MedInnovation
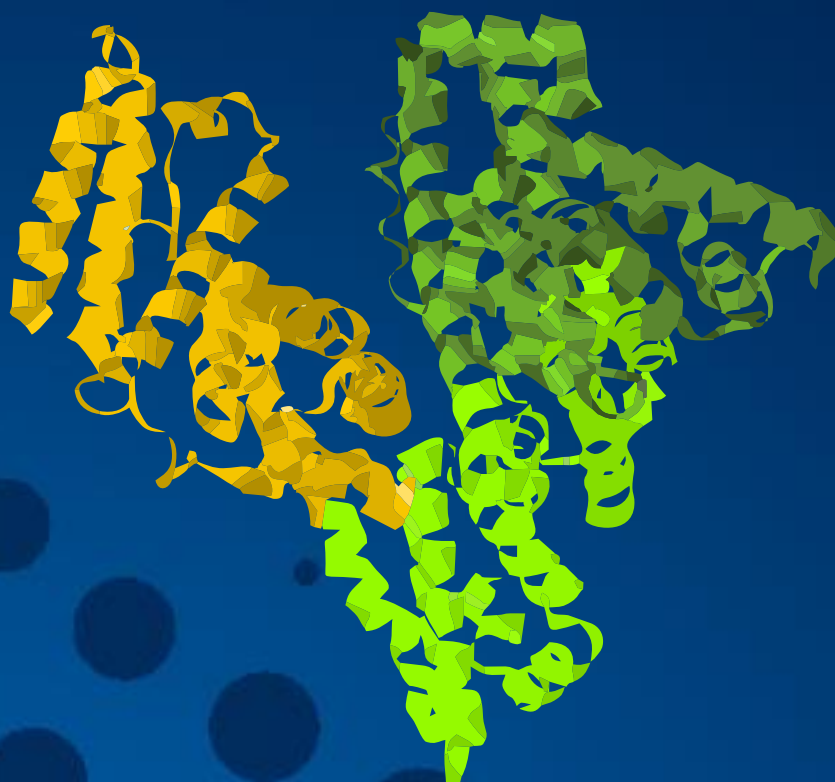## Advanced Albumin Research

Analysis of functional characteristics of serum albumin in intensive care medicine
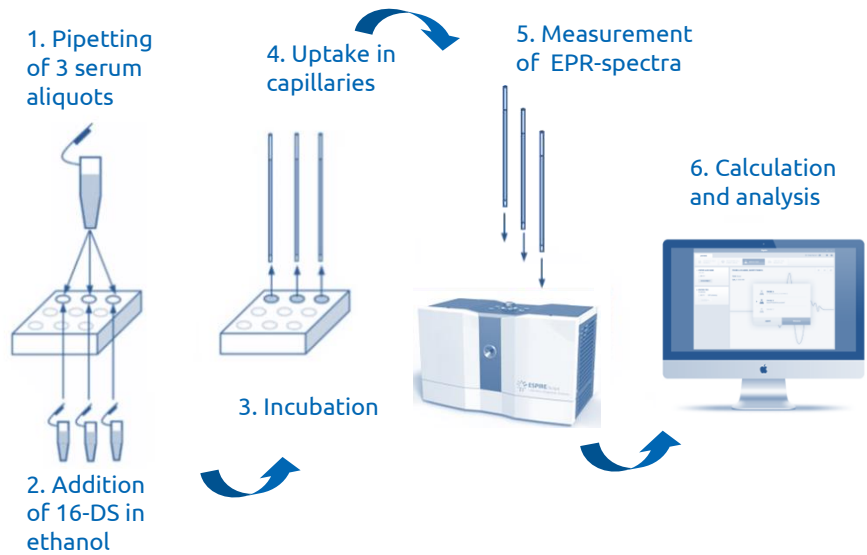
Principles - Albumin

Albumin is the most abundant protein in human blood serum. It is produced in the liver and has a serum half-life of approximately 19 days.

Transporting a large variety of hydrophobic substances like fatty acids, drugs and metabolites is one of its main physiological functions [1].

Beside this it maintains the oncotic pressure and buffers the pH of the blood.

For long chain fatty acids seven binding sites are known [2]. Three of them with high and four with lower affinity [3]. The binding sites with high affinity are described as long and narrow pockets, whereas those with low affinity are short and wide [2].

During the last years low molecular weight biomarkers bound to serum carrier proteins like albumin were intensively investigated, assuming they might have a potential for early disease detection [4, 5, 6].

