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TITLE

INFLAMMATORY MEDIATORS AS BIOMARKERS FOR TESTICULAR CANCER DIAGNOSIS AND FOLLOW-UP

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SUMMARY

Germ cell testicular cancer (GTC) represents 95% of testicular tumors and can be divided in three main categories, which include prepuberal, adolescent-young adult and spermatocytic seminoma (of the elder males). It derives from germ cells at different developmental stages and represents 1% of all the male tumors but it is the most common type of cancer in males between 15 and 34. The incidence is raising all over the world, while more efficient treatments have brought a reduction in mortality. Two types of GTC can be identified: i) Seminomas and ii) Non-seminomas. The classical clinical presentation is a painless lump in the testicle. After identifying testicular mass with ul-trasound, it is necessary to perform radical orchiectomy to have definitive diagnosis. Chest-abdomen-pelvis TC and determination of tumoral serum markers are needed to stage the disease. The markers usually used are beta-HCG, alpha-fetoprotein (AFP) and LDH. Due to low sensitivity and specificity of the currently used markers, it is mandatory to discover novel mol-ecules to be used for both diagnosis and follow-up. Therefore, we have considered molecules involved in inflammation, as cytokines and growth factors. Indeed, cancer is a disease largely accompanied by disfunction of the cytokines network and GTCs are largely infiltrated by immune cells. Moreover, the testicular tumor microenvironment is significantly influenced by high levels of proinflammatory and anti-inflammatory cytokines. The clinical course of a cancer is affected by the interaction of tumor cells with patient's immune system. It is conceivable that immunological parameters may be used as markers of prognostic, diagnostic or predictive value. To sum up, elevated levels of several cytokines and growth factors have been found in patients with cancer, either in the serum or in the tumoral microenvironment and may be of substantial help for GTC characterization.

Classsical markers of testicular cancer

The earliest markers used for diagnosis, staging, prognosis and follow-up of germ cell (GC) cancers are Alphafetoprotein (AFP), beta-human choriogonadotropin (HCG), lactic dehydrogenase (LDH) and placental-like alkaline phosphatase (PLAP); they were introduced into clinical practice the 1970_s and became international standard tools with the worldwide implementation of the immunologically based ELISA measurement technique (1). Nowadays AFP and HCG are the main tumor markers used in diagnosis, staging, prognosis and follow-up of GC tumors (2).

Even though testicular tumors account for only 1% of malignant neoplasms in males, they are an example of malignant tumors that can be cured by combining surgery, chemotherapy, and, if needed, radiation therapy. Clinical admission, in daily urology practice, is usually due to painless testicular mass, while serum tumor markers (HCG, AFP and LDH) and scrotal ultrasonography are used for definitive diagnosis (3).

Histopathologic classification of testicular tumors is primarily based on the histogenesis of these tumors, that can be classified into three categories, which have distinct features: Type I includes teratomas and yolk sac tumors of neonates and infants, which do not develop from a preexistent intratubular germ cell neoplasia in situ (GCNIS). Type II develops from preexistent GCNIS of the testis and include seminomas and mixed nonseminomatous (embryonal carcinoma, teratoma, yolk sac tumor and choriocarcinoma) germ cell tumors (also known as NSGCT). Type III category includes only spermatocytic tumors (previously known as spermatocytic seminoma), which develop without a preinvasive GCNIS in older adults. Over 90% of all invasive GCT develop from GCNIS and to type II neoplasms.

Tumor markers used in clinical and pathologic practice can be classified as:

a) serologic markers secreted by or released from the tumor cells and readily detectable in serum;

b) cell-related markers detectable by immunohistochemistry or molecular biologic techniques applied to tumor tissue that was obtained by biopsy or surgical intervention.

From the historical point of view, testicular tumors played an important role in defining tumors markers, such as HCG, AFP, PLAP and LDH. Serologic tumor markers AFP, HCG and

LDH are currently routinely used in testicular tumors clinical work-up, are included as part of the staging protocol and are also used for formulating the prognosis after treatment.

HCG is produced by placental syncytiotrophoblasts during pregnancy. As a tumor marker, it is a reliable marker for choriocarcinoma originating from the placenta, as well as ovarian and testicular choriocarcinomas. Patients with mixed germ cell tumors containing choriocarcinoma elements are also positive for HCG.

HCG consists of two chains labeled alfa and beta; the alpha subunit has a molecular weight of approximately 14.5 Kd and it is identical to the alpha subunit of three pituitary trophic hormones: two gonadotropins (LH and FSH) and thyroid-stimulating hormone (TSH). The beta subunit has a molecular weight of approximately 22.2 kD and is found only in HCG. It is encoded by the HCGB genes that form a cluster on the long arm of chromosome 19. HCG detected in serum can be further subclassified depending on the analytical technique used, however, most laboratories report either regular intact HCG or beta-HCG.

Beta-HCG is measured routinely in the serum of testicular cancer patients. Elevated concentrations of this marker are found in approximately 50% of patients with NSGCT; the serum levels of beta-HCG vary depending on the tumor stage and the extent of choriocarcinoma elements in each tumor. Pure choriocarcinomas are always positive.

Scattered syncytiotrofoblastic multinucleated giant cells can be seen in approximately 20% of seminomas and in these cases, patients have elevated levels of HCG in the serum, albeit in concentrations lower than those with NSGCT. The half-life of this marker in serum is 2-3 days and the normalization of serum HCG happens in 10-15 days after removing tumor. However, such a normalization does not exclude metastases composed of embryonal carcinoma cells, which don’t produce HCG. Recurrence of NSGCT is associated with rising HCG levels in serum in approximately 25% of cases (4).

Alphafetoprotein is a 70 kD protein found in fetal serum. In the early stages of intrauterine life, it is produced by the yolk sac and, after that by the fetal liver and parts of the gastrointestinal tract; the postnatal liver continues to secrete AFP, and therefore the serum levels of AFP remain elevated during infancy and even in early childhood. The AFP gene is

located on the long arm of chromosome 4. This molecule is a widely used tumor marker, most useful in diagnosing hepatocellular carcinoma, NSGCT of the testis, yolk sac tumor of infancy and childhood, extragonadal germ cell tumors and mixed germ cell tumors of the ovary. AFP is a reliable marker for yolk sac components of germ cells tumors in testicular and ovarian tumors. Overall, it is elevated in the serum of 25% of all patients with testicular germ cell tumors; in the subgroup of patients with NSGCT, serum AFP levels are elevated in 60% of cases.

