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Current Issues of Molecular Diagnostics of Bladder Cancer

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Abstract

Nowadays, it is generally accepted that cancer is a genetic disease. Tumor cells appear due to the accumulation of mutations in critical proto-oncogenes and tumor suppressor genes. Urothelial bladder cancer is a frequent oncological pathology, and therefore it is a significant social problem. The practical relevance of the study is due to the fact that the findings can significantly improve the efficiency of bladder cancer diagnosis. The purpose of the study was to consider all modern methods for diagnosing bladder cancer in one paper. As a research method, the analysis of scientific data obtained from the experimental study of bladder cancer was carried out. According to the data of the investigated sources, a fairly large number of scientists believe that bladder cancer (BC) is one of the most common tumors affecting the urinary tract. It is believed that important prognostic factors include the presence of vascular invasion and tumor complexes in the vessels, which increases the risk of the secondary neoplasm growth even at the pT1 stage. However, when assessing vascular invasion, pathologists often mistake the cracks formed around the tumor complexes for vessels. In this case, the study suggests conducting an immunohistochemical analysis for clear visualization of blood vessels.

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1 Introduction

At this point, the choice of the method of treatment and prognosis of bladder cancer is based on its belonging to a certain category according to the TNM classification (The Tumour, Node, Metastasis) and Grade (G) systems (Abdel-Rahman, 2019). These indications are leading, since they determine the spread of the malignant process and allow indirectly judging its potential aggressiveness (Zhao et al., 2007). At the same time, the long-term results of treatment of patients who belong to the same classification subgroups and received the same treatment differ significantly. Thus, for a complete prognosis of BC, additional information is needed on the properties of the tumour, that is, in addition to the stage and histological grade, individual factors determining the clinical behaviour and biological aggressiveness of the neoplasm should be taken into account (Jordan & Meeks, 2019).

According to the interpretation of modern molecular biology, the implementation of hereditary information is carried out in the chain DNA (deoxyribonucleic acid) → RNA (Ribonucleic acid) → protein. Multiple changes in the genome during oncogenesis lead to disruption of numerous intracellular processes overlapping each other, which is manifested by the development of a new, "tumorous" phenotype with a number of characteristic features that underlie typing of tumours (Jordan & Meeks, 2019). Notably, genetic disorders that lead to the emergence of a tumour are accompanied by changes in molecular signalling cascades, which to a certain extent are specific for each particular tumour and that bring unique additions to the general mechanisms of tumour growth (Attallah et al., 2006). In this regard, to explain the diversity of histologic types of urothelial tumours, the theory of divergence in histogenesis by Yu.I. Afanasyev will serve as an example. When it is extrapolated to oncogenesis, the diversity of new features, difference from the original tissue, and similarity with tumours of other histogenetic types becomes clear (Glimelius et al., 1997; Glimelius et al., 1996).

In the propensity to BC, a substantial role is played not so much by mutations as by normal variations in the genomic set (Attallah et al., 2006). Given that the increased risk of developing bladder cancer is conditioned by the presence of certain allelic variants of genes, enzymes, prooxidants and antioxidants, there is reason to believe that cytochromes P450 and glutathione-dependent enzymes, as well as DNA repair genes, may be an important component of the genetic structure of susceptibility to the development of BC (Shapiro et al., 2011). Diagnostics of BC begins with an assessment of the tumour morphology, which allows establishing its histological type (according to the classification of the World Health Organisation (WHO), there are 12 types of infiltrative urothelial carcinoma and 6 types of noninfiltrating) (Elliott et al., 2019).

The prognosis of the disease state from the histological type of cancer is necessary, but far from a sufficient condition for the diagnosis of BC (however, there are opinions that the morphological variant of urothelial carcinoma does not matter for the invasive and metastatic potential of the tumour) (Zhang et al., 2019). It is believed that a papillary urothelial neoplasm of low malignant potential, as a rule, does not develop into cancer, but patients have an increased risk of developing new papillary formations with a higher malignancy potential (Musquera et al., 2013). Squamous metaplasia is common in highly anaplastic carcinomas. In case of the spindle cell variant, regional, and distant metastases are often located (Leon et al., 2014).

In case of the prevalence of lymph and epithelioma-like variants, the prognosis is relatively favourable. Some authors suggest that such types of urothelial carcinoma as micropolar, sarcomatoid, with glandular differentiation have a worse prognosis. Small cell carcinomas, colloid carcinomas, squamous cell carcinomas are characterised by an aggressive clinical course (Alifrangis et al., 2019). There are known cases of classic verrucous carcinoma, which did not have a high risk of progression due to schistosomiasis. Small cell carcinoma is marked by an aggressive clinical course with early vascular and muscle invasion. However, its combination with other types of BC usually has a more favourable prognosis (Schmitz-Dräger et al., 2015).

An increase in the number of cell layers in the urothelium over 7 is not a sign of neoplasia, but it is usually noted in combination with nuclear atypia and does not cause problems when diagnosing carcinoma (Kamat et al., 2013). Tumour prevalence is assessed in accordance with the TNM classification of BC. Most often, the preliminary clinical stage is established by the cystoscopy, ultrasound investigation, and histological examination of biopsy material (Parsons et al., 2020). In noninfiltrating lesions, the basal layer of the urothelium retains an even, clear contour, to which the continuous basement membrane is adjacent, but it is lost in the areas of invasion (Yoshida et al., 2019). In the areas of invasion, fibrosis and inflammatory infiltration are often observed (Glybchenko et al., 2011). When assessing the extent of the tumour in the lamina propria, which can be difficult with tangential sections, it is necessary to indicate extensive or focal infiltration, but not to use the characteristic "superficial cancer", since 2 stages are mixed – pTa and pT1 (Jayson et al., 2016; Blagosklonny, 2004).

