

COMPARATIVE EVALUATION OF PURINE DYSMETABOLISM IN GASTRIC AND PULMONARY ADENOCARCINOMAS

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Aim: To study the state of purine metabolism in gastric (GAC) and pulmonary (PAC) adenocarcinomas and to assess its clinical and pathogenetic significance. **Patients and Methods:** One hundred and six male patients were examined, among whom were 63 subjects aged 34 to 79 suffering from GAC, and 43 subjects aged 24 to 76 suffering from PAC. In GAC, the ratio of the pyloric, corporeal and antral localization of the tumor and variant of overall gastric lesion accounted to 24:5:1:1; and that of the central and peripheral PAC was 2:1. Serum levels of purine metabolism products (uric acid, oxypurinol, adenine, guanine, xanthine, hypoxanthine) were measured and activities of xanthine oxidase, xanthine deaminase, adenosine deaminase and 5-nucleotidase were analyzed. **Results:** Purine metabolism disorders are observed in all GAC and 91% of PAC patients; among other things, hyperuricemia is observed in $\frac{3}{4}$ and $\frac{1}{2}$ of cases, respectively; moreover, the nature of changes is more pronounced in gastric cancer and, in both groups of patients, these indices reflect the disease course severity, are associated with the neoplastic process localization, have a predictive value, trigger the development of metastases. **Conclusion:** Changes in purine metabolism are involved in the pathogenetic patterns of GAC and PAC, reflect the course of the tumor process, are associated with tumor localization and have prognostic significance. **Key Words:** cancer, adenocarcinoma, stomach, lung, purines, metabolism.

DOI: 10.32471/exp-oncology.2312-8852.vol-42-no-3.15068

Worldwide, the number of people with purine metabolism disorders is increasing. They are accompanied by production of an increased content of uric acid (UA) in the body [1], which belongs to key pathogenetic factors of many oncological diseases [2, 3], and constant hyperuricemia worsens the survival rate of such patients [4, 5]. The role of hyperuricemia in the development of malignant tumors has been proved not only by clinical observations [6], but also by special experimental animal studies [7].

It was found that an increase in UA content in blood is a risk factor of gastric [8] and pulmonary [9, 10] cancer development and further severe course. It is considered that purine bases belong to malignant tumor markers, but their diagnostic and predictive value in gastric (GAC) and pulmonary (PAC) adenocarcinomas remains not understood [11, 12].

The objective of the study was a comparative evaluation of the state of purine metabolism in GAC and PAC with determining the clinical and pathogenetic significance of the detected changes.

PATIENTS AND METHODS

The work was performed in accordance with the World Medical Association Declaration of Helsinki, and

the patients gave their informed consent to the study approved by the Donetsk National Medical University Bioethics Commission.

The data of three Ukrainian Cancer Centers were retrospectively analyzed. One hundred and six men suffering from GAC and PAC were observed. The first group consisted of 63 patients aged 34 to 79 (average of 61.3 ± 1.2 years); the second one — of 43 patients aged 24 to 76 (average of 58.0 ± 1.6 years). Among GAC, the ratio of tumor localization in the pylorus, in the stomach body, its antrum and the total lesion variant was 24:5:1:1. The ratio of central and peripheral PAC was 2:1. The ratio of the incidence of the upper lobar, left upper-lower lobar, lower lobar, mediastinal, middle lobar, right middle-upper lobar localizations was 4:3:3:2:1:1.

As a control group, 30 practically healthy men aged from 20 to 68 years old were examined (average 39.7 ± 1.9 years).

We have proposed an integral indicator of the severity index of cancer (IWT), which was determined by the formula: $IWT = \ln [T + N^2 + (\Sigma M)^2]$, where T is the international indicator of the primary tumor, N is the international indicator of the metastatic lesion of the regional lymph nodes, ΣM is the sum of remote organs with metastases. In GAC, IWT accounted to 4.6 ± 0.3 a.u., and in PAC it was 3.5 ± 1.2 a.u.

