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TRANSCRIPT - GR 07 07 25 **"Extracellular Vesicles in the Cardiovascular Kidney Metabolic Syndrome"** guest speaker Uta Erdbruegger MD, University of Virginia

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Internal Medicine Grand Rounds

- All right. Everyone welcome to medical grand rounds. We are excited to have Dr. Ed. Brueger here to speak with us about extracellular vesicles and the cardiovascular kidney metabolic syndrome. I'll take us through our Cme accreditation slides our objectives for the talk today and for faculty claiming Cme credit today's activity code and the multiple ways in which you can apply for credit. And now, Chief Resident, Dr. Shaina Hassan, will introduce our speaker.
- All right. Good afternoon, everyone. It's my pleasure to introduce our grand round speaker for today. Dr. Uda Ardberger, originally from Germany, Dr. Ardberger obtained her medical degree from the free University of Berlin, and then came to the Us. To complete Residency training in Internal Medicine at Tulane University, followed by a nephrology fellowship at the University of North Carolina. At Chapel Hill.
- She has dedicated her career to translational research with particular interest in the role of extracellular vesicles in vascular and renal disorders, regarding them as novel biomarkers and bioactivators in kidney disease and high blood pressure. And because of their small size and heterogeneous nature, her lab's second research focus is the optimization of ev isolation and characterization in serum and urine.
- On a personal note. Dr. Erdberger is married to Dr. Vieder, one of our transplant pulmonologists, and the couple has Triplet boys together. They enjoy exploring the outdoors, biking, hiking, and visiting big cities. We're very excited to host her for grand rounds today, so please join me in welcoming Dr. Erdberger.
- Let me get started here.
- Can you all hear me? Well, thank you. Thank you very much for the kind introduction I'm now here for over 16 years, and I have really enjoyed working with everyone here. So I want to say, Thank you. Here at the beginning of my talk. And today I hope I'm going to introduce you to some novel communicators in our body called extracellular vesicles. So let's see.
- I don't have any conflict of interest here.
- So let's start with this slide. How do our cells communicate? And I got this actually, from a Khan Academy slideshow? So this is what our kids are taught. So we have a cell which is sending a molecule, a ligand, and then we have a receptor and a target cell, and if they bond. We have a response, and if there's non-target cell, we have no receptor for ligands.
- And you. We have one. Very. We have one birthday party last year for insulin. It's over 100 years old. So so we know about a lot of these communicators in our system from the endocrine, metabolic, neuronal immune systems.
- But today I will introduce you to novel communicators in biology, which are extracellular vesicles. It's actually a generic term. These are particles released naturally from all cells in the body. Have a lipid bilayer, and don't replicate and have

various cargo, and I will go over that. And during the last 2 or 3 decades. Research on this is really exponentially growing. So that will be one topic. I will introduce you to that. Then I will introduce you to a new maybe old concept of the cardiovascular kidney, metabolic syndrome. So you are more familiar with cardiometabolic syndrome. But I will talk how the kidney got into this syndrome and then I will merge these 2 topics and provide some. I call them, instead of a clinical vignette. I give you scientific vignettes examples little stories about these vesicles in the cardiovascular kidney, metabolic syndrome. In regards to obesity, hypertension, cardiorenal syndrome.

- So let's start with extracellular vesicles. I already told you they are seen as novel, intercellular interorgan communicators. It's kind of a new dimension in biology and in the forties they were seen as platelet dust as trash, and now they are more seen as treasures. And so they have. Really, there's an evolution going on.
- And they are these small particles. They have different names. You might read in the literature exosomes, microvesicles, microparticles. I will use this generic term because they're very heterogeneous and the challenge is that they are very small. And so you need special instruments to detect them, and to understand that I have here 2 examples. This is working here very well, so they have the size of the lipids on protein complexes in our blood, so it's not so easy to differentiate them. They also have the size of viruses and bacteria. So so these are novel novel messengers which have been overseen for for decades.
- So where do they come from? There are 2 pathways. How they are generated. One is the blabbing process where you have a reorganization of the phospholipid membrane. And you see these little blabs coming. Oh, no sorry little blabs coming off here and then there is an endosome pathway. Some go in, the Lysosomes are degraded, others end up in the multivesicular body and and are excreted, and to the right you have 1st of all a beautiful scanning em picture to show the blabbing. And here you see one of the 1st images with cryom, with transferin is kind of pushed out through the extracellular vesicles, through the multivesicular body, into the extracellular space.
- However, there are many more pathways, and this is from a nice review, showing all the different types of forms we have. So we're learning more and more, and they are submicrome. The most range tested is between 50 and a thousand nanometers, but we have smaller ones, and some larger ones, too and they are very heterogeneous. They have physical and chemical heterogeneity. So seeing is believing we did cry oem pictures. After that people started to believe in even here at Uva. And then, oh, what are you doing there? These do exist, and they're very heterogeneous in size, in density, so likely cargo in shape.
- So this is from beautiful cry-em pictures of Urinary Evs. And my colleague, Luca Musanto, produced them, and rigorously characterized urinary Evs and urine. As a kidney doctor. I'm very much interested in those. So what are their roles in physiology and pathology? You can also say, health and disease. They're very unique, and people are fascinated because they're used as biomarkers because they can be used for diagnostic and prognostic purposes.
- But also what's very interesting is they might have, and they might have. They have a functional and mechanistic role. They are the messengers between cells and organs.
- And what's the industry gets excited. They have a regenerative and therapeutic role. And that's actually fascinating, coming from stem cells. And you know, instead of

