Exp Oncol 2017
39, 4, 304–307

Experimental ONCOLOGY

ADSORPTION-RHEOLOGICAL PROPERTIES OF BLOOD SERUM IN LUNG CANCER PATIENTS

Y.V. Dumanskiy[1], **, O.Y. Stoliarova*[2], *O.V. Syniachenko*[1], *M.F. Giulmamedova*[1], *Y.A. Potapov*[1]
[1]*Donetsk National Medical University, Lyman 84400, Ukraine*
[2]*National Cancer Institute, Kyiv 03022, Ukraine*

***The aim* and *objectives* of the study were to investigate the state of adsorption-rheological properties of blood (ARPB) in patients with different clinical course of lung cancer (LC), the detection of violations of surface-active, viscoelastic and relaxation properties of blood serum, their association with tumor markers, the evaluation of the prognostic value of initial indexes in the development of complications from radiochemotherapy. *Patients and Methods*: The study included 115 patients with LC at the age from 24 to 80 years (average age 58 years), among whom there were 78% men and 22% women. The parameters of surface (interfacial) viscosity, elasticity, viscoelasticity module, tension and relaxation of blood serum were studied by the oscillating drop method using a computer tensiometer "PAT2-Sinterface", and its volumetric viscosity was investigated using a Low-Shear-30 rotational viscometer. ARPB parameters were also studied in a control group composed from 50 healthy donors. *Results*: Increased levels of volumetrical viscosity, surface tension, surface elasticity and the relaxation time of the blood are typical for patients with LC and depended on the localization of the tumor, its histological variant, differentiation grade, severity of the course of the disease, the number of metastases in the lymph nodes, distant organs and skeleton, involvement of the pleura and ribs, the development of compression pulmonary syndrome, metastasis into the spine, adrenals, brain, and pancreas. The surface-active, viscoelastic and relaxation properties of the blood correlated with the levels of tumor markers (TGFβ1, VEGF, C-reactive protein, α_2-macroglobulin). *Conclusions*: Integral changes of ARPB observed in every fifth patient with LC are involved in the pathogenesis of the disease, have predictive value in relation to the clinical course of disease (volumetric viscosity) and the development of complications from radiochemotherapy (surface viscosity).**
***Key Words*: cancer, lung, blood, serum, adsorption, rheology.**

Lung cancer (LC) is the leader in the structure of cancer morbidity and mortality [1, 2]. An important pathogenetic link supporting the properties of LC is neoangiogenesis (the formation of new vessels) [3]. At the same time, the expression of protein products that are surfactants (surface active substances) or possessing insurfactant properties (surface-inactive) in patients with LC provides the processes of neoangiogenesis and the physicochemical interfacial state of blood serum [4]. There is a close intercommunication between the biochemical components and the functional activity of the cells in tumor microenvironment, capable to secrete surfactants/insurfactants influencing biochemical processes, and thus the adsorption-rheological properties of blood (ARPB) — surface tension (ST), serum viscosity (SV), serum elasticity (SE), serum relaxation (SR), and viscoelasticity (VE) modulus [5, 6].

The interfacial tensiometry method is used to study the ST of cancer cell cultures as a criterion of their aggressiveness [7, 8], as well as to evaluate the effect of antitumor drugs *in vitro* [6], in particular, in the context of the character of the drug load on cancer cells [9, 10]. It has been shown that the inhibitory effect on the viability of tumor cells occurs in the cases when ST of the culture medium decreased [11]. Taking into consideration the different activity of suspensions of normal and tumor cells at interphase ("blood"/"air"), it is considered possible to selecting individual treatment of patients with LC on the basis of similar studies *in vitro* [12]. There is an opinion that ARPB can be used to monitor the effectiveness of antitumor radiochemotherapy [13].

The purposes and tasks of the study were to investigate the state of ARPB in patients with different variants of LC course, to determine the practical significance of violations of surface-active, viscoelastic and relaxation properties of blood serum, their interrelationships with tumor markers, to evaluate the prognostic role of initial indices in the development of complications of radiochemotherapy.

MATERIALS AND METHODS

The work was carried out in accordance with the ethical norms set forth in the Declaration of the Helsinki Medical Assembly, the patients gave informed consent for the study, which was approved by the commission on bioethics of the Donetsk National Medical University.

