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Future of GFR estimation

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Mayo Clinic cooperation

PROLOG

Welcome to numares insider

Volker Pfahlert

Philipp Pagel

Many exciting things have happened at numares since our last issue of the numares insider was published and we would like to share some of them with you.

numares participated in several scientific events, like the recent ASN Kidney Week. There we met a lot of interesting people and had plenty of opportunities to talk about our *AXINON® renalTX-SCORE®-U100* allograft rejection test as well as the upcoming *AXINON® Clearance Check* test for better quantification of glomerular filtration rate. We got very helpful feedback. We were surprised to see how many potential scenarios may be of interest for the *AXINON® Clearance Check* that are not part of nephrology at the first glance.

After several years of successfully leading our US branch in Boston, Claus Botzler has returned to the "mother ship" to serve as our COO and received a warm welcome back home. With Sean Keohane we have found a great successor for heading the US office and he is looking forward to meeting our customers as soon as possible.

All of you are well aware of the importance of using the correct blood collection tubes in order to avoid interference. However, that aspect is not fully under the control of a lab, and clients do send unsuitable material at times. Jonathan Genzen and his team at ARUP have recently published an excellent study on the topic of interference from sampling tubes that we are covering in this issue because we think it's a great piece of work that many of you will be interested in.

Finally, we are proud to announce that our collaboration with Mayo Clinic in Rochester, MN is now official and was covered by press releases on both sides. We believe that this is a great step for numares and we are looking forward to our joint work. We hope that you enjoy our second issue of this publication and welcome your feedback and suggestions for topics that you care about. □

Volker Pfahlert, Chief Executive Officer
Philipp Pagel, Chief Medical Officer

HANDS ON

Why we do not ship or store specimen samples on dry ice

In the last numares insider we discussed the importance of "Keeping your Probe Clean." Although, failure to read a sample is rare, there are complexities in sample management that are important to consider. In addition to contaminants, impacting sample reading, it is important to NOT ship or store samples on dry ice. Patient specimens collected for diagnostic analysis by NMR spectroscopy usually need to be transferred from the doctor's office to a dedicated diagnostic NMR facility. Commonly, diagnostic samples are shipped on dry ice in a frozen state. For NMR analysis, this may impede or invalidate the analysis of the specimen samples:

- Dry ice (CO_2) slowly transforms, from the frozen to the gaseous state, during storage and/or transport.
- Gaseous CO_2 has the ability to diffuse into specimen tubes even when sealed, lowering the pH value.
- Pronounced changes in pH, secondary to dry ice storage or transport can cause large shifts of pH-sensitive NMR signals. This may adversely impact lipoprotein analysis (see figure a).
- During NMR measurement, a warm-up step, heating to 37°C, may allow CO_2 to diffuse out of the samples leading to a significant loss of spectral quality due to signal distortions and broadening. These distortions are amplified until a steady state is reached (see figure b).

The numares software performs a quality check that detects significant pH associated shifts in spectral signal in both directions to insure accuracy of sample measurement. A significant change will stop the analysis.

No analysis of specimens will be possible

To ensure sample accuracy and reproducibility and to limit sample rejections during the QC process within the software, we have the following recommendations.

No dry ice usage for:

- Sample storage
- Sample shipping
- *AXINON®* kits storage □

Katja Barthelmes, Customer Support Specialist

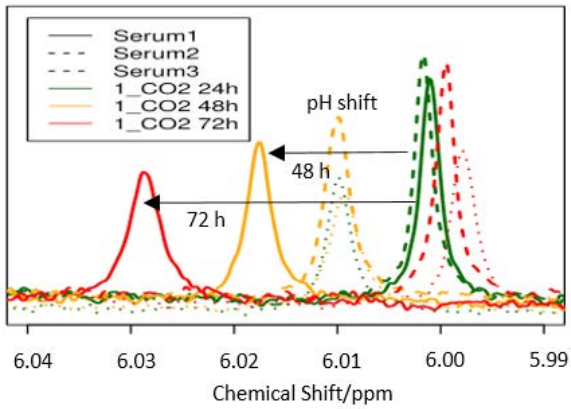

Figure a) Effect of dry ice on samples during shipment or storage. CO_2 diffuses into the samples causing a decrease in pH over time. The NMR signal will experience a shift to higher ppm values and a loss of spectral quality.