The half-life of AFP is approximately 5-7 days, and therefore, one could expect the serum AFP to normalize 25-35 days (five half-lives) after orchiectomy for NSGCT. False positive results could result from liver injury related to chemotherapy or even some non-neoplastic liver disease such as steatohepatitis or chronic viral hepatitis and cirrhosis. In patients receiving chemotherapy, tumor lysis syndrome may include elevated serum AFP levels, even though there are no viable tumor cells in the treated patient's body. Three isoforms of AFP are known to appear in blood: L1 found in the serum of patients with non-neoplastic liver disease, L2 found in patients with yolk sac tumors and L3 in patients with hepatocellular carcinoma. In routine clinical practice, these subtypes are of limited significance (5).

Lactate Dehydrogenase (LDH)

LDH is a ubiquitous enzyme present in nearly all human tissues, from which it is released into the serum. Structurally it is a tetramer composed of four subunits, the most common being LDH-M and LDH-H protein, encoded by two LDH genes, called LDHA and LDHB, respectively. Serum LDH and its main isoenzymes are routinely measured in clinical laboratories. LDH is found inside the cell of most human tumors and it is often detected in cancer patients.

In GCT, LDH with a cutoff of 2,5 upper normal limits was identified as an adverse prognostic factor in patients with good prognosis seminoma, which may suggest an intensification of treatment in this group; advanced age and lung metastasis are included in the new IGCCG update model as additional adverse prognostic factors (6). Owing to its wide distribution in normal and neoplastic tissues, it cannot be used as a diagnostic tumor marker. Nevertheless, serum LDH values can be used as a valuable laboratory finding, reflecting

tumor burden, invasiveness of neoplastic cells, and the extent of tumor spread and metastasis. In patients receiving chemotherapy, serum LDH can be used to monitor the residual total tumor mass and spontaneous and chemotherapy-induced tumor necrosis.

American Joint Committee on Cancer (AJCC) staging system for testicular tumors requires from clinicians to record the serum levels of LDH at the time of diagnosis and periodically monitored them after surgical intervention and chemotherapy. In patients diagnosed with small tumors limited to the testis, serum LDH is within normal limits in 80-90% of cases. As the tumors grow, serum levels of LDH will rise proportionally to the size of the tumor. It is less specific marker reflecting growth rate and tumor volume, its level can be elevated in 80% of advanced seminomas and 60% of non-seminomatous tumors. In clinical stage I TGCT, fewer patients present with elevated serum tumor markers. Patients whose serum tumor markers normalize after orchiectomy are clinically disease free and surveillance or adjuvant treatment can be offered depending on the risk factors for occult metastatic disease. Patients with clinical stage I whose serum tumor markers remains elevated after orchiectomy are categorized as clinical stage IS and these patients should be accepted to have systemic disease and treated with chemotherapy. Most TGCT metastasize via lymphatic route to retroperitoneal lymph node, lung, liver, bone and brain are other sites of metastasis (7).

Patients with metastatic tumors have almost all high LDH serum levels. Likewise, tumor lysis is accompanied by markedly increased levels of serum LDH. Recurrence of tumors is also typically associated with high serum LDH levels (5).

Another molecule used especially in the past for diagnosis, staging and follow-up of GTC was placental alkaline phosphatase (**PLAP**); it is also known as Regan isoenzyme, is one of the four alkaline phosphatases (AP) typically found in the human tissues from which they are released into the circulation. Functionally, they are all hydrolases that have their optimal enzymatic activity in alkaline milieu. Each of these alkaline phosphatases is derived from a different organ and encoded by its gene. In addition to PLAP, other three forms are known as hepatic, bone and kidney AP, intestinal AP and placental like AP. PLAP gene is usually expressed in trophoblastic cells and fetal primordial germ cells. It is not expressed in normal spermatogenic cells of the adult testis (5).

PLAP is encoded by ALPP gene at chromosome 2q37.1. PLAP is a dimer of 65Kd consisting of 535 aminoacids and is thought to play a role in guiding migratory cells and transport specific molecules over the plasma membrane. PLAP is expressed from the ninth week of gestation and its concentration increases continually throughout pregnancy. PLAP can be separated into three distinct isoenzymes corresponding to early, mid and term placenta. In normal human tissues, the expression has also been reported for uterine cervix, fallopian tube, and -to a lower level- the lung.

PLAP expression also occurs in tumors and this is particularly known for testicular germ cell tumors; PLAP expression, often at high levels, has been described to occur in up to 100% of testicular germ cell neoplasia in situ, up to 100% of seminoma, up to 100% of embryonal carcinoma, up to 87% of yolk sac tumors and up to 100% of choriocarcinomas. PLAP expression can also occur in non-germinal cell tumors as adenocarcinoma of lung, urothelial cancer, colorectal adenocarcinoma, as well as mucoepidermoid carcinoma of salivary glands. Germ cell tumors show the highest PLAP expression prevalence than the other types of tumour (2).

The classical tumor markers are used to assist timely diagnosis of GTC, to accurately stage the disease, to assess the prognostic category of metastasized GTC_S, to monitor treatment success and finally to detect relapse during follow-up.

The elevation of each of the marker AFP, betaHCG and LDH in the entire GCT population are clearly less than 50%, and even the elevation of any of the three markers is encountered in less than 60% of patients. The marker elevation rate is significantly associated with histological subgroups, clinical stages, local pathological (pT) stages, with size of the primary tumour and younger age.

The well-known association of marker levels with response to treatment was confirmed, but notably, LDH remained elevated despite cure in more than 30% of patients. At relapse, nearly one-half of the patients had elevated serum markers, however, the pattern of markers changed in almost half of these patients.

Because of the histologic variability of GCT_S, not all the patients do actually have measurable serum levels of these markers. Overall, patients with seminoma have significantly lower elevation rates of all tumour markers than patients with

nonseminoma. Accordingly, the median serum level of betaHCG was significantly lower in seminoma than in nonseminoma because many of the seminoma patients have only slightly elevated serum levels of this marker. While the proportion of patients having elevated LDH levels is significantly higher in nonseminoma (38,7%) than in seminoma (29,1%), the median measured LDH serum levels of the two subgroup are not different from each other. About 30% of seminoma patients have elevated levels of betaHCG or LDH.