115 patients with LC aged 24 to 80 years (mean 57.9 ± 1.1 years) were under the observation, among them there were 78.3% of men and 21.7% of women. None of the examined patients received previously surgical treatment of LC. Localization of LC was right-sided in 59.1% of the cases, left-sided — in 38.3%, bilateral — in 2.6%; in the upper lobes of the lungs — in 23.5% of cases, in the lower lobes — in 13.9%, in the upper-lower-lobe on the left — in 19.1%, the middle-upper-lobe on the right — in 30.4%, the mediastinal — in 13.0%. The central form of LC occurred in 77.4% of cases, peripheral — in 22.6%.

Submitted: May 10, 2017.
Correspondence: E-mail: oncologdopc@gmail.com
Abbreviations used: ARPB – adsorption-rheological properties of blood; CT – chemotherapy; ISI – integral severity index of tumor process; LC – lung cancer; RT – radiotherapy; SE – surface elasticity; SR – surface relaxation; ST – surface tension; SV – surface viscosity; VE – viscoelasticity; VV – volume viscosity.

Small cell histological variant of LC was established in 16.5% of cases, non-small cell histological variant of LC — in 83.5%, including adenocarcinoma — in 40.0%, squamous cell carcinoma — in 38.3%, large cell carcinoma — in 5.2%. IIIA stage of the tumor process was observed in 12.2% of cases, IIIB — in 18.3%, IV — in 69.6%. The degree of differentiation of LC was 1.15 ± 0.108 points, the staging — 6.57 ± 0.065 r.u., the integral severity index of tumor process (ISI) was 3.97 ± 0.056 r.u., which was calculated by the formula:

$$ISI = ln\,[T + N2 + (\Sigma M)2],$$

where ln is the decimal logarithm, T is the international index of the primary tumor, N is the international index of metastasis in regional lymph nodes, and ΣM is the sum of metastases in distant organs. The course of the disease in 12.2% of patients was complicated by exudative pleurisy, in 11.3% — compression syndrome, 10.4% — tracheal invasion, in 5.2% — invasion in the chest wall and compression of the recurrent nerve, in 4.4% — respectively obstructive atelectasis and compression of the upper vena cava, in 3.8% — tumor invasion in the esophagus, in 2.6% — in the ribs.

Metastatic spreading of LC to lymph nodes was found in 96.5% of observations, in distant organs — in 54.8%, in the skeleton — in 51.3%, and the average number of metastases per one patient was respectively 2.42 ± 0.136 a.u., 1.86 ± 0.137 a.u. and 3.03 ± 0.192 a.u.

For the diagnosis of LC and its metastases, the methods of radiography, computed tomography, magnetic resonance imaging and sonography (Multix-Compact-Siemens, Germany; Somazom-Emotion-6-Siemens, Germany; Gygoscan-Intera-Philips, Netherlands; Envisor-Philips, Netherlands), esophagogastroscopy (fibroscope Olympus-GIF-Q20, Japan) were used. The parameters of the blood (SV, SE, ST, SR, and VE) were assessed using a computer tensiometer PAT2-Sinterface (Germany), the volume viscosity of the blood (VV) was examined using a rotary viscosimeter Low-Shear-30 (Switzerland). In our studies, a rapid stress deformation of the expansion of the blood serum surface was used (at t = 1200 s). The integral degree of change of ARPB (Ξ) indices was determined by the formula:

$$\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[\frac{(M_1 - M_2)}{SD}\right]^2},$$

where M1 is the index in the patient, M2 is the average index in healthy persons of the control group, SD is the standard deviation of the index in healthy persons. The modified value was considered to be Ξ > 2 r.u. As control, the parameters of ARPB were studied in the blood serum of 50 practically healthy people aged 18 to 62 years (27 men and 23 women). Using immunoenzyme and biochemical methods (the reader PR2100-Sanofi Diagnostic Pasteur, France; Olympus-AU640 Analyzer, Japan), the levels of TGFβ1, VEGF, osteopontin, osteocalcin, C-reactive protein, fibronectin and α_2-macroglobulin in the blood serum were studied.

