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Association of expression of pSTAT3, pAKT1 with the survival of patients with diffuse large B-cell lymphoma

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Abstract

Aim. To assess the relationship between isolated and combined expression of pSTAT3, pACT1 in tumor cells with the survival of patients with diffuse large B-cell lymphoma (DLBCL).

Methods. The study included 100 patients with the first diagnosed diffuse large B-cell lymphoma, observed in the institute's clinic between 2010 and 2018 who received standard first-line R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine and prednisone) chemotherapy. The relative number of expressing pSTAT3 and pAKT1 tumor cells was determined by using immunohistochemical and morphometric methods. The optimal cut-off level of expression on tumor cells estimated by using receiver operating characteristic (ROC) curve analysis for pSTAT3 was 68% and for pACT1 — 70%. Given these values, all patients with DLBCL were divided into groups with a high and low degree of expression of the biomarkers. As a result, 53 patients were enrolled in the pSTAT3 high expression group (≥68% tumor cells) and 47 patients to the pSTAT3 low expression group (<68% tumor cells). Spearman's correlation coefficient was used to examine relationships. Overall survival and event-free survival were estimated by Kaplan–Meier curves. The log-rank test was used for groups comparison.

Results. The five-year overall survival rate in the pSTAT3 high expression group was 55% versus 87% in the low expression group, p=0.015. A significant difference was found in the assessment of event-free survival: 43% for the group of pSTAT3 high expression, 66% for the group of low expression, p=0.011. A statistically significant value of a high level of pACT1 expression was revealed for 5-year overall and event-free survival (p <0.001 and p=0.003). Overall survival rate was 81% for the pACT1 low expression group and 43% for the high expression group while event-free survival rate was 64 and 41%, respectively. Also, patients with $pAKT1^{+}/pSTAT3^{+}$ (high level) co-expression had extremely low rates of overall and event-free survival rates compared with the $pAKT1^{-}/pSTAT3^{-}$ (low level) group (p=0.001; p <0.001).

Conclusion. The pSTAT3 and pAKT1 biomarkers can be used as additional prognosis criteria for diffuse large B-cell lymphoma.

Keywords: diffuse large B-cell lymphoma, pAKT1, pSTAT3, hyperexpression.

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Background

Diffuse large B-cell lymphoma (DLBCL) is among the most common forms of aggressive lymphoid neoplasia. This disease is characterized by a heterogeneous group of non-Hodgkin lymphomas that differ by morphology, molecular genetics, and immunophenotypic criteria. The disease is characterized by a variable clinical course (that is, response to therapy) and prognosis [1,2].

Existing clinical systems for assessing prognosis (such as international prognostic indices) are successfully used in clinical practice for early stratification of DLBCL patients and selection of an adequate therapeutic approach. However, in 30–40% of patients, they do not accurately predict the further course of the disease, since they mainly rely on the clinical characteristics of patients [2–4]. This determines the need for additional biomolecular predictive factors.

Many pathologically activated signaling pathways (JAK/STAT3, PI3K/AKT/mTOR, among others) are known to be involved in the pathoge-

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nesis of DLBCL. The expression rate of key molecules that mediate these pathways affects the biological behavior of neoplastic cells and can determine the disease prognosis. In addition, components of various signaling cascades function as potential targets for therapies [5].

STAT3 protein is an important player in JAK/STAT3 signaling pathway [6]. This protein mediates the expression of multiple genes in response to various stimuli and thus regulates important cellular processes including mitotic cell division and apoptosis. Some studies have shown that hyperexpression of this transcription factor is directly related to proliferation and malignant transformation of cells. On the contrary, repression of *STAT3* gene leads to cell-cycle arrest, apoptosis, and tumor regression [7].

The signaling protein pAKT1 is involved in many vital cellular processes. In oncogenesis, dysregulation of its expression promotes tumor progression, neoplastic cells survival, and intensification of angiogenesis. The PI3K/AKT/mTOR cascade has been shown to be constitutively activated in 25–50% of DLBCL cases [8]. At the same time, the prognostic value of pSTAT3 and pAKT1 expression in this disease has not been sufficiently investigated. The findings in literature in this area are contradictory [9, 10]. Moreover, no work on this area have been found in Russian scientific literature.

