Plasma Albumin Alterations in Gastrointestinal Cancer Patients

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Abstract
Albumin plays a prime role in most functional characteristics of plasma and immune state of organism. Patients with gastrointestinal cancer exhibit a poorly functioning immune system. This study work investigated the possibility of using the fluorescent probe ABM for detecting structural and functional alterations of blood plasma albumin in pathological states. The aim of this research work was to evaluate the changes of blood albumin in correlation with some immunological parameters in patients with gastrointestinal cancer. 46 patients with gastrointestinal cancer and 24 healthy donors were examined in this study. Lymphocyte subpopulations were determined by flow cytometry using the appropriate specific monoclonal antibodies in the peripheral blood. Patients were tested one day before the operation and ten days after surgical treatment. The fluorescent probe - amino derivative of benzanthrone, ABM (developed at Daugavpils University, Daugavpils, Latvia) was used to characterize the blood plasma albumin. Before surgical treatments, the average ABM intensity in the patients' blood plasma was decreased compared to that seen with the samples from the healthy donors. The lymphocyte distribution among subsets differed not only between observed groups of patients, but also before and after surgical treatment. ABM fluorescence intensity was found to correlate with the absolute number of CD38⁺ cells and with some immunological parameters both before and after surgical treatment. To conclude, measures of ABM fluorescence values for lymphocytes and blood plasma could potentially be used in clinical immunological screening (instead of more expensive routine test) to provide a snapshot of immune status of individuals.

Keywords: Blood plasma albumin; Immune diagnostics; Fluorescent probe ABM; Gastrointestinal cancer; Lymphocytes

Introduction
The principal function of plasma albumin is to transport a wide variety of fatty acids and metabolites via the main binding regions [1,2]. Membrane damage in different pathologies may be a consequence not only of lipid peroxidation, but also of protein alterations. Albumin plays a prime role in most functional characteristics of plasma and immune state of organism [3]. The fluorescent probe - aminoderivative of benzanthrone, ABM (developed at Riga Technical University, Riga, Latvia) was used to characterize the blood plasma albumin. Patients with gastrointestinal cancer exhibit a poorly functioning immune system, that is characterized by decrease in T-lymphocyte proliferation [4,5] and reduced CD4⁺/CD8⁺ ratio [6,7]. This research investigated the possibility of using the fluorescent probe ABM for detecting structural and functional alterations of blood plasma albumin in pathological states. ABM reflects such properties of lymphocytes as physical-chemical state of their membrane, membrane micro viscosity, and proliferating activity of lymphocytes, lipid metabolic activity and some phenotypical characteristics of lymphocytes. The examined changes of the studied parameters could be used for assessing the alterations of the cellular mechanisms of immunity, and, therefore, can be applied as preliminary screening test in immune diagnostics.

Aim
The aim of our study work was to evaluate the changes of blood albumin in correlation with some immunological parameters (T-lymphocytes counts, CD4⁺/CD8⁺ ratio etc.) in patients with gastrointestinal cancer.

Material and Methods
ABM
A 46 patients with gastrointestinal cancer (30 colorectal in stages II-IV; 16 gastric in stage III)
Table 1: Spectral characteristics of ABM and subpopulations of T-lymphocytes in colorectal cancer patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>F(PI)</th>
<th>F(Ly)</th>
<th>Lymphocytes (abs.)</th>
<th>CD16+ (abs.)</th>
<th>CD38+ (abs.)</th>
<th>CD4+/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Before surgical treatment</td>
<td>1.44±0.12</td>
<td>0.28±0.03</td>
<td>2664±238</td>
<td>359±31</td>
<td>91±8</td>
<td>1.28±0.12</td>
</tr>
<tr>
<td>2. After surgical treatment</td>
<td>1.24±0.11</td>
<td>0.36±0.05</td>
<td>2180±187</td>
<td>169±16</td>
<td>277±34</td>
<td>1.56±0.15</td>
</tr>
<tr>
<td>3. Control group</td>
<td>1.87±0.13</td>
<td>0.25±0.02</td>
<td>2300±218</td>
<td>288±27</td>
<td>566±48</td>
<td>1.91±0.16</td>
</tr>
<tr>
<td>p&lt;0.05 between groups</td>
<td>1-2, 1-3, 2-3</td>
<td>1-2, 2-3</td>
<td>1-2, 2-3</td>
<td>1-2, 1-3, 2-3</td>
<td>1-2, 1-3, 2-3</td>
<td></td>
</tr>
</tbody>
</table>

Notes: F (PI) - fluorescence intensity in blood plasma; F (Ly) - fluorescence intensity in lymphocytes. Values are shown in terms (mean±SE).