using stem cells with all the side effects. You use these evs from the secretome of the stem cells. All organs, all cells, produce them. They are found, and right now studied in all biofluids.

- But the initial focus was in immunology, actually, in the eighties. They started to see them as Antigen, presenting vesicles or particles. Cancer is studying them in metastasis, in neurology they can cross the blood brain barrier because they are so small and then therapy.
- And I'm not a market analyst. But when you look it up, it's pretty impressive. Their growth market growth is 30 to 40% over the next years. It's going to be a billion dollar market for these ev research products. More is on therapy. But about 15% is in biomarker development.
- Rigorous analysis is really needed in our labs, and it relies on many different technologies. So I have introduced this over the last 16 years. We have particle analyzers to identify size and count them. We visualize them with cryom super resolution imaging. You see this down here, beautiful images and then single ev analysis I started with early on with flow cytometry. Then you have the bulk analysis of plain old Western blood and all the Omics you can imagine proteomics, metabolomics, transcriptomics. Fortunately, we, the societies, especially from the International Society of Extracellular physically have a lot of recommendations and standardization to bring this field forward and have rigorous analysis. You know you need to know what you are studying. I told you they are very small. Are you studying a V or studying a protein? So you need to be really, really rigorous here.
- So now that was number one. Now I'm talking about the cardiovascular kidney, metabolic syndrome, and then I will merge the 2 topics. So you know, we are all familiar with cardiometabolic syndrome. But about a year ago I was at the American heart, and there was a Presidential advisory and introduced this cardiovascular kidney, metabolic syndrome, and you can imagine as a kidney doctor. I'm pretty happy that it has such a prominent role and, as you know, we are dealing with an epidemic. You can name it obesity, metabolic syndrome diabetes. And as a kidney doctor, diabetes is the number one reason why we see our patients on dialysis, and when you have diabetes you double or triple your cardiovascular risk. So in in many ways.
- So you know, we have this high burden of cardiovascular disease in the population. I'm on service right now. I see it all the time. Right now. We have so many bad diabetes, complication, and sepsis. So we need to improve the cardiovascular kidney metabolic health and related outcomes. And there is a critical need actually to better define this syndrome to find an approach to cardiovascular kidney metabolic staging that promotes prevention. So we don't want to start at the end. We we need to look back at the beginning, find prediction algorithm and also find strategies to for the prevention and management and and include the kidney more. So make it more more obvious.
- So they are very short. There are these 5 stages 0, no Ckm risk factors. Then you have stage one excess or dysfunctional adiposity, and then stage 2. You have all the metabolic risk factors or moderate to high risk chronic kidney disease. It's all silent diseases. And then you start with the subclinical cardiovascular diseases. And and finally, the clinical and progressive diseases.
- So how do we measure. Actually, this increased cardiovascular risk. I'm coming more and more. Yeah, I'm becoming more and more interested in that. And you might remember the latest American heart hypertension guidelines came out in

2017. Actually, we'll have the newest one coming this year, and at that time they recommended this atherosclerotic cardiovascular disease risk calculator. If it was more than 10, you should treat your patients in the early stages of hypertension and more recent, I think the prevent online calculator was added, and it includes sex, age, cholesterol, smoking medication, even the Zip code. So the social health determinants are becoming also very, very important. I found that quite clever to include the Zip code and there is a calcium risk course. But if you think about it, this is already late disease, you know there is already calcification. There is already disease, so I don't know who of you uses all these calculators.

