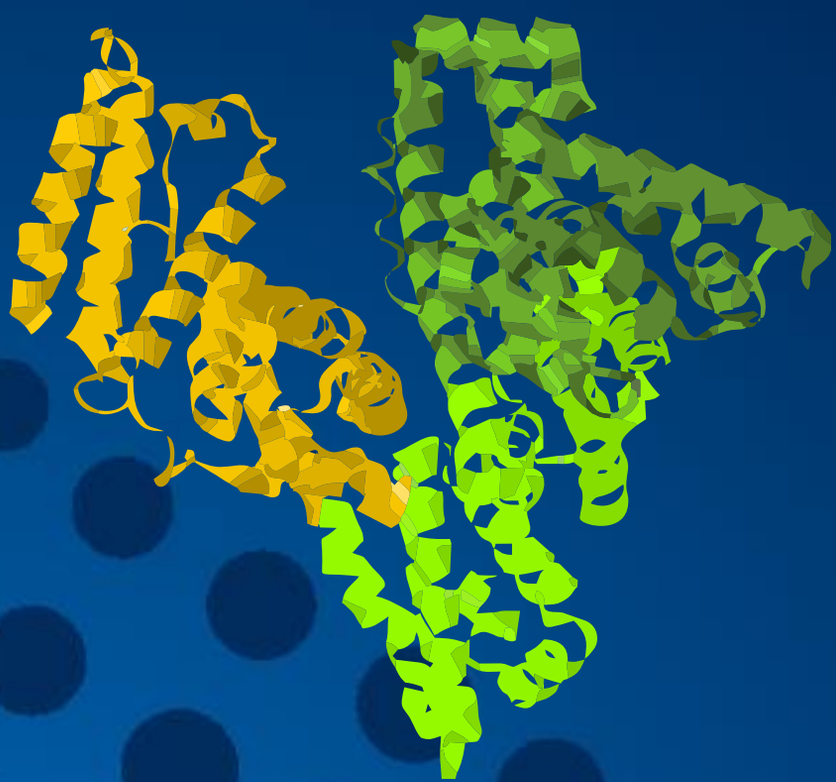
MedInnovation

Advanced Molecular Diagnostics



Analysis of functional characteristics of serum albumin

Method

Principles - Albumin

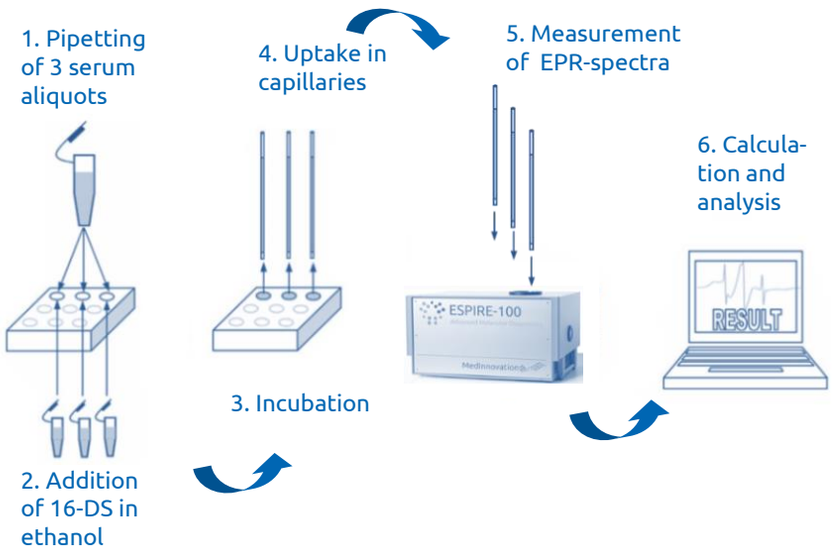
Albumin is the most abundant protein in human blood serum. It is produced in the liver and has a serum half-life of approximately 19 days.

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].

