METHODS

Laser Correlation Spectroscopy in an Investigation of the Subfractional Composition of Plasma from Patients with Gastric Bleeding, Craniocerebral Injury, and Intoxications

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Experimentation on quasielastic light scattering makes it possible to obtain information on the diffuse broadening distribution of the intensity of light scattering of objects, and, correspondingly, on the size distribution of particles in the sample [3]. Laser correlation spectroscopy (LCS) of blood serum employing the methods of reconstruction of on the size distribution function assists in solving a number of immunological and virological problems [1,2]. The advantages of LCS are a minimal volume of sample required, simplicity of sample preparation, and prompt output of data ready for computer processing.

In the present study the possibilities of LCS as an additional diagnostic method in emergency situations of acute pathogenic interventions were tested. Etiopathogenetically diverse forms of pathology were chosen as biological models.

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MATERIALS AND METHODS

The subfractional composition of the blood plasma was studied in 26 patients with profuse gastroduodenal bleeding, 19 patients with craniocerebral injury, and 13 patients with acute alcohol intoxication (AAI) ranging in age from 20 to 63 (inall, 34 male and 24 female patients). The examination data from 45 blood bank donors served as controls. In addition, results on 37 patients with chronic hepatitis in remission and 149 patients with chronic chlorophos intoxication, examined at the Research Institute of Hygiene and Occupational Diseases, Russian Ministry of Health, are also presented in our study. Blood (0.1 ml) was taken from a finger and immediately mixed with 0.3 ml 0.85% NaCl in a 1.5-ml centrifuge tube. The mixture was centrifuged at 1500 g and room temperature for 15 min. The supernatant was then placed in an 0.4 ml Eppendorf tube, hermetically sealed, and stored in a refrigerator at -12°C prior

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to measurement. Special investigations showed that no visible changes in the spectrum occur after 4-5 weeks of storage under these conditions. The samples were thawed in an incubator at 37°C for 30 min and centrifuged at 1500 g for 15 min. Measurements were performed with a spectrometer assembled at the Department of Molecular and Radiation Biophysics, Institute of Nuclear Physics, Academy of Sciences, St. Petersburg. To this end a sample (0.2 ml) was placed in a measuring cell of the spectrometer and cumulation of the spectrum was performed during 5-6 min (up to 10,000 cumulations). The cumulated spectrum was regularized during measurement using special software with an IBM PC/AT-286 computer. The data were stored in the computer as numerical values of spectrum subgradients in a range of measured particles from 1 to 10^4 nm. Classifikator, multivariate analysis software, was used for statistical processing of the groups of spectra.

RESULTS

The results of the investigations may be presented in three forms. First, planar printing of the compared groups of spectra with the confidence area within 2σ outlined, which provides a general evaluation of the differences between the analyzed groups. Second, presentation of the data in the form of tables, which contain the absolute value or percentage of overlapped spectra from multivariate analysis. Third, averaged histograms of an analyzed reference group, which represent a visual assessment of the contribution of particles of a certain size in a spectrum characteristic. The percentage contribution of a subfraction with a certain hydrodynamic radius is presented in numerical form.

As is seen from the multivariate plot (Fig. 1, *a*), most of the subfractional spectra of the plasma from patients with gastroduodenal bleeding and healthy donors are separated from each other by confidence areas. The spectra outside the areas differ from those within the areas, and so, they cannot be assigned to either of the reference groups.

TABLE 1. Comparison of Blood Plasma Spectra from Patients with Gastric Bleeding and Healthy Donors Based on the Data of Volumetric Analysis.

Group of subjects	Confidence interval			
	donors	patients	outside of area	number of patients
Donors	40	4	1	45
Patients	3	20	3	6

Note. Here and in Table 2 figures are the number of spectra in the studied group which fell within the confidence area in the multivariate analysis.