- I know the cardiologists like to use the calcium risk scores, and then I think it's time for novel biomarkers. I mean, we're all looking for it, but which one which can help us, you know, to find early disease, and these are a few studies which make me think, what are we missing here? So I mean, the 1st one is an old study, but it's repeated that diabetes has 2 to threefold risk for cardiovascular disease, but only 40 50% is actually accounted for by the classics, hyperglycemia, hypertension. And then really recent, it just popped up. In the last couple of months there was a large nationwide registry study from Denmark. They looked at 150,000 people diabetics, and then they looked at, I think, 3 or 400,000 controls matched controls, and they found a twofold more cardiovascular event decades before type 2 diagnosis. So they have risk factors already decades before they go on this path. And then this one I found the 3rd one I found also interesting. Being in shape is better than being thin.
- So this is a large, a very comprehensive study to study the relationship between aerobic fitness and longevity. So you know, obesity is not bad, it's just. There are other factors, fitness, etc. So what are we missing here? Sorry I'm repeating myself. So we have these traditional risk factors, and then we have non-traditional risk factors, including also sleep disorders and gut microbiota. That's getting excited. Diet style, psychological factors, vitamin d deficiency etc. But other circulating factors. I was talking about proteins, rna and metabolites so, and this is now a really exciting time. And I bet you have had these talks about all the new drugs we have. It's like a new toolbox after decades of limited options. So I really like this slide, though I have to renew it. It's quite now a little old. But you see that decades long we didn't have a lot of options. And now we have all these drugs beside Ace inhibitor we can utilize but we are still learning how to use them. And we're using them in patients who have disease. So I think we need to understand the cardiovascular risk much sooner that we can intervene sooner.
- So what do we need to tackle these challenges? I mentioned it better biomarkers for defining disease stages also for the Ckms syndrome. Early detection before the end organ damage happens. You know, microarpineuria is our end. Organ damage left ventricular hypertrophy is all end organ damage personalization of care, you know not. Everyone reacts to each drug. Actually, not everyone can even afford every drug. And people have side effects. We are seeing all these side effects. Now, we need novel targets. We need to continue, understand unknown mechanism. We need novel, more targeted treatment tools. And we need more funding.
- So my proposal is that extracellular vesicles are potential novel messengers overseen. So far, I'm not saying they're the only ones. But this is not my research. Interest. So you well, sorry you you will hear about them today.

- So let's start with the scientific vignettes, and I'm going to talk about the different roles in obesity and insulin resistance. We have some work, and then some work. I started actually with my work started in hypertension and look at blood Evs and urinary Evs, and then talk about the cardiovenal syndrome.
- And before I do that, one more word about the cargo, so you have these vesicles floating around billions. I mean, we have trillion cells. You can imagine how many vesicles we have. And so these vesicles have. Their cargo depends really on their contacts, on their disease and on the stimulus. And you see here nicely that all these cells have different cargo and then have different functions. So it's almost overwhelming here.
- So some people approach the cargo analysis with a bulk analysis. They take all the evs, isolate them, and then put them together and do proteomics, transcriptomics, metabolomics. You name it. And this is a study looking at proteomics and phosphoproteomics, of circulating evs in diabetes, and shows insight in diabetes, pathobiology, by defining signatures of these genes of these proteins, and you have here the normal glucose tolerance really differentiates from the Prediabetes. And then the diabetes. So this work is evolving. You know, these are thousands of proteins which are analyzed.
- The same happens with Rna, so the other one was proteomics in the blood. This is now Mrna analysis in the urine. So a lot of small rna, including microrna tns, I mean a whole range of small rnas. But Mrnas are also found. The messenger Rnas are also found beside the non-coding ones.
- And this is a work from a Diabetes group in Finland, and they identified 6 signature, Mrna Genes, which correlated with diabetes, outcome, and they could deconvolute the data and and say that most of these came from the tubular proximal tubular cells.
- But they are now in the process of validating so, and beside bulk analysis, you can also do single ev analysis, either by flow, cytometry, just particle tracking and super resolution. I showed you those images. I have done a lot of flow cytometry here, with the help of the flow core. And the beauty is that these Evs, they have an origin. Okay. So in comparison to other proteins floating around, you don't know where they're coming from.
- But these Evs, when they are shed from their cells, they have cargo from their origin, so you could. Oh, maybe I can identify some coming from the glomerulum and from the tubulum. If I have more glomerular disease, and then I can add on some damage marker. So this is one of the research project I have.
- And I call them disease and site specific biomarkers. And we developed a panel where you combine podocyte and tubular markers. And now there are beat assays. Fabian Bolt is working on that, studying over almost 50 different evs of the immune cells in the urine. So with the beat technology. But it's single ev analysis.
- So there's 1, unfortunately, only one ev biomarker success story. Many candidates you can imagine identified only if you made it in the clinic. It's not easy. This one is in prostate cancer. It's an Xo Dx prostate intelliscore, you know, it's challenging to predict which men are likely to have high-grade prostate cancer when the psa is in the gray zone and they have actually a microrna genomic marker signature, and they call it exosome. So in the industry they use a lot the word exosome, less extracellular vesicles and they found a score, and if you're over a cut point you do a biopsy. If not, you can spare biopsy. But there is still clinical inertia. The doctors don't use it. People don't have knowledge about it. So there are roadblocks, but I