Regarding pT-stages, elevation rates of betaHCG and LDH are significantly higher in GCT patients with advanced pT-stages (>pT1) than in those with organ confined tumours (pT1), but notably, the rates of AFP are not (1). Only 50% of all GCTs express one of the three markers: seminoma does not express AFP and teratoma, the most differential subtype, lacks all markers expression and an informative biomarker of this subtype is still missing (8).

There is an unmet need for biomarker development in the following areas of clinical practice:

1)Improving diagnostic performance at disease outset.

2)Using biomarkers to aid in identifying which stage I patients will relapse and should be offered adjuvant chemotherapy, sparing those who do not require it.

3)Improving early detection of relapse, particularly in those patients who are non-secretors of the traditional TGCT markers; this may allow a reduction in the radiation burden on those young patients and reduce imaging costs.

4)Identifying patients with residual active disease in post-chemotherapy masses.

Recent data suggest a promising role for circulating microRNAs (miRNAs) in addressing such questions; miRNAs are non-protein coding RNAs that regulate the expression of protein-coding genes (2).

Both blood cell counts and neutrophil to lymphocyte ratio (NLR) are statistically significantly higher in patients with testicular cancer, compared with control group, therefore, these indexes can be used as a simple test in the diagnosis of testicular cancer besides the classical biomarkers (9). NLR of 4 was associated with an adverse overall survival and, in the preorchiectomy setting, a high NLR is associated with advanced cancer stage and poor prognosis. A significant decrease in NLR after

orchiectomy, especially a value of less than2, indicated localized disease; on the contrary, the absence of a significant reduction after orchiectomy, in particular, an NLR value greater than 2, indicated non-localized disease. Furthermore, the NLR value was significantly higher in patients with intermediate and poor prognosis than in patients with good prognosis (8). NLR is easily measurable laboratory marker used to appraise systemic inflammation.

These markers are maybe related with many circumstances such as thyroid function abnormalities, renal and/or hepatic dysfunction, diabetes mellitus, hypertension, chronic obstructive respiratory disease, metabolic syndrome, malignancy, B12 and folic acid deficiency, inflammatory diseases (8)

The chronic inflammatory process and the high burden of disease cause a great presence of inflammatory markers in advanced tumors. Inflammation-based markers can easily be detected from routine blood tests and, for this reason, might be useful prognosticators. Single markers such as leukocytes, platelets, hemoglobin and non-circulating blood cell markers (albumin, PCR) have been shown to have prognostic information.

A retrospective study of 539 testicular survivals showed that patients with CRP≥ 1,5 mg/L had a larger risk of developing a non-germ cell second tumor and a higher risk of cardiovascular disease than survivors with CRP<1,5 mg/L; this suggest that CRP may serve as a potential marker of cardiovascular events in long-survival testicular cancer.

Systemic inflammation could influence response to treatment. The neutrophil count is often increased and it is known it plays a role in cancer progression: the absolute count of neutrophils >8000/μL was associated with higher percentages of progression and exitus (2).

Beyond NLR and PCR, derived neutrophil-to-lymphocyte ratio (dNLR), platelet-to-lymphocyte (PLR), lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII) and albumin-to-globulin ratio (AGR) theoretically provide a more reliable, objective and robust indication of the host immune response, oncologic status and outcome, indeed, all these indexes were significantly altered in patients with higher tumor stages. All the evaluated pre-orchiectomy inflammation markers demonstrated value in predicting metastatic disease (10).

Thrombocytosis has been found to be related with significantly shorter survival in many cancers and it is seen in 25% of patients with testicular GCT and it is found to be associated with poorer chemotherapy response in metastatic patients. Thrombocytosis can be used to predict the response to chemotherapy. It is defined as a platelet count greater than $400^{\times}10^{9}$ platelets/L. Advanced germ cell testicular cancer can be cured with conventional cytotoxic cisplatin-based chemotherapy in around 80% of cases, but there is a minority of patients that are either resistant at first presentation or relapse after an initial response to treatment. Patients with thrombocytosis were found to have worse chemotherapy response compared to patients without thrombocytosis and thrombocytosis was shown to be an independent prognostic factor for chemotherapy response in multivariate analysis. Definitely, this index can be used to predict the response to chemotherapy in this patient population (11).

Another potential biomarker is VEGF, marker of angiogenesis in the development of blood vessels in tumors; VEGF is considered to be involved in tumor development and metastasis in GCTs (12).

Cytokines and testicular cancer

Cancer is a disease largely accompanied by a disfunction of the cytokines network: the cytokines ability to affect self-renewal cellular capacity, migration, senescence or apoptosis is often impaired in cancer because there is an aberrant activation of the immune/inflammatory response (13).

The cytokines exert their functions through the binding to membrane receptors; they exert anti-tumoral effects through the direct suppression of the growth and survival of the cancer cells (block of the cellular cycle, apoptosis stimulation, inhibition of the cellular motility), modifying the behaviour of the stromal non-immune cells and stimulating the immune response against malignant cells; on the other hand, some cytokines and chemokines promote the recruitment of immunosuppressive cells in the tumoral environment (14) and may also act directly on cancer cells regulating invasiveness and progression (15, 16).

They can be divided in several categories: TH1/proinflammatory (IFN-gamma, IL-12, IL-1beta, TNF-alfa and IL-17) and TH2/anti-inflammatory (IL-4, IL-5, IL-6, IL-10 and IL-13),

chemokines (IL-8, CCL5 and MCP-1 and growth factors (IL-7, G-CSF, GM-CSF, VEGF, EGF, IGF-1 and basic FGF) (17).

Cytokines, receptors for growth factors and components of downstream signal pathways are frequently over-expressed in cancer (13).