2 Materials and Methods

This paper presents a comparative analysis of studies selected for the final summary on the diagnosis and treatment of bladder cancer, as well as medical advances in the fight against this disease as of today. As a way to reduce all the results obtained in this area to a common denominator, comparative analysis and modelling were applied. To study the statistics of diseases and the effectiveness of certain methods of treatment, a mathematical approach was utilized (Garden et al., 1997; Chapelier et al., 2004).

The main methods for detection of the BC are morphological, molecular and genetic. Also, recently, such methods of laboratory diagnostics as the determination of BTA (bladder tumor antigen), NMP 22 (nuclear matrix protein), UBC antigen (urinary bladder cancer), urine telomerase, and other tumor markers have become frequently used. In order to predict the development and optimize treatment methods for a particular tumor, at present, a large amount of basic research is focused on the search for molecular markers that may indicate a predisposition to the appearance of carcinomas. This is done in order to have the opportunity to make the earliest possible diagnosis. Such methods are used to determine the levels of tumor markers: cancer screening; differential diagnosis of cancer and benign processes; assessment of the prevalence of the process (in combination with methods of radiation diagnostics); prognosis; evaluation of the effectiveness of therapy; monitoring of patients with the aim of early detection of relapses and disease generalization (Grunnet & Sorensen, 2012; Wu & Wu, 2002).

So far, these methods have been inferior to instrumental studies, since the cost of test systems is quite high, there is a proportion of false results, and it is also impossible to determine the stage and prevalence of the process by searching for tumor markers. In terms of treatment methods, one of the surgical methods of treating superficial transitional cell BC is its transurethral resection (TUR). There is reason to believe that in the case of studies of neoplastic angiogenesis, tumor transformation, and the emergence of resistance, more effective methods of treatment will be developed (Michal et al., 2000; Lähdevirta et al., 1988).

In order to determine the appropriate treatment strategy for BC, the European Organization for Research and Treatment of Cancer (EORTC) has developed a scoring system and risk tables for the prediction of recurrence and progression. The basis of this system is the clinic pathologic parameters of the tumor. Nevertheless, the division of tumors by morphological characteristics does not fully reflect the clinical potential; therefore, in recent years, much attention has been paid to the search for additional factors for predicting the disease state. Identification of these factors should lead to the development of a holistic prognostic system, the use of which in clinical practice would allow identifying tumors with different clinical course, to determine a high probability of progression, recurrence, and metastasis of BC. Such a system can include biochemical, immunohistochemical, and genetic markers. One of the most promising areas is the identification of molecular genetic changes in the genetic apparatus of a cell, which underlie its malignant transformation, and their use as clinical markers that determine the nature and prognosis of the disease (Dharmayuda et al., 2021; Kurtieva et al., 2021).

3 Results and Discussions

It is believed that a tumor infiltrated into the stromal with a "wide front" is less aggressive than the one with "tentacular" growth. It is also possible to isolate other forms of invasive tumor growth: micro papillary, microcytic, and nested (Yang et al., 2014). Adverse factors in the prognosis of invasive carcinomas include: multiplicity of lesions, tumor size more than 3 cm, the presence of background changes in the form of pervasive carcinoma, increases the recurrence risk. It is assumed that urothelial bladder cancer is characterized by infiltrating growth already at the stage of disease detection. In this case, the prognosis is especially unfavorable. Urothelial bladder cancer is fundamentally different from progressive superficial carcinomas in its molecular pathogen etic mechanisms of development (Sun et al., 2017).

One of the most significant prognosis factors after the disease state, according to most researchers, is the grade of tumor differentiation (G), which is directly proportional to the recurrence rate (Chan et al., 2020). Today, the identification of certain markers that allow judging the properties of a tumor are moving from the category of additional to the category of necessary ones, providing an opportunity to choose the most effective methods of treatment and predict the development of tumors (Unoki et al., 2009). All tumors are characterized by a change in the proliferative properties of their cells. As a result of dysfunction of suppressor genes, the proliferative activity of tumor cells changes, which increases with tumor progression. When studying the proliferative activity of tumors, antibodies to the proteins Ki-67 (Molecular Immunology Borstel (MIB1)) and PCNA (proliferating cell nuclear antigen (PC10)) are usually used.

Bladder cancer, as well as many other solid tumors, is characterized by intense angiogenesis associated with an increase in the content of vascular endothelial growth factor (VEGF). Assessment of tumor angiogenesis can also help to identify groups with an increased risk of metastases, requiring adjuvant treatment after surgery (Unoki et al., 2009). To select the optimal strategy for the treatment of transitional cell ladder cancer, in addition to its classification according to the TNM and G systems, a number of properties are also used to predict the development of the disease and the sensitivity of the tumor to therapeutic measures. However, in 20-30% of cases, the treatment outcomes do not coincide with the expected ones. It became obvious that the establishment of one or several markers does not provide the necessary basis for predicting the disease course and, accordingly, making a decision on further treatment of the patient (Shiryaev et al., 2020).

The primary task of diagnostics is to identify patients with a high risk of cancer progression who require the most radical approach, the use of a complex of surgical methods in combination with the appointment of chemotherapy and immunotherapy. Along with this, it is fundamentally important not only to determine, but also to comprehensively evaluate the signs of bladder cancer, revealed by morphological, molecular, and genetic analyses. It is also necessary to assess their relationship. According to many researchers, who believe that BC accounts for 70% of all tumors of the urinary tract and 4% of all oncological diseases (Yang et al., 2014; Widana et al., 2021). At the time of diagnosis, 70-85% of patients have superficial bladder cancer (SBC). This group includes tumors that are limited to the mucous and sub mucous layer (pTa, pTis, and pT1). The standard treatment strategy for SBC is transurethral resection (TUR) of the tumor, chemotherapy, and immunoprophylaxis. However, up to 85% of SBC recurs after treatment, with 10-30% developing into invasive and disseminated cancers.