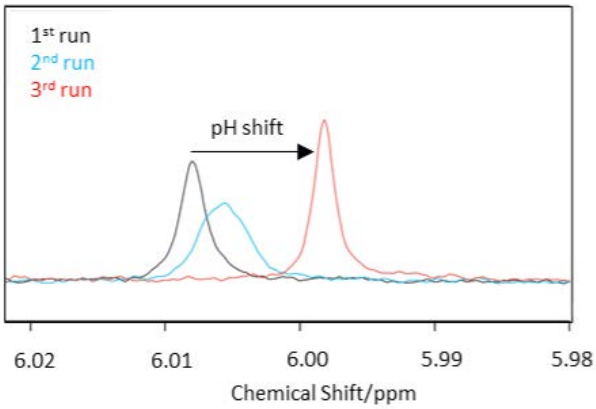

Figure b) Effect of dry ice on samples during remeasurement. Heating to 37°C will cause CO_2 to diffuse out of the samples causing an increase in pH over time. The NMR signal will experience a shift to lower ppm values and a significant loss of spectral quality.

If assistance is needed, please contact:
technicalsupport@numares.com
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HANDS ON

Could tubes play a role?

Interferences in nuclear magnetic resonance based lipoprotein analysis

Besides measuring the components of standard lipid panels, lipoprotein analysis by NMR offers additional insights of lipid physiology in our blood. This extra knowledge is of great clinical relevance and makes NMR-lipoprotein measurement a powerful tool to help clinicians in managing cardiovascular disease and disease risk. However, like all other clinical analytical methods, NMR-lipoprotein measurement can sometimes be affected by a variety of factors.

Generally, errors in clinical diagnostics can occur during all stages of the laboratory testing process but up to 70 % of all problems occur in the pre-analytical phase[1]. This phase refers to everything that happens before the actual sample analysis in the clinical laboratory.

Clinical laboratories depend on healthcare professionals to collect quality blood samples. *Picture: iStock*

It comprises several steps like patient preparation, sample collection and sample transportation. One of the most common types of pre-analytical errors are interferences caused by the presence of foreign compounds in the blood sample.

When addressing the origin of pre-analytical errors, one may think of irregularities introduced during the process of blood drawing, sample stability issues or interferences caused by ingestion of drugs, food supplements or alcohol. However, another aspect has to be considered in this context – the role of phlebotomy tubes. While most clinical-use tubes are designed to minimize pre-analytical variables, interferences can occur depending on tube type and the different additives or gel components which might be adsorbed to the tube surface.

Until recently, there was only limited knowledge about how phlebotomy tubes affect clinical NMR measurements. Now a comprehensive study has been published in January 2019 in Clinica Chimica Acta[2]: Using numares' NMR-based lipoprotein test, Laura L. Needham, Jonathan R. Genzen and their colleagues from ARUP laboratories examined the performance of more than 20 different tube types from Greiner Bio-One, Becton Dickinson and Sarstedt and analyzed their impact on lipoprotein subclass analysis by NMR. Their work has shed more light on the influence of common contaminants that are often found in diagnostic blood samples.

Needham *et al.* demonstrated that the NMR-based lipoprotein measurement is quite robust and **not** impacted by a number of common contaminants routinely found in patient specimens. There was prior empiric data that NMR spectra could be impacted by the use of alcohols used to disinfect gloves, the patient skin or the sample during collection.

