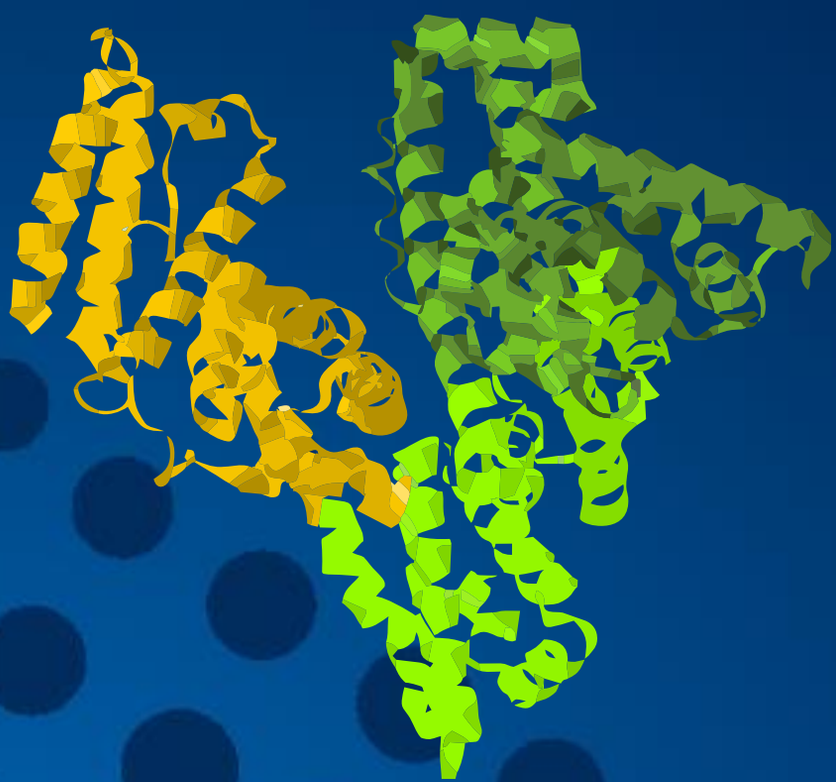
MedInnovation

Advanced Molecular Diagnostics



Analysis of functional characteristics of serum albumin

A test for cancer diagnosis and monitoring

Principles - Albumin

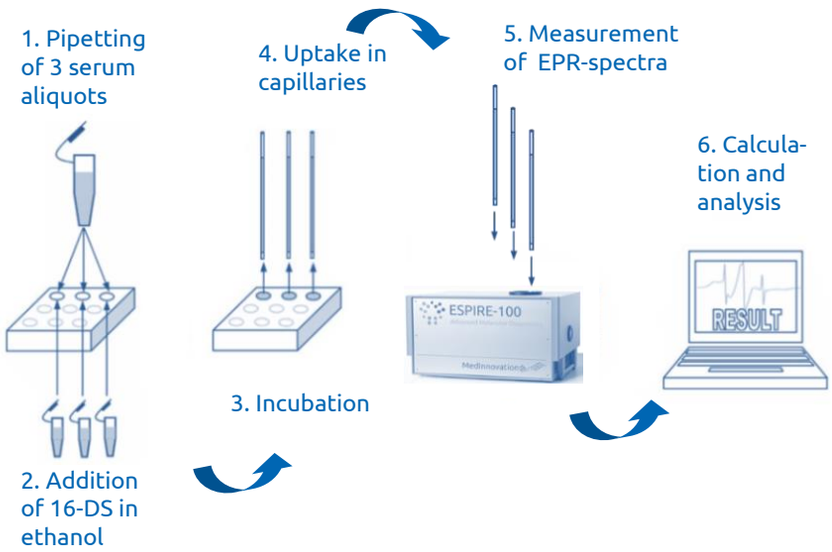
Albumin is the most abundant protein in human blood serum. It is produced in the liver and has a serum half-life of approximately 19 days.