Superficial forms of bladder cancer have a pronounced tendency to relapse. It has been shown that relapses of SBC develop with a high frequency even after 3 years of relapse-free course of the disease and may be characterized by a growing malignant and metastatic potential. In this regard, one of the key problems faced by a physician in the treatment of patients with SBC is an adequate assessment of the risk of recurrence in this patient. Many studies have shown the insufficient predictive value of individual clinic pathologic criteria recommended by the EORTC for the overall risk assessment system. It is believed that the depth of neoplasm invasion plays one of the key roles in determining the survivability of patients. However, despite the combination of I (non-invasive) and T1 (minimally invasive) carcinomas into one group of superficial bladder cancer, the difference between them is extremely important. In the group of I-tumors progression is considered a relatively rare event, while T1-tumours, even in the early stages, are potentially highly malignant.

However, histological diagnosis of minimal invasion is usually difficult. Often, micro invasive sites are not detected at the light-optical level, and this can explain the more aggressive behavior of a group of urothelial carcinomas classified as no infiltrating. The use of molecular genetic markers can help in a clearer division of tumors into Ta and T1 subgroups. No less acute is the problem of choosing treatment strategy in patients with a high risk of progression and recurrence (T1G3 group). This problem can also be solved by creating a system of additional prognostic factors, which would distinguish tumors with a high risk of recurrence and progression from less aggressive neoplasms and apply a differentiated treatment approach.

In addition to assessing the clinic pathologic parameters of the tumor, an important aspect of clinical practice is solving the problem of personifying the prognosis. Admittedly, during the transformation of a normal cell into a malignant one, a large number of various changes accumulate in its genetic apparatus, both spontaneously and induced by carcinogens. The combination of such changes leads to the fact that the malignant potential of a tumor in each individual patient can vary significantly. That is why the "behavior" of a tumor cannot be unambiguously predictable and described only by phenotypic manifestations such as size, type of growth, etc. Changes that occur in the cell nucleus during malignant transformation are the primary event in relation to the malignant properties that are acquired by it. Therefore, the identification of these lesions and the establishment of links between them and the clinical behavior of the tumor is a research priority for modern oncology. The need to introduce these developments into clinical practice for the purpose of a more thorough and personalized prognosis of the disease state is also obvious.

Today one of the most pressing challenges in clinical oncology is the early diagnosis of malignant neoplasms. The main line of research is the search for tumor markers that would allow diagnosing the onset of a tumor process with high reliability long before the appearance of clinical symptoms, especially in at-risk patients. An ideal tumor marker should meet the following criteria: be produced only by malignant cells; be organ-specific; appear in high concentrations in body fluids; its concentration should correlate with the size of the tumor, with the stage of the disease; with the prognosis and effectiveness of treatment. Indications for detecting the levels of tumor markers: cancer screening; differential diagnosis of cancer and benign processes; assessment of the prevalence of the process (in combination with radiation diagnostics); prognosis; evaluation of the effectiveness of therapy; monitoring of patients with the aim of early detection of relapses and disease generalization (Table 1).

Table 1
A combination of tumor markers associated with certain oncological diseases

Malignant neoplasms	Tumour markers		
	Main	Secondary	Additional
Gastric cancer	CA72-4 (Carbohydrate antigen 72-4), CEA (Carcinoembryonic antigen)	TK (Thymidine kinase) or TPA (Tissue polypeptide antigen)	
Colon cancer	CEA (Carcinoembryonic antigen), CA19-9 (Carbohydrate antigen 19-9)	TPA (Tissue polypeptide antigen)	TK (Thymidine kinase)
Pancreas cancer	CA19-9 (Carbohydrate antigen 19-9), CA50	CEA (Carcinoembryonic antigen)	AFP (Alpha-fetoprotein)
Gallbladder cancer	CA19-9 (Carbohydrate antigen 19-9)	AFP (Alpha-fetoprotein)	TK (Thymidine kinase) or TPA (Tissue polypeptide antigen)
Liver metastases	CA19-9 (Carbohydrate antigen 19-9), CEA (Carcinoembryonic antigen), AFP (Alpha-fetoprotein)	TK (Thymidine kinase)	
Small-cell lung cancer	NSE (Neuron-specific enolase)	TK (Thymidine kinase) or TPA (Tissue polypeptide antigen)	

Lung cancer	CYFRA 21-1 (Cytokeratin-19 fragment), SCCA (Squamous cell carcinoma antigen), CEA (Carcinoembryonic antigen)	TK (Thymidine kinase) or TPA (Tissue polypeptide antigen)	
Breast cancer	CA15-3, TPA (Tissue polypeptide antigen), TPCA	CEA (Carcinoembryonic antigen)	MCA (Mucin-like Carcinoma-associated Antigen)
Chorionepithelioma	β-HCG (Beta-human chorionic gonadotropin)	TK (Thymidine kinase)	
Papillary carcinoma	β-HCG (Beta-human chorionic gonadotropin)		
Teratoid tumour	AFP (Alpha-fetoprotein), β-HCG (Beta-human chorionic gonadotropin)		
Ovarian carcinoma	CA125 (Cancer antigen 125), CA72-4 (Carbohydrate antigen 72-4)		
Uterine corpus cancer	CA125 (Cancer antigen 125), CYFRA 21-1 (Cytokeratin-19 fragment)		
Cervical cancer	SCCA (Squamous cell carcinoma antigen), CYFRA 21-1 (Cytokeratin-19 fragment)		
Prostate cancer	PSA (Prostate-specific antigen), Free PSA (Free Prostate-specific antigen)	TK (Thymidine kinase)	
Testicular cancer	AFP (Alpha-fetoprotein), β-HCG (Beta-human chorionic gonadotropin)		
Bladder cancer	TPA (Tissue polypeptide antigen), CEA (Carcinoembryonic antigen), CYFRA 21-1 (Cytokeratin-19 fragment)	TK (Thymidine kinase)	
Neuroblastoma	NSE (Neuron-specific enolase)		
Malignant melanoma	NSE (Neuron-specific enolase), TK (Thymidine kinase)		
Phaeochromocytoma	NSE (Neuron-specific enolase)		
Carcinoid	NSE (Neuron-specific enolase)		
Leukaemia	B2M (Beta-2 microglobulin), TK (Thymidine kinase)		
Malignant lymphoma	B2M (Beta-2 microglobulin), TK (Thymidine kinase)		

