Dr. BobbieJean Sweitzer: Hello. I’m BobbieJean Sweitzer, Professor of Anesthesiology at Northwestern University and an Associate Editor for Anesthesiology and you are listening to an Anesthesiology podcast designed for physicians and scientists interested in the research that appears in our journal.

Today we are speaking with two authors of publications that appear in the March 2019 issue of the journal. With us is Dr. Tim Rahmel. Dr. Rahmel is the lead author of an article titled “Aquaporin 5-1364A/C Promoter Polymorphism Is Associated with Pulmonary Inflammation and Survival in Acute Respiratory Distress Syndrome.”

He’s a Professor in the Department of Anesthesia, Intensive Care Medicine and Pain Therapy, University Hospital Knappschaftskrankenhaus in Bochum, Germany. Welcome, Dr. Rahmel.

Dr. Tim Rahmel: Hello, Dr. Sweitzer, and thanks for having me.

Dr. BobbieJean Sweitzer: And joining Dr. Rahmel is Dr. Wolfgang M. Kuebler who wrote an accompanying editorial “Acute Respiratory Distress Syndrome: Biomarkers, Mechanisms, and Water Channels.”

Dr. Kuebler is from the Institute of Physiology, Charité – Universitätsmedizin in Berlin, Germany, and Keenan Research Centre for Biomedical Science, St. Michael’s Hospital and Departments of Physiology and Surgery, University of Toronto with the latter two both in Toronto, Canada. Welcome Dr. Kuebler.

Dr. Wolfgang M. Kuebler: Hello and thank you for having me.

Dr. BobbieJean Sweitzer: So, let’s start with you, Dr. Rahmel. What were the primary and secondary goals of your study?

Dr. Tim Rahmel: The primary goal of the study was to assess the impact of aquaporin 5-1364A/C single-nucleotide polymorphism on pulmonary inflammation in patients suffering from ARDS. As secondary goal, we tested the hypothesis that the polymorphism is also associated with 30-day survival.

Dr. BobbieJean Sweitzer: So, maybe we should start with your telling us more about aquaporin 5 and especially how it relates to ARDS. I’m going to confess that I had not heard of this before I read your paper. Interestingly, beneath the ability of aquaporin 5 to regulate the transport of water across membranes, aquaporin 5 also impacts on the key mechanisms of inflammation including immune cell migration. Therefore, aquaporin 5 could also be a key player for pulmonary inflammation in ARDS.

After the presence of bilateral opacities on chest imaging, usually x-ray, then the fact that the lung edema cannot be fully explained by cardiopulmonary or fluid overload, so there may be some degree of cardiac failure, but it does not suffice to fully explain the extent of lung edema that is evident.

And finally, reduced oxygenation and based on the degree of this oxygenation deficit, you can further subclassify ARDS in mild, moderate or severe.

Dr. BobbieJean Sweitzer: Thank you. So, Dr. Rahmel, ARDS can result from both direct insult to the lungs as well as indirect causes. What were the inciting causes of ARDS in your patients? And did you include all ideologies?

Dr. Tim Rahmel: Yes, of course. Aquaporin 5 is a water channel and belongs to a larger family of small integral membrane proteins. Aquaporin 5 forms pores in the cell membrane mainly facilitating the water transport of the cell. So, the question is, of course, where’s the link to the immune system?

Interestingly, beneath the ability of aquaporin 5 to regulate the transport of water across membranes, aquaporin 5 also impacts on the key mechanisms of inflammation including immune cell migration. Therefore, aquaporin 5 could also be a key player for pulmonary inflammation in ARDS.

Accordingly, we tested the hypothesis that a special gene variant of the aquaporin 5 promoter region in patients with ARDS is associated with, first, the pulmonary inflammation as demonstrated by bronchoalveolar lavage characteristics of, second, 30-day survival.

Dr. BobbieJean Sweitzer: So, what gene variant were you looking for in this study?

Dr. Tim Rahmel: The cumbrous name is aquaporin 5-1364A/C promoter single-nucleotide polymorphism. On sequencing the aquaporin 5 promoter region among healthy patients, we previously described this functional important and common polymorphism in the aquaporin 5 gene promoter.

Substitution of cytosine for (sounds like: adenine) at the position 1364 in the promoter region was associated with a reduced (inaudible) activation of the aquaporin 5 gene; therefore, we find lower mRNA and protein expression in the C-allele carriers of the aquaporin 5 polymorphism.

Dr. BobbieJean Sweitzer: I think you said it was common. How common is this?