think we will overcome this and So finally, I can start with my 1st scientific vignette evs and insulin sensitivity and vascular function obesity. And you know the adipose tissue is a very active, metabolically active and complex and metabolically active endocrine organ. So you can imagine that a lot of Evs are secreted and they are tested in the blood. But also a lot of studies have been done from tissue culture, from adipose or hepatocytes, or adipose tissue macrophages, mostly rna have been studied, and I have 2 functional in vivo experiments. I'd like to share with you just to tell you how fascinating this work is. They took extracellular vesicles from adipose tissue macrophages, and injected them into lean mice, and they developed and decreased insulin sensitivity. So this is an in vivo experiment in an animal. But of course it has to be shown that this happens in humans as well, and one group actually used a microrna exosomal microrna. This is where the money comes where the people want to use therapy and injected them, and the glucose tolerance improved. So so there are a lot of new avenues for therapy.

- So I worked here. I was lucky to start collaborating with Steve Marlin when he was at Uva. He's an exercise physiologist, and that's when I started here so a long time ago, and we did single Ev analysis of these patients, and he studied in particular obesity and prediabetes, and used exercise as an intervention, and we measured Evs in obese and correlated with the fat mass, and there is a strong correlation, and it's not so you oh what is going on here?
- So it's a marker of obesity. Now, it's a correlative finding so. But others found that as well, and they also dug a little deeper. They found that in particular Endothelly derived and platelet derived. These were associated with obesity. So maybe correlating. Yeah, showing a correlation, but also showing maybe an effect on vascular
- I don't know what's going on. So Natalie Eitner, one of his graduate students put these patients on a treadmill, but before she assessed their fitness and she found that endothelial plater derived evs were higher in obese adults, with poor fitness, and she could differentiate between poor and very poor fitness, so kind of really sensitive biomarkers, and she put them on the treadmill, and she found that endothelial derived Evs. You know that these patients have a lot of endothelial dysfunction and damage. They decreased and were directly correlated with increase of vo. 2. So exercise. You know what it's good. But this is kind of showing you some hints for the Pathophysiology. So again, sensitive marker of fitness, but possibly also the vascular function which is affected.
- So then, when we have patients with obesity, metabolic syndrome, they do an insulin clamp. I don't know who of you does insulin clamps, but to measure the insulin resistance and secretion, it's very standardized. And so they infused insulin, and we measured Evs before and after, and the Evs counts were dropping.
- Natalie had done is actually also with an oral glucose tolerance. Test, you know, all the pregnant women have to do that, and we found a similar phenomenon, and when she did a single bout of exercise this actually potentiated effect, we know that exercise improves insulin sensitivity, and these reductions in insulin mediated Evs, they correlated directly with reductions of vascular stiffness. So there is again this connection with insulin evs and vascular function and this is all descriptive and correlative. So we did one functional test with the help of Brent Isaacson and his team. This was Miss Melissa Luce, and they take mesentery arteries which are the small resistance vessels which are important for your blood pressure regulation, and we injected these evs from metabolic patients into the vessel and expose them

to insulin, and you know insulin is one of the strongest vasodilator brings all the nutrition to our organs, and when you look here now, you see, when you give insulin in a dose response. Yeah you see that the vasodilation increases. This is what's expected when we added healthy Evs. It potentiated this vasodilation. So the healthy Evs were potentiating this but metabolic syndrome. It blunted it. So there's something bad in these vesicles which made the vascular dysfunction.