1. Pipetting of 3 serum aliquots

2. Addition of 16-DS in ethanol

3. Incubation

4. Uptake in capillaries

5. Measurement of EPR-spectra
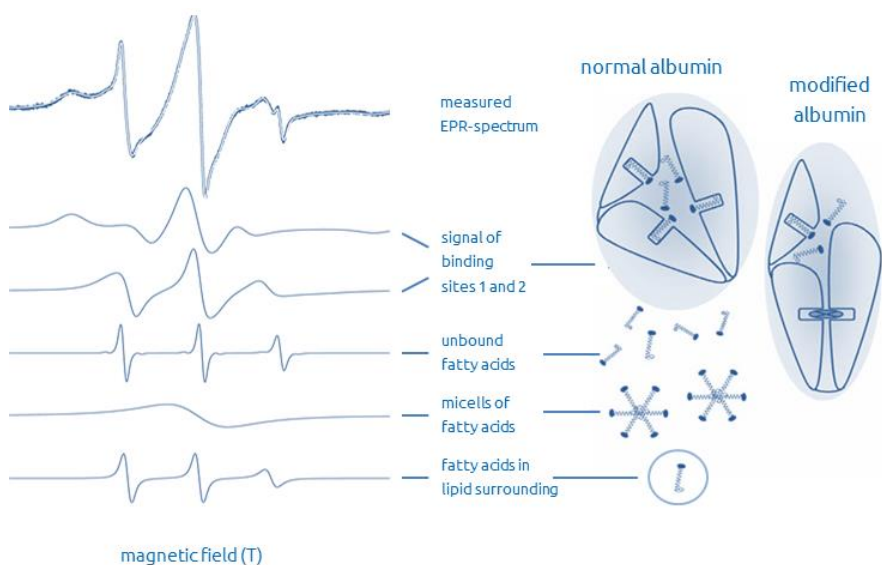
6. Calculation and analysis

EPR technology

The albumin-functionality-test uses electron paramagnetic resonance spectroscopy (EPR) to estimate the functionality of albumin in human serum.

It is based on a comparison of three different albumin/ethanol solutions, which simulate binding, transport and release conditions in vitro [7, 8]. By adding a spin-labelled fatty acid, the binding sites of albumin can be investigated.

Albumin transport parameters binding efficiency (BE) and detoxification efficiency (DTE) can be estimated by simulating the EPR spectra. DTE describes how well metabolites and toxins can be eliminated, also if there exists an increased accumulation of this substances in the organism. Additionally the binding efficiency (BE) describes capacity of the fatty acid binding sites.

measured EPR-spectrum

signal of binding sites 1 and 2

unbound fatty acids

micells of fatty acids

fatty acids in lipid surrounding

normal albumin

modified albumin

magnetic field (T)

Equipment

ESPIRE Scope
Laboratory Diagnostic Analyzer

Applicable in standard laboratory routine – easy to handle.

Automated device – generating parameter control algorithms, automated measurement procedure, signal registration and pre-processing of spectra as an integral process.

Provides high accuracy, stability and sensitivity – at a high throughput rate.

Guarantees comparable results in the analysis of several aliquots of one sample.

Especially designed for the analysis of probes of biologic materials, where molecular conformation changes depending on temperature, pH and other factors occur.

All algorithms programmable and provide a wide range of routine as well as scientific applications.

ESPIRE AlbuMini
Automated Liquid Handling System

Accurate contact and non-contact sample preparation system for handling of chemical or biological solutions or suspensions

Avoids human pipetting errors, shows highly reproducible results and increases productivity

High resolution touch panel display with an intuitive user friendly interface

High precision of pipetting with less than 2% (10-14µl)

Diagnostic Kit

- Set of solutions of 16-doxyl-stearic acid in ethanol (three different concentrations with different cap color)
- 96-well microtiter plates for sample incubation
- Lid for microtiter plate
- Glass capillaries
- Wax on undercoat for capillary sealing
- Laboratory film for microtiter plate's wells closure during incubation
- Package

Fields of application

- Cancer diagnostic an monitoring
- Determination of albumin transport parameters in patients with liver disease or sepsis
- Quality control of commercial albumin solutions

DTE in patients with sepsis or SIRS

In a retrospective and masked pilot study the transport properties of albumin of patients in an intensive care unit were investigated.

Five patients developed a SIRS* and five a sepsis.