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 these 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].

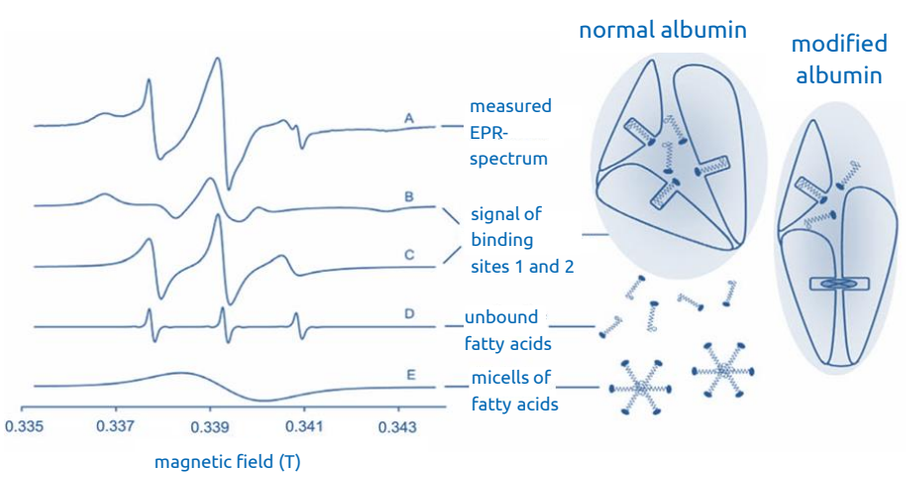


EPR technology

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

- 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

Albumin-functionality-test

The albumin-functionality-test offers a possibility for easy and fast detection of active tumor growth, independently from type or location.

The variety of albumin function for all transport stages is based on the high conformational flexibility of this protein.

In the case of cancer, several specific peptides and lipids are present in the blood circuit in very low concentrations [5, 9].

These molecules preferentially accumulate to serum albumin and influence the albumin function by changing its conformation.

Under pathological conditions there are several modifications of conformation mobility of albumin, resulting in modified transport and altered detoxification characteristics.

A comparison of the structure of normal albumin and albumin saddled with cancer peptides or lipids reveals a dramatic extent of conformational changes.

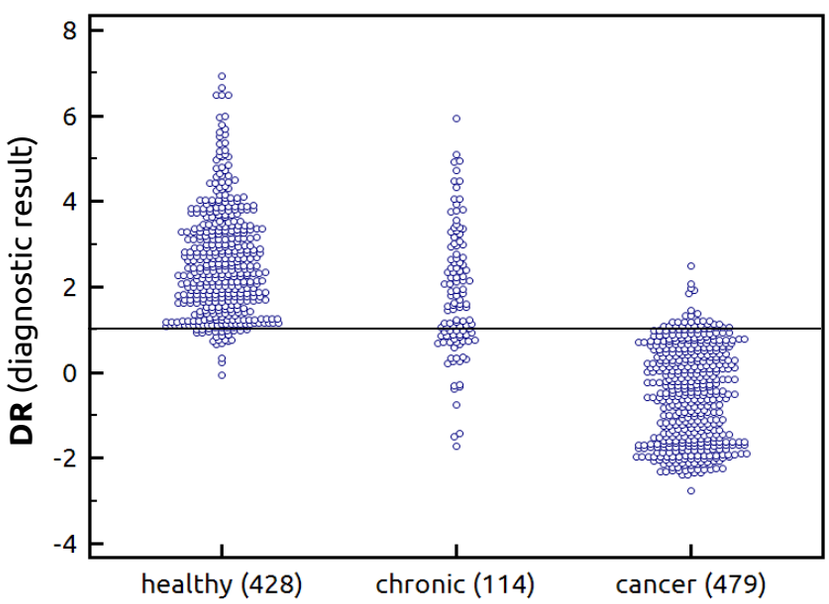
These modified binding characteristic can be detected by Electron Paramagnetic Resonance Spectroscopy (EPR).