The study aimed to assess the association of isolated and combined expression of pSTAT3 and pAKT1 in tumor cells with survival of DLBCL patients.

Materials and methods

This retrospective study included 100 DLBCL patients registered (from 2010 to 2018) at the clinic of Kirov Research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia. The inclusion criterion for the study was diagnosis of DLBCL confirmed by morphology and immunohistochemistry, in accordance with the classification of hematopoietic and lymphoid tissue tumors of the World Health Organization (2017). All subjects received standard first-line therapy according to the R-CHOP scheme. The median age of patients was 58 years (range=24–83 years). The study was approved by the local ethical committee of the Kirov Research Institute of Hematology and Blood Transfusion of the Federal Medical and Biological Agency of Russia (protocol No. 10 dated 04/14/2020).

Histological and immunohistochemical (IHC) studies were performed on archival material of primary biopsy of lymph nodes/tumor formations in DLBCL patients. IHC analysis was performed

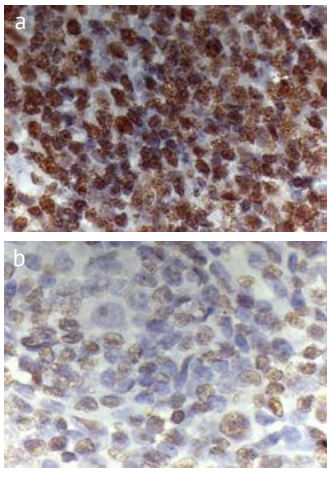

Fig. 1. Lymph node. Immunohistochemical staining of tumor cells with pSTAT3 antibody: (a) level of nuclear pSTAT3 expression ≥68%; (b) level of nuclear expression pSTAT3 <68%; ×1000.

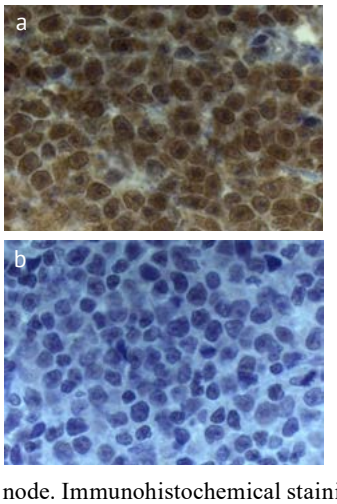

Fig. 2. Lymph node. Immunohistochemical staining of tumor cells with pAKT1 antibody: (a) level of nuclear expression of pAKT1 ≥70%; (b) level of nuclear expression of pAKT1 <70%; ×1000.

in all patients prior to treatment. pSTAT3- and pAKT1-positive tumor cells in biopsy specimens were identified using antibodies for pSTAT3 (phosphorTyr705, GeneTex) and pAKT1 (phosphor-Ser 473, GeneTex). Morphometric assessment of the relative content of tumor cells was performed visually by double blind analysis using an Axio-Scope.A1 light microscope (Carl Zeiss Microscopy GmbH, Germany). Cells were counted in a 10-field view for each sample using ×10 eyepieces and ×100 camera lens.

The optimal cutoff threshold for assessing the level of protein expression was calculated by receiver operating characteristic (ROC) analysis. Overall (OS) and event-free (EFS) survival rates were calculated using the Kaplan-Meier method with graphical plots of corresponding curves.

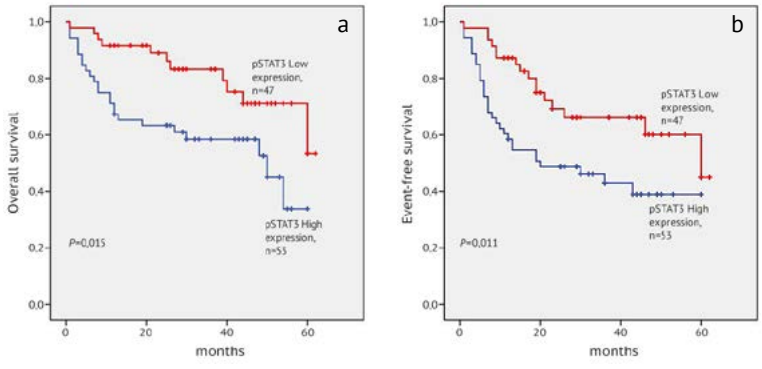

Fig. 3. Overall (a) and event-free (b) survival rates of patients with diffuse large B-cell lymphoma, depending on the degree of pSTAT3 expression.