Statistical analysis of the data was carried out using computer variational, nonparametric, correlative, regressive, one- (ANOVA) and multifactorial (ANOVA/MANOVA) dispersion analysis (Microsoft Excel and Statistica-Stat-Soft, USA). Mean values (M), their standard deviations (SD) and errors (m), Pearson's correlation coefficients (r), regression criteria (R), dispersions of Brown — Forsythe (BF), Student (t), Wilcoxon — Rao (WR) and the reliability of statistical indices (p) were estimated. The values $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The indices Ξ > 2 r.u. were found in 20.9% of the patients with LC, which are conditionally included in the main group. If in healthy people the VV parameters were 1.3 ± 0.03 mPa • s, SV — 15.5 ± 0.23 mN/m, SE — 42.8 ± 0.68 mN/m, ST — 42.8 ± 0.25 mN/m, SR — 114.4 ± 3.03 s, VE — 23.7 ± 1.04 mN/m, the values of VV, SE, ST and SR were significantly higher in patients with LC, which (> M + SD healthy), were respectively detected in 100.0; 5.2; 18.3 and 16.5% of cases (Fig. 1, 2).

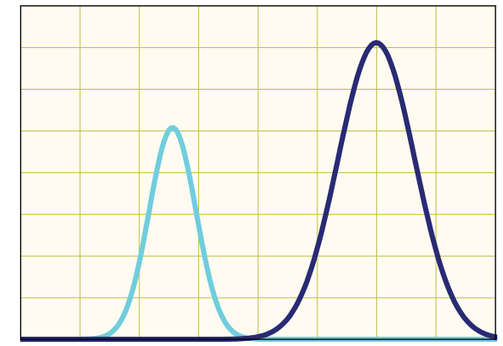

Fig. 1. Histograms of the VV index in patients with LC (dark curve) and healthy people (light curve)

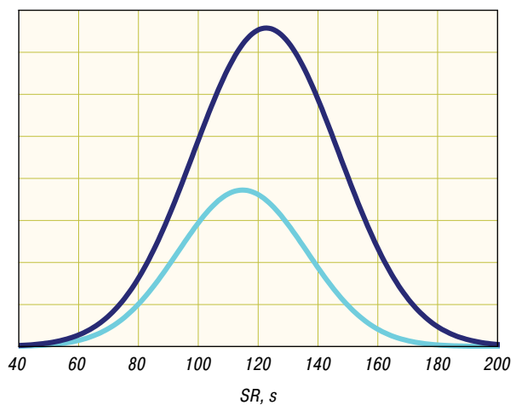

Fig. 2. Histograms of the SR index in patients with LC (dark curve) and healthy people (light curve)

According to the findings of the multifactorial dispersion analysis of Wilcoxon — Rao, the localization of LC (WR = 1.58, p = 0.019), the presence of compression pulmonary syndrome (WR = 1.64, p = 0.013), the number of lymph node metastases per one patient (WR = 2.04, p = 0.001) and to distant organs (WR = 3.55, $p < 0.001$)

influence the integral state of ARPB. According to the Brown — Forsythe analysis, the parameters SV (BF = 2.69, $p = 0.013$), which in men proved to be significantly (by 8%) large ($t = 2.87$, $p = 0.005$) are associated with the sex of patients with LC. Indices of VV are affected by the small-cell form of LC (BF = 5.46, $p = 0.021$), the presence of compression pulmonary syndrome (BF = 4.16, $p = 0.044$), adrenal metastases (BF = 6.99, $p = 0.009$) and the ribs (BF = 7.37, $p = 0.008$), the number of metastases in lymph nodes per a patient (BF = 5.39, $p = 0.022$), SV is affected by development of exudative pleurisy (BF = 2.69, $p = 0.013$), tumor invasion in the ribs (BF = 3.33, $p = 0.003$) and metastasis in the brain (BF = 2.74, $p = 0.012$), SE is affected by metastases in subclavian lymph nodes (BF = 1.79, $p = 0.041$), the sternum (BF = 1.82, $p = 0.036$), the humerus (BF = 2.60, $p = 0.002$) and the spinal column (BF = 3.20, $p < 0.001$), ST is affected by the index of differentiation of LC (BF = 2,26, $p = 0,047$), SR is affected by metastases in the spine (BF = 4.61, $p = 0.038$) and pancreas (BF = 3.22, $p < 0.001$), VE is affected by metastases in the spine (BF = 1.74, $p = 0.037$) and non-small cell LC (BF = 2.34, $p = 0.003$).