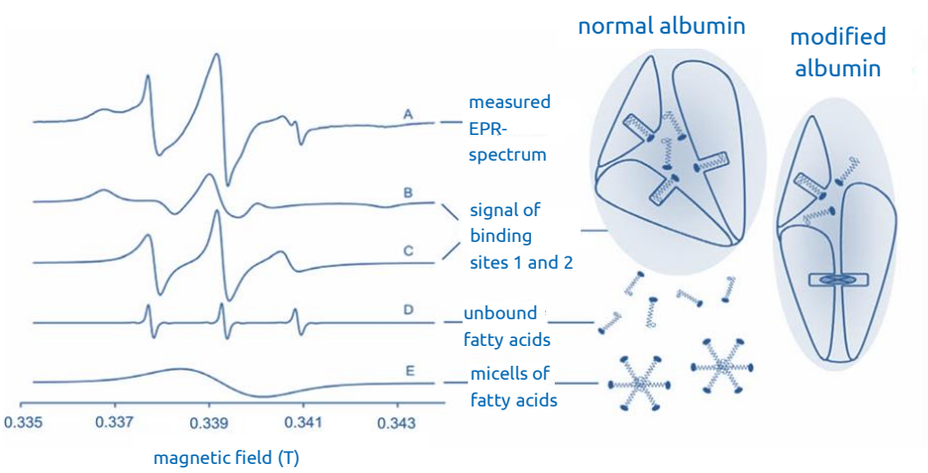


EPR technology

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.
- 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.

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

Applications

- Cancer diagnosis & monitoring
- Quality control of commercial albumins
- Estimation of albumin transport and detoxifi-cation parameters in patients with several diseases

Transport parameters

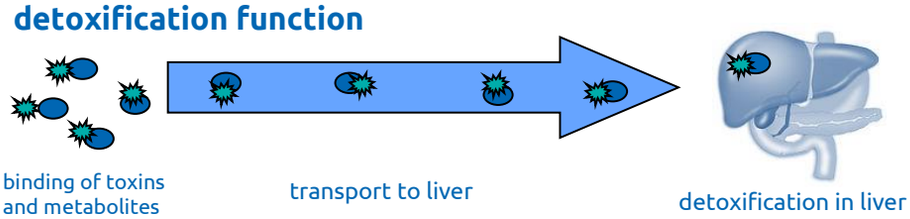
The albumin-functionality-test based on EPR technology provides different parameters for comprehensive evaluation of the albumin functionality.

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 function of the investigated albumin solution.

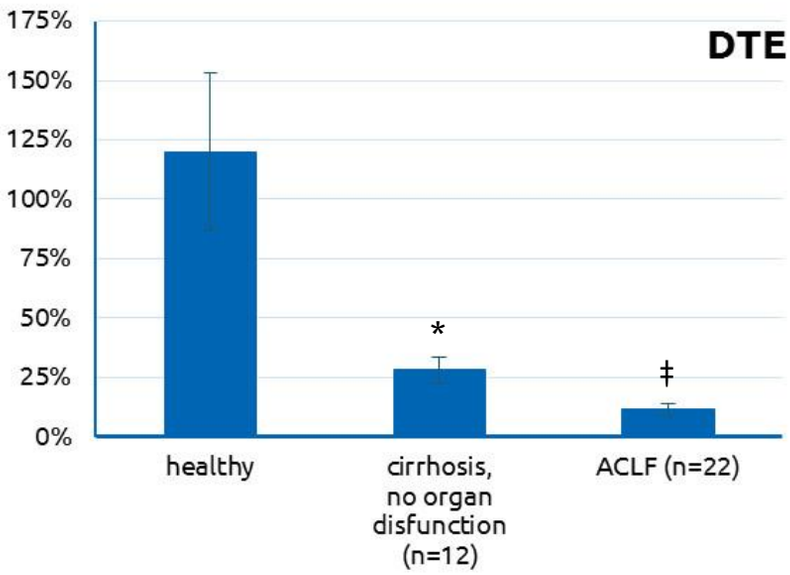
detoxification function



Study results

In a study investigating patients with different liver diseases, the albumin-functionality-test showed that the detoxification efficiency of these patients is reduced, in comparison to a healthy control group [9].