The inflammatory answer, that involves proinflammatory cytokines, chemokines, adhesion molecules and enzymes, plays an important role in the initiation, promotion, progression and tumoral metastasis. (13)

The macrophages are an essential component of the inflammation promoting and are related to cancer. The tumor-associated fibroblasts (TAFs) are the main cellular component related to cancer growth; these cells may trans-differentiate from the mesenchymal stem cells. Other relevant cells are the endothelial cells and the dendritic cells. The dysfunction of the stromal cells brings an increased production of cytokines and induces the development of mesenchymal stem cells. (18)

Inflammation and cancer are connected by two pathways: an extrinsic pathway resulting from conditions that cause smoldering inflammatory response, not resolved; and an intrinsic pathway driven by oncogenes and tumor-suppressor genes that activate the expression of transcription programs of proteins related to inflammation. (19) Both the pathways lead to the activation of many transcription factors like NF-kb and STAT3 in the cancer cells with consequent expression of many cytokines, chemokines and prostaglandins. (19)

Different types of immune cells, included the macrophages associated to the tumor (TAMS), dendritic cells (DCS), T and B cells have been found in tumoral tissues, indicating that the inflammation associated to cancer involves either innate immunity or acquired immunity; the immune cells are source of growth factors, cytokines and proteases that, after the secretion, act on multiple cellular types and matrix components in paracrine manner; neutrophil granulocytes are also present, with either antitumoral or pro-tumoral function and they are present on the edges of the cancer and in the not necrotic areas. (20)

Various studies report a significant correlation between high levels of TAMS and cancer aggressivity; these cells, for example, produce growth factors like EGF, VEGF that promote the tumor proliferation and metalloproteinases that promote cellular motility. (21)

In tumors, the term ‘reactive stroma’ has been used to describe the existence of a tumoral microenvironment modified, characterized by inflammation accompanied by tissue and matrix remodelling; various types of immune cells either about innate immunity or acquired immunity have been found in the reactive stroma together with non-tumoral cells like TAFs that express large quantities of matrix proteins, and endothelial cells. (22)

The cytokines have key effects on cancerogenesis: on one side, they can be involved in the activation of effector immune mechanisms that limit the cancer growth, on the other side, they can be involved in promotion of malignant transformation, tumoral growth, invasion and metastasis.

They are mainly produced by stroma and immune cells in response to molecules secreted by cancer cells or as part of inflammation that frequently accompanies the tumor growth; the cancer cells themselves produce cytokines in the same environment. (23)

At least the 20% of all the types of cancer arise in areas of infection and chronic inflammation, like part of a normal host response; the DNA damage caused by chronic inflammation is probably the mechanism through which the infections may cause cancer. Also, those types of cancer that don’t arise like consequence of a chronic inflammatory state, exhibit an extended inflammatory infiltrate, with a distinct expression of cytokines in the tumoral environment. (24, 25) Indeed, the tumoral microenvironment contains macrophages, neutrophils, mastcells, suppressive cells derived from myeloid lineage, dendritic cells, NK cells and B and T lymphocytes. (26)

Elevated levels of many cytokines have been found in patients with cancer, either in the serum or in the tumoral microenvironment. (27)

An association between a high concentration of IL-6 and poor prognosis has been detected in 16 different types of cancer: diffuse large B-cell lymphoma, renal cell cancer, pancreatic cancer, colorectal cancer, gastric cancer, neuroblastoma, metastatic malignant melanoma, non-Hodgkin’s lymphoma, nasal-pharyngeal carcinoma, lung cancer, metastatic breast cancer, bladder cancer, advanced gastric cancer, prostate cancer, squamous cell carcinoma of the neck, esophageal squamous cell carcinoma. It is one of the cytokines pro-tumorigenic best characterized. (28)

The serum concentrations of IL-10 have been associated with poor prognosis in 10 types of cancer: diffuse large B-cell lymphoma, colorectal cancer, gastric cancer, Hodgkin’s disease,

pancreatic cancer, bone sarcoma, hepatocellular carcinoma, metastatic melanoma, renal cell carcinoma, non-small cell lung cancer. (29)

Beside their value as biomarkers to support , and in the monitoring of disease activity, cytokines can be considered potential therapeutic targets for the treatment of cancer. (30)

List and features of the main cytokines

IL-1

The main source are the macrophages, neutrophils, endothelial cells, B cells and dendritic cells, although cancer cells can contribute to increase its levels; target cells are B cells, NK cells and T cells. It has pyrogenic and proinflammatory activity and stimulates proliferation and differentiation of the bone marrow cells. (31) It also promotes the tumoral growth, angiogenesis and the metastasis formation in various types of cancer like breast cancer, melanoma, non-small cell lung cancer and colon adenocarcinoma. (32)

TGF-beta (transforming growth factor-beta)

TGF-beta is expressed at high levels in many malignant tumors, where it is related to poor prognosis. (33) It has different effects depending on the stage of the tumor: it has an anti-cancer role in the early stages and a role of promotion of progression, invasion and metastasis in the late stages, through an immunosuppressive activity inhibiting the anti-tumoral properties of the T cells. More recently its role has been identified in the drug resistance. The main sources are the macrophages, monocytes and T cells and the target cells are the macrophages in which it induces the production of other cytokines. TGF-beta 1 is a potent inducer of the epithelial-mesenchymal transition (EMT), which has a bonding role between cancer and inflammation and can contribute to the generation of myofibroblasts and fibrosis. In general, the inflammatory cytokines induce metalloproteases activity in the TME (tumor microenvironment).

IL-6

It is one of the soluble factors more abundant in the TME. The main sources are TH lymphocytes, macrophages and fibroblasts and the target cells are activated B lymphocytes and plasma cells. (34) There is a strong association between its serum con-centration and the entity of the tumoral mass, the tumoral stage and the disease progression. It promotes tumorigenesis regulating the metabolism of the cancer cell, increasing growth and self-renewal, promoting apoptosis resistance and regulating angiogenesis; it is involved in the recruitment of neutrophils and promotes migration and proliferation of white cells in the tumoral tissue. Resident fibroblasts produce matrix metalloproteases and degrade the extracellular matrix by action of such cytokine. It stimulates the tumor growth because activates STAT3 that regulates the expression of some cyclins and down-regulates the expression of the kinase cyclin-dependent inhibitor p21, thus promoting the entry in the cellular cycle IL-6 also induces EMT in various types of cancer cells. It plays an important role in the generation and maintenance of the cancer stem cells. (35)

IL-10

It shows immunosuppressive activity inhibiting the antitumoral properties of the T cells. The main source is represented by TH2 cells and monocytes and the main target cells are the B cells and the macrophages. (36) The tumoral cells produce big quantities of IL-10 that contributes to the tumoral progression; in many types of cancer its serum levels are related to the disease severity. IL-10 has also been described to stimulate tumoral development. (37)

IL-4

It is a cytokine that in normal conditions acts like anti-inflammatory compound; a strong correlation between tumoral progression and IL-4 produced by the lymphocytes TH2 infiltrating the tumor has been found in many types of malignant tumors like non-small cell lung cancer, breast cancer, renal cancer, prostate cancer and other cancers. This cytokine supports cellular proliferation and survival and may contribute to suppress immune antitumoral response. (38)

IL-7

It is a pro-inflammatory cytokine that regulate the development and the homeostasis of T cells. (39) The main source are the stromal cells of the bone marrow and the epithelial cells, the target cells are the stem cells and the main function is to act like growth factor for B and T cells.