Analysis of multiple regression showed a direct dependence of VV on the integral state of clinical signs of LC ($R = +2.28$, $p = 0.025$), and on the character of tumor metastasis and the number of metastases in lymph nodes ($R = +2.74$, $p = 0.007$). VV directly correlates with ISI ($r = +0.231$, $p = 0.013$) and the number of metastases in the lymph nodes ($r = +0.360$, $p < 0.001$), and SR and VE values are inversely correlated with the number of metastases in distant organs ($r = -0.189$, $p = 0.043$ and $r = -0.238$, $p = 0.011$), which is reflected in Fig. 3 and 4.

There are no correlations between separate indexes of ARPB and such tumor markers as osteopontin, osteocalcin and fibronectin. In turn, with all the parameters of ARPB without exception, there are direct relations between the level of VEGF, which, in addition to the prognostic factor of high aggression of LC [14–16], is an important component in the development of neoangiogenesis in such patients [17]. In addition to the above, VV positively correlates with TGFβ1 ($r = +0.197$, $p = 0.035$) and C-reactive protein ($r = +0.293$, $p = 0.002$), and ST and SR directly correlates with TGFβ1 (respectively $R = +0.209$, $p = 0.025$ and $r = +0.191$, $p = 0.042$) and inversely with α_2-macroglobulin ($r = -0.190$, $p = 0.042$ and $r = -0.266$, $p = 0.004$).

In deceased patients with $\Xi < 2$ r.u. the life expectancy was on average six months longer than in the main group of the examined persons, respectively amounting to 11.3 ± 1.41 months and 17.9 ± 6.96 months, but the differences were unreliable. The life expectancy of such patients was influenced by the initial VV parameters (BF = 1.94, $p = 0.018$) and SV (BF = 1.71, $p = 0.048$), and a direct correlation was established with SE, ST and VE levels ($r = +0.279$, $p = 0.007$, $r = +0.245$, $p = 0.019$, $r = +0.360$, $p < 0.001$).

According to the results of ANOVA/MANOVA, the character of tumor metastasis in the lymph nodes (WR = 1.92, $p = 0.048$) and the skeleton (WR = 1.71, $p = 0.049$) affects the integral state of ARPB in patients

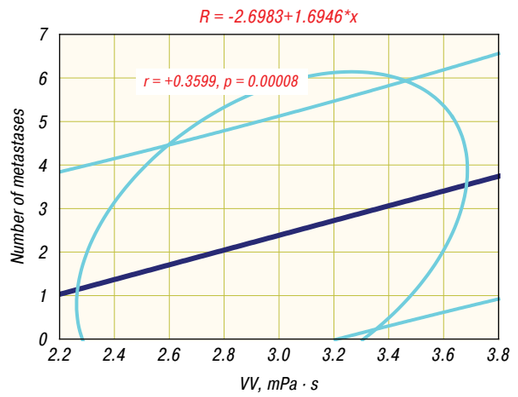

Fig. 3. Correlation-regression relationships of the VV indication with the number of metastases in the lymph nodes of the patients with LC

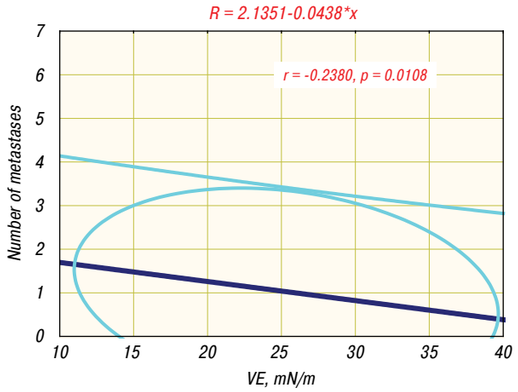

Fig. 4. Correlation-regression relations of the VE indication with the number of metastases in the distant organs of the patients with LC