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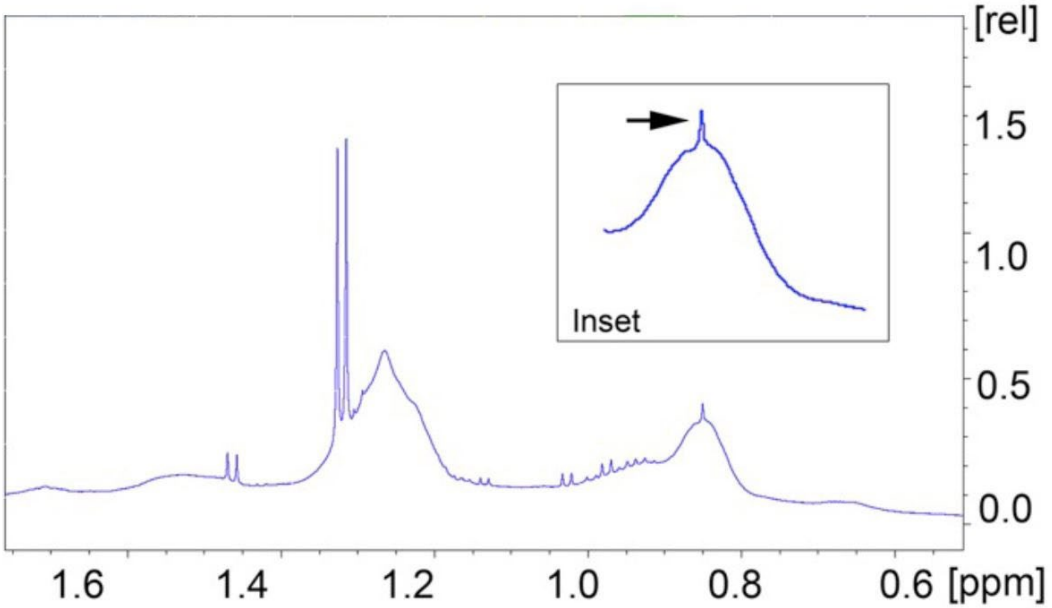
This graphic (modified from Needham et al., 2019) shows a region of the NMR spectrum of human serum incubated in a serum separator tube (SST) by Becton Dickinson. A characteristic interfering peak (indicated by arrow in magnified view) is observed in the region where lipoprotein particle numbers and sizes are measured. x-axes are in parts per million (ppm); y-axes are in relative units.



In this study, blood alcohol and antiseptic use alcohols (isopropyl alcohol) or common agents such as glycerin and propylene glycol present in skin creams and cosmetics did not cause any noteworthy interferences. Even at extremely elevated concentrations only slight interferences were shown for small LDL particle numbers. In contrast, the study revealed relevant interferences from some types of blood collection tubes not recommended for use in this test. For example, the widely used serum separator tubes (SST) by Becton Dickinson showed strong interferences for subclass-specific parameters like small LDL particle number and VLDL particle size. In general, significant phlebotomy tube associated interferences in NMR analysis occurred in a variety of subclass specific parameters (LDL-p; SLDL-p; LVLDL-p; HDL-p; LHDL-p; VLDL-s; HDL-s) as well as in more common lipid parameters (CHOL; TRIG; LDL-C).

As expected, tube-associated interference worsened with decreased tube fill volumes. However, the factors responsible for these observed interferences could not be fully clarified. Given the fact that standard lipid parameters measured by conventional chemistry assays did not show significant interferences across the different tube types, the study excluded the possibility that lipids were absorbed to a different extend by the respective tube compounds. Instead, it is more likely that interfering substances may leach into the specimen and cause problems with NMR measurement. Substances worth considering as possible interfering agents are anticoagulants, clotting agents or other tube type-specific components like rubber stoppers, plasticizers, surfactants, gels, etc.

As stated above, the pre-analytical phase is where the majority of laboratory errors occur. The study by Needham *et al.* stresses the importance of educating physicians to only use approved blood sampling tubes to minimize contaminants during phlebotomy for NMR-based lipoprotein analysis. Of course, it is difficult to eliminate all pre-analytic variables, but the standardized use of validated phlebotomy tubes provides an easy opportunity to ensure a more accurate testing process. □

Christian Karger, Product Management

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2. Needham LL *et al.* Phlebotomy tube interference with nuclear magnetic resonance (NMR) lipoprotein subclass analysis. Clin Chim Acta. 2019 Jan; 488:235-241.

