# Customer Insights

- Predicting the Health of Future Generations Using High Resolution NMR

NMR

Innovation with Integrity

# Investigating Human Health: Applying Standardized Nuclear Magnetic Resonance (NMR) Spectroscopy for Research to Human Diseases

High resolution NMR spectroscopy enables scientists to generate spectra for analysis to quantify markers with statistical models of health or disease states at University Medicine Greifswald

Matthias Nauck's laboratory at University Medicine Greifswald uses standardized nuclear magnetic resonance (NMR) spectroscopy for epidemiological research.

"NMR spectroscopy is the leading analytical method to use in our biobanking studies. We know that test results with NMR spectroscopy in 2018 could be comparable with NMR spectroscopy results in 2028, taken from the same patient cohort and the data will be 100% comparable."

## Activities at the Institute of Clinical Chemistry

Matthias Nauck is a Professor at the Institute of Clinical Chemistry at University Medicine Greifswald (Universitätsmedizin Greifswald) – one of the oldest higher education institutions in Germany. Research at the university covers a broad spectrum of fields, with five key areas of research: Community Medicine and Individualized Medicine, Environmental Change: Responses and Adaptation, Cultures of the Baltic Sea Region, Plasma Physics, and Proteomics and Protein Technologies.

Professor Nauck's role is split between a focus on patient care and a range of scientific research projects. The university hospital has over 1000 beds and cares for additional patients in the area of Greifswald. Professor Nauck and his team within the Institute of Clinical Chemistry and Laboratory Medicine are involved in patient care here, as well as across additional hospitals in the local area. Professor Nauck explains:

*"We conduct about 3 million analyses per year at the Institute of Clinical Chemistry, with our routine lab working 24/7. I also have a special interest in new-born screening, which I address here in Mecklenburg state and in collaboration with our partners in Szczecin, Poland."*

He adds: "*Our other interest is point-of-care testing. We are heavily involved in the quality assurance and quality management of point-of-care testing in the region.*"

## NMR and biobanking

The key function of biological membranes – the structure surrounding cells and intracellular compartments – is to protect the cell and regulate the entry and exit of specific molecules. Membrane

proteins are incorporated into the lipid bilayer which makes up the membrane structure, enabling the specific passage or transport of selected substances across the membrane. Cell communication is another vital role of membrane proteins: many act as sensors and receptors, to receive incoming signals at the outer surface of the membrane and transduce across the bilayer. Membrane proteins serve key cellular functions in all cells, ranging from substrate sensing in prokaryotes to voltage-gated ion channels in nerve cells in the human brain.

Biobanking is defined as the practice of creating large-scale repositories of human biological material (for example blood, urine, tissue samples and DNA) designed to further medical research. Professor Nauck has collaborated with Bruker in the biobanking area, he explains further:

*"We developed a system with Bruker for lipoprotein diagnostics, involving all the subclasses, in collaboration with Freiburg University. This is promising, and now we are looking at how changes in these lipoproteins are reflected in clinical and sub-clinical states. In the biobanking area, independent from the disease risk and biomarker research, our problem is that samples which are stored over decades can change. We can never be sure that the sample will remain unchanged over time. The high reproducibility of nuclear magnetic resonance (NMR) spectroscopy makes it a promising area where we can characterize our stored samples and state if these are OK, or if there are some changes leading to artifacts. Artifacts are interesting but not helpful for patients, which is important to know to perform future research."*

## Bringing standardized NMR spectroscopy to Greifswald

In the core laboratory at Greifswald, Professor Nauck and his team perform analyses in hematology, hemostasis and in traditional clinical chemistry, as well as in specific immunoassays; all are performed in a 24/7 working environment. The team have very short turnaround times for analyses. For example, a sample is transported from the emergency ward via a pneumatic tube system covering the distance of 300 meters in 30 seconds. The sample is then placed immediately into their track system which connects many analyzers. This ensures a high degree of standardization and completely automates the processes in the laboratory. Professor Nauck explains further:

*"When we implemented a track system in our core laboratory, one of the key aspects was the standardization we felt we could achieve across all our procedures. In the past, when we were working with stand-alone analyzers, we had more anomalies, but having applied the track system we know that our sample preparation, centrifugation and all the timed steps of our work are identical at 9am and 9pm. Therefore, standardization is very important."*

## Long term epidemiological research: the SHIP study

Professor Nauck introduced NMR spectroscopy to the laboratory at Greifswald upon their initiation of epidemiological research in Greifswald. The University of Greifswald started the SHIP study (Study of Health in Pomerania) in 1997 [1].