The transport parameter DTE in patients with sepsis or SIRS was compared to ICU patients without sepsis or SIRS (labeled as normal).
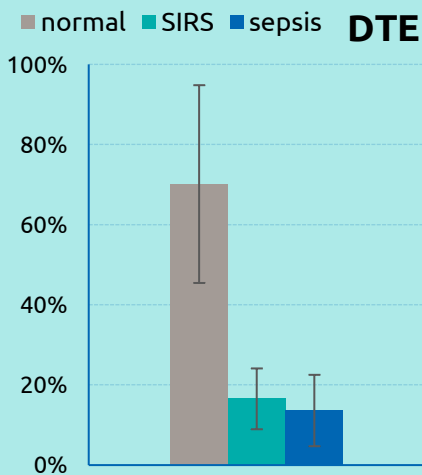
With values of less than 20% the detoxification efficiency is distinctly reduced in both groups in comparison to the physiological values and ICU control.

* Definition before sepsis-3

Time course of DTE

From patients in an intensive care unit blood was taken at the day of entry and 60, 36 and 12 hours before diagnosis by a standard method.

There are significant differences (P = 0.009, u-test) in the progress of the DTE between sepsis and SIRS.

While the DTE values of the patients with SIRS remain almost constant, the values of the patients with sepsis decrease over time.

Thus it might be possible, to predict the progress of a sepsis with high probability.
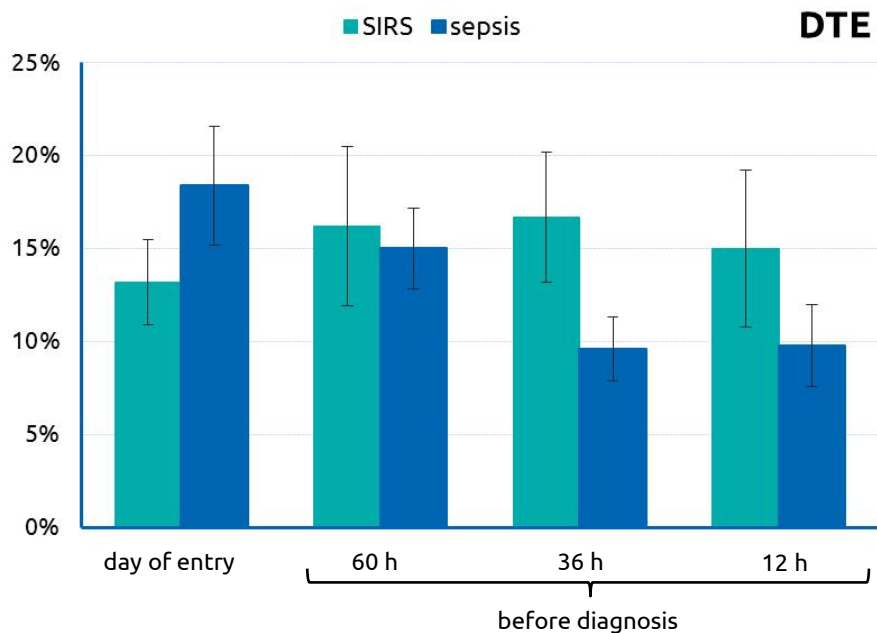
This can be realized by daily determination of the albumin transport parameters initiated from day 1 of the patient monitoring.

Hence a tendency will be timely recognized, and systemic measures could be initiated earlier.

These first findings are currently verified in a study [University Rostock und Fraunhofer IZI; DRKS00025079].

Comparison with other parameters

A comparison of other clinical parameters and parameters of our albumin-functionality-test was made, to find out whether the albumin-functionality-test show equal or better results in differentiation of sepsis from SIRS.
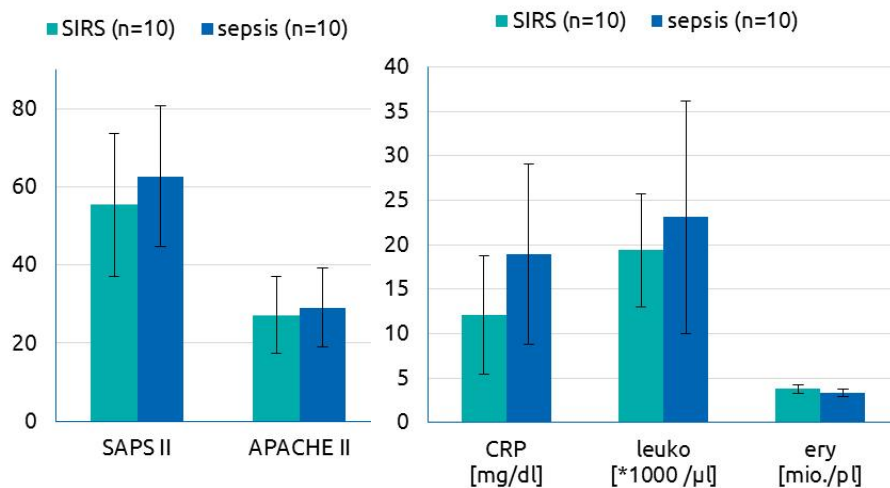