In the early stages of the disease, with a small tumour mass, a significant concentration of waste products in the blood and other biological fluids of the body cannot be expected. False negative test results are possible. Treatment (surgery, chemotherapy, radiotherapy), which leads to complete removal of the tumour and devitalisation of tumour cells, must necessarily be accompanied by a decrease in the levels of tumour markers to normal. As a result, tumour markers in combination with clinical, radiation, endoscopic and other modern diagnostic methods help in solving urgent problems of clinical oncology. It is necessary in the future to search for new highly specific markers and effectively combine existing ones to increase the efficiency of malignant tumours diagnostics and monitoring of cancer patients.

The factors predisposing to the occurrence of bladder cancer are the contact with carcinogenic substances: b-naphthylamine, benzidine, toluidine, 2-methylalanine (used in the production of dyes); smoking, the use of

certain drugs, such as phenacetin, and schistosomiasis. The risk of developing BC is increased in patients who have had pelvic irradiation. Recently, other methods of laboratory diagnosis of BC have been used, in particular, the determination of BTA markers (bladder tumour antigen), NMP 22 (nuclear matrix protein), UBC antigen (urinary bladder cancer), urine telomerase, etc.

Superficial transitional bladder cancer is currently considered a mucosal disease. In this regard, only surgical removal of the tumour is not enough to treat the patient or obtain long-term remission. Among the surgical methods of treatment of superficial transitional cell bladder, the TUR is the procedure of choice. However, relapses of the disease within 5 years after TUR (transurethral resection) develop on average in 60-70% of patients. In addition, in some patients, a recurrent tumour becomes invasive or has a lower degree of differentiation (tumour progression). Progression in papillary carcinoma is noted in 1 in 10-20% of patients, and in highly malignant tumours of T1G3 and cancer in situ – in 30-50%.

The factors influencing recurrence, tumour progression, and treatment outcomes are: the number of tumours by the time of TUR, their size, the frequency of recurrence within 1 year after TUR, stage (CISTATI), grade of tumour cells differentiation (G). Depending on these parameters, groups are distinguished: low, high and intermediate risk of relapse. Recent molecular studies have established that patients with BC in "normal" urothelium have chromosomal aberrations, changes in the total DNA content, impaired p53 expression, and proliferation intensity, similar to those observed in tumour cells. That is, there is a general genetic instability of the urothelium, which can be considered as the cause of the multifocality of tumour growth in the entire transitional epithelium.

Tumour markers – a number of laboratory tests currently used to suspect bladder cancer based on the detection of a number of substances in the urine: a test for the presence of a specific antigen BTA (bladder tumour antigen) – the sensitivity (reliability) of the method is 67%, BTA TRAK test – the sensitivity of the method is 72 %, test for nuclear matrix protein (NMP-22) – sensitivity of the method is 53%, determination of haemoglobin chemiluminescence – sensitivity of the method is 67%. Most of these tests have been developed recently and have not yet found widespread use in clinical practice. The advantage of the BTA test is its simplicity, the possibility of performing it on an outpatient basis, as well as by the patient himself. Also noteworthy is the method for determining hyaluronic acid and hyaluronidase in urine, since the reliability of the method reaches 92.5%. Due to the high cost of test systems, the presence of a certain proportion of false results, the inability to diagnose the state of the process and determine the strategy of further treatment, these methods are inferior to clinical investigations.

More than 10 million patients with primary malignant neoplasms are diagnosed annually in the world. Despite the improvement of existing diagnostic methods and the introduction of new ones, in 60-80% of cases these diseases are detected at the stages III-IV, which negatively affects the prognosis. At present, a huge number of fundamental studies are focused on the search for molecular markers of predisposition to carcinomas for early diagnosis, on the one hand, and the identification of factors that allow predicting the development of the disease and optimising treatment methods.

Today, a lot of data has been accumulated confirming the influence of growth factors, in particular VEGF (vascular endothelial growth factor) and EGF (epidermal growth factor), which are responsible for mitogenic activity of cells, on the development and progression of malignant neoplasms. Therefore, they are promising targets when using targeted therapy. Researchers have revealed functionally significant polymorphism G+405C in VEGF and polymorphism A+61G in EGF (epidermal growth factor). EGF is one of the ligands of the epidermal growth factor receptor (EGFR), expressed on the surface of both normal and transformed epithelial cells and is a crucial transmembrane tyrosine kinase receptor in the regulation of cell growth and differentiation. The EGF/EGFR interaction is a functionally important autocrine pathway that promotes tumour development. Increased expression of EGF enhances tumour invasion and metastasis, triggering a whole cascade of intracellular processes that initiate cell proliferation and a number of other biological processes responsible for tumour progression: adhesion and invasion of transformed cells, activation of antiapoptotic response.