Dr. Tim Rahmel: These polymorphism are in about – in a representative population two-third for the normal AA genotype and one-third in the (inaudible) cytosine allele is found. The largest amount is in the heterozygous AC-allele carriers and just a small amount of about 5% is found in the homozygous CC-allele carriers.

Dr. BobbieJean Sweitzer: So, we’re going to talk a little bit more about that gene a little later in this discussion, but first let’s talk about your study participants and the setting for this study. Can you tell us about that?

Dr. Tim Rahmel: Yes, of course. All patients for the study were admitted to our intensive care unit in the years from 2009 and 2013 and considered eligible if they fulfilled the formal criteria of ARDS. Most patients that were included in the study were referred from other intensive care units for possible ECMO therapy to our center after a rapidly progressive ARDS course. In total, 136 patients with ARDS were included in our study.

Dr. BobbieJean Sweitzer: So, Dr. Kuebler. Can you perhaps give us a primer on ARDS? I think obviously even the non-intensivists listening to this are familiar with this term, but I’m not sure that I’m even up to date on the latest information. Specifically, what are the criteria to establish a diagnosis of ARDS?

Dr. Wolfgang M. Kuebler: So, the diagnostic criteria were initially introduced by Ashbaugh in his first description in 1967 of ARDS and then were updated, I think, in the late 80’s or early 90’s.

But right now we actually use the diagnostic criteria that has been updated in 2012 by the so-called Berlin definition and those comprise sudden onset, which basically means within one week of a known clinical insult, that can be associated to the development of ARDS.

Then the presence of bilateral opacities on chest imaging, usually x-ray, then the fact that the lung edema cannot be fully explained by cardiopulmonary or fluid overload, so there may be some degree of cardiac failure, but it does not suffice to fully explain the extent of lung edema that is evident.

And finally, reduced oxygenation and based on the degree of this oxygenation deficit, you can further subclassify ARDS in mild, moderate or severe.

Dr. BobbieJean Sweitzer: Thank you. So, Dr. Rahmel, ARDS can result from both direct insult to the lungs as well as indirect causes. What were the inciting causes of ARDS in your patients? And did you include all ideologies?

Dr. Tim Rahmel: So, ARDS was involved in 110 cases, so 81% of the entire cohort directly by a bacterial pneumonia and in 26 cases 90% of the cohort by a primary extrapulmonary sepsis with a secondary bacterial pneumonia leading to ARDS.

Dr. BobbieJean Sweitzer: So, Dr. Kuebler, is there a difference in ARDS outcomes and responses to therapy based on underlying causes of this condition?

Dr. Wolfgang M. Kuebler: That is an interesting question. There are a series of studies which suggest that outcomes and therapeutic effectiveness may vary depending on the cause of ARDS. For example, patients with direct lung injury ARDS, so with an insult which primarily affects the lungs, has a higher mortality than those with indirect ARDS. So, patients where ARDS develops subsequent to a systemic inflammatory disease such as pancreatitis or sepsis or transfusion.

Studies from the late 90’s and early 2000’s even suggests that patients with direct versus indirect ARDS respond differently to, for example, ventilator maneuvers such as high PEEP or prone positioning or size. And also the effectiveness of such measures seems to depend on the severity of the disease. So, there is quite a different response depending on the initial trigger of the disease as well as the course of the disease.

More recently, Carolyn Calfee introduced the concept of subphenotype in patients by clinical parameters into two phenotypes: one was hyperinflammatory and one was hypoinflammatory. The hyperinflammatory phenotype is more frequently associated with sepsis and has a worse outcome, but in
contrast to this hypoinflammatory phenotype seems to respond better to, for example, high PEEP ventilation or treatment with statins.

So, again, there are differences not only in terms of outcome but also in therapeutic response.

**Dr. BobbieJean Sweitzer:** It sounds like quite a broad spectrum that's all caught under one simple term.

**Dr. Wolfgang M. Kuebler:** That, I think, is a very big problem in the whole ARDS field. We're really talking about a syndrome here and not a specific disease and we lump a lot of different conditions which share a common pathology and maybe a common pathway under this big umbrella.

But whether we can target it with one single approach or whether we have to stratify it depending on the cause of the disease or the patient or the profile, how the disease develops is really not (sounds like: unclear). It has divided the field, really, in the lumpers and the splitters: What do you want to do? How do you want to approach it?

**Dr. BobbieJean Sweitzer:** So, Dr. Rahmel, how did you actually determine these genotypes? Was this just a simple blood test? And also, how did you measure pulmonary inflammation? Was it the biopsies or some other mechanism?