Table 2: Spectral characteristics of ABM and sub-populations of T-lymphocytes in gastric cancer patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>F(PI)</th>
<th>F(Ly)</th>
<th>Lymphocytes (abs.)</th>
<th>CD38+ (abs.)</th>
<th>CD4+ / CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Before surgical treatment</td>
<td>1.46±0.09</td>
<td>0.16±0.02</td>
<td>2660±2.85</td>
<td>702.24±2.0</td>
<td>1.26±0.11</td>
</tr>
<tr>
<td>2. After surgical treatment</td>
<td>J.31±0.07</td>
<td>0.29±0.06</td>
<td>1920±1.60</td>
<td>380.16±1.7</td>
<td>1.52±0.14</td>
</tr>
<tr>
<td>3. Control group</td>
<td>1.87±0.13</td>
<td>0.25±0.02</td>
<td>2300±1.30</td>
<td>565.80±2.1</td>
<td>1.91±0.16</td>
</tr>
<tr>
<td>p&lt;0.05 between groups</td>
<td>1-2, 1-3, 2-3</td>
<td>1-2, 1-3</td>
<td>1-2, 2-3</td>
<td>1-2, 1-3, 2-3</td>
<td>1-2, 1-3, 2-3</td>
</tr>
</tbody>
</table>

Notes: F (PI) - fluorescence intensity in blood plasma (in AU); F (Ly) - fluorescence intensity in lymphocytes (in AU). Values are shown in terms (mean±SE).

Results

ABM binding with plasma albumin

In the gastrointestinal cancer patients, the ABM emission spectra maximum (i.e. 650 nm) in patients’ blood plasma was not altered in comparison to that seen in healthy donors. In contrast, with the respect to fluorescence intensity, before surgical treatments, the average ABM intensity in the patients’ blood plasma was decreased compared to that seen with the samples from the healthy donors. Specifically, the fluorescence in colorectal and gastric groups (Table 1 and 2) were decreased by 23% and 22%, respectively. At 10 days after their operations, the average ABM fluorescence intensity in colorectal and gastric groups was decreased further by 13.9% and 10.3%, respectively (Table 1 and 2).

The lymphocyte distribution among subsets differed not only between observed groups of patients, but also before and after surgical treatment. Results among the patients of colorectal cancer group (Table 1) indicate that, before surgery, the absolute count CD38+ cells, and the CD4+/CD8+ ratio were significantly decreased (by 84% and 33%, respectively) compared to corresponding control subject value. Somewhat unexpected, relative percentages of C16+ cells in this group were greater (by 25%). After surgical treatment, the absolute count of lymphocytes and CD16+ cells were reduced by 18% and 53%, respectively. The absolute count of CD38+ cells and CD4+/CD8+ ratio increased (by 205% and 22%, respectively) compared to pre-surgical values, but did not reach control levels.

The results from the gastric cancer group patients (Table 2) show, that before surgery, the absolute count of CD38+ cells and lymphocytes were each increased (by 15.7% and 24.1%, respectively) compared to control values. After surgery, the absolute count of lymphocytes was reduced (by 18.2%) relatively to pre surgery levels. This shift was sufficient enough to yield a value that was now lower than control levels (by 5.2%). As with the results in the other patient subgroup, the absolute count of CD38+ cells was decreased and CD4+/CD8+ ratio was increased (by 45.9% and 21.9%, respectively) compared to pre-surgical values, but did not reach control levels.

These results (different ABM spectral characteristics) can be explained by different structural characteristics of albumin in observed patient groups. ABM fluorescence in pre-operation in colorectal and gastric patient groups was found to correlate with the absolute count of CD38+ cells (r=0.926, r=0.798, respectively). Before surgical treatment in gastric cancer group it was held with respect to the relative count of CD8+ cells (r=0.804). After the operations both CD4+/CD8+ ratio increases in both groups of gastrointestinal cancer as compared with pre-operation indices. Absolute count of CD38+ cells in gastric cancer patients blood was observed to be decreased, but in colorectal patients-increased relative to pre-operation values. ABM fluorescence intensity correlated with some immunological parameters both before and after surgical treatment. Before operation and 24 healthy donors were examined in this study. Lymphocyte subpopulations: CD3+ common T-cells, CD4+ helper cells, CD5+ cytotoxic cells, CD16+ natural killer cells, CD38+ activated T- and B-cells, also activated natural killer cells were determined by flow cytometry using the appropriate specific monoclonal antibodies (Becton Dickinson, USA) in the peripheral blood. These patients were tested one day before the operation and ten days after surgical treatment. Plasma albumin, as in earlier studies, was detected using the ABM fluorescent probe [8-10] developed in Daugavpils University, Daugavpils, Latvia. In the current study, blood plasma (200-fold diluted) or cells (5·10^6 per assay) incubated without probe were used as each patient’s personal “blank” in each experiment. The ABM (resulting concentration in sample=19.6 pmol/1) was added into 1 ml aliquot of the patient’s blood plasma (or 1 ml solution containing the cells) at temperature of 18-20°C and the mixture then was allowed to sit for ~ 5 min. The time interval between cell/plasma isolation and fluorescence measurement was held constant for all samples (i.e., 3 hr). Resulting fluorescence parameters were then registered on a Spectroflow JY3 spectrophuorometer (ISA JobinYvon Instruments S. A., Longjumeau, France) at an excitation wavelength of 470 nm and an emission wavelength of 520-700 nm. To register luminescence, the sample was placed in a cuvette (1·10^4·40 mm^2) fixed at an angle of 30° to the excitation light beam. Fluorescence intensity was then recorded and reported in terms of arbitrary units (AU). The final intensity value for each patient’s sample was then generated after accounting for (i.e., subtracting out) the value associated with their personal “blank”; this approach thereby eliminated any potential contributions from any autofluorescing constituents in the plasma sample. Data were analyzed using either Student T-test or Wilcoxon-Mann-Whitney U-test. Significance was considered at < 0.05 [11].
in all patient groups fluorescence intensity correlated with the number of CD38+ cells (p <0.05), supporting the view that fluorescence intensity depends on the level of activated lymphocytes. In both groups (colorectal and gastric cancer) the ABM fluorescence in cell suspension was found to correlate with ratio CD4/CD8 (r =+0.843, r=+0.756, respectively).