VEGF is a glycoprotein and is one of the most important stimulators of angiogenesis in a variety of healthy and cancer-affected tissues. VEGF performs its functions through tyrosine kinase receptors located in the membrane of endothelial cells. Angiogenesis is one of the main factors in the survival and spread of tumour cells. The family of vascular endothelial growth factors, VEGF, plays a key role in the development of a new vascular network during tumour growth. The appearance of new vessels contributes to the progression of the

disease, increasing the rate of tumour growth and its ability to metastasise. Assessment of tumour angiogenesis is important for predicting the course of the disease and prescribing chemotherapy in many malignant tumours.

Analysis of data on VEGF labelling (position G+405C) in patients with bladder cancer (lung cancer) did not find any differences in the frequency distribution of molecular markers GG/GC/CC of the VEGF gene between the group of patients (0.31:0.56:0.3) and control group (0.31:0.53:0.16). However, there were significant discrepancies in the distribution of molecular markers between the groups of patients with squamous cell lung cancer (LC) and adenocarcinoma. Among patients with adenocarcinoma, the GG marker was found 2 times more often than in patients with squamous cell lung cancer, and carriers of the CC marker were not identified in the group of patients with adenocarcinoma. It was also shown that the CC marker occurs 2 times more often in patients with lung cancer with the presence of metastases (N1-3) compared to patients with tumours without metastases (N0).

It was found that the frequency of detecting carriers of the CC marker in patients with malignant ovarian carcinoma was lower than in the control group (11.0%), and the frequency of detecting carriers of the GG marker in the group of patients was 1.5 times higher than in the control group. Moreover, in patients with stages III-IV of the disease, the GG marker was found 4 times more often than in patients with stages I-II of tumour development. Several studies showed the absence of an association of polymorphism in the +405 VEGF position with the occurrence of some types of cancer, including LC (lung cancer) and OC (ovarian cancer), but revealed its effect on the degree of tumour differentiation in patients with melanoma, prostate cancer, breast cancer, as well as survival in patients with RL and OC. The highest expression level of the VEGF protein was in peripheral blood mononuclear cells with the GG marker, the intermediate level – in the cells with the GC marker, and the lowest – in the cells with the CC marker. There is also evidence that an increased level of VEGF expression is more often observed in patients with high-grade tumours.

The progression of solid tumours largely depends on the degree of vascularisation and angiogenesis of malignant tissues. Of the whole spectrum of pro-angiogenic factors, the vascular endothelial growth factor (VEGF) is the most important. The suppression of VEGF functions leads to regression of neoplastic vessels and restriction of tumour growth. Clinical trials of complex antiangiogenic chemotherapy of various neoplastic formations have demonstrated promising results. Currently, the drug bevacizumab has entered wide clinical practice in the treatment of breast cancer, peri-rectal cancer, and grade III-IV gliomas. Unfortunately, in most cases anti-angiogenic therapy does not lead to a complete recovery, but only slows down the development of the tumour. Resistance mechanisms include potentiation of alternative proangiogenic signalling pathways and activation of an invasive tumour cell population.

Despite the fact that chemotherapy remains the most effective tool in the treatment of tumours, its main disadvantages are high nonspecific cytotoxicity, which limits the dose administered, and the development of MDR (Multidrug resistance) of tumour cells. The study suggests that tumour growth and metastatic spread may depend on the degree of development of microvessels in malignant tissue, and blocking angiogenesis will be an effective means of limiting the growth of solid tumours. This strategic approach has now become the mainstay in the treatment of several types of cancer. Of the many pro-angiogenic factors involved in physiological and pathological angiogenesis, the most important effector is vascular endothelial growth factor (VEGF). In a number of cases, it has been shown that increased VEGF expression correlates with a poor prognosis and a high likelihood of relapse. The advantage of this approach is that anti-VEGF therapy suppresses the activation of receptors involved in angiogenesis and, therefore, signal transduction in genetically stable endothelial cells. Clinical studies have shown encouraging results with combined anti-angiogenic and chemotherapy or radiotherapy; development of this direction may open up new prospects for increasing the effectiveness of tumour therapy. Currently, highly specific drugs aimed at selective suppression of angiogenesis are being developed and undergo clinical trials; of these, bevacizumab is the most widely used.

As is well known, blood vessels consist of three main types of cells: endothelial cells – cells lining the tubular structure of a blood vessel; smooth muscle cells that regulate intravascular pressure; pericytes – satellite cells that support the viability of endothelial cells. VEGF-C and D regulate the development of lymphatic vessels. The gene VEGF-A, which is also called VEGF, was first characterised as a factor of vascular permeability, but later its mitogenic activity in relation to endothelial cells was discovered. The gene of the VEGF-A molecule is located on the 6th chromosome; its nucleotide sequence has high homology with the

PDGF gene (Platelet-derived growth factor). VEGF-A is a potent mitogen for endothelial cells, but it does not induce proliferation of other cell types.

VEGF binds to the tyrosine kinase receptors VEGF-R1 (Flt1 (Fms related tyrosine kinase 1)) and VEGF-R2 (KDR (Kinase insert domain receptor)); a murine form known as Flk1 (Fetal liver kinase 1). Activation of these receptors triggers the phosphorylation of proteins involved in signal transduction. VEGF expression increases vascular permeability, which leads to an increase in interstitial and intratumoural pressure, facilitating the penetration of tumour cells into the vascular bed; in addition, VEGF disrupts the entry of therapeutic agents into tumour tissues. Chaotic arrangement of tumour vessels leads to uneven oxygen supply into the surrounding tissues, resulting in the emergence of local foci of hypoxia, resistant to radiation therapy.