An integral discrimination function (DR – **D**iagnostic **R**esult) can be calculated from the parameters achieved from the EPR spectra.

In case of a DR value less than 1.0 the albumin conformation has changed and there is a very high probability of an active malignant process.

If the DR value is higher than 1.2, no active tumor growth exists. A high risk of active tumor growth is indicated for DR values in the threshold range from 1.0 to 1.2.

In clinical studies the albumin-functionality-test showed – in dependence of the location – a high sensitivity (about 90%) and specificity (about 90%) [10 - 14].



Field of application

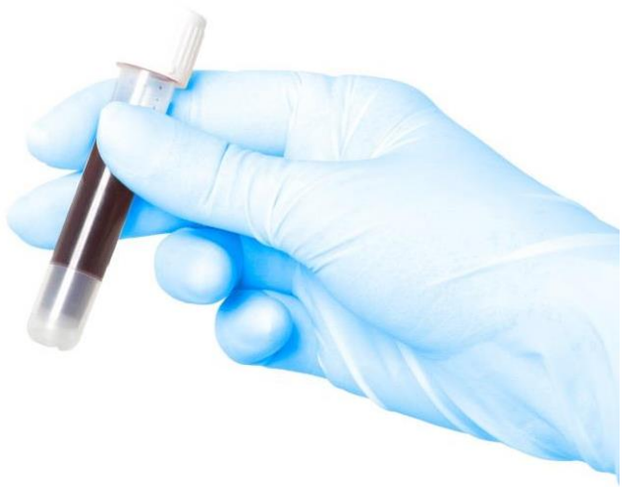
- therapy monitoring and relapse control
- early detection of cancer in populations with elevated risk (contact with carcinogenic substances, accumulation of cancer in family history)

Analytical requirements

For an adequate analysis we require:

- At least 2 ml blood stored at 8°C. The whole blood should attain to MedInnovation GmbH within 24 hours after sampling.

- "Pure" (centrifuged) serum should attain to us within at least 4 days after sampling. Centrifugation should be done at 1,000 – 1,500 x g at room temperature. Serum or EDTA plasma must be transferred into a separate and labeled tube.



- Blood withdrawal systems with anticoagulant (except EDTA) or gel are not allowed and if possible please don't use vacuum collection systems.

- Storage of samples for more than 4 days must be at less than -20°C, repeated freeze-thaw cycles of serum / plasma is not permissible.

- It is allowed to have a light breakfast.

- Blood withdrawal should be done in the morning.

Criteria for exclusion

Acute exacerbations of chronic inflammatory diseases, such as Crohn's disease, ulcerative colitis or rheumatoid arthritis distort test results.

For regeneration of serum albumin it is necessary to keep a time lag of four weeks before blood sampling.

After surgery, chemo and/or radiation therapy, it is also necessary to keep a time lag of four weeks before blood sampling. This also applies to diseases with inflammatory processes.

Because at the moment* the albumin-functionality-test does not give no information about the location of the cancer, it is inadvisable to use it in a screening situation.