- So this is all exciting. So we said, What's in these vesicles? And we did an Rna cargo analysis, a transcriptome looking at small Rnas micrornas and we cleaned them up from all the lipids. And we've we got about 20 differentially expressed micrornas and their difference between before and after insulin simulation. And we are currently in the face of validating that and testing these individual microparticles. So I told you, these are incomplete stories. The 1st story is not over yet, so we got a grant, and our overarching hypothesis is that insulin modifies ev physiology cargo to impact the vascular insulin sensitivity. So we have questions like, does insulin alter the cargo and how does exercise affect it?
- How is the vascular function? Can we develop new biomarkers of vascular insulin sensitivity? Earlier biomarkers? So now, can this tell us earlier? The only test. Yeah, we don't have really good tests to understand this vascular dysfunction and then finding new vascular pathways to which we can target.
- So that's scientific vignette number one obesity.
- Now we come to the next one hypertension. So actually, when I came here, I started with hypertension. So why studying extracellular vesicles, you know over 100 million people have it in the Us. One in 3 Americans. To the 2017 guidelines only 50 have controlled blood pressure. You know it. It's a silent disease, and it's really the major modifiable risk factor. You can really accomplish something for your patients and for the kidney.
- But there's still a big debate. What is the correct blood pressure when to start and and how to lower so? And and also we don't have good end organ damage marker the same here in hypertension. It's already late what we measure.
- So and we need this marker to address questions. So that's why I thought about these these vesicles. And you know, hypertension is a multifactorial disease. The immune system is involved. The central nervous system is involved. Vasoactive substances and the kidney. I could give another talk. Why, the kidney is important in hypertension, but early on people, even in 2,003 we have a study. They studied circulating, endothelial extracellular vesicles, because you can imagine there is stress to the endothelium in the high blood pressure damaged vessels, and they release it. And it is there is a correlation with higher endothelial derived evs. And these folks actually focus on endothelial derived Evs with blood pressure and correlated with the degree of blood pressure. So a clinically actually meaningful biomarker, it tells you correlates with your blood pressure grade. And we did that recently with more advanced flow cytometry instrument, where we measure smaller ones. We had 62 patients with obesity undergoing exercise again. And you see how heterogeneous humans are. This is our trouble. You know, we we are dealing with all these people with different genes and different risk factors. And yeah, so, however, here we could also show that the endothelial one correlated with blood pressure.
- So, however, these Evs can also give you hints for pathophysiology and Sabrina Lasavia was a postdoc in my lab, and we did human study, but also animal study, and the most one of the most common used models. Most models for hypertension

is angiotensin. 2 induced hypertension. I saw it a few times this week in the Icu. We use it to bring the blood pressure up. We do it to introduce hypertension to the mice, and Sabrina isolated these Cvs and checked for endothelial leukocyte platelet cells. So all the circulating vessels, and she found that also the leukocyte derived elevated, and in particular the T cells and the T cell derived disease, correlated also with the blood pressure even better than the endothelial derived.

- And then she took the Evs also from the kidneys of these mice, and she could isolate T cell. Sorry. Leukocyte derived evs from the kidneys, and they also correlated.
- This is in line with some studies ongoing. Since the early 2 thousands that the immune system in particular T cells are involved in hypertension. They get activated through Angiotensin Ii and oxidative stress, and they produce cytokines, and they, the T cells lead to sodium and water reabsorption.
- So we are getting hints for pathophysiology doing this.
- So there are a lot of vasoactive factors potentially ev cargo from vasoconstriction to vasodilation. I'm not going through all of them, but you know, nitric oxide as a vasodilator endothelium as a vasoconstrictor and we know that a lot of these are already identified in Evs, like endothelium, nitric oxide function, 81 receptor. So all the players of the Renin-angiotensin system, but a lot of unknown factors. So we still don't know exactly what. So there are more potential for sure. And as a kidney doctor I had the chance to work together with Jin Wei and Rui Sheng Liu from Florida. They do really fascinating micropuncture studies. So you know, we have 1 million glomeruli in each kidney, and they are able to isolate a glomeruli. It's really fascinating with the afferent vessel, and we injected evs from these hypertensive mice into the vessel and measured their luminal diameter. And when you injected these Evs, together with Angiotensin Ii. There was an enhancement of angiotensin. 2. When you injected evs from hypertensive mice compared to controls. And we did that also. In humans, we actually took blood from hypertensive patients and injected it in the mice glomeruli and had the same effect. So the the top is animals. And this is with humans. And here we cleaned up the Evs, you know, made sure it's really pure. Ev. And we have the same effect so quite fascinating. There is an enhancement of Angiotensin 2 with these vesicles in the kidney and Jin Wei. Actually he did an in vivo experiment. We injected the Evs in the mice, opened up the belly, took an ultrasound, measured the blood pressure and blood flow in the kidney and he found that the renal blood flow was reduced after the enhancement you can imagine with the vasoconstriction and the mean arterial pressure increased. So we are wondering how is this happening? You inject these Evs. And then there is an enhancement of Angiotensin 2. Now you know, my 1st slide said, you have a ligand at the receptor. So Angiotensin 2 has an Angiotensin one receptor, and they're actually knockout models. And we got hint from this paper from circulation in 2015. There's a knockout mice of Angiotensin, one receptor, and when you give them Angiotensin 2, they don't rise. Their blood pressure makes sense. Now there's no receptor, but they generated Evs, which contained the Angiotensin one receptor, and they could resuscitate more or less the blood pressure after they gave the Evs.
- So we actually couldn't do that. I didn't get access to these mice, but we studied the activation of at one receptor, and I'm not going to detail. But we on the right. Here we were able to understand that with Angiotensin 2. Sorry with adding the Evs from

Angiotensin, 2 induced mice. We could activate anti one receptor. So we think here the Evs are transferring a receptor so that a function can happen.

