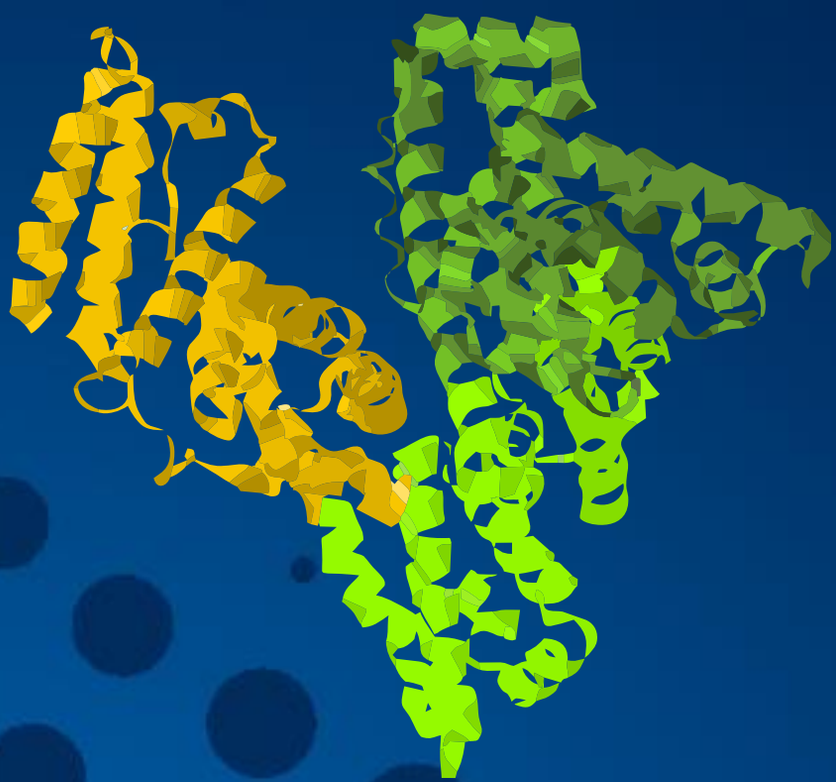
MedInnovation

Advanced Molecular Diagnostics



Disease progression monitoring for Sepsis

Analysis of functional characteristics of serum albumin

Method

Principles - Albumin

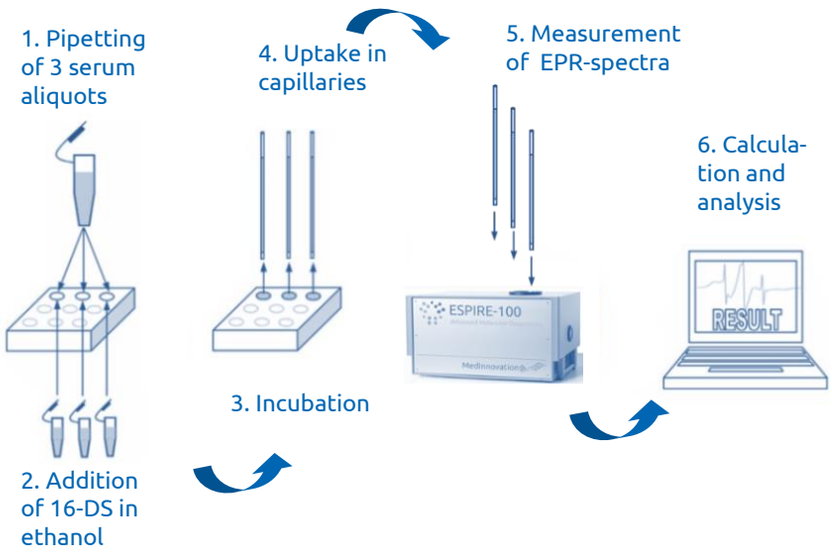
Albumin is the most abundant protein in human blood serum. It is produced in the liver and has a serum half-life of approximately 19 days.

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].