IL-8

This inflammatory cytokine is involved in various cellular processes that induce chemotaxis and invasion. The main source are the macrophages, and the target cells are neutrophils. (40) It has an important role in angiogenesis through the stimulation of migration of the endothelial cells and the suppression of the programmed cell death. It is elevated in most of the patients that suffer from prostate cancer, breast cancer and colon cancer.

TNF-alpha

It is the prototype of the proinflammatory cytokine. It plays an important role in the regulation of the immune response either innate or acquired through its ability to condition many cellular types. It is considered an important regulator of the leukocyte movement, stimulating the activation and the recruitment of neutrophils and monocytes to the sites of inflammation and recently it has been demonstrated to affect the tumoral cells via an autocrine action. (41) It has effects either on endothelial cells or on the tumor growth and the metastasis through its effects on myeloid cells associated with cancer. It promotes cell survival, invasion and angiogenesis. It is produced either by malignant cells or by other cell types in the tumoral microenvironment and it has been found in different types of human cancer like prostate cancer, ovarian cancer, hepatic cancer and breast cancer; high plasmatic levels are present in advanced cancer. High serum levels indicate poor prognosis in ovarian, renal, pancreatic, breast cancer and in chronic lymphocytic leukaemia. (42)

IFN-gamma

It is a key promoter of the macrophage activation and induction of the expression of MHC-II molecules. The more important source are TCD8+, TCD4+ and NK cells. It possesses antitumoral activity. (43)

IL-12

It exerts an essential role in the interaction between the arms of innate and adaptive immunity. Produced by phagocytes, B cells and dendritic cells as a result of the binding with infective agents, it acts on T and NK cell; it is also the main cytokine responsible of cellular differentiation of the CD4+ versus TH1, allowing a powerful production of IFN-gamma. It has a powerful antitumoral activity. (44)

VEGF

It is overexpressed in various solid human tumors. It determines tumoral angiogenesis, survival, invasion and migration and immune suppression in the tumor sites next to neoangiogenesis. Almost all solid tumors overexpress VEGF and high serum and intratumoral levels are associated to poor prognosis. (45)

GM-CSF

It promotes the inflammatory response supporting the activation of immune cells. (46)

CCL5/RANTES

It is an inflammatory cytokine that determines the recruitment of leukocytes in the inflammatory sites; it is expressed by several hematopoietic and non hematopoietic cells. The chemokines and their receptors are key components of inflammation related to cancer because it affects many pathways of tumoral progression, included leucocyte recruitment and function, cellular senescence, proliferation, survival, invasion and tumoral metastasis. It is expressed in various types of human cancer, including breast, prostate, ovarian and

cervical, Colon, gastric cancer, melanoma, multiple myeloma, Hodgkin's lymphoma and acute T-cell lymphoblastic leukemia. In the context of tu-moral TME, sources of CCL5 are infiltrating leukocytes, mesenchymal stem cells and fibroblasts associated with cancer. (47)

Cytokine	**Source**	**Action**
IL-1	Macrophages,neutrophils,endothelial cells, B cells, dendritic cells	Pro-inflammatory
TGF-beta	Macrophages,monocytes, T cells	Anti-inflammatory
IL-6	Lymphocytes TH, macrophages,fibroblasts	Pro-inflammatory
IL-10	Lymphocytes TH2, monocytes	Anti-inflammatory
IL-4	Lymphocytes TH2	Anti-inflammatory
Il-7	Stromal cells of the bone marrow, epithelial cells	Pro-inflammatory
IL-8	Macrophages	Pro-inflammatory
TNF-alfa	Macrophages,monocytes and lymphocytes TH1CD4+	Pro-inflammatory
IFN-gamma	TCD8+,TCD4+,NK cells	Pro-inflammatory
IL-12	Phagocytes, B cells, dendritic cells	Pro-inflammatory
VEGF	Vascular endothelia cells,lymphatic endothelial cells	Pro-angiogenic activity,mitogenic and anti-apoptotic effect on endothelial cells
GM-CSF	Activated T and B cells,monocytes, macrophages, endothelial cells,fibroblasts, neutrophils,eosinophils, epithelial cells, mesothelial cells, chondrocytes, Paneth cells, tumor cells	
CCL5/RANTES	In the context of tumoral TME: leukocytes, mesenchymal stem cells and fibroblasts associated with cancer	Pro-inflammatory

Cytokines and diagnosis/prognosis of testicular cancer

Testicular seminoma and non-seminoma belong to type II testicular germ cell tumors (TGCTS) and they derive from embryonic/fetal primordial germ cells (PGCS)/gonocytes that undergo maturation arrest during their development; cells with altered differentiation are susceptible to abnormal cell division and can undergo malignant transformation. Ultimately, the gonocytes turn into germ cell neoplasm in situ (GCNIS) that remains quiescent in the seminiferous tubule until puberty and adolescence; when these cells begin to proliferate, the GCNIS progresses to invasive tumor. (48)

The cells of GCNIS share several features with embryonal stem cells (ES): The GCNIS holds totipotent/pluripotent cells that have capacity of self-renewal and aberrant differentiation in many types of tissues; in addition, they share morphological features, immunological profile, genetic background and functional properties (cell cycle control and DNA repair mechanisms) with gonocytes. (49)

Testicular germ cell tumors are largely infiltrated by immune cells (CD8+ cells, regulatory T cells and B cells). The testicular tumor microenvironment is significantly influenced by high levels of proinflammatory cytokines like IL-1beta, IL-6 and TNFalfa and anti-inflammatory cytokines like TGFbeta1; there are also IL-2 and IFN-gamma and chemokines like CCL-5. T cells and macrophages are cells characterizing testicular tumors (50).