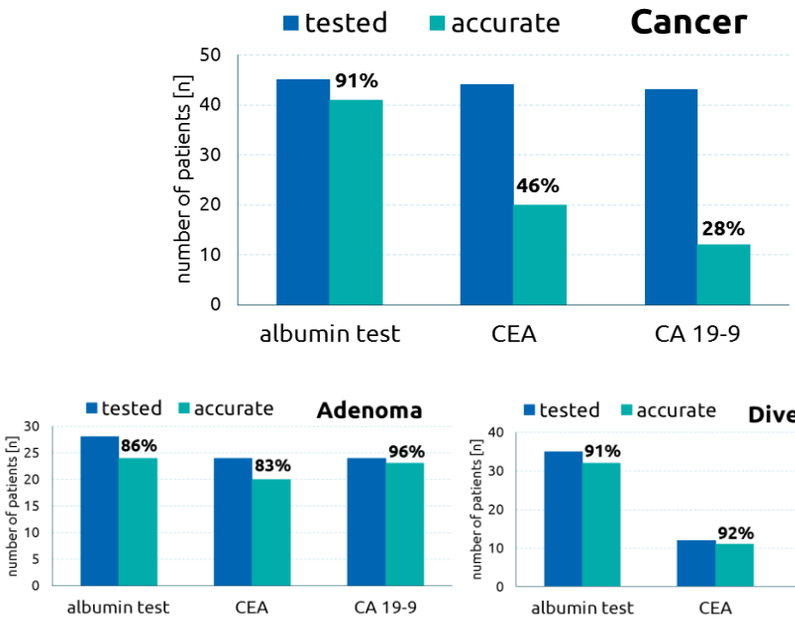
* See page 7

Study results

Comparison with tumor markers

In a study investigating patients with diseases of the large bowel the albumin-functionality-test was compared to the tumor marker CEA (Carcinoembryonic antigen) and CA 19-9.

In case of colorectal carcinoma the albumin-functionality-test showed clear advantage (91% accurate) compared to conventional tumor markers CEA (46% accurate) and CA 19-9 (28% accurate).



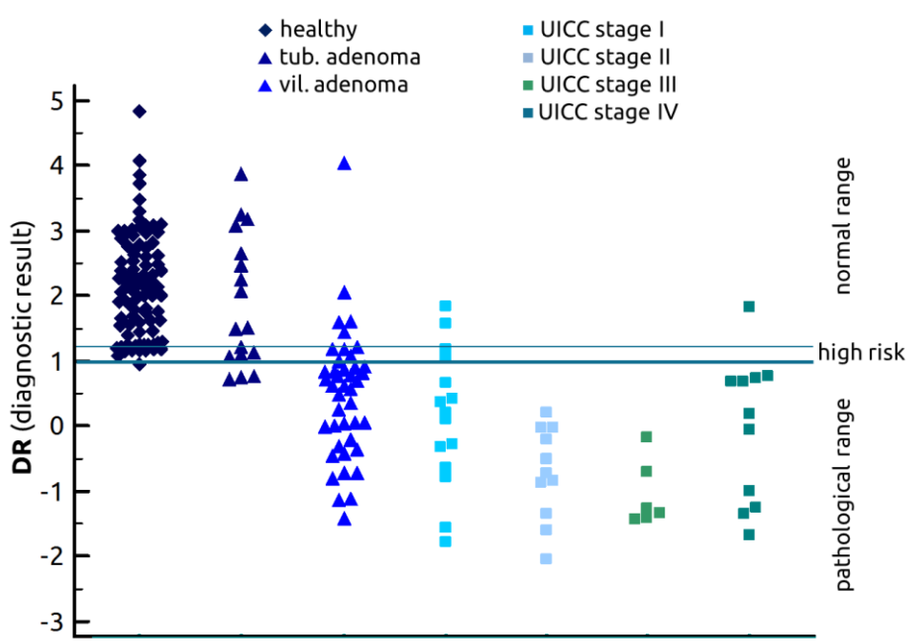
In case of adenoma and diverticulitis the albumin-functionality-test and the conventional tumor marker CEA and CA 19-9 are comparable in their specificity.

Patients with colo-rectal diseases

Studies with patients with malignant and benign colorectal diseases were conducted in Berlin, Bochum and Moscow.

A clear correlation between the DR value and the malignancy and the risk to malignant degeneration is recognizable.

Further investigations are on the way to enhance the number of cases to verify the first results.