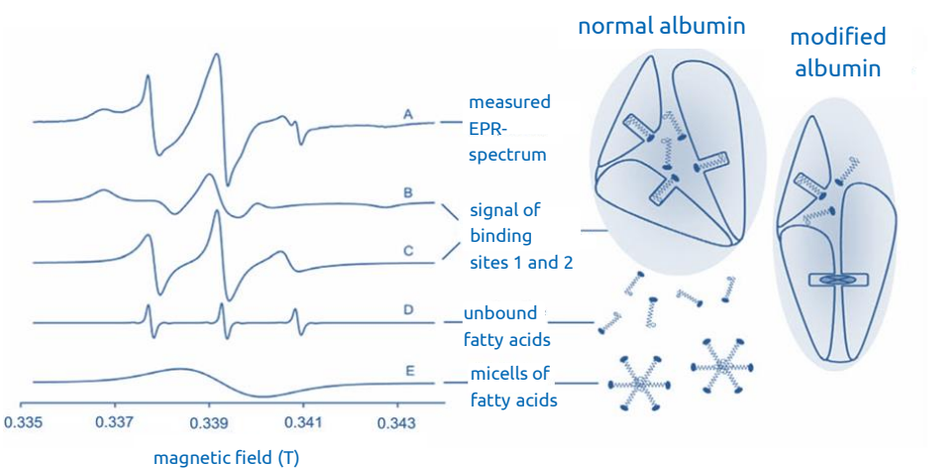


EPR technology

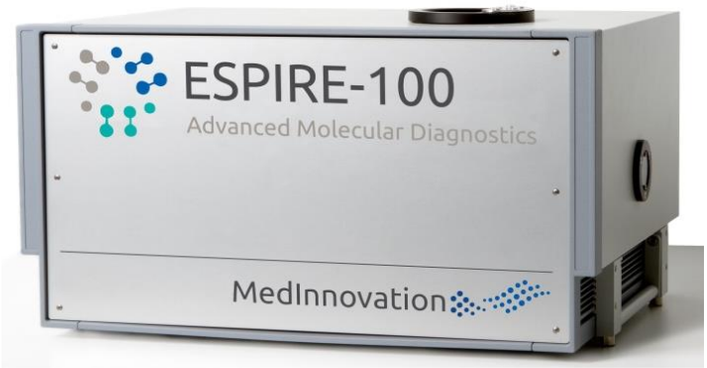
The albumin-functionality-test uses electron paramagnetic resonance spectroscopy (EPR) to estimate the functionality of albumin in human serum.

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.

Binding constants, binding capacities and biophysical parameters of both binding site types in the three different serum-ethanol-fatty acid solutions can be estimated by simulating the EPR spectra and hence the transport parameters (BE = binding efficiency, RTQ = real transport quality, DTE = detoxification efficiency) can be calculated.



Equipment



Device (EPR-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.
- All algorithms programmable and provide a wide range of routine as well as scientific applications.

Accessories box

The accessories box is a component of device and contains:

- Guidance tube
- Micropipetter
- Cup for Micropipetter (as compensation)
- Rubber caps for capillary positioning
- Glass tube cleaning equipment (4 syringes, 4 needles, Hellmanex III)
- Screwdriver for Guidance tube

Diagnostic Kit

- Set of solutions of 16-doxyl-stearic acid in ethanol
- 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
- Package

Transport parameters

The albumin-functionality-test based on EPR technology provides different parameters for comprehensive evaluation of the albumin functionality.

The **detoxification efficiency** (**DTE** in %) is a functional parameter for evaluation of the quality of albumin as transport vehicle in competitive situations.

It describes how well toxins can be eliminated and harmful substances can be delivered to the target tissue, also if there exists an increased accumulation of this substances in the organism.

Additionally the **binding efficiency** (**BE** in %) describes the fatty acid binding sites and the **real transport quality** (**RTQ** in %) the transport ability of the investigated albumin solution.

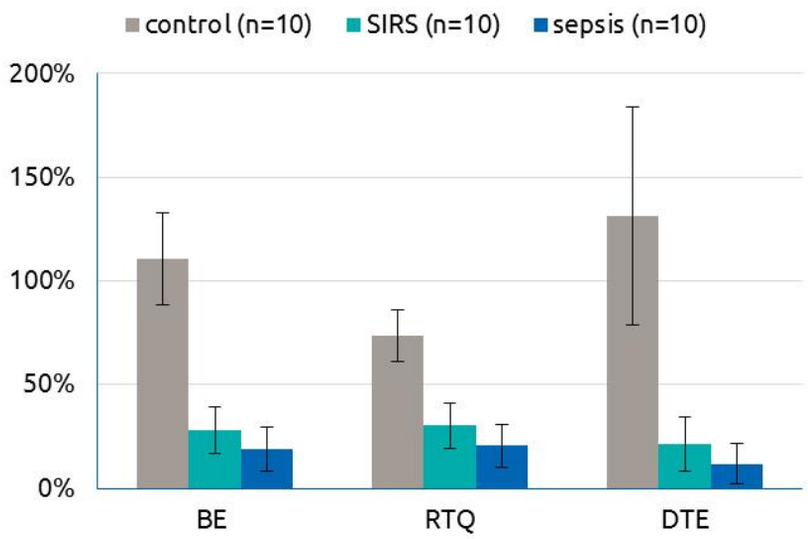
Furthermore two different kinds of **apparent binding constants** can be calculated:

1. for different binding sites independent of ethanol concentration (**kb1** – high affinity, **kb2** – low affinity)
2. a global one for each of the ethanol concentration (**KB A**, **KB B**, **KB C**).