Diagnosis of cancer and its metastases was based on the findings of imaging techniques (X-ray, computed tomography, sonography), bronchofibrosopic, esophagogastroduodenal examination and cytological (histological) analysis. We used a Multix-Compact-Siemens X-ray machine (Germany), a Somatom-Emotion-6-Siemens computer tomograph (Germany), a Gygoscan-Intera-Philips magnetic resonance

Submitted: March 02, 2020

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Abbreviations used: 5N — 5-nucleotidase; Ad — adenine; AD — adenosine deaminase; BF — uniformity criterion of Brown — Forsyth dispersion; D — single factor analysis criterion of variance; GAC — gastric adenocarcinoma; Gu — guanine; Hx — hypoxanthine; IWT — severity index of cancer; OP — oxypurinol; PAC — pulmonary adenocarcinoma; UA — uric acid; WR — Wilcoxon — Rao multivariate analysis of variance; Xa — xanthine; XD — xanthine deaminase; XO — xanthine oxidase; τ — non-parametric Kendall correlation coefficient.

tomograph (Netherlands), an Envisor-Philips sonograph (Netherlands), Olympus-GIF-Q20 fiberscopes (Japan), EXERA-II-Olympus (Japan), Fujinon-FG-1Z (Japan). Morphologically in all cases the tumors were adenocarcinomas.

To assess purine metabolism, Olympus-AU640 semi-automatic biochemical immunoturbidimetric analyzers (Japan) and BS200 (China), SPECORD-S600 spectrophotometers (Germany) and SF46 (Russia). Serum levels of purine metabolism products were studied — UA, oxypurinol (OP), adenine (Ad), guanine (Gu), xanthine (Xa), hypoxanthine (Hx), xanthine oxidase (XO) activity, xanthine deaminase (XD), adenosine deaminase (AD) and 5-nucleotidase (5N). The OP content was determined by the method [13], and purine bases were determined by direct spectrophotometry in the aqueous extract of the venous blood serum thermocoagulant (the studied purines in solutions have the property of maximum ultraviolet absorption at a wavelength defined for each metabolite) [14].

Statistical processing of the obtained results was performed using computer variance, nonparametric, correlation, single (ANOVA) and multivariate (ANOVA/MANOVA) dispersion analysis ("Microsoft Excel" and "Statistica-Stat-Soft", USA programs). We estimated the average values (M), their standard errors and deviations, the parametric correlation coefficients of Pearson (r) and the nonparametric of Kendall (τ), the criteria of Brown — Forsythe (BF) and Wilcoxon — Rao (WR) dispersion (D), Student (t) and the reliability of the statistical indices (p). The critical significance level for checking the statistical hypotheses was considered to be 0.05.

RESULTS AND DISCUSSION

Integral purine metabolism disorders have been found in 100.0% of the GAG and in 90.7% of the PAC patients. The indices of serum purine metabolism in the healthy persons and cancer patients are shown in the Table. Hyperuricemia ($< 420 \mu\text{mol/l}$) was observed in 74.6% and 46.6% of the GAC and PAC patients, respectively (the differences are not statistically significant). The common feature for both diseases was an increase in UA, OP, XO and

AD blood levels compared to the healthy persons. In GAC, the UA content was by 92% higher; OP — 4.7 times higher, XO activity — 2.1 times higher, and AD activity — 7.1 times higher than the corresponding values in control group. In PAC, the UA content was by 70% higher, OP — 4 times higher, XO activity — by 80% higher, and AD activity — by 4.6 times higher than the corresponding values in control group ($p < 0.001$ for all comparisons).

It should be noted that GAC was also accompanied by a significant increase in the XD concentration by 33% ($p = 0.032$) and by 14% in that of Ad ($p = 0.036$), the latter being 12% higher than in PAC patients ($p = 0.028$) (Table, Fig. 1). In addition, in GAC, the uricemia values prevailed by 12% ($p = 0.011$) (Fig. 2) and those of AD activity prevailed by 54% ($p = 0.042$). Consequently, purine metabolism disorders in GAC patients were observed more often than in PAC cases, and shifts in the indices under study were more pronounced.