**Dr. Tim Rahmel:** To your first question, how we determined the genotypes, therefore DNA whilst extracted from whole blood and for genotyping, a polymerized chain reaction was performed followed by pyrosequencing.

In this context, pyrosequencing is a well-known method of DNA sequencing or determining the order of nucleotides in the DNA, respectively. Based on the principle sequencing by synthesis, the template DNAs immobilize in solutions of the four different nucleotides sequentially added and removed from the reaction.

When the nucleotide solution complements to the first (sounds like: unpaired base) of the template, a detectable light is produced based on a reaction when pyrophosphate is released. Hence, the sequence of the solutions that produce a signal allows the determination of the sequence of the template.

To your second question, how we measure pulmonary inflammation, in the bronchoalveolar lavage of every patient, total protein concentrations, albumin concentrations, lactate dehydrogenase activity and white cell concentrations were measured as surrogates for pulmonary inflammation.

In addition, three different cytokines, in detail, TNF-alpha, interleukin-6 and interleukin-10 were measured in (sounds like: BIL of antiserum). Concentrations were measured as surrogates for pulmonary inflammation.

**Dr. BobbieJean Sweitzer:** So, if I can summarize, it was better to have the variant gene than the more common gene.

**Dr. Tim Rahmel:** Yes.

**Dr. BobbieJean Sweitzer:** So, before we get to the results from your study, can you tell us a bit about what other studies have shown us about the polymorphisms of aquaporin 5?

**Dr. Tim Rahmel:** Yes, of course. We know that the 1364A/C single-nucleotide polymorphism in the promoter region of aquaporin 5 is associated with an altered aquaporin 5 expression and also impacts on 30-day survival in sepsis.

Due to the role of aquaporin 5 mediating key mechanisms of inflammation in the immune cell migration, we suspected the single-nucleotide polymorphism to affect key mechanisms of pulmonary inflammation and in new cell migration also in ARDS and also this may contribute to an altered mortality risk among the genotypes.

**Dr. BobbieJean Sweitzer:** So, Dr. Kuebler, do we know anything about other genotypes or specific variant alleles and outcomes in patients with ARDS?

**Dr. Wolfgang M. Kuebler:** Yes. There are a series of genetic studies, actually some of them are also from the group of Dr. Rahmel and Dr. Adamzik in Bochum, but also from others, who have shown associations between individual genotypes and outcome in ARDS patients.

For example, different alleles of the platelet activating factor acetylhydrolase that are associated with ARDS survival and this acetylhydrolase inactivates platelet activating factor which is an inflammatory mediator and one of the key mediators in ARDS. So, pathophysiologically this association somehow makes sense.

Also, there are associations of polymorphisms in genes, for example, in coding for angiotensin-convert enzymes that are associated with risk for an outcome of ARDS.

And the same has been shown for quite a variety of genetic polymorphisms in various genes that are related to inflammation or vascular responses such as interleukin 8 or hypoxia-inducible factor 1st or vascular endothelial growth factors.

And then, of course, that's then fueled the interest in early detection of such genetic polymorphisms for a guided therapy in ARDS patients.

**Dr. BobbieJean Sweitzer:** So, now that we have some background, Dr. Rahmel, can you share with our listeners what you found with your study? And, I guess, let's start first with the DNA testing.

**Dr. Tim Rahmel:** Regarding distribution of the genetic variations of the aquaporin 5 polymorphism, we observed the frequency of 68% for the AA genotypes and 32% for AC and CC genotypes in line with the expected distribution.

**Dr. BobbieJean Sweitzer:** And what about the lung lavage results?

**Dr. Tim Rahmel:** In bronchoalveolar lavage, total protein concentrations and albumin concentrations were 2.4-fold lower, lactate dehydrogenase activity 2.8-fold lower and leukocyte concentrations 3.4-fold lower in C-allele carriers compared to the normal AA genotype patients.

Furthermore, neutrophil count and interleukin 6 concentrations were also lower in C-allele carriers, hence our results are compatible with an attenuated pulmonary inflammation in AC and CC genotypes.

**Dr. BobbieJean Sweitzer:** It sounds like quite a broad spectrum that's all caught under one simple term.

**Dr. Tim Rahmel:** In our cohort regarding survival, it was an advantage to be an AC or CC genotype, yes.

**Dr. BobbieJean Sweitzer:** Was there a difference between the AC and CC or did you have enough numbers to look at that? Or you just grouped them together versus the AA?

**Dr. Tim Rahmel:** Thank you for this important question regarding, I think, a gene dose effect. (Inaudible) there are differences between these both subgroups is CC, homozygous CC and AC genotypes; however, our data just allows us to speculate on a gene dose effect or a difference between the AC and CC genotypes due to a lower means (inaudible) in both groups.