Professor Nauck explains the SHIP study in more detail:

*"In 1997, the SHIP study was started in Greifswald, looking at 4,400 individuals living in this region. We collected blood, urine and other biomaterials with highly standardized procedures. 10 years later we set up the SHIP trend study with newer instruments for the diagnostics of these patients. The number of probands is limited, but it is sufficient to look at common diseases such as coronary heart disease and diabetes. However, when looking at rarer diseases, such as cancers, then much higher numbers of probands are required. This was one of the driving forces behind the German National Cohort."*

This SHIP study has been running for over 20 years and the team is still investigating the population included in this study. Professor Nauck explains:

*"We took samples from individuals at the start of the study and have invited them to our study center every 5 years since, so we have more than one bio-sample for these individuals. We are now interested in looking for biomarkers that can detect risk of diabetes, for example. We can then compare samples from our probands which are 20 years, 15 years, 10 years and down to 5 years old. "*

## NMR in epidemiological research

If NMR is compared to other techniques such as mass spectrometry (MS), which is much more sensitive, it is not possible to standardize MS measurements year on year. There would be systematic shifts which would

not be helpful in epidemiological approaches, which are the basis of measuring the metabolome of individuals at several intervals across their lifetime.

*"We are changing the view from the past… to measure results during the hospital stay of a patient to see if this is helpful in an actual situation"*

Professor Nauck and his team have completed numerous studies with analyses performed using data from the SHIP study and some from a later phase in the GANI_MED (Greifswald Approach to Individualized Medicine) project. This has allowed Professor Nauck to apply for approval to go directly into patient care so the results are available when the patient is in the university hospital. Professor Nauck explains:

*"We are changing the view from the past and now have the time to calculate and measure results during the hospital stay of a patient to see if this information is helpful in an actual situation."*

## The German National Cohort

The German National Cohort is now the biggest German epidemiological study, recruiting overall 200,000 individuals in 18 centers across Germany. In this project, Professor Nauck was responsible for the quality of the samples in the study. Therefore, he established sample procedures at each study center with identical equipment and standard operating procedures (SOPs). The study has already recruited more than 180,000 of the planned 200,00 probands. From each proband the team collect more than 100 aliquots, at the very least, so the number of aliquots in the biobank is higher than 20,000,000. The serum, plasma, urine and other biomaterials are stored in part at the Helmholtz Zentrum München, in the gas phase of liquid nitrogen at -180 °C, which are the optimum conditions to keep the samples stable over long periods.

*Professor Nauck sees NMR spectroscopy as the leading analytical method, providing the confidence that results from 2018 will be 100% comparable with results from the same patient in 2028.*

The aim of the German National Cohort study is to look at individuals developing diseases, some of whom are already ill, and follow-up the development of diseases, as well as checking biomarkers from a clinical chemistry point of view to see if anything can be said about the progression of these diseases. The aim is for NMR measurement to be implemented in this study to form the basis of reference ranges. The team plans to start analysis during the follow-up period, in order to have different time points in future to compare how each individual changes in view of health status and metabolomic state.

Professor Nauck sees NMR spectroscopy as the leading analytical method for such a prolonged study. Looking forward, NMR spectroscopy provides confidence that the test results from 2018 will be 100% comparable with results taken from the same patient cohort in 2028. This is important to Professor Nauck's team when they are looking for biomarkers with only slight changes in state, as he explains:

*"Historically, in clinical chemistry, we used tumor markers which increased thousand-fold in the specific situation. Now, however, we are continuously looking for sub-clinical deviations. These are, by definition, significantly smaller changes, meaning we require a very standardized procedure to detect them. NMR spectroscopy is very useful to ask and answer these questions in relation to biomarkers of specific clinical outcomes. When performing classical laboratory analyses, in most cases, we look at the results to determine if there is no specific result or if it is a pathological result. In the development*

*of individualized medicine (also known as personalized medicine or precision medicine) we are looking for sub-clinical changes. We do not have clarity that an individual is healthy or ill, instead we have many differential states."*

It is now Professor Nauck's aim to detect the minimal changes from a 'healthy state' to a 'disease state' in order to inform preventive medicine approaches. The goal is to identify those individuals at risk of a specific disease and to detect those people earlier, then the focus is on keeping the individuals healthy for longer. Therefore, it is no longer a case of looking for healthy or ill, but tiny clinical variations that indicate a significant change.

