that was great.

**Alexander Niculescu**

01:09:11Thank you, thank you very much. Pleasure being with you, I heard Uva is a beautiful place it is, it's raining today, but it is beautiful. Yeah, I think my youngest daughter is looking to apply there for college. She heard good things about it. So I might visit at some point. Thank you very much. Please share my email address. If people have additional questions or thoughts or suggestions for us love to hear from you. Learn from you. So please send me an email.

- 01:09:38Thank you very much. Yep. Thank you. Bye,