NEWS

Development of new diagnostic tests: numares and Mayo Clinic Laboratories announce collaboration

Mayo Clinic Building

Pictures: Mayo Clinic

numares and Mayo Clinic Laboratories have announced a collaboration to develop clinical diagnostic tests that will measure clusters of risk factors as opposed to individual biomarkers. The unique testing will use nuclear magnetic resonance (NMR) technology, focusing on cardiovascular disease, kidney disease and liver cancer — among a few other specific diseases.

"We hope to exchange knowledge and investigate these new diagnostic paths to improve patient lives by leveraging NMR spectroscopy to quantify patient metabolites and diagnose certain conditions," says Volker Pfahlert, Ph.D., CEO of numares. The envisioned solutions will measure and analyze metabolic "constellations" (clusters of risk factors) derived from clinical diagnostic tests performed on patient samples, such as blood or urine. As such, these solutions can be used to monitor a patient's overall health to help diagnose, treat or prevent diseases.

The numares model

For many medical questions, a single biomarker of sufficient utility does not exist. However, sometimes, combinations of several biomarkers — considered in relation to each other — can provide clinically actionable information. To find meaningful combinations of biomarkers, metabolites must be accurately and precisely quantified, as it is done with NMR technology.

Identifying relevant biomarkers and their relationships to each other requires a huge amount of data processing. The numares approach uses artificial intelligence (AI) to analyze data from clinical studies and machine learning to distinguish which metabolite constellations are meaningful, and then models mathematical equations for the interpretation of the biomarker sets.

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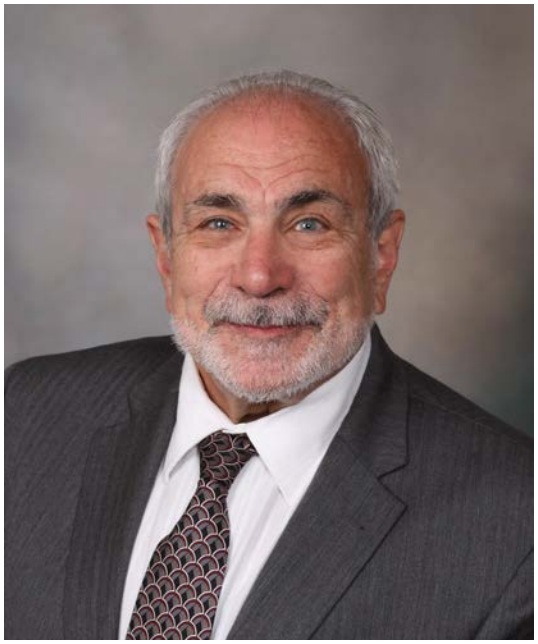
"Our unique approach is similar to finding constellations in the sky," says Dr. Pfahlert. "The medical information is not so much about the brightness and color of each individual star as it is about the position of each star in relation to the others."

AXINON® renalTX-SCORE®-U100 is the first commercially available and CE-marked product using metabolic constellations. This test helps physicians identify kidney transplant recipients at risk of transplant rejection, based on a constellation of metabolites measured in the patient's urine. The company also has efforts underway exploring metabolite constellations for the diagnosis of bladder cancer, the early detection of liver cancer, and a more accurate noninvasive measurement of kidney function.