## Metabolomic aging study

Disease risk profiles depend on a number of factors, with chronological age being the most critical. Aging implicates a wide range of biochemical processes, and metabonomics has emerged as a new tool for characterizing these biochemical changes. Professor Nauck has published research about the metabolic age of patients [2], as he explains:

*"We are able to measure the metabolic age of patients using one urine spot sample. We looked at our SHIP probands and the metabolic age adapted from the NMR spectra, and made the observation that patients with diabetes mellitus (type II) are older in view of the metabolic age. We know these people are at an increased risk of developing diseases or death. We are optimistic that, in a personalized medicine approach, it is now possible to define metabolic age using a urine sample and classify patients at an increased risk and separate from patients with a decreased risk, to ensure we can help patients in preventing progression of diseases. Another point is, from a pre-analytical aspect, urine is quite easy to work with as it is not fresh. Urine products are stable and it is simple to send samples to the lab and get this information back."*

## Examples of subclasses of Lipoproteins work

1. Professor Nauck and his team developed, together with Bruker, an algorithm to analyze lipoproteins in a detailed manner to feed information about their subfractions [3]. It has been known for some time that these associations are more predictive of coronary heart disease than the classical cholesterol concentrations.

   In addition to these specific lipoproteins, it is possible to measure and quantify many other elements in the blood. Professor Nauck is performing NMR spectroscopy analyses in patient care, which is included in clinical studies. This information is added to determine a more detailed picture of these patients in order to classify them for risk of developing a specific disease.


2. Quantification of many analytes is now possible from urine samples, for example, due to NMR spectroscopy. Professor Nauck and his team looked for the creatinine concentration and ratio to check if this information is clearer and has more added value than classical creatinine concentration. Therefore, the team is now checking different aspects in patients to see if additional information can be attained using the metabolome. To perform this kind of research, it is essential to have standardization and good quality clinical data from patients. Professor Nauck and his team established this procedure from the GANI_MED research project which was started several years ago in Greifswald [4]. The GANI_MED research project is an attempt to establish an individualized medicine program at a university hospital. Using state-of-the-art diagnostics and novel therapeutic interventions that take into account the specific characteristics of an individual patient or a well-defined patient group, individualized medicine aims to increase the efficiency of treatment strategies, avoid adverse reactions, and to reduce health care expenditures. Professor Nauck and

several colleagues are still using this structure so they have high quality clinical data from patients to combine them with NMR spectroscopy results.

## The bridge to routine use

Instead of providing the clinician with hundreds of pages of information, Professor Nauck and his team are now able to look at the analysis of the 'omics data and provide a simple guide as to the condition of a patients' organs. Professor Nauck explains the color system developed and the benefit of NMR as a technique:

*"Green shows that the organ is OK, yellow shows some limitations and red indicates a poor condition. The clinician then knows whether to look further at the test results to provide additional detail for clinical treatment. When we are looking at 'omics technologies, there are many techniques available. We prefer NMR spectroscopy because we do not have to complete sample preparation in advance. Additionally, we store the samples after measurement so they could be used for additional analysis. The reproducibility of NMR spectroscopy results over time is very important for us because we have to compare old results with new results, to detect small deviations and confirm we have comparable data."*

*'We are observing significant improvement in our operability since installing the AVANCE IVDr. The instrument can truly be applied in a 24/7 mode'*

## Working with Bruker

Bruker has facilitated numerous collaborations and enabled groups to work together. For example, London is an important partner to the team at Greifswald. Professor Nauck has a new collaboration with Bangkok to exchange knowledge and samples, in order to show the benefit of NMR spectroscopy results for other research groups. Another aspect is that Professor Nauck collaborates with different epidemiological studies where they exchange samples and learn to reproduce investigations in different cohorts, to ensure findings are relevant for patients and clinicians.