with LC. As ANOVA shows, the number of metastases in the lymph nodes per one patient (correspondingly BF = 3.79, $p = 0.002$ and BF = 3.30, $p = 0.005$) is related to the levels of VV and VE. In turn, the process of metastatic spreading in the bone is determined by the parameters of viscosity of blood (BF = 2.26, $p = 0.043$), and in distant organs — VE of the serum (BF = 2.93, $p = 0.016$). Correspondingly, ISI differently depends on VV and VE ($R = +2.24$, $p = 0.027$ and $R = -2.42$, $p = 0.017$, respectively), metastatic spreading into the lymph nodes depends only on VV ($R = +3.65$, $p < 0.001$), and metastatic spreading into distant organs depends on VE ($R = -4.98$, $p < 0.001$). Metastases in the osteoarticular apparatus are closely related to the relaxation properties of the blood ($R = +3.30$, $p = 0.001$). We suppose that the indices in the blood serum VV > 3.5 mPa • s (> M + SD patients) reflect the severity of the unfavorable course of LC.

Radical radiation therapy (RT) of the primary tumor (> 60 Gy) was received by 5.2% of the number of examined patients, radical RT in combination with chemotherapy (CT) — 21.7%, palliative RT of the lungs — 10.4% of patients, palliative RT with CT — 36.5%, palliative RT for distant metastases — 10.4%, palliative RT in combination with CT — 36.5%, palliative RT for distant organs — 10.4%, in combination with CT — 15.7%. In general, RT was received by all patients, and CT was received by 85 (73.9%) of them. At the same time, the power of treatment was evaluated. Thus, the average doses of chemotherapeutic drugs {< M + SD} were defined as minimal (1 point), the doses {M + SD ↔ M + 2SD} — as moderate (2 points), {M + 2SD ↔ M + 3SD} — as high (3 points) and {> M + 3SD} — as very high (4 points). The power of RT (Ω) and CT (Ψ) was calculated by the formula:

$$\Omega(\Psi) = (a + b + c + d) : n,$$

where a, b, c, d — respectively the number of zones of irradiation or preparations in 1, 2, 3 and 4 points, n — the total number of irradiation zones and the total number of used drugs. The average parameters Ω were 1.39 ± 0.076 r.u., and Ψ — 0.37 ± 0.032 r.u.

As evidenced by the multifactorial analysis of Wilcoxon — Rao, the character of complications of radiochemotherapy for LC is closely related to the overall condition of ARPB ($WR = 4.57$, $p < 0.001$). The number of complications from the treatment per a patient is significantly influenced by the initial indices in blood VV (BF = 20.01, $p < 0.001$), SV (BF = 4.86, $p = 0.001$), SE (BF = 4. 20, $p = 0.003$), ST (BF = 6.31, $p < 0.001$), and SR (BF = 3.07, $p = 0.020$), it is demonstrated by ANOVA. At the same time, the values of VV have a dispersion effect on the development of radiation pneumofibrosis (BF = 5.06, $p = 0.026$), the formation of myelodepression (BF = 9.68, $p = 0.002$) and the appearance of acute disturbance of cerebral circulation of different degree of severity (BF = 27,77, $p < 0.001$), the parameters SE and SR — on the appearance of acute radiation pneumonitis (respectively, BF = 5.37, $p = 0.022$ and BF = 4.67, $p = 0.033$), ST and VE — on acute tubulointerstitial nephritis (BF = 8.62, $p = 0.004$ and BF = 4.42, $p = 0.038$) and acute radiation esophagitis (BF = 8.12, $p = 0.006$ and BF = 8.21, $p = 0.005$).

Analysis of multiple regression shows a direct dependence of complications of RT and metastatic spreading of tumor processes on the SV level ($R = +3.98$, $p < 0.001$) and feedback with VE ($R = -3.33$, $p = 0.002$). Taking into account the statistical processing of the obtained data of the study, a conclusion has been made which has a practical focus: the indices of SV > 20 mN/m (> M + SD of patients) are prognostic-negative for possible complications of further radiotherapy of patients with LC.