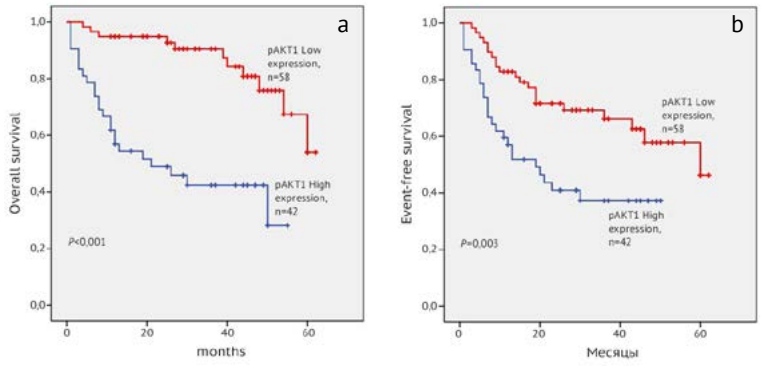

Fig. 4. Overall (a) and event-free (b) survival rates of patients with diffuse large B-cell lymphoma, depending on the degree of pAKT1 expression.

The probability of an unfavorable outcome of the disease was assessed by the risk ratio (RR), with indication of the 95% confidence interval (CI). Comparative analysis of survival rate was performed using a log-rank test. The correlation analysis adopted was Spearman correlation coefficient (*r*). Statistical analysis was performed using IBM SPSS Statistics for Windows Version 19 (IBM Corp., Armonk, N.Y., USA). Differences between indicators were considered statistically significant at $p<0.05$.

Results. According ROC, the optimal cutoff threshold for pSTAT3 and pAKT1 expression in tumor cells was set at 68% and 70%, respectively. Considering these values, all DLBCL patients were distributed into groups as high and low level expression of these markers. As a result, the group with pSTAT3 hyper-expression (≥68% of tumor cells) had 53 patients, while the group with low expression of pSTAT3 (<68% of tumor cells) had 47 patients (Fig. 1).

The group with a hyper-expression of pAKT1 (≥70%) had 42 patients, while the group with low expression of pAKT1 (<70%) had 58 patients (Fig. 2).

The long-term results of therapy for DLBCL patients (OS and EFS) were assessed be considering the different levels of expression of pSTAT3 and pAKT1. The 5-year OS (Fig. 3a) in the pSTAT3 hyper-expression group was 55% (*Me*=50 months) versus 77% (*Me* was not achieved) in patients with low expression of the protein (RR=2.3, 95% CI=1.16–4.87, $p=0.019$). When analyzing EFS (Fig. 3, b), in cases with high expression of pSTAT3, the 5-year OS was 43% (*Me*=20 months) versus 66% in cases with low expression of the protein (*Me*=60 months, RR=2.2, 95% CI=1.17–3.96, $p=0.014$).

The 5-year OS in patients with high pAKT1 expression (Fig. 4a) was 43% (*Me*=21 months), while that of the patients with a subthreshold expression level of the protein was 81% (*Me* was not achieved, RR=5.3, 95% CI=2.51–11.25, $p<0.001$). The same trend was observed for EFS (Fig. 4b), since 5-year OS was 41% (*Me*=19 months) in cases with high pAKT1 expression and 64% in cases with low expression of the protein (*Me*=60 months; RR=2.3, 95% CI=1.31–4.23, $p=0.005$).

The correlation analysis revealed a moderate positive relationship ($r=0.368$; $p<0.01$) between the expression of pSTAT3 and pAKT1. In this regard, evaluation of the combined expression effects of these markers on OS and EFS in DLBCL patients was necessary (Fig. 5).