As to be seen in the figures and table, SAPS II, APACHE II and count of leucocytes show nearly equal values for both groups. CRP, count of erythrocytes, BE, DTE and KB A show differences but without reaching significance.

Only RTQ (real transport quality) and kb2 (apparent binding constant of second binding site of albumin) show significantly different values.

Thus parameters of albumin-functionality-test show lower P-values (u-test) than other clinical parameters, except for count of erythrocytes.

Using logistic regression, the values between patients with sepsis and SIRS differ significantly by combining RTQ with kb2 and KB A in one equation.
As a result of that, statistical analysis shows a P-value of 0.023, which is better than all single parameters.
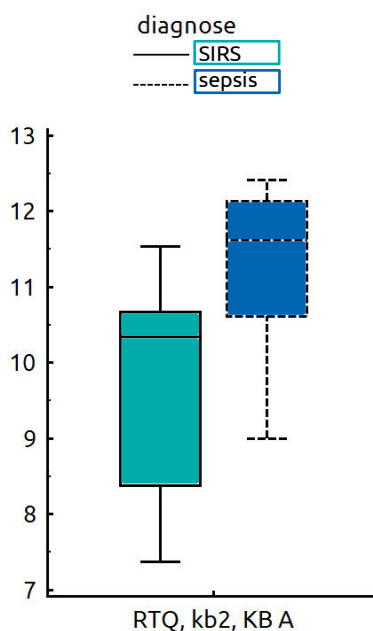
For patients with sepsis or SIRS other clinical parameters like:
- SAPS II (Simplified Acute Physiology Score)
- APACHE II (Acute Physiology And Chronic Health Evaluation)
- C-reactive protein
- Procalcitonin
- blood count

were also available.

As procalcitonin was available only for 50% of the patients, it was not used for statistics.

	u-test			u-test
SAPS II	0.473		**BE**	0.082
APACHE II	0.650		**RTQ**	**0.049**
CRP	0.141		**DTE**	0.131
leuko	0.821		**kb2**	**0.049**
ery	0.059		**KB A**	0.096

significant P<0.05

Conclusions

Although only in an initial feasibility study with small group sizes, parameters of the albumin-functionality-test have shown the potential to be useful parameters in patients with sepsis and SIRS and in the differentiation between these two groups.

Further studies are needed to verify these findings.

Conclusions

In patients with sepsis the determination of the detoxification efficiency from the albumin-functionality-test could be used for a disease progression monitoring.

To verify these results further studies were initiated.

Together with University of Rostock and Fraunhofer IZI (DRKS00025079) patients from an ICU with sepsis, septic shock and without these complications were compared in a pilot study and followed by a long term study.

CytoSorbents Europe GmbH is performing a study regarding the performance of CytoSorb® Adsorber (NCT04963920). Albumin-functionality-test will be used as one diagnostic method.

In all of these studies not only albumin transport parameters will be analyzed, but also parameter combinations. The aim is to yield the best diagnostic performance.
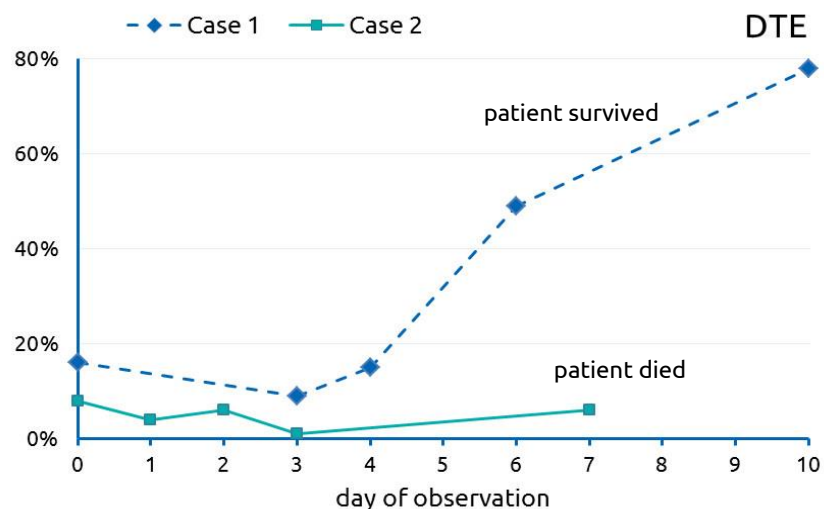
Long term monitoring