Study results

A pilot study was carried out to investigate whether the transport parameters of our albumin-functionality-test are able to describe and to differentiate between patients with SIRS (Systemic Inflammatory Response Syndrome) and sepsis.

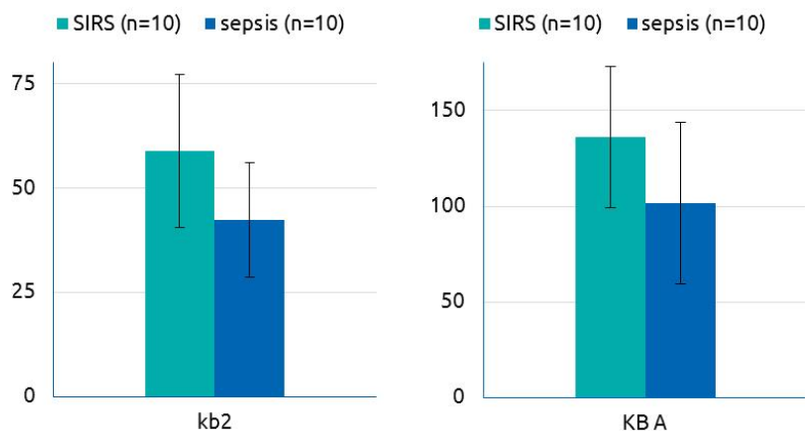
For comparison a control group of healthy people was measured. All samples were measured blindly.



All three transport parameters show a clear differentiation between healthy people and patients with sepsis or SIRS, whereby the last two groups show strongly reduced values with less than 30% of physiological values.

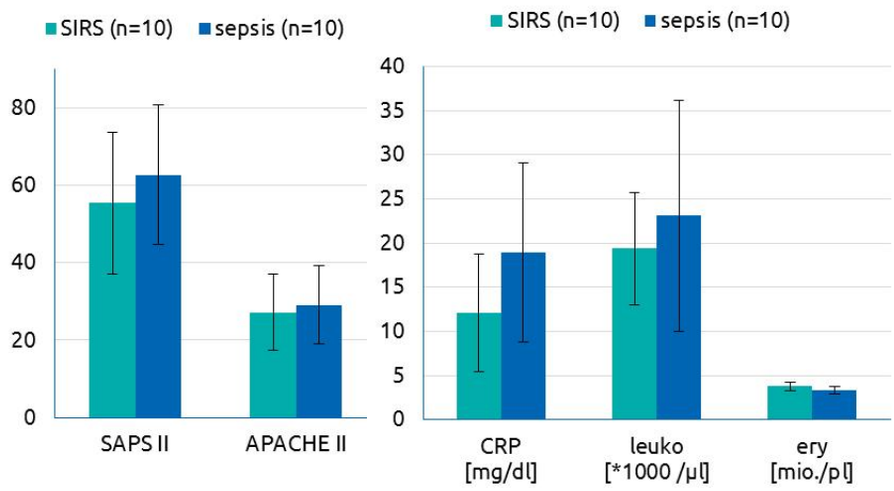
Additionally, in all three parameters patients with sepsis show lower values than with SIRS, but u-test shows significance only for RTQ (see table).

Furthermore kb2 (apparent binding constant of low affinity binding sites) and KB A (global apparent binding constant at lowest ethanol concentration) show the same effect.



Comparison with other parameters

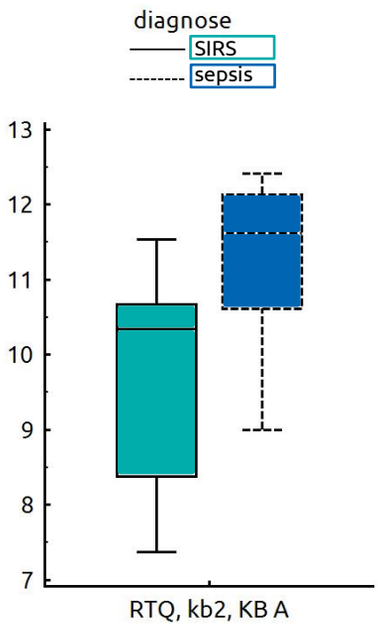
A comparison of other clinical parameters and parameters of our albumin-functionality-test was made, to find out whether the albumin-functionality-test show equal or better results in differentiation of sepsis from SIRS.