In patients with acute-on-chronic liver failure (ACLF), this parameter showed a further reduction in comparison to patients with liver cirrhosis without organ dysfunction.



* p<0.001 compared with healthy volunteers
‡ p<0,01 compared with cirrhosis

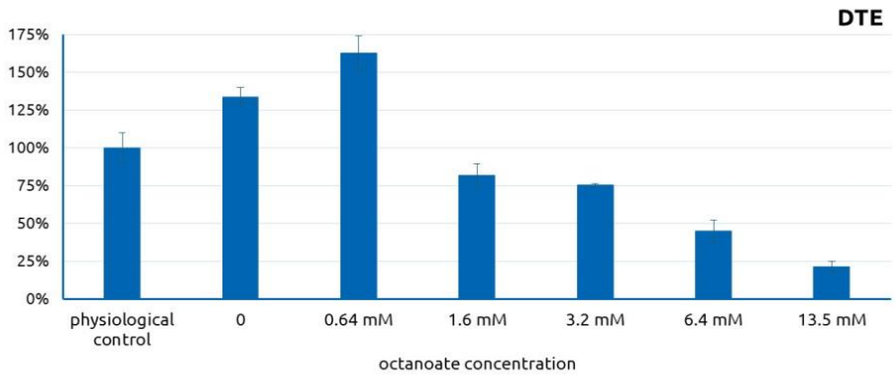
Commercial albumin

Quality control

In vitro experiments showed that the addition of a hydrophobic ligand to an albumin solution changed the transport properties of albumin, dependent on the ligand concentration.

Octanoate was used as a ligand, because it is a common stabilizer in commercial albumin solutions.

The commercially used concentrations range between 4mM and 20mM.



At octanoate concentrations above 3mM a distinct reduction of the detoxification efficiency (DTE) of the albumin was observed.

However, at concentrations below 1mM the DTE was increased in comparison to the physiological values.

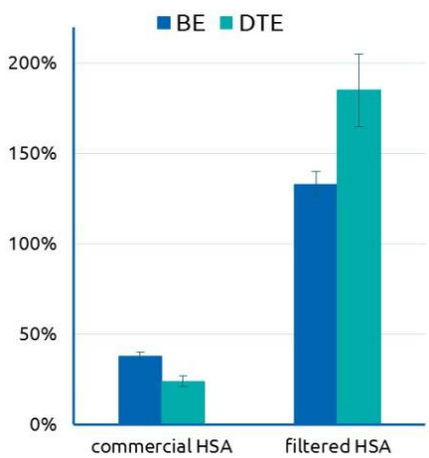
This could be explained with influences of nutrition, drugs or inflammatory processes in the normal population, which prevent it to be maximal.

Filtering of albumin

Commercial human albumin solutions (HSA) show reduced transport parameters before filtering.

After a filtration of the albumin solution with the Hepalbin™ – Adsorber (Albutec GmbH), the concentration of stabilizers could be reduced.

First results showed strikingly improved detoxification efficiency and binding efficiency (BE) of the filtered albumin.



albumin solutions were diluted to 45 g/l

These parameters are regenerated to physiological values and above.

Conclusions

Commercial albumin solutions used for patients with various diseases like liver failure, hepatorenal syndrome or ascites, might be applied with caution.

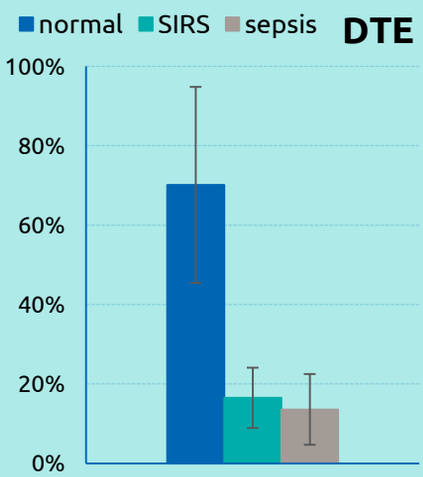
Not only these albumin solutions are not able to bind as much toxins as expected in the patient blood, but also add unwanted molecules (stabilizers) to a weakened organism.