"The approach of identifying 'constellations' of metabolites for diagnostics will play an important role in the future of precision medicine," says ***Allan Jaffe, M.D.****, division chair for Clinical Core Laboratory Services in the Department of Laboratory Medicine and Pathology at Mayo Clinic. "Collaborating with numares, we want to convert the diagnostic capability of these metabolic constellations into clinical tests that will help patients who have undiagnosed diseases."*

"LDL cholesterol, the notorious 'bad' cholesterol associated with heart disease, is contained within LDL particles which are a better indicator of risk," says ***Jeff Meeusen, Ph.D.****, co-director of Cardiovascular Laboratory Medicine at Mayo Clinic. "The numares lipoprotein method measures both LDL cholesterol and LDL particles."*

Mayo Clinic Laboratories to validate testing

Mayo Clinic Laboratories is the global reference laboratory of Mayo Clinic. The organization provides advanced laboratory testing and pathology services to support 4,000 health care organizations around the world. The first test Mayo Clinic Laboratories will offer using numares' technology is the measurement of lipoproteins. Bernhard Schirmers, Ph.D., chairman of the supervisory board and partner of numares' lead investor SHS Beteiligungsgesellschaft, says, "This strategic collaboration underpins the potential of our NMR approach to take large amounts of relevant data and find meaningful results. We have two organizations, combining talents, to help patients who are on medical journeys but have not found answers yet." □

Gina Chiri-Osmond, Mayo Clinic Public Affairs
Julia Hertlein, numares Public Relations

INSIDE

The future of kidney function assessment°

The patient Jack is 63 years old and was diagnosed with diabetes 10 years ago. Since his initial diagnosis he has been on Metformin therapy and regularly sees his general practitioner (GP) as part of a chronic care management program. Jack's GP knows that diabetics are at a higher risk for kidney impairment and thus is concerned about his kidney function. He orders GFR testing at each visit. Jack's last three eGFR values have steadily been going down, suggesting that kidney function is getting worse. Now Jack is at a point where his eGFR and kidney function is so low that a safe use of Metformin might not be possible anymore and his doctor considers taking him off Metformin. For Jack this would be a huge problem because then he would need to inject himself with Insulin resulting in much higher cost and inconvenience.*

However, this decision would not be correct. How can that be? eGFR is an estimation of glomerular filtration rate and has weaknesses in precision and reliability. But let's start from the top. Harmful substances and waste products from the metabolism are removed from the blood through glomerular filtration or tubular secretion by the kidneys. Loss of kidney function can lead to edema, high blood pressure, disorders of the nervous system, anemia and bone resorption. If the loss of kidney function is permanent and proceeds steadily over months and years, a patient is diagnosed with chronic kidney disease (CKD). When finally end-stage renal disease (ESRD) is reached and the kidneys have almost no function left, patients require dialysis or organ transplantation.

For assessment of kidney function the glomerular filtration rate (GFR) is the most important parameter. It is useful in many medical conditions, e.g. management of chronic kidney disease (CKD), diabetes management, patients with chronic congestive heart failure and dosing of nephrotoxic drugs. It can either be measured with tracer based clearance methods or estimated using specific formulas.

The gold standard for measuring GFR (mGFR) is still Inulin clearance [1,2], a procedure that was introduced in 1935. Inulin is an exogenous polysaccharide that is physiologically inert and freely filtrated meaning that it is not secreted, reabsorbed, synthesized or metabolized by the kidneys and is therefore the perfect marker for glomerular filtration. Since measuring Inulin clearance is very time-consuming, elaborate and inconvenient, it has been replaced with Iothalamate [3] or Iohexol as exogenous markers. These are radioactively marked contrast agents that both fulfill the requirements for markers of glomerular filtration rate.

Pictures/Graphic: iStock

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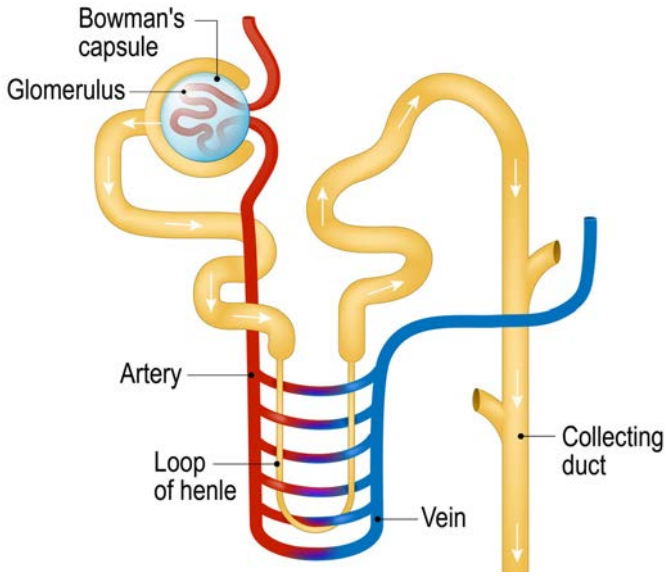
They are easier and faster to measure than Inulin, but have the negative impact of radioactivity. Since measuring GFR is either very elaborate or goes ahead with radioactivity, it is only performed very rarely in clinical routine.