Increased expression of VEGF by tumour cells can play an important role in the pathogenesis of malignant neoplasms, since blood supply is one of the dominating factors of tumour growth. VEGF triggers neoplastic angiogenesis, as a result, the density of microvessels increases, and the malignant tissue receives more nutrients. VEGF secretion by tumour cells leads to an escalation of the synthesis of pro-angiogenic factors; Since VEGF triggers angiogenesis, new vessels begin to supply the malignant tissue with oxygen and nutrients, the tumour grows and produces more VEGF. In addition, VEGF by the principle of positive feedback increases the expression level of the VEGF-R2 receptor by endothelial cells of tumour microvessels, which stimulates cell growth and proliferation of endothelial cells.

The sunitinib, a VEGF-R inhibitor, temporarily improves the penetration of temozolomide into the tumour. Antiangiogenic therapy with bevacizumab has become the standard approach in the treatment of colon, lung, and breast cancer. It was shown that bevacizumab suppresses the activation of VEGF-R receptors and stimulates apoptosis of tumour cells: the median decrease in the level of phosphorylated VEGF-R2 receptor in the tumour was 66.7% and the median increase in the level of apoptosis of tumour cells was 128.8%. The level of VEGF expression correlates with the parameters of tumour progression and vascular density in malignant neoplasms, and in addition, it allows predicting the tumour response to antiangiogenic and cytostatic therapy, but not to patient survival. Preclinical and clinical studies have shown that anti-VEGF drugs, in particular, bevacizumab, are quite effective in the treatment of malignant tumours of the brain, intestines, breast, and lung. Antiangiogenic therapy made possible to significantly increase the effectiveness of the treatment of malignant neoplasms, in particular, insular gliomas. Combination therapy with bevacizumab significantly increased the likelihood of response to treatment (as measured by magnetic resonance imaging) and the 6-month progression-free survival rate. In most cases, this approach has reduced the dose of corticosteroids and, therefore, reduced side effects on the patient's body. Similar results were obtained in studies of drugs that inhibit the functional activity of the VEGF-R receptor. Unfortunately, for a significant proportion of patients with insular gliomas, such therapy does not lead to remission and recovery. Nevertheless, the use of anti-VEGF drugs to suppress angiogenesis in tumour tissue has demonstrated promising clinical results and significant potential in the treatment of metastatic and primary tumours. Experimental data have shown that tumour resistance to antiangiogenic therapy inevitably develops. Probably, the resistance mechanisms include activation of alternative proangiogenic signalling cascades together with an increase in the expression of growth factors, as well as an increase in the contribution of perivascular invasive growth to tumour progression. Apparently, antiangiogenic therapy can stimulate glioma cells to migrate outside the tumour focus, activating invasive tumour growth; this cell population is able to grow in the absence of angiogenesis by absorbing existing microvessels.

There is reason to believe that the study of neoplastic angiogenesis, tumour transformation, and resistance will lead to the development of more effective treatments. The most promising strategy seems to be the complex anti-angiogenic therapy, including suppression of alternative pro-angiogenic pathways, together with anti-invasive drugs that prevent local invasion and metastatic spread. Vascular endothelial growth factor (VEGF) is the main regulator of microvessel growth. It promotes vasodilation, increases vascular permeability, influences the severity of the inflammatory response and has a number of extravascular effects. Oncological processes, diabetes mellitus, rheumatoid arthritis, endometriosis, hypertension, vascular atherosclerosis and a number of other pathological conditions are accompanied by an increase in the VEGF content in the blood and tissues.

Blockade of the VEGF/VEGF-R axis causes an increase in blood pressure, and the VEGF infusion – its decrease. Thus, the VEGF system is actively involved in the regulation of blood pressure. Theoretically, in patients with arterial hypertension, which is characterised by a decrease in the number of microvessels, the

VEGF concentration should be reduced. However, clinical and experimental studies revealed an increase in the VEGF content in those examined with high blood pressure. This contradiction may be associated with the complexity of the structure and functioning of the VEGF/VEGF-R system, includes various isoforms and variants of ligands and receptors that have both pro and antiangiogenic properties. It is possible that the increase in VEGF expression reflects the activation of compensatory-adaptive mechanisms in response to an increase in blood pressure. A prolonged decrease in the concentration or blockade of VEGF in adults leads to a deterioration in the survival of endothelial cells, dilution of microvessels (a decrease in the tissues of terminal arterioles and capillaries) and an increase in blood pressure. Tumour necrosis factor (TNF) or cachectin is capable of causing a cytotoxic effect on a tumour cell. The term TNF means its antitumor activity associated with haemorrhagic necrosis. It can cause haemorrhagic necrosis of some tumour cells, but does not damage healthy cells. It is formed by macrophages, eosinophils. Tumour necrosis factor alpha is not detected in the blood serum of healthy people; its values increase in the presence of infection and bacterial endotoxins. With inflammation of bone tissue and other inflammatory processes, TNF-α is determined in the urine, affects tumour cells due to apoptosis, generation of reactive oxygen and nitrogen species. It inhibits tumour cells and cells affected by the virus, takes part in the development of the immune system's response to the antigen, inhibits the development of lymphocytes, and causes a radioprotective effect. TNF can act as a hormone, entering the bloodstream, stimulates the development of phagocytes, increases blood coagulation, reduces appetite.

In tuberculosis and cancer, TNF is an important factor in the development of cachexia. TNF plays an important role in the pathogenesis of septic shock, rheumatoid arthritis, proliferation of the endometrium, necrotic brain lesions, multiple sclerosis, pancreatitis, nerve damage, liver damage by alcohol, diagnosis and prognosis during the treatment of hepatitis C. An increased level of TNF provokes chronic heart failure, exacerbation of bronchial asthma. The concentration of TNF increases with obesity, while it is clearly expressed in the adipocytes of the visceral adipose tissue. TNF reduces the activity of the insulin receptor tyrosine kinase, delays the action of intracellular glucose transporters in muscle and adipose tissues. An increase in TNF expression in the amniotic fluid signals a violation of intrauterine development of the foetus. The first stages of studying the TNF gave reason to think that it performs the function of providing antitumor protection in the body, but further studies have shown that it has a wide range of biological activity and is involved in physiological and pathological processes.