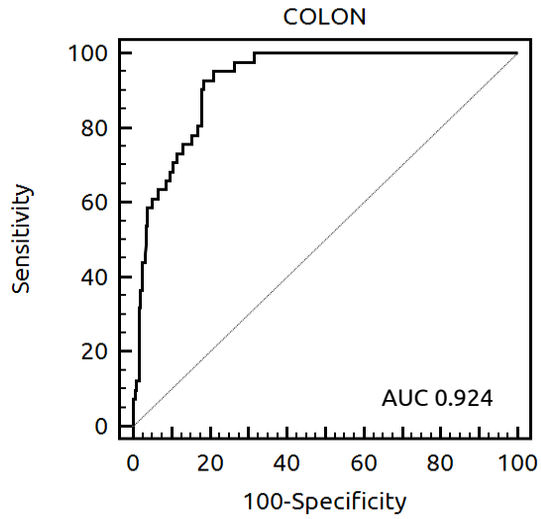
After a validation study regarding benign and malignant colorectal diseases, the albumin-functionality-test could be used for early detection of colorectal cancer in risk groups and for prolongation of colonoscopy intervals.

Experimental results

First results for determination of cancer location

In a proof of concept project we have developed algorithms for four cancer types (colon, pancreas, prostate and mamma carcinoma).

For the classification of colon cancer the ROC curve is shown, consisting of 41 colon cancer cases and 322 patients with either pancreas, prostate or mamma carcinoma, as well as patients with benign colorectal diseases and healthy controls.

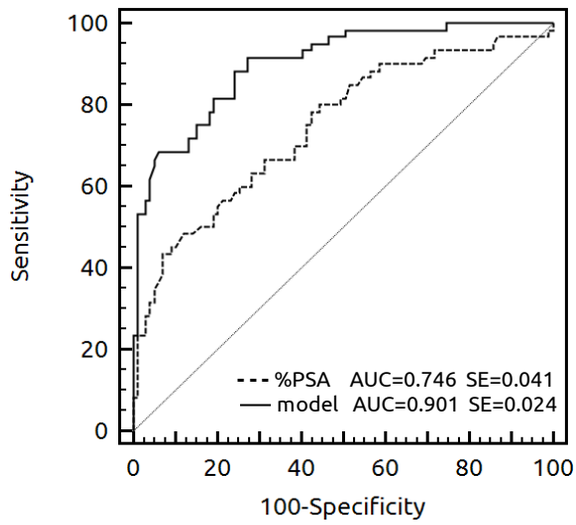


First analyses of biophysical parameters achieved from EPR spectra have shown that a discrimination of different tumor localizations is possible.

Further investigations with larger groups and more cancer localizations are on the way to verify these first results.

Albumin-functionality-test improves clinical diagnosis of prostate cancer

A comparison of ROC analysis of free-to-total PSA ratio (%PSA) and a parameter combination of %PSA, total PSA, albumin concentration and DR (model) of 60 prostate cancer patients and 99 age-matched healthy controls is shown.



The Study was conducted with the Department of Oncology and Cancer Epidemiology University Hospital Lund, Sweden, 2010 (unpublished data).

It is possible to increase the efficiency of prostate carcinoma diagnosis without invasive method by a parameter combination of PSA related values and the albumin-functionality-test.

All values could be ascertained from the same blood sample.

Literature

1. Peters, Academic Press, New York 1995
2. Bhattacharya et al. JMB, 2000
3. Simard et al., PNAS, 2005
4. Kawashima et al. , J Proteome Res 2010
5. Lowenthal et al. , Clin Chem 2005
6. Mehta et al. , Dis Markers 2003
7. Taboada et al., Chemical Physics, 2007
8. Reed et al., JBC, 1989
9. Xu et al. , JAMA 1998
10. Seidel et al. , Z Med Phys 2005
11. Kazmierczak et al. , Clin Chem 2006
12. Gurachevsky et al. , Clin Chem Lab Med 2008
13. Gelos et al. , Int J Colorectal Dis 2010
14. Mörgel et al. , Clin Oral Investig 2012

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