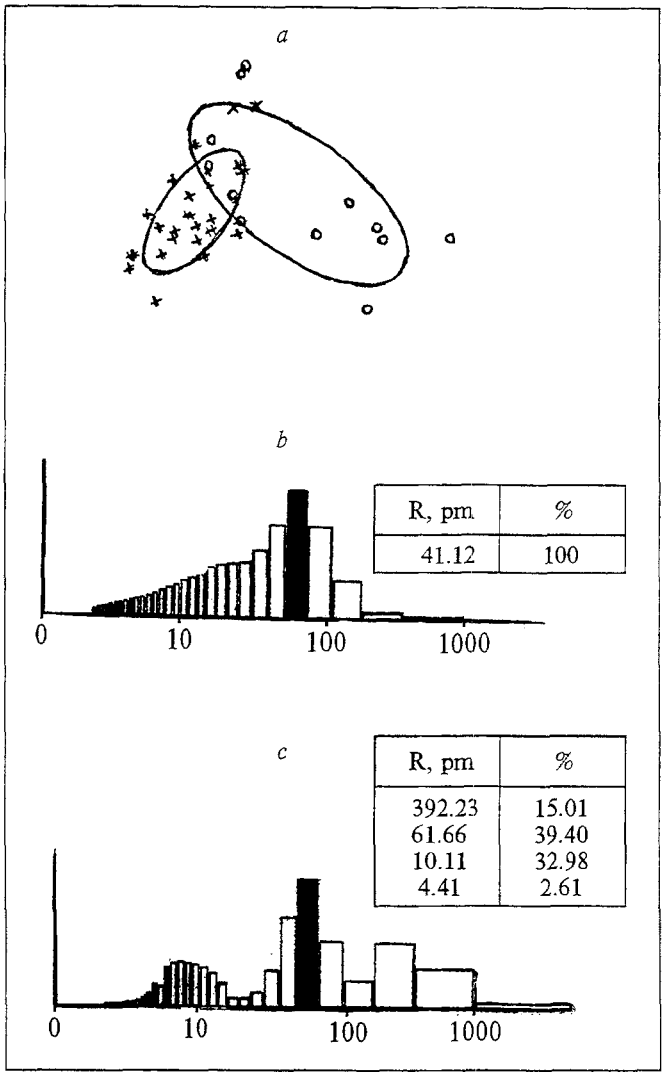
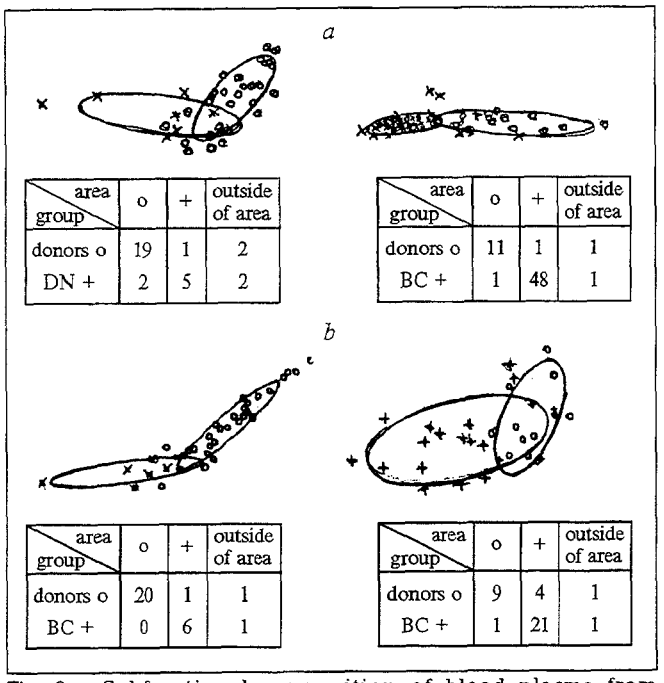


Fig. 1. Subfractional composition of blood plasma from patients with gastric bleeding and clinically healthy donors. *a*, plot of groups of plasma spectra with bordered confidence areas within 2σ: crosses: patients with gastric bleeding; circles: healthy donors. Averaged histograms and numerical information on contribution (in %) of subfractions with certain hydrodynamic radii of particles in plasma from clinically healthy subjects (*b*), patients with gastric bleeding (*c*), and patients with gastric bleeding, whose spectra fell outside the confidence areas of both reference groups (*d*).

The quantitative evaluation of the data is presented in Table 1. The spectra of just 4 out of 45 samples from healthy donors were similar to those from patients with bleeding, and one sample did not correspond to any reference group. Three out of 26 blood samples from patients with gastroduodenal bleeding were similar to those from healthy donors, and three others did not resemble samples either from healthy donors or from patients with bleeding.

The essence of the differences between the spectra from bleeding patients and healthy donors becomes clear upon a study of the averaged plasma



area / group	o	+	outside of area
donors o	19	1	2
DN +	2	5	2

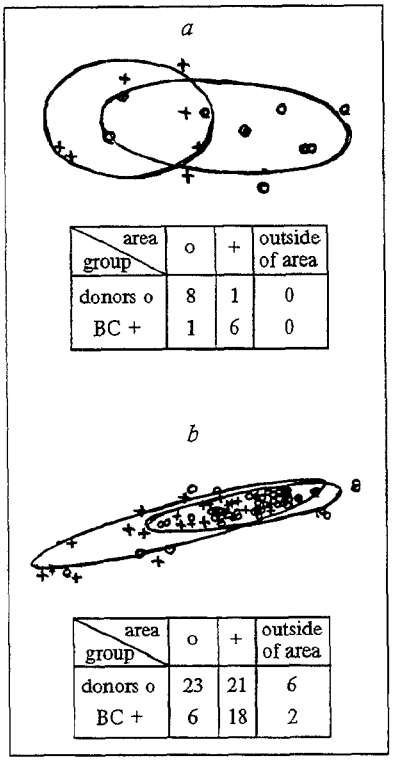
area / group	o	+	outside of area
donors o	11	1	1
BC +	1	48	1