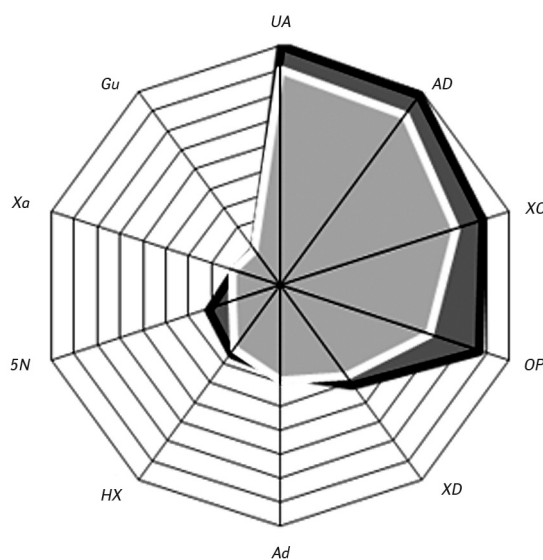


Fig. 1. The changes in frequency of the purine metabolism indices (%) in the GAC (black curve) and PAC (white curve) patients

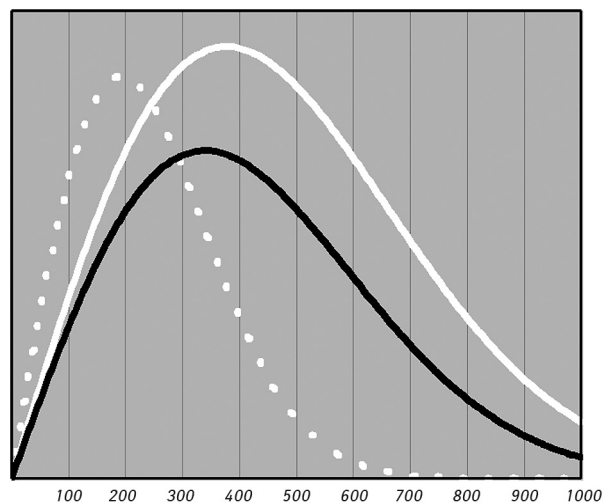


Fig. 2. Rayleigh histograms of the uricemia index in the GAC (black curve), PAC (white curve) patients and in healthy persons (dotted curve)

Table. Indices of blood purine metabolism in healthy persons and cancer patients (M $\pm$ SE)

Indices	Groups of examined subjects		
	Healthy persons (n = 30)	GAC (n = 63)	PAC (n = 43)
UA, $\mu\text{mol/l}$	271.1 $\pm$ 3.2	520.9 $\pm$ 13.7*	461.1 $\pm$ 19.6*,**
OP, $\mu\text{mol/l}$	27.7 $\pm$ 0.2	115.9 $\pm$ 8.2*	98.7 $\pm$ 10.7*
Ad, a.u.	122.6 $\pm$ 7.0	140.1 $\pm$ 4.5*	124.6 $\pm$ 4.8**
Gu, a.u.	175.5 $\pm$ 7.7	182.6 $\pm$ 5.1	181.1 $\pm$ 6.2
Xa, a.u.	147.0 $\pm$ 4.7	149.7 $\pm$ 3.4	146.3 $\pm$ 4.7
Hx, a.u.	164.1 $\pm$ 7.6	172.0 $\pm$ 4.9	171.7 $\pm$ 6.6
XO, nmol/ml $\times$ min	3.5 $\pm$ 0.1	7.5 $\pm$ 0.5*	6.3 $\pm$ 0.7*
XD, nmol/ml $\times$ min	6.1 $\pm$ 0.1	8.1 $\pm$ 0.6*	7.4 $\pm$ 0.6
AD, nmol/ml $\times$ min	1.6 $\pm$ 0.1	11.4 $\pm$ 1.5*	7.4 $\pm$ 0.7*,**
5N, nmol/ml $\times$ min	5.7 $\pm$ 0.1	5.9 $\pm$ 0.1	6.0 $\pm$ 0.2

Note: * differences between similar indices in healthy persons and cancer patients are statistically significant ($p < 0.05$); ** differences between similar indices in the GAC and PAC patients are statistically significant ($p < 0.05$).

