Changes in Serum Albumin Measured by Electron Spin Resonance: In Vitro Diagnostic EPR Test

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Introduction. Serum albumin is the major blood protein that binds fatty acids, as well as a variety of other metabolic intermediates and hormones. The relatively long half-life of albumin and its ability to bind a variety of compounds results in albumin being able to provide a "historical" record of those metabolites circulating within the blood. Thus, normal physiologic and pathologic processes result in distinct conformational and allosteric changes to this protein, what can be measured by EPR of spin probed albumin. EPR measurement of these changes can serve as a marker for active pathological processes.

Material and Methods. The functional changes that occur to albumin are assessed with use of 16-doxyl stearic acid spin probe. The test procedure requires approximately 0.2 ml of serum or plasma and is completed within 20 minutes, including 10 min incubation of three 50 µl aliquots of the serum with different amounts of spin probe (5-10 mmol/L) and ethanol (10-14 µl), and 3 x 3 min measurements of EPR spectra. Spectra processing includes de-convolution of five different components (Fig. 1), from which two refer to spin probe bound in different albumin sites and one to free spin probe. Two other components reflect minor moieties of spin probe aggregated in micelles and associated with serum lipids. The main parameters estimated from there are the ratio of free and bound in different albumin sites spin probes and effective a-tensors of the albumin bound molecules. This allows an assessment of a conformational change of albumin and its ability to bind fatty acids.

In Vitro Diagnostic EPR Test and Laboratory EPR Analyzer, CE-certified for in-vitro diagnostics, were developed based on conventional EPR spectroscopy of spin probed serum albumin. The test provides an assessment of the conformational changes to the protein, as well as the efficiency with which albumin bind and transport fatty acids.

Cancer Diagnosis and monitoring. Studies in animals and humans have demonstrated that EPR analysis of albumin conformational changes provides approximately 95% specificity and 90% sensitivity for diagnosis of cancer (Fig. 2). In addition, measurement of serial changes to albumin conformation enables the effect of treatment to be assessed. Presumably, the systemic influence of the malignancy, due to the release of low-molecular-weight mediators from tumor cells, alters the composition of metabolites normally carried by serum proteins. [1] These alterations to albumin are readily measured by EPR spectroscopy.

Discussion. Spin-probe EPR spectroscopy is known to be a perfect tool for studying protein features in almost native conditions. [2] Nevertheless, it has hardly ever been used for clinical applications, in contrast to NMR. Main reasons for that are the problems of different molecular interactions complicating EPR spectra in such objects as human tissue and difficult processing of many-component EPR spectra. Our results show, that such problems are solvable, and the potential of EPR technique can be applied for practical medicine. Clinical evaluations of the EPR based test of serum albumin showed high diagnostic relevance of specific modifications measured in blood proteins under development of pathologic processes. [3]

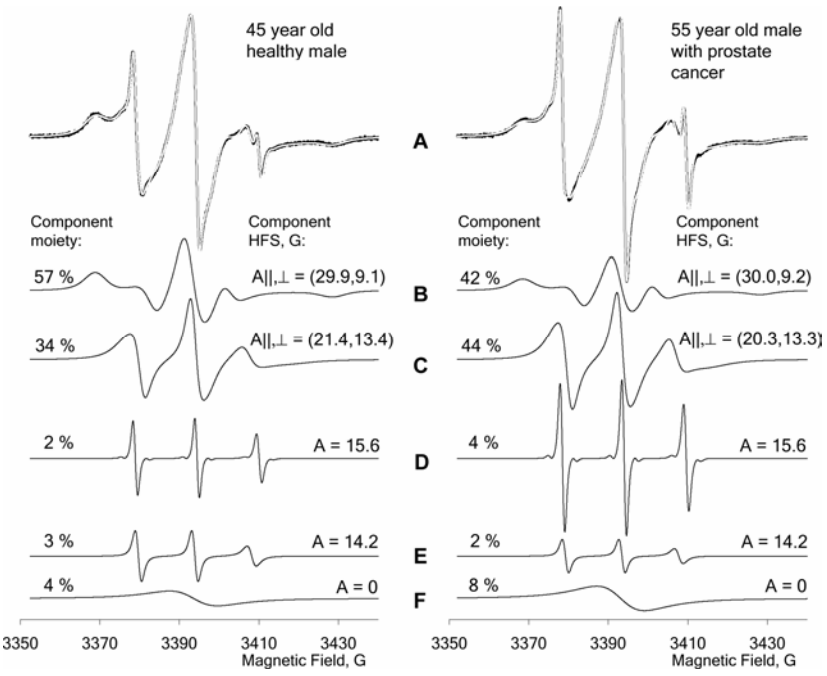

Fig. 1. A – Experimental EPR spectrum of 16-doxyl stearic acid (16-DS) in serum (bold curve) and simulated one (thin white curve). B – Spectrum component corresponding to 16-DS bound to high affinity fatty acid binding site on albumin. C – Spectrum component corresponding to 16-DS bound to second, low affinity fatty acid binding site on albumin. D – Spectrum component corresponding to unbound 16-DS in serum. E – Spectrum component corresponding to 16-DS molecules associated with serum lipids. F – Spectrum component corresponding to 16-DS micelle aggregates (shown at 5 times actual intensity).

References

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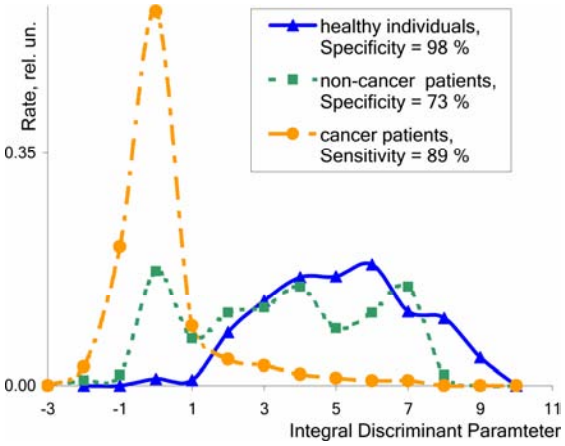

Fig. 2. Distribution of Integral Discriminant Parameter (developed based on EPR spectral data) in groups of patients. Healthy group (included 586 patients) is compared to 128 patients with diverse non-cancer diseases and 537 patients with cancer of diverse sites (in most cases blood was collected before start of treatment). Shown specificity and sensitivity levels correspond to cut-off value of 1.0.