However, there's no statistical difference between the AC and CC genotypes because the rare occurrence of the CC genotypes. We have only five patients with the CC genotype; hence, definite conclusions regarding a strict gene dose effect cannot be drawn with our data.

**Dr. BobbieJean Sweitzer:** Is there anything about these patients with these gene variants if they don’t have ARDS that are different in either lung function or any other way?

**Dr. Tim Rahmel:** They don’t have different characteristics without ARDS. So, we just find different mortality rates when patients are suffering from ARDS or sepsis and we have several things that we thought this could be. For example, aquaporin 5 would address to alter the lung fluids concentration or effect on pulmonary edema and due to other studies we have performed, we know that inside that it's more the effects on the immune system. But without hyperinflammation we don't get these genotypes clinically differed.

**Dr. BobbieJean Sweitzer:** Interesting. So, Dr. Kuebler, were you surprised by the results of Dr. Rahmel's study? And why or why not?

**Dr. Wolfgang M. Kuebler:** Yes and no. So, initially when aquaporins were discovered in the 1990’s, they attracted early interest from the ARDS community because it was believed that their function as water channels could play an important role in lung edema formation.
But when people did the experiments with corresponding knockout animals, they didn’t find differences in cardiogenic edema formation and also in edema resolution. And so, at that stage, it was thought that the case for aquaporins in ARDS was pretty much closed. But a few years later now the team of Dr. Adamzik in Bochum identified a predictive role of the aquaporin 5-1364A/C polymorphism in sepsis and was able to link it to a role of aquaporin 5 in immune cell migration. And this, of course, then, brought aquaporins back on the map in the context of systemic inflammatory disease and then the next step was to go back to ARDS and that’s what Dr. Rahmel very elegantly did; and, so somewhat this is now a logical extension of this work.

Dr. BobbieJean Sweitzer: Dr. Rahmel, in your paper I think you write about the discrepant findings of aquaporin 5 variants in different studies. Can you expand – I think maybe that’s what Dr. Kuebler was telling us a bit about, but can you expand on these findings, diverse findings for us?

Dr. Tim Rahmel: Yes, of course. Other studies, especially the study of Zhang and colleagues reported that a greater aquaporin 5 expression is made protective in the maintenance of the pulmonary barrier function and also in acute lung injury. However, we found more or less the opposite; we found an aggravated pulmonary inflammation in association with the AA genotypes that are related to higher aquaporin 5 expression. Strikingly, Zhang and colleagues also showed that the aquaporin 5 deficiencies associated with a decline activation of inflammatory pathways like NF-kappa B which is, therefore, in line with our results.

Dr. BobbieJean Sweitzer: Are there reasonable explanations and/or mechanisms that explain these divergent outcomes?

Dr. Tim Rahmel: Taking all these results into account and in addition to what Dr. Kuebler has said, the aquaporin 5 promoter polymorphism and also the aquaporin 5 expression in ARDS might represent a double-edged sword, so you can say this.

On the one hand, the AA genotype aggravates the immune cell migration to the infected tissues which is associated with a better bacterial eradication and less bacterially evoked harm. On the other hand, situations with exaggerated immune system and therefore a hyperactive immune system, cell migration is also aggravated in the AA genotypes that may evoke greater release of proteases reactive oxygen species that potentially damages the host’s tissue. And the latter hypothesis is in line with our results demonstrating lower protein and albumin concentration as markers for pulmonary inflammation as well as lower lactate dehydrogenase activity, a marker for pulmonary damage in our bronchoalveolar lavage characteristic in the AC and CC genotypes.

Dr. BobbieJean Sweitzer: So, Dr. Kuebler, is there an evolutionary explanation or rationale as to why a mutation like this, so aquaporin 5-1364A/C, may have both positive and negative effects?

Dr. Wolfgang M. Kuebler: Yes, I guess so. So, evolution is in a large part driven by infection and the development of appropriate immune responses. And the AA genotype of the aquaporin 5-1364A/C promoter may originally have conferred an evolutionary advantage because of the higher expression of aquaporin 5 and, therefore, a higher migration of immune cells to the site of injury and infection which help the body to defend itself against invading pathogens. However, in our days now where we live with antibiotics which, in some cases, at least, can kill the bacteria for us where we live also with sterile modes of inflammatory signals or stimuli such as mechanical ventilation in the critically ill, this may actually turn in a disadvantage because now we still have the increased infiltration and migration of immune cells and inflammatory cells, but they don’t need to fight the invading pathogens anymore, but they still kill the host tissue in the lung and cause ARDS.