In a prospective study with patients of an intensive care unit blood samples were collected from the day of entry until leaving ICU and transfer to another department.

Two different patients are exemplarily depicted.
Clinical conditions:

Case 1: patient with sepsis (E. coli) after nephrectomy in consequence of kidney cancer, liver failure and encephalopathy, therapy with antibiotics

Case 2: patient with sepsis (C. albicans and C.spp), lymphoma with left lung affection, therapy with Fungizone®


Results from long term monitoring with albumin-functionality-test :

Case 1: patient showed increased detoxification efficiency in the course of monitoring, indication of selective effect of the antibiotics, good clinical prognosis confirmed, patient has survived

Case 2: patient showed nearly constant low detoxification efficiency in the course of monitoring, indication of missing enhancement by drug addition, confirmation of the negative tendency by death of the patient

In the above-mentioned study with University of Rostock and Fraunhofer IZI (DRKS00025079) ten patients of intensive care medicine without any suspicion for sepsis or septic shock were compared to ten patients with sepsis and six patients with septic shock.
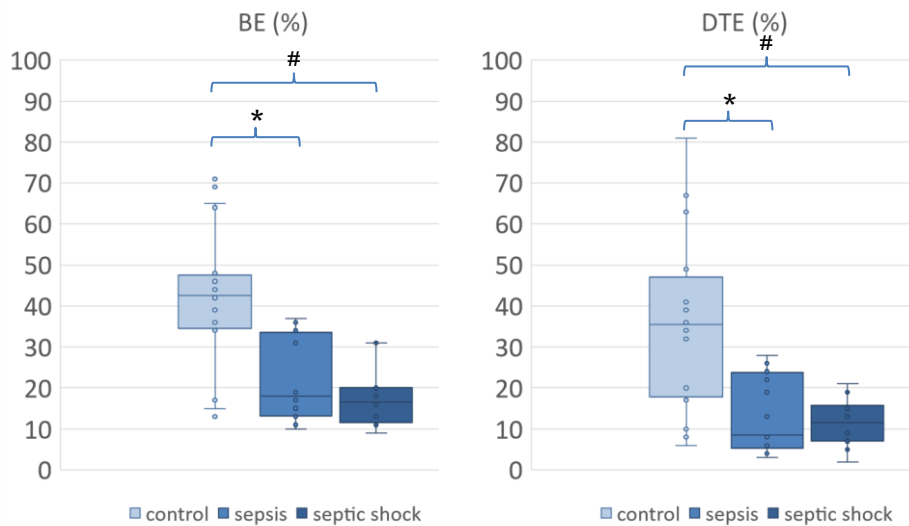
Mann-Whitney-test (independent samples) *P<0,01, # P<0,03

All patients show strongly reduced transport parameters in comparison to healthy individuals (100%). Binding efficiency as well as detoxification efficiency show further significant reduction in patients with sepsis or septic shock in comparison to patients without these complications.

A multiple linear regression analysis with the variables age and sex, inflammation related parameters PCT (procalcitonin) and CRP (C-reactive protein), liver related parameters bilirubin and albumin concentration and kidney related parameters creatinine and urea showed that urea and CRP are the best joint predictors for BE and DTE.

Date were submitted to publication.

The pre-study with University of Rostock and Fraunhofer IZI (DRKS00025079) was performed to acquire all relevant parameters needed to plan and conduct a monitoring study with patients from intensive care units. It was performed with the latest generation of devices.

In the course of this study transport parameters were compared between different patient populations. Followed by an analysis of lab parameters, age or involvement of organ disfunction.

Conclusion

All necessary parameters for sample size calculation and development of a study protocol for a bigger study monitoring patients from an intensive care unit are now available.

Literature

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