CONCLUSIONS

RT in every fifth patient is accompanied by integral changes in ARPB, which are manifested by an increase of the levels of VV, SE, ST and SR, depending on the sex of patients (VE), tumor localization, its histological form, degree of differentiation and severity of the course (VV, ST, VE), the number of metastases in lymph nodes, distant organs and skeleton (VV, VE), involvement of the pleura and ribs in the process (SV), the development of compression lung syndrome (VV, SR), metastatic spreading in the spine (SE, SR, VE), adrenal glands, the brain (SV), and the pancreas (SR). Surface-active, viscoelastic and relaxation properties of blood correlate with the levels of tumor markers such as VEGF, TGFβ1, C-reactive protein, α_2-macroglobulin, and indices VV and SV possess prognostic significance with respect to LC course and development of complications of radiochemotherapy respectively.

REFERENCES

1. Ceniceros L, Aristu J, Castanon E, ***et al***. Stereotactic body radiotherapy (SBRT) for the treatment of inoperable stage I non-small cell lung cancer patients. Clin Transl Oncol 2015; **55**: 213–9.

2. Gradalska-Lampart M, Karczmarek-Borowska B, Radziszewska AU. Lung cancer in Podkarpackie region in the years 2002–2011. Pneumonol Alergol Pol 2015; **83**: 109–19.

3. Yamada K, Maishi N, Akiyama K, ***et al***. CXCL12-CXCR7 axis is important for tumor endothelial cell angiogenic property. Int J Cancer 2015; **23**: 172–8.

4. Zhou L, Pan Y, Xing Y, ***et al***. Effects of Feijining Decoction on vascular endothelial growth factor protein expression and changes of T cell subsets in Lewis lung carcinoma-bearing mice. Biomed Rep 2015; **3**: 403–7.

5. Giannopoulou E, Siatis KE, Metsiou D, ***et al***. The inhibition of aromatase alters the mechanical and rheological properties of non-small-cell lung cancer cell lines affecting cell migration. Biochim Biophys Acta 2015; **1853**: 328–37.

6. Rojewska M, Prochaska K, Olejnik A, Rychlik J. Adsorption properties of biologically active derivatives of quaternary ammonium surfactants and their mixtures at aqueous/air interface II. Dynamics of adsorption, micelles dissociation and cytotoxicity of QDLS. Colloids Surf B Biointerfaces 2014; **119**: 154–61.

7. Butler CM, Foty RA. Measurement of aggregate cohesion by tissue surface tensiometry. J Vis Exp 2011; **50**: 165–9.

8. Winters BS, Shepard SR, Foty RA. Biophysical measurement of brain tumor cohesion. Int J Cancer 2015; **114**: 371–9.

9. Laskar P, Samanta S, Ghosh SK, Dey J. *In vitro* evaluation of pH-sensitive cholesterol-containing stable polymeric micelles for delivery of camptothecin. J Colloid Interface Sci 2014; **430**: 305–14.

10. Munyendo WL, Zhang Z, Abbad S, ***et al***. Micelles of TPGS modified apigenin phospholipid complex for oral administration: preparation, *in vitro* and *in vivo* evaluation. J Biomed Nanotechnol 2013; **9**: 2034–47.

11. Jiang L, Shen C, Long X, ***et al***. Rhamnolipids elicit the same cytotoxic sensitivity between cancer cell and normal cell by reducing surface tension of culture medium. Appl Microbiol Biotechnol 2014; **98**: 10187–96.

12. Preetha A, Banerjee R, Huilgol N. Dynamic surface tensiomeSRy of tissues using Langmuir films. Colloids Surf B Biointerfaces 2015; **40**: 35–43.

13. Bloy N, Pol J, Manic G, ***et al***. Radioimmunotherapy for oncological indications. Oncoimmunology 2014; **3**: 954929.

14. Domigan CK, Warren CM, Antanesian V, ***et al***. Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. J Cell Sci 2015; **128**: 2236–48.

15. Lin Q, Xue L, Tian T, ***et al***. Prognostic value of serum IL-17 and VEGF levels in small cell lung cancer. Int J Biol Markers 2015; **30**: 165–75.

16. Ostheimer C, Bache M, Güttler A, ***et al***. Prognostic information of serial plasma osteopontin measurement in radiotherapy of non-small-cell lung cancer. BMC Cancer 2014; **21**: 858–65.

17. Akin Kabalak P, Çiledag A, Demir N, ***et al.*** Prognostic significance of serum vascular endothelial growth factor and angiopoietin-2 in patients with lung cancer. Tuberk Toraks 2015; **63**: 71–7.