- So are you ready for another vinette? Okay so far, I've talked about blood circulating. And now I'm talking about urine, and I think urinary weeks can be storytellers and hopefully seen as they are, not. Hopefully, they are seen as liquid biopsy, because we think they mirror what happens in the kidney? And there is a beautiful study that took kidney.
- They mashed up kidneys from animals and understood their protein. And then they did urinary evs from these mice, and did also proteomics, and it correlated pretty well, you know, so you could argue the proteins you find in the Evs. Reflect what's in the kidney.
- And then to the right. Charles Blade did another cool study. He took ostomy, urine, urine from the ostomy coming directly from the kidney, compared it to urine, which comes the regular way, and compared the proteome, and it was pretty overlapping, so we are pretty sure that most of the Evs in the urine come from the kidney, so they have a potential to be really good biomarkers.
- So I was involved with a friend who's using the dash, diet, dietary, approach to stop hypertension, who in the room uses dash diet?
- Very good. A few. Okay.
- So it started in early 2 thousands. Actually, I interviewed at Duke at that time, and one of the authors is from Duke, and there was all the hype about dash diet. And it's actually they did early on randomized, controlled feeding trials, comparing different dietary patterns including the dash diet on blood pressure level. Initially, it didn't include a low salt, but later it included also a low salt, and this dash diet is rich in fruits, vegetables, low fat had then in this study in 2,001 reduced sodium significantly lowered the blood pressure compared to a typical American diet. And besides the low sodium, it's also rich in potassium. And that's also the trick, and it has other benefits on on lipids, and not only on blood pressure inflammation, so it has a lot of good benefits.
- So Dana did a study in New York. She did an inpatient nutritional study, where she admitted a patient with type, one hypertension, not on medication and transitioned them from American diet to a dash diet, and measured every day the urine, and of course they had high. They had 5 gram of protein and a low potassium, and you can measure in the urine actually, the ratio between potassium with sodium and potassium. And when you switch them on the dash diet the ratio flipped.
- Okay?
- So she gave me the urine. Let's say, measure the Evs. Can they reflect because there are no mechanistic studies? You can't do what I showed you in the animals. You can't do that with humans. So you need to find. And Evs seem to be kind of a good biomarker to reflect what's going on in these channels. So we isolated Evs.
- And you know, in our urine we have a lot of healthy protein. It's called temhosphol, and we cleaned it off because it can interfere with the Evs. But we had still enough Evs. This was done by Luca Musante, who was in my lab, and then we did proteomics, and we identified 1,800 proteins and 22 were upregulated, 25 down, regulated, and we singled out 2 proteins when there was a change from American to Daesh diet. In these 9 patients the sodium chloride abundance increased and the aquaporin decreased. The people actually started to pee so aquaporin goes down they pee more.

- And then here is also visualized that when your blood pressure goes down the Ncc. Went up. So the protein, correlated with the blood pressure response. And the same was true for aquaporin. Aquaporin went down, and the the protein consistent in the Evs also. So we had to validate it. You do that, you know you have to do. Test the phosphorylation of these sodium sodium chloride co-transporters, and we could validate this. And Samantha was sitting here helped this with my carding. They did beautiful Western blots here. And then
- I put this slide into. Tell you again how this happens. This potassium switch. So what happens is that so this is what should happen. And then I tell you how the Evs are reflecting it.
- When you have a high potassium diet. It's kind of down regulates the sodium chloride co-transporter, and that leads to an activation to more sodium upregulate here. Negative charge and potassium is streamed out. So we have Caleuresis, natural rhesis and blood pressure goes down. But if you listen properly in my study, the sodium chloride water went up and not down, they should be down regulated.
- So we think that we need to consider another mechanism. So these vesicles can reflect exactly what's going on in the kidney or the opposite, which might be a waste mechanism. So Ncc. Is down regulated. So the Evs are kind of a shedding mechanism to get rid of these sodium fluoride co-transporters.
- I hope that was clear enough. So some people call it also the potassium switch and it almost works like a diuretic. And actually there is a study where people use Hctz which affects this, and they also found an increase in sodium chloride co-transporters. So we are not the only one finding this. So yes, Evs might reflect, the sodium channel changes ion channels in the kidney and help us to understand how these nutritional changes have an effect.
- So now we come to the final final and exciting clinical. No scientific vignette.
- These Evs, I told you, are intercellular communicators, but also intra organ. Okay? And in the kidney. Actually, they can communicate between the different parts of the kidney. You know, we have the nephron unit, but we have the glomerulum. We have the tubular system. We know these vesicles work intraglomerulum and vasculitis. They have been found to have function, glomerular, tubular, you know, when you have proteinuria? Why do you develop fibrosis. They have shown that these evs from podocytes can induce fibrosis. We have tubular interstitial communication after acute kidney injury. Why do we have more interstitial fibrosis. So evs are also contributing.
- Then we come to this. So this is the intranephron communication, and then we can have the interorgan communication, and we all deal every day this week. Cardiorenal syndrome hepatorenal syndrome, you know. But we have no no good tools. It's Hepatorenal is a diagnosis of exclusion. I mean even on Cardiorenal. Also, we don't know. Is there an additional cardiorenal
- physiology going on? You know our patient had atn? What else is, you know we don't know and we can't. We don't do a biopsy, and the biopsy wouldn't help us. It would just show us the tubular dead cells, but it wouldn't help us to understand the physiology.
- So I'm working together with Susmita Zahu, who works in cardiovascular medicine at Mount Sinai. She's also an ev biologist, and we know each other for a long time, and we wanted to tackle the renal cardiac axis as a kidney doctor. Of course I'm interested in that access. You know. Cardiorenal syndrome is a bi-directional disease. People with heart disease can develop kidney disease.