In most cases an estimated GFR (eGFR) based on serum Creatinine is sufficient to indicate the actual renal function [4]. Currently more than 50 such estimation formulas exist [5]. Each of these were developed in a specific patient group and is therefore particularly suitable for this group and has its weaknesses in other patient groups at the same time. The most commonly used formulas are MDRD and CKD-EPI for adults and the Schwartz-formula for children [6-8]. Creatinine is a degradation product of Creatine, which is mostly present in muscles. Although Creatinine is mainly removed from the blood stream by glomerular filtration, it is not an ideal marker for glomerular filtration because at high plasma levels of Creatinine also some tubular elimination occurs as a compensation mechanism. This has the consequence that only if a patient has lost about 50 % of kidney function, serum Creatinine will rise significantly. As a result, eGFR formulas have a Creatinine-blind area from 40-70 ml/min/1.73m^2 and therefore, patients with a moderate loss of kidney function are often overlooked based on Creatinine (eGFR). Additionally, serum Creatinine levels are influenced by muscle mass, age, activity and other (patho-)physiological situations. eGFR determination based on serum Creatinine is therefore very imprecise and often leads to false classification of patients according to the CKD stages.

In order to overcome these disadvantages, eGFR formulas based on Cystatin C, another serum marker, have been promoted [9]. Cystatin C is an endogenous protein of 120 amino acids. It is produced at a relatively constant rate by most nucleated cells and eliminated through glomerular filtration. In contrast to Creatinine, Cystatin C is not significantly secreted by the tubuli and will rise earlier with the loss of kidney function. Furthermore, its serum concentration is not influenced by age and muscle mass. So Cystatin C based eGFR formulas have a better performance than Creatinine in many cases. However, glucocorticoid therapy, autoimmune diseases and hyperthyroidism lead to elevated levels of Cystatin C and consequently to a lower eGFR, independently of kidney function.



The glomerular filtration rate

In healthy adults about 1500 liters of blood flow through the glomeruli of the kidneys every day, resulting in 180 liters of primary urine through glomerular filtration. In the subsequent tubules, recyclable substances and about 90 % of the water contained in the primary urine are reabsorbed and urinary substances are actively excreted by tubular secretion. This results in about 1 to 1.5 liters of final urine. The glomerular filtration rate (GFR) is volume of primary urine produced by the nephrons of both kidneys in a defined period of time. It is a measure for the quality of kidney function.

Chronic kidney disease (CKD) is the chronic loss of kidney function and proceeds over months and years until finally end-stage renal disease (ESRD) is reached. There are five stages of CKD [10]:

- Stage 1: Kidney damage with normal kidney function (eGFR ≥ 90 ml/min/1.73 m^2) and persistent proteinuria.
- Stage 2: Kidney damage with mild loss of kidney function (eGFR 60-89 ml/min/1.73 m and persistent proteinuria).
- Stage 3: Mild to severe loss of kidney function (eGFR 30-59 ml/min/1.73 m^2).
- Stage 4: Severe loss of kidney function (eGFR 15-29 ml/min/1.73 m^2).
- Stage 5: Kidney failure requiring dialysis or transplant for survival, end-stage renal disease (eGFR < 15 ml/min/1.73 m^2).