Many cytokines and growth factors play crucial physiological roles in testis. IL-6 and TNF have direct effects on differentiation of spermatogenic cells and testicular steroidogenesis. The anti-inflammatory cytokines of TGFbeta family are involved in testicular development; Activin A, member of TGFbeta family, can affect gonocytes development (51). Either it or NODAL, another member of the same family, influence the normal growth of the fetal testicle (52) . Hence, pathways controlled by the family members of TGFbeta are involved in signaling that leads to damaged differentiation of teratocarcinoma and, generally, are involved in carcinogenesis of testicular tumors. (53)

Moreover, IGF-1 increases the production of testosterone in Leydig cells, ensures the homeostasis of Sertoli cells and stimulates the spermatogonium production. (54) The expression levels of VEGF are related to the risk of metastasis in seminoma (55) and members of FGF family have an effect promoting the proliferation of terato-ma/teratocarcinoma. (56)

Cytokines potentially useful for testicular cancer diagnosis

One main histological feature of testicular cancer is profound angiogenesis. In this context, a marked elevation of average serum levels of PTN (pleiotrophin) (20 fold) and FGF-2 (7 fold) has been demonstrated in patients with testicular cancer; to a lesser extent, VEGF (vascular endothelial growth factor) serum levels are increased, especially in teratoma, and EGF, either in seminomas or non-seminoma even in early tumor stages.

PTN and FGF-2 may represent promising new diagnostic markers for testicular cancer with high sensitivity even in early-stage testicular cancer. (57)

Transforming growth factor-beta (TGF-beta) emerges as one of the key regulators of tumor development and progression. It influences all crucial steps of cancer progression, including migration and invasion, with a prominent influence on the process of epithelial-mesenchymal transition (EMT). The mechanisms of TGF-beta action in cancer are complex: at the beginning of tumor development, TGF-beta attenuates cancerous proliferation, limiting tumor growth; during cancer progression, these tumor sup-pressive effects reverse, and TGF-beta promotes migration, invasion and formation of distant metastasis. These paradoxical TGF-beta actions could result from interplay with the activity of microRNAS, which emerge as important modulators and mediators of TGF-beta effects in cancer cells. Abnormalities in the expression of these small, non-coding RNAs are involved in the control of cell development, proliferation, growth, differentiation and apoptosis; abnormalities in their expression or function contribute to the development of multiple disorders, including cancer.

TGF-beta signaling is crucial for both the physiology and pathology of testis, contributing to its development and functioning. In fetal testis, TGF-beta attenuates proliferation, indicating that aberrances of the TGF-beta pathway may lead to tumor development. Expression of TGF-beta1 and TGF-beta2, as well as TGFBR1 and TGFBR2, was increased in testicular tumors compared with peritumoral non-neoplastic testis tissues. TGF-beta1, together with EGF and FGF4, synergistically contribute to differentiation of seminoma cells into non-seminoma-like cell type.

Since seminomas naturally progress into non-seminomas, this may suggest that TGF-beta stimulates the progression of testicular tumors. Furthermore TGF-beta-induced signaling stimulates proliferation of TCam- 2 seminoma cells. (53)

Cytokines as prognostic biomarkers of testicular cancer

Seventy percent of all patients with testicular germ cell tumors (GCT) are initially diagnosed with localized disease (stage I). If the cancer is limited to the testicle, the therapy involves surgical removal of the affected testicle, followed by a strict surveil-lance program including regular blood tests, x-rays and computerized tomography.

Despite the excellent prognosis of localized tumor, 15-30% of patients experience dis-ease recurrence during surveillance. An option to reduce the risk of recurrence is the administration of one cycle of adjuvant chemotherapy after orchiectomy, which substantially reduces the risk of disease recurrence.

Attempts have been made to stratify patients, who are at higher risk for disease recurrence based on different pathological characteristics (the size of the tumor and rete testis infiltration or the presence of lympho-vascular invasion and of embryonal carcinoma). However, the discovery of new markers that more reliably predict the risk of recurrence are of utmost importance.

Lobo and co-workers have examined 422 seminomas,172 embryonal carcinomas, 65 yolk sac tumors, 29 teratomas, 7 choriocarcinomas and 9 germ cell neoplasia in situ (GCNIS) (58)

The expression of CXCL12 was observed in a large number of patients with non-seminoma but was absent in normal tissue, GCNIS or seminoma and its presence in stromal/inflammatory cells might also influence the final tendency for tumor cells to migrate and invade. CXCL12 expression was also associated with a higher risk for dis-ease recurrence in patients with metastatic non-seminoma after first line chemotherapy. (59)

Beta-catenin is also a prognostic factor of unfavorable outcome in this kind of tumor; the immunoexpression pattern is significantly lower in seminomas when compared to non-seminomas. Higher expression is associated with relapse and a tendency to show overall poorer relapse-free survival. (58)

Vascular endothelial growth factor (VEGF) and thymidine phosphorylase (TP/platelet-derived endothelial cell growth factor (PD-ECGF) are involved in in-creased angiogenic activity and disease progression in solid tumors.

VEGF protein levels were higher in testicular germ cell tumors compared with non neoplastic testes. VEGF expression and microvessel count were correlated with metastasis

in seminoma; conversely, four variables (VEGF expression, microvessel count, the presence of venous invasion and the presence of embryonal carcinoma elements in the primary tumor) were correlated with metastasis in non-seminomatous testis lesions. (60)

A correlation has been found among overall survival, progression-free survival and circulating cytokines in metastatic testicular germ-cell tumor patients. Plasma cytokines could be potential biomarkers for identification of high-risk patients. The study by Svetlovska et al. (49) included 92 metastatic chemotherapy-naïve TGCT patients treated with platinum-based chemotherapy, plasma was isolated before first administration of chemotherapy and the concentration of 51 plasma cytokines were analyzed using multiplex bead arrays.

Elevated plasma levels of interferon (IFN)-alfa2, IL-2Ralfa, IL-16, hepatocyte growth factor (HGF) and monocyte chemotactic protein (MCP)-3 were significantly associated with worse progression-free survival and overall survival (OS). Moreover, elevated levels of stem-cell growth factor (SCGF)-beta were also associated with worse OS. Patients with elevated levels of all 6 cytokines experienced significantly worse outcomes compared to patients who had fewer than 6 cytokines elevated.