- People have kidney disease can develop heart disease, and sometimes they are overlapping. You have acute forms, and you have chronic forms.
- But we were actually interested in understanding. How does a kidney communicate with the heart? Why do my patients on dialysis. They all die of cardiovascular complications. And why have they accelerated cardiovascular disease? So we are proposing that circulating evs from chronic kidney disease patients are cardiotoxic we are distinct molecular cargo.
- So Smita, and with the help of cardiologist here at Uva. So Dr. Bergen helped a lot. We collected, and Tusha helped us recently to get some patients with Ckd and heart failure and with and without. So we took patients, samples patients. This is a pilot study Ckd patients between Stage 3 and 5, and with and without heart failure took the Evs. Cleaned them up, and then Zosmita exposed them to cardiomyocytes. I wish we would have that functional test in nephrology, because these cardiomyocytes they can almost you can almost do an ekg, and they move. But she showed that when you add the Evs from the Ckd patient compared to controls, and also compared to the ev free fraction, there was increased apoptosis and cardiotoxicity and she then also did these. I call it the ekg of the myocardial cells. It's called ion optics analysis. It shows the calcium transients and contractility of primary red cardiomyocytes, and she could show that they are really depressed when you use the extracellular vesicles.
- She then also in her team, injected actually Xicheng and Nicole did that. They injected Evs into the heart of mice, and then looked at increased tunnel testing so increase in apoptosis.
- So this is in vitro, showing already that there is some cardiotoxicity. They then developed a mouse model. Because you, you need to bring it to another level to really prove that Evs coming from the kidney are cardiotoxic.
- And you need to convince the reviewers that the Evs are really coming from the kidney. So she took kidneys from Ckd. Model induced with aniline. And because of the time. I'm not showing how effective that was. They had Ckd and developed heart failure, and she took the Evs from these kidneys. You kind of mash them up, and then you isolate the Evs. And she did the same thing, added them to the cardiomyocytes and injected them in the heart, and she found the same. They found the same, finding that these Evs were cardiotoxic and finally, they have done more testing. But we don't have so much time. Actually, this work is in review right now, so you can read it when it's out. But this is actually the most exciting part, you know. We 1st showed that they are cardiotoxic, and they might come from the kidney.
- But what's in the Evs coming from patients with Ckd, so we did transcriptomics again and focused on the analysis of small Rna particular, Mrna, and they selected the top 10 differentially expressed micrornas in Ckd. Evs.
- You see that here they they did the analysis here and picked the the ones which were most prominent.
- And we had a pathway analysis, and we found a really beautiful signature that these Evs from patients with Ckd carry distinct micrornas. And you see that here, here's a healthy control. You see the heat map and it does look different to the Ckd patients or Ckdevs on the right side. However, look at this. What's happening here? So I have patients who look like healthy. And then I have patients who truly look different. So this is what I'm talking about. We are all different. Some patients have these risk factors, some develop heart failure and others not. And when we looked

at the when, we confirmed these 10 differentially, mostly expressed we did. Qpcr, so you identify these individual micrornas in your plasma of your patients, and we found differences between 1st control and the Ckd patients, but also between patients with and without heart failure. And that's our goal. And that's where we will move forward to understand which micrornas are really cardiotoxic and which ones can be target. You can silence these. You can use these for therapy. That's why, microrna analysis is actually quite interesting.