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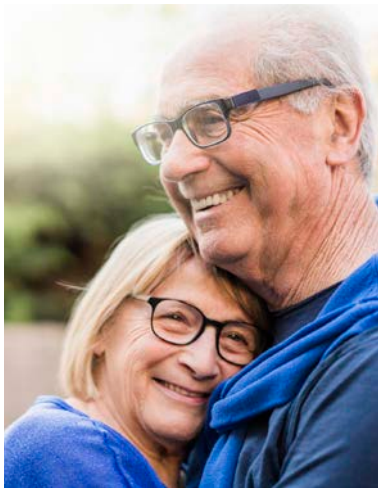
If eGFR is significantly incorrect, this can lead to serious consequences. For example, if eGFR is too high (this is challenging especially in the Creatinine blind area), loss of kidney function is missed and the patient does not get the adequate therapy. So instead of getting better, kidney function will worsen over time until it is finally so bad that eGFR will indicate it. This unnecessarily prolongs time to treatment and the chance of early intervention is missed. Overestimation of kidney function is also problematic for dosing of nephrotoxic drugs. If kidney function is significantly lower than indicated by eGFR, a patient might receive a higher dose of a nephrotoxic drug leading to (permanent) kidney damage and serious harm. On the contrary, underestimation is also a serious problem, for example in the case of our patient Jack who is taken off Metformin because of his low eGFR. Instead of simply taking Metformin, he will have to inject himself with Insulin. A much more expensive and unpleasant therapy. What if his true GFR was actually higher and he could safely stay on Metformin for now?

There is an additional need for having a precise and true estimation of GFR. Our vision was to simply take a blood sample and get a GFR value that is almost as good as mGFR. With single markers this is not feasible, but by using the constellation of several biomarkers, it is. *AXINON® Clearance Check°* is a serum test that does exactly this. It uses a set of serum metabolites previously reported to be altered by kidney (dys) function and combines them to yield a much improved assessment of GFR (GFR_{NMR}). The concentrations of all of these metabolites reflect different reasons of the kidney dysfunction, such as metabolic acidosis or oxidative stress. By combining the concentrations of metabolites and GFR_{NMR} *AXINON® Clearance Check°* provides a deeper insight into kidney function. So instead of just seeing to which extent the kidney function is impaired, it may even be possible to see what might be the reason and what could be the best treatment for a patient.



To get a deeper understanding of Jack's kidney function, his doctor ordered the innovative AXINON® Clearance Check° test. His results showed that actually his GFR is still at 50 ml/min/1.73 m² and he can stay on Metformin. His physician also noticed that Jack's Dimethylsulfone level is elevated, hinting into the direction of oxidative stress as reason for kidney impairment. □

Doris Zeugner, Product Management

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10. (KDOQI), K.D.O.Q.I., KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kindney Int, 2013. 3(1).

° For Research Use only in the United States. numares' products are not yet available for sale within the United States; they have not yet been approved or cleared by the U.S. Food and Drug Administration.

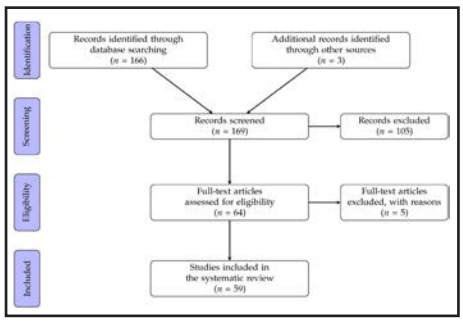
** All photos used in this article are stock photos, that were used to illustrate the contents. The depicted persons are models. All persons listed in this article are purely fictitious and any resemblance to existing persons is purely coincidental.*

#numares@Social Media

Welcome to the social media world of numares! By following the LinkedIn (.com/company/numares-ag) profile of numares, you will get a continuous flow of news around our products, developments and the company itself. Here is an excerpt from the latest news:

numares management team member **Philipp Pagel** and HR manager **Floxie van der Sterren** welcome **Sean Keohane**, new Vice President of Marketing & Sales in the U.S.! Sean succeeds **Claus Botzler**, former U.S. Sales President. Sean will be responsible making numares' disruptive diagnostic technology combining **#metabolomics** and **#MGS** available to US customers. Congrats, Sean, and all the best for the upcoming challenges in the **#precisiondiagnostics** and **#precisionmedicine** market!