A correlation was found among progression free-survival, OS and circulating cytokines in TGCT, suggesting an association between plasma cytokines and baseline clinicopathologic features in these cancers. Thus, plasma cytokines could be used for identification of high-risk patients who are candidates for personalized approaches.

The association between high levels of circulating cytokines and poor outcome has been described in many cancer types, including breast, gastric, prostate, head and neck, renal cell, colorectal and hematologic malignancies.

Pure seminoma but not nonseminoma also showed a strong immunohistochemical reaction for both SCF and c-kit protein on the surface of the tumor cells. VEGF expression predicted metastasis in seminomas and HST-1 (a member of the fibroblast growth factors) expression was associated with nonseminomas and with tumor stage. IGF-1/IGFR and SDF-1/CXCR4 signaling pathways are important mediators of TGCT pathogenesis, as they are involved in many metastatic processes, such as cell proliferation, migration and angiogenesis; moreover, IGF-2, FGF-8 and FGF-9 have a pro-survival effect on teratoma.(61)

Cytokines as predictive biomarkers of testicular cancer

The clinical course of a cancer is influenced by the interaction of tumor cells with patient's immune system. It is thus conceivable that immunological parameters may be used as markers of prognostic, diagnostic or predictive value.

The onset and progression of $TGCT_S$ depend on the testicular niche and therefore even on the growth factors and growth factor-activated pathways present in the neoplastic testicular microenvironment.

Overactivation of the c-MET/HGF system is a feature of many cancers. Type II testicular germ cell tumor (TGCT) cells express the c-MET receptor, forming non-seminomatous lesions that are more positive compared with seminomatous ones.

NT2D1 non-seminomatous cells (derived from an embryonal carcinoma lesion) increase their proliferation, migration and invasion in response to HGF.

PI3K/AKT is one of the signaling pathways triggered by HGF through the c-MET activation cascade.

HGF is present in the testis and male reproductive tract and its level circulating is inversely correlated with the progression free survival of TGCT patients.

Even if the embryonal carcinoma cells from patients are positive for HGF, NT2D1 cells don't secrete or express this factor and this observation highlights the importance of the microenvironment in the modulation of embryonal carcinoma cellular physiology. (62)

HGF (Hepatocyte growth factor) increases the proliferation, migration and invasion of NTERA-2 clone D1 (NT2D1) non-seminoma cells; the receptor of this protein is c-MET and it has been demonstrated the over-expression either of HGF or of c-MET in biopsies of non-seminoma lesions. (63)

CXCL12 is a chemokine produced by somatic cells that influences early mammalian germline cells at stages considered of relevance for the initiation of testicular germ cell tumors ($TGCT_S$).

The CXCL12 signaling may contribute to the "dedifferentiation" of PGC_S into GCNIS cells. This cytokine is known to mediate normal cell migration and is implicated in many neoplasias, including the overexpression of its canonical receptor, CXCR4, in various cancers.

Activins are involved in multiple biological functions including the control of inflammation, fibrosis, developmental biology and tumorigenesis; they are dimers of inhibin beta subunits and act via a classical TGF-beta signalling pathway. Its bioactivity is regulated by two endogenous inhibitors, inhibin and follistatin.

Activins A and B act through the serine/threonine kinase pathway common to other TGF-beta members and the activin signalling is increased in human testicular and ovarian cancers. (64)

The potential involvement of activin A signaling in $TGCT_S$ has been demonstrated through immunohistochemical localization of activin type I (ALK2 and ALK4) and type II receptors (ActRIIB) in GCNIS, seminoma and non-seminoma cells. Nuclear phosphor-SMAD2/3 signal is present in all seminoma samples, indicating active activin/TGFbeta signaling in these tumors. (65)

Activin A stimulates expression of MMP2 and MMP9 in a seminoma-derived cell line. MMP2 and MMP9 metalloproteinases are well-known stimulators of tumor invasion and progression, which suggest that activin/TGFbeta signaling may contribute to TGCT development from GCNIS.

Furthermore, elevated TGCT risk is associated with a SNP in INHA gene, which encodes the inhibin alpha subunit.

The TGF-beta pathway has been investigated in human germ cell tumors. The ligands TGF-beta1, TGF-beta2 and TGF-beta3, as well as inhibin and activin, have been shown to be expressed in malignant germ cell tumors. Specific snp variants in the TGFB1 gene are associated with elevated risk of developing testicular germ cell tumor.

In addition, BMP signaling activity is robustly present in most yolk sac tumors, a differentiated tumor type. (66)

IL-6 archetypically signals through the JAK/STAT signaling pathway, with STAT3 phosphorylation indicative of active signaling via the IL6R. Dysregulated IL6 production has been identified in several cancers, often positively correlated with tumor growth and invasive behaviors (64).

The unique immune environment of testicular germ cell neoplasia comprises B cells and dendritic cells as well as high transcript levels of IL-6 and other B cell supporting or T helper cell type I (Th1)-driven cytokines; T cells are known to be the major component of inflammatory infiltrates associated with testicular cancer.

Within GCNIS and seminoma, in addition to T cells, high numbers of dendritic cells and B cells have been found, the latter additionally organized in cell clusters. Transcripts encoding pro-inflammatory cytokines (IL-1beta, IL-6 and TNF-alfa), anti-inflammatory cytokines (TGF-beta1), Th1-driven cytokines (IL-2 and IFN-gamma) as well as chemokines (CXCL-13, CXCL-10 and CCL-5) are all significantly increased in testicular germ cell neoplasia, suggesting the presence of a pro-tumorigenic environment, in particular the presence of focal lymphocytic infiltrates without neoplastic cells in the biopsies.

One of the most important outcomes is the pivotal role of IL-6 in testicular cancer that opens potential novel early biomarker and target for therapeutic intervention in testicular germ cell neoplasia.

Intriguing findings were made on the pivotal role of B cells and IL-6 in $TGCT_S$ and the pro-inflammatory, potentially pro-tumorigenic cytokine environment associated with these tumors; a 130-fold increase of IL-6 transcript levels has been detected in testis cancer samples analysed and this suggests a pro-proliferative role of this cytokine. There is a significantly elevated levels of CXCL13 detectable in the same neoplasia samples, which is a highly potent B cell chemoattractant that can also promote follicles formation of immune cells; B cell clusters can moreover be a source of IL-6 and IFN-gamma, acting in a potential autocrine positive feedback loop.