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 healthy individuals.



With values of less than 20% the detoxification efficiency is distinctly reduced in both groups in comparison to the physiological values.

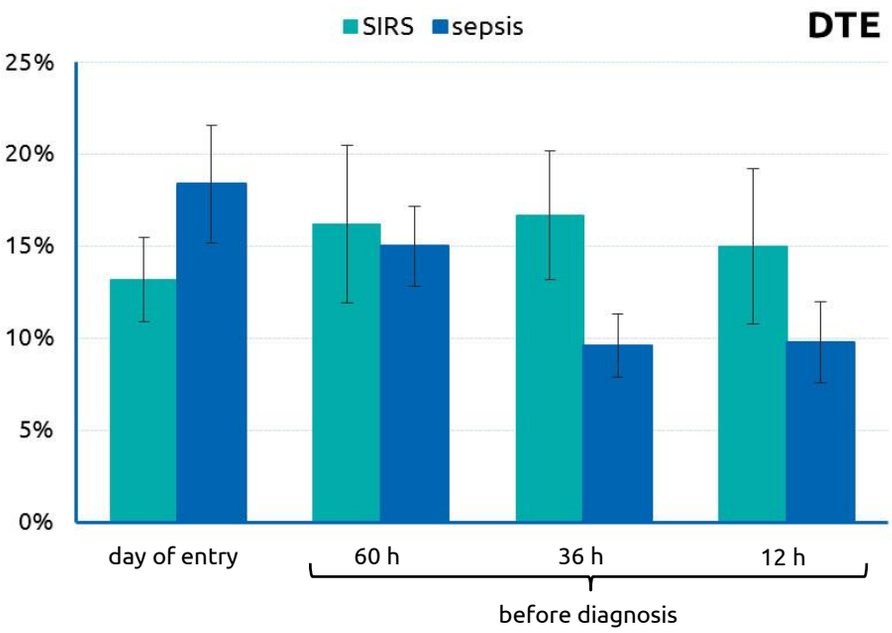
Time course of DTE

From these patients 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.

So 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

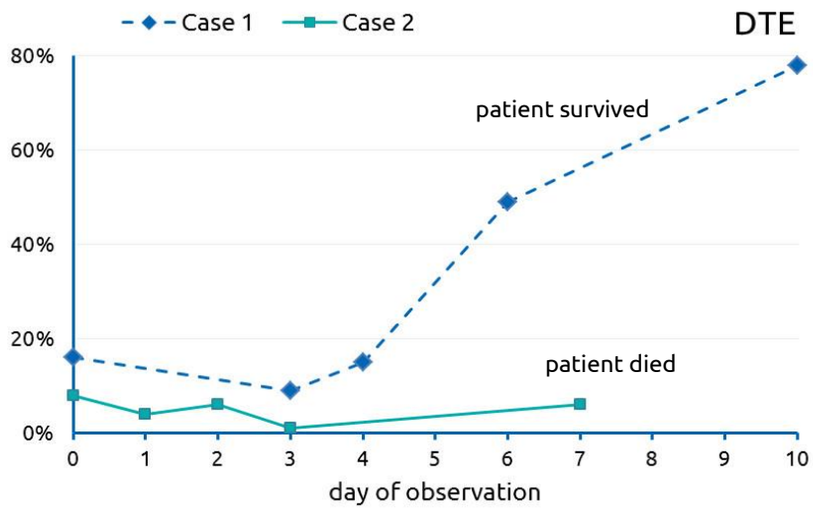
Time course of DTE

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.

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®



long term monitoring:

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 by the albumin-functionality-test could be used for a disease progression monitoring.

Fields of application

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

Literature

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MedInnovation
Advanced Molecular Diagnostics

MedInnovation GmbH – Groß-Berliner Damm 151, 12487 Berlin Germany
phone: +49 (0)30 720 126 31 – fax: +49 (0)30 720 126 36
www.medinnovation.de