- So coming to an end here. So my title was extracellular vesicles and the cardiovascular kidney metabolic syndrome, so I had the question, are these vesicles, biomarkers, novel biomarkers, messengers of increased cardiovascular risk? I think so. I think this is one way to explore this field and help us maybe not the only ones. But I think we have some evidence that they have a functional role.
- So if I summarize this and conclude, we are all aware of this epidemic of cardiovascular kidney, metabolic syndrome. And I'm very happy that the American Heart Association includes now the kidney, because we need to check microbiuria in our patient, the Egfr. We need to work together with cardiologists, endocrinologists. It's a multidisciplinary approach discuss diet exercise. I hope I got you excited about exercise and diet. Who, I think, I asked, who is ordering high potassium in their patients? Who is doing that for hypertension treatment?
- Dr. Tusha, of course. So I think we have a lot of opportunities here and there are not good early biomarkers. That's a little frustrating. We need to identify, I think the individual risk early end organ damage in order to provide more personalized care.
- And I think these extracellular vesicles are novel intercellular interorgan messengers. I hope they have the potential to be that I hope I convinced you and got you excited about it. I'm convinced you will hear more about them.
- And then our data here suggests that subtypes of blood evs correlate with obesity, hypertension.
- Very sensitive biomarkers that evs are involved in insulin vascular action, and urinary evs contain sodium and water channels which can be used to understand diet changes, and that Evs from the kidney are likely cardiotoxic in the renal cardiac. Actually, there is data where people have studied the other the cardio-renal axis, and have found identified micrornas as well.
- So thank you very much for listening. I want to do a little advertisement. I'm conducting a hands-on ev workshop later this month. So we have all the international experts on extracellular vesicle characterization here. It's a long week course. So if you know someone who wants to do this, please reach out to me, and then, of course, I want to thank you. And then my lab, a lot of them are here. I'm very grateful for the help former and previous lab members, Dr. Okusa. And then I have a lot of member mentors and collaborators in particular here at Uva, but also outside the flow. Core facility has been super helpful. And then the funding. Thank you so much.
- Thank you for that excellent talk. So I'll open up the questions. One of the questions I have you demonstrated beautifully how Evs can be great messengers can give us an idea of like what's going on physiologically. Is there also space in which you see Evs playing a role also in like drug delivery, or even within you know if we talk about like the cardiorenal axis and someone with Ckd having cardiotoxic elements in an modifying that in some way or doing drug delivery in that fashion. Yeah, you are hitting it, I mean, if they are used. I told you for therapy. So stem cell derived. But people drug load them. So they are nanoparticles over the years. People have used

liposomes but they can induce autoimmunity, and you can imagine if you use vesicles, they are natural carriers. They have all the receptors outside. So yes, people isolate. Evs actually very promising are red Blood cell derived Evs. They load them with

- Rna silencing Rna to target micrornas, which are negative, having a pathologic negative impact. Yes, in one study I still need more funding, but we wanted to use them also to load them with some chemotherapeutic agent, for example, for bladder cancer, where you can do local therapy. Yes, people drug load them with cancer drugs.
- So yes, this is where big business is.
- Yeah.
- And I hope the you know, the Manning biotechnology center will also include some Ev research.
- This is an opportunity.
- Oh, Ruta. Thank you so much for that wonderful talk. And you know it's it's really it's really great to see how this has evolved over the 16 years, and starting with with hypertension, actually, vasculitis. And I'm moving on into cardiometabolic kidney syndrome. So are these these these vesicles? Can they be in in. And this is this goes back to the the 1st question about whether you can use drug delivery. Can they be targeted specifically to specific organs? So in cardiac disease, can you target them? Yes. So people are working on adding on receptors.
- Either there are groups who find ways for cells to produce Evs with special receptors. So you can modify. So I think the future of ev therapy is actually engineered Evs. They are engineered from cells, and you can modulate these cells, you can make sure that then these evs express your receptor of interest. Yes and another question in Ckd patients, they oftentimes have cognitive dysfunction. Have there been any studies with regards to Evs and cognition in Ckd patients?
- Oh, I have to. I have to see if that has been done. I mean, there is a lot of work. I don't know about that study, but I'll look it up for you, because they might cross the brain blood barrier. So they are really interesting molecules to think about. I know Sam Bahamo here from at Uva, in the Neuroscience department. They look at the microbiota evs from the microbiota effect in people with Alzheimer's disease.
- So I mean, it's this, this is this intraorgan communication. Yes, I'm sure you know, we don't know what uremia is, you know, we we can't grab it. Well, and sometimes we dialyze patient kind of okay. If the patient doesn't get better with dialysis, it's not, you know, we didn't decide if Uremia plays effect. So you know, like cardiotoxic, they might be brain toxic.
- I mean, they have accelerated vascular disease. So you don't know in these patients. Is it the vascular, or is it the Evs that might contribute to it? You know?
- Yeah. okay, thank you so much. I'm really happy that you all can thank you.