As shown by the performed Wilcoxon — Rao multivariate analysis of variance, the integral indices of the GAC and PAC course (localization, differentiation grade, the nature of its invasion, metastatic spread into the lymph nodes, remote organs and bones) are influenced by OP content ($p < 0.001$, $WR = 4.75$ and $WR = 4.07$, respectively), Ad content ($WR = 4.70$ and $WR = 5.80$) and AD activity ($WR = 2.84$ and $WR = 8.70$). In addition, the signs of GAC are closely related to Gu ($WR = 2.08$, $p = 0.018$), and PAC is closely related to UA ($WR = 11.55$, $p < 0.001$).

In both variants of adenocarcinoma, there have been observed direct Pearson correlations of the IWT index with blood UA ($r = +0.836$, $p < 0.001$ and $r = +0.935$, $p < 0.001$), Ad ($r = +0.566$, $p < 0.001$ and $r = +0.405$, $p = 0.007$) and AD ($r = +0.277$, $p = 0.031$ and $r = +0.273$, $p = 0.048$) levels (Fig. 3, 4). We emphasize that GAC is also characterized by a positive relationship between IWT and Gu ($r = +0.381$, $p = 0.002$). Correlation associations of the neoplastic process severity are accompanied by dispersion relations with the same indices of purine metabolism. Thus, IWT is influenced by UA ($D = 40.53$, $p < 0.001$ and $D = 61.52$, $p < 0.001$, in GAC and PAC, respectively), Ad ($D = 4.51$, $p < 0.001$ and $D = 5.29$, $p = 0.004$) and AD ($D = 2.53$, $p = 0.021$ and $D = 3.17$, $p = 0.035$) levels in blood. It should also be noted the IWT dependence on XD activity in GAC patients and on oxypurinolemia in case of PAC.

We selected those indicators of purine metabolism that simultaneously had BF relations and nonparametric Kendall correlations with individual signs of the neoplastic process course. In the GAC

patients, direct correlations of tumor invasion of the spleen were established with UA level ($BF = 76.08$, $p < 0.001$; $\tau = +0.195$, $p = 0.024$), as well as those of invasion of the pancreas with Ad ($BF = 4.12$, $p = 0.046$; $\tau = +0.174$, $p = 0.044$), those of gastric body lesion with AD ($BF = 3.52$, $p = 0.047$; $\tau = +0.179$, $p = 0.041$), those of metastatic spread into the adrenal glands with XD ($BF = 5.16$, $p = 0.027$; $\tau = +0.210$, $p = 0.015$). In PAC, UA is associated with the development of metastases to the lymph nodes ($BF = 12.56$, $p < 0.001$; $\tau = +0.476$, $p < 0.001$) and the osteoarticular system ($BF = 11.78$, $p < 0.001$; $\tau = +0.418$, $p < 0.001$); Ad is associated with exudative pleurisy ($BF = 3.38$, $p = 0.013$; $\tau = +0.261$, $p = 0.014$); XO is associated with nerve root compression syndrome ($BF = 9.02$, $p = 0.005$; $\tau = +0.222$, $p = 0.020$), AD is associated with metastatic spread into remote organs ($BF = 4.26$, $p = 0.004$; $\tau = +0.262$, $p = 0.013$).