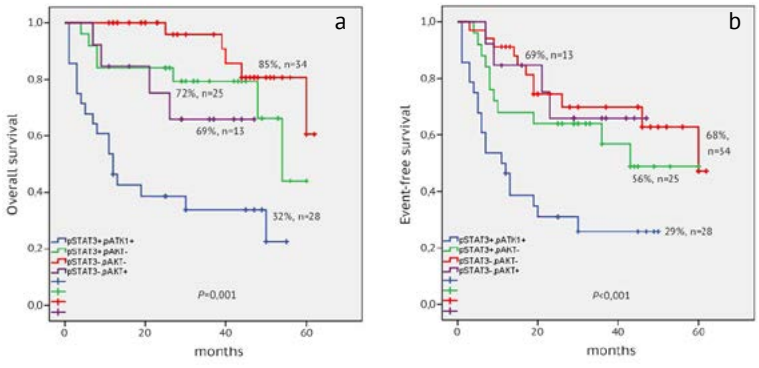

Fig. 5. Overall (a) and event-free (b) survival rate of patients with different combinations of pSTAT3 and pAKT1 expression.

These data indicate that indicators of the 5-year OS rate in the patient groups were significantly different (p=0.001). The worst OS (32%; *Me*=12 months; RR=5.2, 95% CI=2.57–10.22, p=0.001) was registered in the group of patients whose tumor cells were characterized by simultaneous hyper-expression of pSTAT3 and pAKT1. On the contrary, the highest OS (85%) was found for patients with low combined expression of these markers. OS for the patients with double positive status of pSTAT3/pAKT1 expression was significantly lower than similar indicators in groups with isolated hyper-expression of pSTAT3 and pAKT1 (32% versus 69% and 72%; p=0.001).

Analysis of EFS also revealed intergroup differences (p<0.001). In patients with a high degree of co-expression of pSTAT3 and pAKT1 tumor cells, EFS was 29% (*Me*=17 months), which was significantly lower than those of other groups (RR=3.2, 95% CI=1.81–5.88, p=0.001).

Discussion. The study revealed an association between degree of expression of pSTAT3 and pAKT1 proteins in tumor cells and survival rate of DLBCL patients who received standard first-line multi-agent chemotherapy. It was established that the risk of lethal outcome was 2.3 times higher in DLBCL patients with hyper-expression of pSTAT3 in tumor cells than in patients with low expression of the protein. The risk of development of the disease according to EFS was 2.2 times higher in patients with high expression of pSTAT3. This was presumably due to the fact that excessive synthesis of this transcription factor in neoplastic cells constitutively stimulates cell cycle, leading to tumor progression. This data is consistent with results in some literatures, in which uncontrolled activation of JAK/STAT3 signaling pathway correlates with low OS and EFS in DLBCL patients [7]. However, some scientific works did not agree with this fact [11].

It was revealed that pAKT1 is a predictor of low survival in DLBCL patients. In patients with a super threshold expression level of this protein, OS was 1.9 times lower and risk of death was 5 times higher compared to patients with low degree of pAKT1 expression. A similar trend was observed in terms of EFS. The data obtained is consistent with reports in literature, which confirm the significant role of PI3K/AKT/mTOR pathway dysregulation in the pathogenesis of DLBCL [10].

The combined effect of pSTAT3 and pAKT1 expression on prognosis of the disease was established. In patients with simultaneous super threshold expression of the proteins, OS and EFS values were, on average, 1.5 times lower compared to patients with isolated hyper-expressions of pSTAT3 and pAKT1. This may indicate a close relationship in the function of the various signaling pathways and existence of synergistic and modulating effects on course of the disease.

Conclusions

1. Super threshold values of pSTAT3 and pAKT expression are associated with low overall and event-free survival rates in patients with diffuse large B-cell lymphoma.

2. The combined hyper-expression of pSTAT3 and pAKT in tumor cells of diffuse large B-cell lymphoma significantly increases the risk of an unfavorable outcome following treatment with R-CHOP protocol.

3. pSTAT3 and pAKT biomarkers can be used as supplementary prognosis criteria for diffuse large B-cell lymphoma.

Authors' contributions. V.A.R. supervised the work, developed, designed, and reviewed the manuscript, analyzed and interpreted the data; E.V.V. collected the data, performed the practical part of the research, analyzed and interpreted the data, drafted the manuscript; D.A.D. developed the scientific aspect, verified the critically important intellectual content of the article;

S.V.S. collected the clinical data of patients; I.V.P. coordinated clinical and laboratory works.

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Conflict of interest. The authors declare no conflict of interest related to the article presented.

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