*'We trust each other. It's the ideal combination – Bruker's strong competence in NMR spectroscopy and our expertise in clinical and epidemiological studies'*

Many of the working groups applying NMR spectroscopy are using Bruker instrumentation. Professor Nauck elaborates further:

*"We trust each other. Bruker has know-how in NMR that I do not have, but we have the experience in epidemiological work to perform clinical studies and looking for new biomarkers. It is an ideal combination to have Bruker's strong competence in NMR spectroscopy with our expertise in clinical and epidemiological studies, sample processing and biobanking steps (such as sample handling) which are all important elements in avoiding mistakes. From this point of view, we have a great collaboration. Our goal is to improve medicine and there are different ways to do this. For us, quality is key and so we constantly reevaluate steps to ensure our results are sustainable."*

The built-in software has proved an additional benefit for Professor Nauck as when he uses Bruker's data processing, the data is sent to Bruker automatically, removing the manual step in the process.

## Instrumentation

Professor Nauck has been working with Bruker and NMR spectroscopy since 2004. He purchased his first NMR spectrometer with a 400 MHz magnet but now has replaced this with the AVANCE IVDr system, a 600 MHz instrument. Professor Nauck explains the importance of resolution:

*"We founded a company called Baltic Analytics, where we have been using a 600 MHz instrument for the last 10 years. We have completed many publications focused on the stability of samples, so we have a lot of experience in pre-analytical approaches to avoid mistakes. The resolution of the 600 MHz is much better than 400 MHz and has developed in line with the progress in medicine. When we are collaborating with other groups interested in NMR spectroscopy, they are all working from a 600 MHz platform, so from this*

*point of view the standardization is very important and something we have to fulfill. Finally, to be compatible with other research groups, it is essential we also meet this standard."*

When looking at the different instrument vendors, Professor Nauck chose Bruker for the company's approach to instrument development:

*'The resolution of the 600 MHz is much better and has developed in line with the progress in medicine'*

*"Bruker is outstanding and is driving the research questions. It wants to improve its products and develop applications with clinicians. We are working with Bruker for research purposes only, but more and more we are looking to translate into clinical patient care. This changes the collaboration with Bruker as the performance and availability of the analytical platform is much higher when we are involving these systems in patient care."*

Bruker has strived to improve the applicability of its systems for operators, making it easier to apply for routine use, which will be one of the precursors for the future. Professor Nauck explains his plans for next steps *in instrumentation:*

*"We are now observing significant improvement in our operability since installing the AVANCE IVDr. The system can be used both in partnership with our existing technology as well as in comparison for analyses. In applying the system, temperature and stability ensures it is easier for technicians to use the system as a whole. The instrument can truly be applied in a 24/7 mode."*

## Future of NMR

The medicine landscape is changing and Professor Nauck and his team are on the way to improving this in terms of individualized medicine. They are also trying to improve the health status of the population, whilst looking at disease prevention. Professor Nauck feels that for both sides, laboratory diagnostics is needed to accompany these ideas:


*"For patient care in the hospital, we need biomarkers that can classify the patients that need to be operated on. We need it for clear diagnostic decision making and when we want to adjust some therapeutic decisions, such as when we need a higher dose or lower dose. This decision making could be independent from some of the established laboratory analyses, so we have to apply measurements like the NMR system to measure reliable biomarkers that are important for such decisions."*

Another approach is prevention. Professor Nauck is leading the way in measuring the metabolic status of individuals living in the local area, then re-measure the metabolic status 5 years later to see if the development of the metabolic stage is normal or if there are abnormal deviations. It is important to take learnings at the beginning and help people avoid development of specific diseases in the future.

For more information on the University Medicine Greifswald (Universitätsmedizin Greifswald), please visit https://www.medizin.uni-greifswald.de/de/home/.

For more information on the AVANCE IVDr NMR system, please visit https://www.bruker.com/products/mr/nmr/avance-ivdr/overview.html.

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