After statistical processing of the performed studies, the following practice oriented conclusions have been made: in GAC and PAC, the UA values $> 630 \mu\text{mol/l}$ and $> 590 \mu\text{mol/l}$, respectively, as well as Ad $> 176 \text{ a.u.}$ and $> 155 \text{ a.u.}$ ($> M \pm$ standard deviations of the patients) are risk factors for severe course of the neoplastic process (IWT $> 6.8 \text{ a.u.}$ and $> 4.2 \text{ a.u.}$). The degree of prediction of the model result were calculated as 75.0%, 88.9%, 66.7%, and 77.8%.

Disturbances in purine metabolism are a consequence, not a cause, of mutations in genes responsible for the regulation of cell proliferation and differentiation. The Ad end-products are allosteric inhibitors of 5-phosphoribosyl-1-pyrophosphate aminotransferase. The first step is catalyzed by 5N and cytosolic isoenzymes, in the presence of which Hx is synthesized [15]. These processes most actively occur just in tumor cells [16, 17]. AD catalyzes the transfer of γ -phosphate from adenosine triphosphate to adenosine and further Ad, thus acting as a potentially important regulator of the concentrations of this extracellular nucleotide, and an increase in AD activity is a consequence of mutations in the genes responsible for the regulation of cell proliferation and differentiation.

In patients with gastric and lung cancer, an increased activity of the purine metabolism enzyme XD in blood contributes to UA overproduction, which, in turn, is considered to be one of the factors of proinflammatory cytokine synthesis stimulation and activation of enzymes (e.g. cyclooxygenase-2) [18]. In turn, an excessive UA concentration in tumor cells contributes to XD deregulation supporting cell proliferation and metastatic spread of the neoplastic process, thereby closing the vicious circle [19].

To sum up, we observed the integral disturbances in purine metabolism in all patients with GAC and 91% PAC, including hyperuricemia in $\frac{3}{4}$ and $\frac{1}{2}$ cases. Moreover, the changes in UA, Ad and AD in gastric cancer are more pronounced. In both groups of patients, these indicators reflect the severity of the course of the

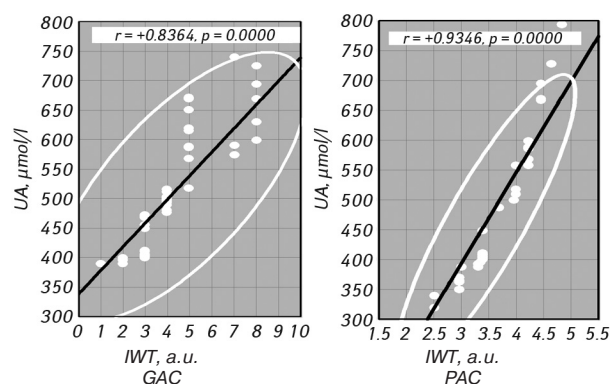
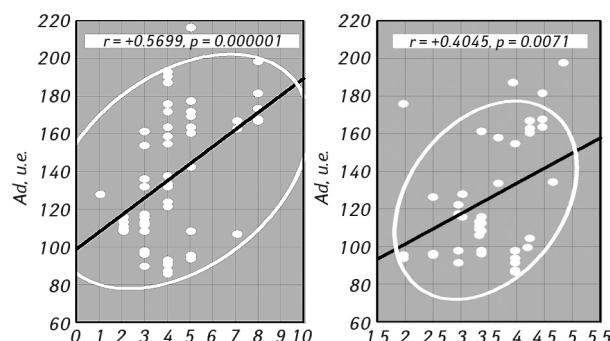


Fig. 3. Direct Pearson correlations of the UA level with the IWT index in the GAC and PAC patients



disease, are associated with the localization of the tumor and have prognostic significance. High UA, Ad and XD levels in GAC patients' blood participate in pathogenetic patterns of tumor invasion into the spleen and the pancreas, in the processes of metastatic spread into the adrenal glands; and in case of PAC, the UA, AD, Ad and XO concentrations determine the development of metastases in the lymph nodes, bones and remote organs, formation of exudative pleurisy and nerve root compression syndrome.

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