TNF, TNF-β – two similar proteins (approximately 30% of amino acid residues are homologous) – exhibit similar activity of the inflammatory response, immune and tumour processes. TNF was first detected in the serum of mice injected with bacterial products that induce tumour cell necrosis. TNF-β or lymphotoxin was detected in the lymph nodes of immunised rats. The source of TNF-β is an activated macrophage, TNF-β-activated T-cell. Through the same specific receptors on the cell surface, both factors cause lymphoma cell lysis, necrosis of sarcoma induced by methylcholanthrene, activate polymorphonuclear lymphocytes, and exhibit antiviral activity. TNF synthesis is not observed in unstimulated cells and is not detected in the blood serum of healthy animals and humans. Activation of macrophages by lipopolysaccharides (LPS) or other stimulants leads to the induction of TNF synthesis in 15-20 minutes. Dexamethasone pre-treatment of macrophages prevents LPS-induced TNF synthesis at the level of inhibition of mRNA translation (Matrix ribonucleic acid).

TNF synthesis is a necessary autocrine factor for the growth and differentiation of macrophages themselves. The regulating role of TNF on the proliferation of macrophages during their differentiation has been revealed. In the early stages of differentiation, mouse bone marrow macrophages synthesise endogenous TNF. To date, there is no complete and unambiguous picture of the mechanism of TNF antitumor action in vivo. Several ways by which TNF destroys the tumour or stops its growth are considered (Figure 1):

a) The first of them is the direct effect of the TNF protein on the target tumour cell triggered inside the cell, the end result of which is cell apoptosis (cytotoxic action) or cell cycle arrest (cytostatic action). In the latter event, the cell also becomes more differentiated and expresses a number of antigens.
b) The second – a cascade of chemical reactions that includes the activation of the system of blood coagulation and local inflammatory reactions caused by TNF-activated endothelial cells and lymphocytes, which leads to the so-called "haemorrhagic" necrosis of tumours.

c) The third way – blocking angiogenesis, leading to a decrease in the germination of a rapidly growing tumour by new vessels and, as a consequence, to a decrease in blood supply up to necrosis of the tumour centre.
d) And, finally, the fourth path, which predetermines the destruction of the tumour, is the effect of cells of the immune system, the cytotoxicity of which was closely related to the presence of TNF molecules on their surface or the process of maturation/activation of these cells is associated with the response to TNF.

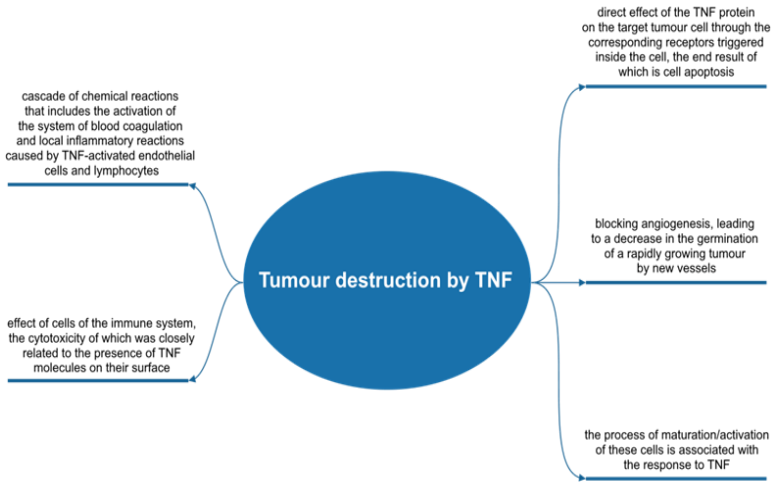

Figure 1. Tumour destruction by TNF

TNF is not only a powerful apoptosis inducer; it also affects the transition of cells from the cell cycle phase. In general, the fate of a cell depends on many factors that are mostly unknown. Even before the 1990s century, very few people knew about programmed cell death (apoptosis). Necrosis was well-known, and now the choice of five types of cell death is at question. The activity of TNF-α, IFN-γ (gamma-interferon) and TNF-β at concentrations of 100-10000 U/mL on 10 human lines of bladder tumour cells was studied. IFN-α was active against five out of ten lines, while IFN-γ was active against one line, and TNF against five out of ten lines. The maximum synergistic effects were obtained from the combination of IFN-α and TNF in nine out of ten cell lines. It is concluded that IFN-α and TNF are active as separate agents and are even more active when used together in vitro in human bladder tumour cell lines.

It has been shown that TNF is one of the main participants in transplant rejection reactions. Today TNF-α is believed to play a key role in the pathogenesis of many chronic inflammation and rheumatic diseases, in particular Crohn's disease, rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis. However, it should be borne in mind that TNF plays an important role in the body's immune defence against infections and in controlling tumour growth. Therefore, therapy with antibodies against TNF can increase the risk of developing serious infections and malignant neoplasms. A meta-analysis of data from clinical trials of two drugs of licensed anti-TNF antibodies, which were used to treat patients with rheumatoid arthritis, was performed to assess their effect on increasing the risk of developing serious infections and malignant neoplasms.