#numares #metabolicconstellations #NMR #MagneticGroupSignaling



numares article "**#Metabolomics #Biomarkers** of **#ProstateCancer**: A Systematic Review" has been published in MDPI Diagnostics as part of the Special Issue Diagnostic Biomarkers in Prostate Cancer.

TransPot #metabolomics #MGS #metabolicconstellations #precisionmedicine #precisiondiagnostics #cancer #oncology

Today, **#numares** is in Cologne! We are working on the distribution of the AXINON IVD system, offering clinical laboratories access to diagnostic tests based on **#metabolicconstellations** and **#MGS**. Let us improve patient care by using more than one biomarker in several indications! □

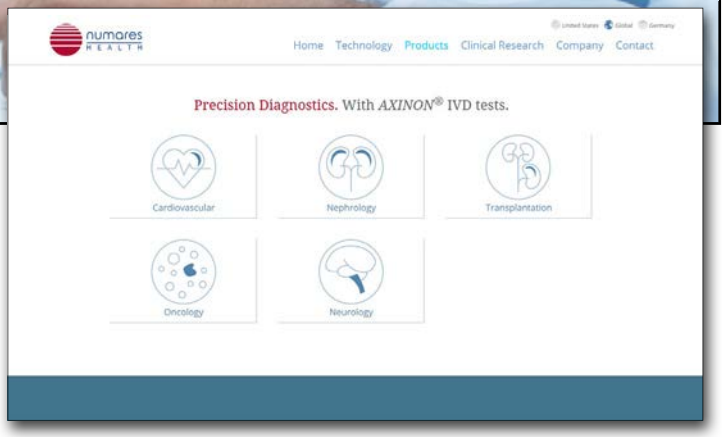
#Machinelearning #AI #precisionmedicine #diagnostics

Learn more at
https://lnkd.in/gGMf9EW

Christiane Proll, Social Media Marketing

NEWS

Launch of numares new corporate website



These days we are happy to launch numares' new corporate website "numares.com".

In the "technology" section you will find detailed information on the numares approach, using metabolic constellations and our proprietary *Magnetic Group Signaling (MGS®)* technology enabling NMR and Metabolomics to be used in assessing and evaluating diseases, e.g. in oncology, nephrology, cardiovascular diseases as well as transplantation medicine.

The "products" section gives you an overview of all avaible numares tests, integrable on any *AXINON® System*. For them and future products we illustrated the underlying medical needs of our IVD development efforts.

The new section "Clinical Research" introduces our approach and efforts in the development of novel diagnostic products in valuable partnerships.

Finally, the company section gives you an abstract on standard information, all media activities like news in the press and social media, but also current information on milestones, upcoming events and job opportunities.

The website meets current technological standards. Besides being consumer-friendly by reaching required contents with minimal clicks, the website is optimally displayed on desktop as well as on mobile devices by a responsive design and appropriate functionalities. □

Christiane Proll, Digital Marketing

Pleased to meet you

European Atherosclerosis Society (EAS) Congress

May 26-29, Maastricht (The Netherlands)
https://eas2019.com/

American Transplant Congress (ATC)

June 1-5, Boston, MA
https://www.atcmeeting.org

European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Congress
June 13-16, Budapest (Hungary)
http://web.era-edta.org/

Annual Meeting & Clinical Lab Expo of the American Association for Clinical Chemistry (AACC)
August, 4-8, Anaheim, CA
https://19congres.com/aacc/

American Society of Nephrology (ASN) Kidney Week

November 5-10, Washington, DC
https://www.asn-online.org/education/kidneyweek/

American Heart Association (Scientific Lessons)

November 16-18, Philadelphia, PA
https://www.heart.org

Pictures: iStock

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Julia Hertlein, Marketing

LEGAL

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