area / group	o	+	outside of area
donors o	20	1	1
BC +	0	6	1

area / group	o	+	outside of area
donors o	9	4	1
BC +	1	21	1

Fig. 2. Subfractional composition of blood plasma from patients with AAI and craniocerebral injury. *a*) plot of groups of plasma spectra with bordered confidence areas within 2σ; crosses: AAI; circles: craniocerebral injury. Averaged histograms and numerical information on contribution (in %) of subfractions with certain hemodynamic radii of particles in plasma from patients with AAI (*b*) and craniocerebral injury (*c*).

histograms. The plasma subfractional composition in healthy donors is generally characterized by a bimodal distribution of particles, which includes particles of 4-12 nm and 90-120 nm contributing



area / group	o	+	outside of area
donors o	8	1	0
BC +	1	6	0

area / group	o	+	outside of area
donors o	23	21	6
BC +	6	18	2

Fig. 3. Subfractional composition of blood plasma from patients with AAI and chronic hepatitis. *a*) plot of groups of plasma spectra with bordered confidence areas within 2σ; circles: AAI; crosses: chronic hepatitis; *b*) plot of gro-ups of plasma spectra with AAI (circles) and chronic chlorophos in-toxication (crosses).

TABLE 2. Comparison of Blood Plasma Spectra from Patients with Craniocerebral Injury (CCI) and Acute Alkohol Intoxication (AAI)

Group of patients	Confidence interval			
	CCI	AAI	outside of area	number of patients
With CCI	16	2	1	19
With AAI	1	9	3	13

40-50% and 50-60% to light scattering, respectively. Large structures are not generally detected in these spectra.

In samples from patients with gastroduodenal bleeding (Fig. 1, *c*), a trimodal distribution is predominantly recorded, the main contribution (74.59%) in light scattering being made by relatively large (290.34 nm, in average) and medium-sized (28.73 nm) particles. Small particles with a mean radius of 7.29 nm account for just 25% (a mode with a mean radius of 2.65 nm can be discounted, since its weight contribution is just 0.25%). The major light-scattering components of the plasma may be assumed to be small immune and globulin-lipoprotein complexes.

Analysis of 6 spectra which lie outside the areas characteristic for both healthy donors and patients with bleeding (Fig. 1, *d*) allows us to zero in on their atypical nature. From averaged histograms, the particle size in these samples increased, on average, to 422 nm, while the main medium-sized globular fraction dissociated into two modes (61.78 and 16.80 nm), its contribution to light scattering being increased to 59.51%. When comparing the LCS results with clinical examination data, we found that 5 out of 6 these patients had started intensive therapy before blood sampling: the patients had received polyglucin, albumin, and plasma preparations, which could have been responsible for the atypical spectra, which fell outside the cross-sectional areas characteristic of healthy donors and patients with bleeding. These observations suggest that LCS is sensitive enough to pick up the changes in homeostatic parameters.

For comparisom of the LC-spectra of plasma samples from patients with craniocerebral injury and AAI, no overlapped areas in confidence intervals in plotted groups of spectra were found (Fig. 2, *a*), which may be attributed to essential differences in homeostatic parameters in patients with such distinct etiopathogenetic variants. Only two out of 19 patients with craniocerebral injury were the spectra of plasma samples similar to those of patients with AAI, and just one patient with AAI had a plasma spectrum similar to that of patients with craniocerebral injury (Table 2).

The averaged histograms of plasma spectra from patients with AAI (Fig. 2, *b*) and craniocerebral injury (Fig. 2, *c*) demonstrate different distributions of particles suspended in the plasma.

Thus, LCS offers the possibility not only of distinguishing between the spectra of blood plasma from healthy and diseased individuals, but also of differentiating reference groups with diverse pathology, thus making it a practical tool for differential diagnosis, in particular, in states which cannot be properly studied by traditional methods.

There is much of interest in the data obtained from the comparison of plasma spectra from patients with AAI and either chronic hepatitis in remission or patients with chronic chlorophos intoxication, i. e., pathologies which have a common element, in our case, liver damage (Fig. 3, *a*, *b*). Six out of 13 spectra from patients with AAI were found to fall within the area characteristic of spectra from patients with chronic hepatitis and chronic chlorophos intoxication. The liver disturbances which are common for these states, though different in etiology and pathogenesis, may be assumed to result in similar changes in the blood plasma, and this accounts for the similarity of the spectra.

LCS may be of special importance as a method for large-scale population screening, provisional preclinical diagnosis, establishment of risk groups, and the differential diagnosis of emergency states (coma, shock, collapse) and exogenous and endogenous intoxications.

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