The use of TNF as a drug is limited due to toxic side effects. Thus, TNF causes the release of free fatty acids and suppression of lipoprotein lipase in the culture of adipocytes with a specific activity of 107 U/mg, suppresses glycerol-phosphate dehydrogenase at the level of transcription, a protein that binds fatty acids, suppresses fatty acid synthetase and acetyl-CoA carboxylase. All this leads to an increase in blood triglyceride levels and cachexia. TNF is a mediator of endotoxic shock in gram-negative infections and, in toxic doses, causes fever, weight loss, hypotension, and metabolic acidosis. However, there is a different sensitivity of

humans and animals to the toxic effects of TNF. Perhaps humans are the most sensitive. TNF is not detected in the blood serum of healthy people. The presence of TNF in the serum of patients with meningococcal infection of 10 U/mL is accompanied by shock; TNF concentration of more than 440 U/mL leads to death.

A clinical study was carried out in 21 patients with locally advanced or metastatic solid tumours for whom no other standard therapy was available. They were injected with TNF in the morning as a 20-minute intravenous infusion at a dose of 75 μg/day for 5 consecutive days (one cycle). In the absence of progression, the next two cycles were done at 14-day intervals, while the dose was increased in the 2nd cycle – up to 100 μg/day, in the 3rd cycle – up to 150 μg/day. Among these patients, assessed for clinical and immunological responses to treatment, 4 patients gave complete and 4 partial responses. It was shown that TNF treatment is not associated with an increase in the number of circulating lymphocytes. The CD4/CD8 ratio in patients treated with TNF showed significantly lower values than before treatment.

The tolerance and activity of local, TNF and IFN-α2b administration in locally advanced, hormone-resistant prostate cancer (LA-HRPC) have been investigated. 10 patients with LA-HRPC (Locally advanced, hormone-resistant prostate cancer) (T4N0M0, n=3; T4N0M1, n=5; T4N1M1, n=2) were treated with recombinant TNF, injected locally into the prostate tumour at 4-week intervals (maximum four cycles) combined with subcutaneous injections of 5.106 IU (International Units) IFN-α2b. Despite a significant intake of TNF into the systemic circulation 2 hours after intraprostatic administration (up to 416 pg/ml; p=0.0034), TNF (and IFN-α2b) were well tolerated by patients (1-2 degree of toxicity according to WHO classification). TNF caused prostate tumour cell necrosis in all patients, resulting in a significant decrease in prostate volume in 9 out of 10 cases. Significant short-term (more than 4 hours) increase in serum levels of prostate-specific antigen (PSA) (on average by 65%; $p<0.001$) and polypeptide tissue-specific antigen (TPS) (on average by 85%; p=0.001) and IL-8 (on average by 2687%; $p<0.009$) after TNF showed a cytotoxic effect in vivo. In the long term, serum PSA levels decreased by 18-87%, reaching the lowest baseline level after 7 weeks.

Intra-prostatic administration of TNF to patients is possible with moderate toxicity with LA-HRPC and thus may be a new treatment of choice for these patients. Studies have shown that the atypical variant of the TNF allele (308A) causes increased gene transcription compared to the typical variant of the TNF allele (308G), which leads to an increase in TNF secretion by macrophages in vitro and an increase in the concentration of TNF in blood serum in vivo. It has been proven that the atypical variant of the TNF allele (308A) is associated with the adverse consequences of various infectious and inflammatory diseases, in particular, the generalised form of meningococcal infection, cerebral malaria. Therefore, the atypical variant of the TNF promoter part (308a) can be considered as a separate risk factor for the development of generalised forms of purulent-septic diseases of various aetiologies. The atypical variant of TNF (308A/G) is a precursor to generalised forms of purulent-septic conditions in children. Carriers of this variant of the TNF gene, when a localised bacterial infection appears, should immediately be prescribed powerful antibiotic therapy.

4 Conclusion

Studies by many authors have shown that IL-6 (Interleukin-6), IL-10 (Interleukin-10), TNF and its receptors TNF-R1 and TNF-R2, depending on the level of regulating spermatogenesis. In particular, it was found that TNF and its receptors regulate spermatogenesis (normal proliferation), prevent delayed sperm development and the development of testicular tumours. Studies by many authors have shown that IL-6, IL-10, TNF, depending on the level, and their receptors TNF-R1 and TNF-R2 regulate spermatogenesis and its disorders. In addition to these groups of cytokines in the follicular fluid, cytokines have been identified that are involved in the induction of angiogenesis (vascular endothelial growth factor – VEGF). For women with malignant neoplasms, the pro-inflammatory factors of immunity are significantly pronounced. Thus, women with cancer of the cervix and uterine corpus in the blood, have increased levels of IL-1α, IL-6, IFN-γ, TNF-α and IL-1RA (Interleukin-1 receptor antagonist). With dysplasia of the cervical epithelium, an increase in the serum IL-1α content and a decrease in the levels of antibodies to IFN-γ and TNF-α are noted.

Several authors suggest that bladder cancer (BC) is one of the most common tumours affecting the urinary tract. Bladder cancer accounts for 6-8% and 2-3% of all malignant tumours in men and women, respectively. In 75% of cases, BC is diagnosed at an early stage, for which the standard treatment is transurethral resection (TUR) of the bladder followed by adjuvant chemotherapy or immunotherapy. Frequent relapses and the

associated progression, which are observed in superficial bladder cancer in 60-70% and 15%, respectively, require lifelong observation and measures aimed at preventing relapse. The study of the content of TNF-α, IFN-γ and IL-1 in the blood serum and urine of patients was carried out before instillation of A-bacterin, 24 and 48 hours after it. It was found that the differences in the content of IFN-γ (5.0±1.5 and 5.3±1.2 pg/ml) and IL-1 (21.0±2.7 pg/ml and 24.9±6.0 pg/ml), respectively, in the blood and urine of the examined patients were insignificant ($p>0.05$). The level of TNF-α in urine (19.1±3.1 pg/ml) significantly exceeded its content in blood (17.6±6.0 pg/ml), although the latter indicator differed considerably.

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