So, while a strong inflammatory response may have had an evolutionary advantage, it may now result in a higher mortality in diseases associated with hyperinflammation such as ARDS.

Dr. BobbieJean Sweitzer: Very interesting. Dr. Rahmel, Dr. Kuebler mentioned the renin–angiotensin system earlier and I understand that patients, I think, taking ACE inhibitors and ARBS have a lower mortality with aspiration pneumonia and I think in your paper you mentioned the involvement of the renin–angiotensin system and the pathogenesis and clinical outcome of ARDS. What is the connection between the single-nucleotide polymorphism and the renin–angiotensin system?

Dr. Tim Rahmel: The link is that the renin–angiotensin system signaling and also is linked to inflammation and fibrosis and, in part, lung function and is potentially associated with a (inaudible) outcome in ARDS.

In another context, our group previously described the aquaporin 5 polymorphism as the renin–angiotensin system regulation in young and healthy humans as well in patients with coronary heart disease. So, this is also for ARDS or sepsis quite an interesting association.

However, we previously showed that the aquaporin 5 polymorphisms not associated with altered plasma angiotensin or serum aldosterone concentration in patients experiencing severe sepsis. Nevertheless, research on this topic may offer a better understanding of the role and interaction of renin–angiotensin system and aquaporin 5 in ARDS pathophysiology.

Dr. BobbieJean Sweitzer: Are there any clinical trials looking at the use of ACE inhibitors or ARBS in treating patients with ARDS?

Dr. Tim Rahmel: Definitely yes, but our group is, at the moment, not looking at this interaction. So, we are focusing on immune cell migration because we have these results from patients that are suffering from sepsis and so we are not continuing to investigate the interaction of the renin–angiotensin–aldosterone system on aquaporin 5 and inflammatory diseases at the moment.

Dr. BobbieJean Sweitzer: I see. So, Dr. Kuebler, can we use this information we know now about this variant genotype or phenotype to actually guide therapies for individual patients with ARDS?

Dr. Wolfgang M. Kuebler: This is probably the Holy Grail of precision medicine in ARDS, but I think it’s still a way to go. We have, at present, identified individual genetic polymorphisms for ARDS, such as the aquaporin 5 promoter alleles, but we are still far from an all-encompassing picture of the whole genetic risk of an individual patient because so far we have not coherently synthesized the known individual polymorphisms into a global risk stratification assessment and probably there are still more polymorphisms which predict outcome which we have not yet identified.

And even if we do, we also have to consider that the genetic profile of a patient is only one piece to the puzzle when we aim for precision medicine and we have to consider other factors such as the type and severity of injury and the individual patient response pattern which are probably of equal relevance for the prediction of outcome and response to therapy.

And I think this is clearly the goal where we want to go, but we have to take a lot of different aspects into consideration here such as disease triggers, patient genotype and acquired traits rates if we have to achieve a reliable and comprehensive stratification.

Dr. BobbieJean Sweitzer: So, Dr. Rahmel, how close do you think we are to using the information such as what you’ve discovered about this variant genotype in the actual clinical care of patients with ARDS? And what are the next steps?

Dr. Tim Rahmel: I just totally agree what Dr. Kuebler said and also thank you for this important question. There are still some steps to take; nevertheless, the facts of aquaporins on shaping or potentially shaping the immune response could be a very interesting starting point for new therapeutic targets, especially in the context of (inaudible), it seems, in my opinion, appropriate to try to counteract the mortality differences between the genotypes that we have described.

However, we still need a better mechanistic understanding, for example, to define the right time and the right indication for such a therapy and we need a more profoundly knowledge about the mechanisms that are linked of aquaporin 5 and immune cell migration. And furthermore, we need to profoundly task the substances that potentially influence the aquaporin 5 expression in the context of our polymorphism and ARDS. So, there are several steps, but I think at the moment we can say it’s a quite interesting starting point for potential new therapeutic targets.
Dr. BobbieJean Sweitzer: Yes. I think like most good studies we just kind of move this along the spectrum towards a better understanding and knowledge, but also raises further questions and opens up more opportunities.

So, I hope today’s discussion will interest many of our listeners and lead you to read this important article to learn more. Thank you, Drs. Rahmel and Kuebler, for discussing your work with us today. I wish you well as you continue your efforts to enhance the practice of anesthesiology and strive to improve the care of our patients.

{Music}

Host: You’ve been listening to the Anesthesiology journal podcast, the official peer-reviewed journal of the American Society of Anesthesiologists. Check anesthesiology.org for an archive of this podcast and other related content.

THE END