**Discussion**

It is known that different disease-associated biomarkers that bind to albumin can cause changes of the protein structure and function. Tumour cells release a variety of bioactive proteins and peptide fragments into the blood that may reflect important disease-related information. It may reveal effects of the tumour on the organism itself. Tumour-derived proteins enter the circulation and bind to transport proteins. Sequestration of these peptides by carrier proteins (albumin) protects these markers from clearance and helps amplify their concentration in the circulation [12]. In cancer, the blood plasma content of important unsaturated fatty acid (i.e., oleic, arachidonic acids) is increased - these natural constituents and their counterparts also increasingly occupy binding sites on albumin [5,13]; and, albumin can bind an array of drugs, including ibuprofen, indomethacin, etc. (and their metabolites) commonly ingested by cancer patients [13]. Some conformational alterations in albumin are observed at early stages of most cancers. In late stages of cancer, the levels of pathological metabolites increase and the albumin ultimately cannot bind them all. These induced structural/functional alterations in the albumin could then manifest as “shifts” away from the normal “main” binding sites with high affinity for the ABM to other sites with lower affinities and specificities. Such shifts would be in agreement with the observations of others [2,14,15] that albumin molecules are known to contain different binding sites (i.e., classes) that differ in affinity [5,10,16] for ABM and various other probes. Albumin is a single source of ABM fluorescence in blood plasma. Probe binds to albumin with high selectivity. The results of the current investigation are also in agreement with Electron Spin Resonance (ESR) spectroscopy studies to measure structural/functional changes in serum albumin of cancer patients [12]. Analysis of the ESR spectra, using spin probe, revealed substantial differences in spectrum variables between patients and healthy individuals. For example, the fraction corresponding to patients and the increasing width of the spectral line indicated an alteration in albumin conformation that limited the movement of spin probe bound to this site. In general, the ESR spectral line shape allowed several different values to be deduced, e.g., albumin capacity to bind spin probe, polarity of spin probe binding site, and probe mobility [12].

We observed that before the operation the accounts of lymphocytes and natural killer cells (CD16+) were increased in patients with gastrointestinal cancer in comparison with healthy donors. Absolute count of CD38+ cells (the CD38+ cells mainly consist of activated T- and B-lymphocytes) increased with an increase of F in cell suspension in colorectal cancer patient group, but in gastric patient group decreases relative to pre-operation values. The numbers of natural killer (CD16+) cells was not related with ABM fluorescence intensity F and the functional activity of the whole lymphocyte subpopulation. Increase of ABM fluorescence intensity in cell suspension after the operation is due to compensatory rise of functional activity in response to a decrease in the count of the lymphocytes subsets.

Changes in the fluorescence parameters of the cancer patients’ lymphocytes could be reflective of changes in one/more inherent characteristics of their cells. In the studies here, the phenotypical character of the cells was evaluated by examining changes in the lymphocyte populations (i.e., their numbers) themselves. These flow cytometry studies indicated that there were significant changes in lymphocyte (and subpopulations) levels among the gastrointestinal cancer patients, unfortunately, the variations in total lymphocytes levels never clearly and consistently tallied the corresponding changes in ABM fluorences (Table 1 and 2).

**Conclusion**

Our results suggest the measures of ABM fluorescence intensity values for lymphocytes and blood plasma could potentially be used in clinical immunological screening (instead of more expensive routine test) to provide a snapshot of immune status in these cancer patients. Fluorescence behavior of ABM could be of use as potential indicators of alterations of cellular immunity in individuals. Compared to many commonly used diagnostic protocols this fluorescence-based method is less expensive and not very time consuming, technically simple and 100 times more sensitive than standard absorbance based method.

**References**