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 and kb2 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
SAPS II	0.473
APACHE II	0.650
CRP	0.141
leuko	0.821
ery	0.059

	u-test
BE	0.082
RTQ	**0.049**
DTE	0.131
kb2	**0.049**
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.

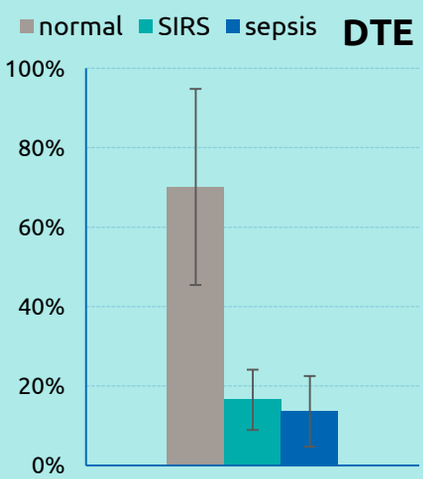
Time course of DTE

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.

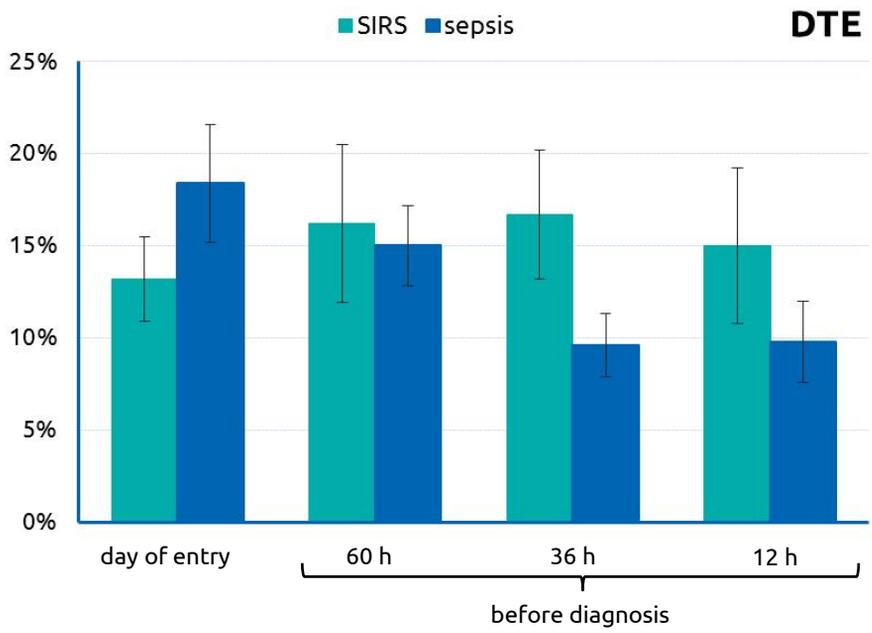
These values confirm the findings of the former study (see page 4).

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 will have to be verified in a study with larger numbers of patients.

Case reports - Sepsis

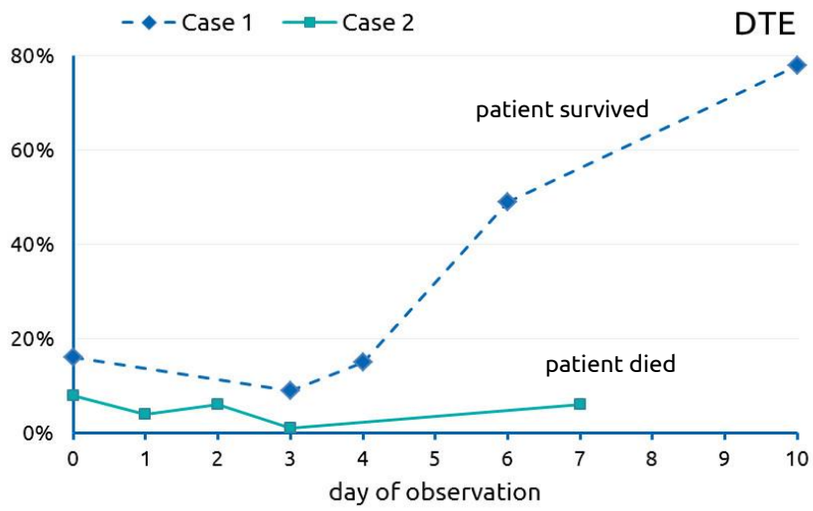
Long term monitoring

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

Conclusions

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

All fields of application of albumin-functionality-test

- Disease progression monitoring and prognosis of SIRS / Sepsis
- Disease progression monitoring and prognosis of acute liver failure
- Examination of the efficiency of liver dialysis systems
- Quality control of commercial albumins

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