The pro-inflammatory, pro-tumorigenic cytokine environment is likely to facilitate tumor progression and invasion (67).

Conclusion

Elevated levels of several cytokines have been found in patients with cancer, either in the serum or in the tumoral microenvironment. Beside their value as biomarkers to support the diagnosis and the monitoring of disease activity, cytokines can be considered potential therapeutic targets.

Transcripts encoding proinflammatory cytokines, anti-inflammatory cytokines as well as chemokines are all significantly increased in testicular germ cell neoplasia.

Thus, since testicular tumor microenvironment is significantly affected by high levels of proinflammatory and anti-inflammatory reactions, several molecules could be potentially useful for testicular cancer diagnosis (PTN, FGF2), or as prognostic biomarkers (CXC12, Beta-catenin, HST-1, IGF-1, SDF-1, IGF-2, FGF-8, FGF-9) or as predictive biomarkers of testicular cancer (HGF, CXCL12, Activin signalling, BMP signal-ling, IL-6)

References

1) Dieckmann KP, Simonsen-Richter H, Kulejewski M, Anheuser P, Zecha H, Isbarn H, Pichlmeier U. Serum Tumour Markers in Testicular Germ Cell Tumours: Frequencies of Elevated Levels and Extents of Marker Elevation Are Significantly Associated with Clinical Parameters and with Response to Treatment. Biomed Res Int. 2019 May 28;2019:5030349. doi: 10.1155/2019/5030349. PMID: 31275973; PMCID: PMC6558624.
2) Paolo Pedrazzoli, Giovanni Rosti, Eleonora Soresini, Silvia Ciani, Simona Secondino, Serum tumour markers in germ cell tumours: From diagnosis to cure, Critical Reviews in Oncology/Hematology, Volume 159,2021, 103224, ISSN 1040-8428, https://doi.org/10.1016/j.critrevonc.2021.103224.
3) Ersan Arda, Gurkan Arikan, Hakan Akdere, Murat Akgul, and Ilkan Yuksel. Predictive and prognostic impact of preoperative complete blood count based systemic inflammatory markers in testicular cancer. Int Braz J Urol. 2020 Mar-Apr; 46(2): 216–223. Published online 2020 Jan 10. doi: 10.1590/S1677-5538.IBJU.2018.0820
4) Gerl A, Lamerz R, Mann K, Clemm C, Wilmanns W. Is serum tumor marker half-life a guide to prognosis in metastatic nonseminomatous germ cell tumors? Anticancer Res. 1997 Jul-Aug;17(4B):3047-9. PMID: 9329598.
5) Hu X, Chen R, Wei Q, Xu X. The Landscape Of Alpha Fetoprotein In Hepatocellular Carcinoma: Where Are We? Int J Biol Sci. 2022 Jan 1;18(2):536-551. doi: 10.7150/ijbs.64537. PMID: 35002508; PMCID: PMC8741863.
6) Beyer J, Collette L, Sauvé N, Daugaard G, Feldman DR, Tandstad T, Tryakin A, Stahl O, Gonzalez-Billalabeitia E, De Giorgi U, Culine S, de Wit R, Hansen AR, Bebek M, Terbuch A, Albany C, Hentrich M, Gietema JA, Negaard H, Huddart RA, Lorch A, Cafferty FH, Heng DYC, Sweeney CJ, Winquist E, Chovanec M, Fankhauser C, Stark D, Grimison P, Necchi A, Tran B, Heidenreich A, Shamash J, Sternberg CN, Vaughn DJ, Duran I, Bokemeyer C, Patrikidou A, Cathomas R, Assele S, Gillessen S; International Germ Cell Cancer Classification Update Consortium. Survival and New Prognosticators in Metastatic Seminoma: Results From the IGCCCG-Update Consortium. J Clin Oncol. 2021 May 10;39(14):1553-1562. doi: 10.1200/JCO.20.03292. Epub 2021 Mar 17. PMID: 33729863; PMCID: PMC8099394.

7) Ilktac A, Dogan B, Ersoz C, Akcay M, Akbulut H. The relationship of neutrophil to lymphocyte ratio with testicular cancer. Int Braz J Urol. 2020 Jan-Feb;46(1):101-107. doi: 10.1590/S1677-5538.IBJU.2019.0321. PMID: 31851466; PMCID: PMC6968911.
8) Dieckmann KP, Radtke A, Geczi L, Matthies C, Anheuser P, Eckardt U, Sommer J, Zengerling F, Trenti E, Pichler R, Belz H, Zastrow S, Winter A, Melchior S, Hammel J, Kranz J, Bolten M, Krege S, Haben B, Loidl W, Ruf CG, Heinzelbecker J, Heidenreich A, Cremers JF, Oing C, Hermanns T, Fankhauser CD, Gillessen S, Reichegger H, Cathomas R, Pichler M, Hentrich M, Eredics K, Lorch A, Wülfing C, Peine S, Wosniok W, Bokemeyer C, Belge G. Serum Levels of MicroRNA-371a-3p (M371 Test) as a New Biomarker of Testicular Germ Cell Tumors: Results of a Prospective Multicentric Study. J Clin Oncol. 2019 Jun 1;37(16):1412-1423. doi: 10.1200/JCO.18.01480. Epub 2019 Mar 15. PMID: 30875280; PMCID: PMC6544462.
9) Yuksel OH, Verit A, Sahin A, Urkmez A, Uruc F. White blood cell counts and neutrophil to lymphocyte ratio in the diagnosis of testicular cancer: a simple secondary serum tumor marker. Int Braz J Urol. 2016 Jan-Feb;42(1):53-9. doi: 10.1590/S1677-5538.IBJU.2014.0593. PMID: 27136467; PMCID: PMC4811226.
10) Bumbasirevic U, Bojanic N, Simic T, Milojevic B, Zivkovic M, Kosanovic T, Kajmakovic B, Janicic A, Durutovic O, Radovanovic M, Santric V, Zekovic M, Coric V. Interplay between Comprehensive Inflammation Indices and Redox Biomarkers in Testicular Germ-Cell Tumors. J Pers Med. 2022 May 20;12(5):833. doi: 10.3390/jpm12050833. PMID: 35629255; PMCID: PMC9143453.
11) Nakao S, Minaguchi T, Itagaki H, Hosokawa Y, Shikama A, Tasaka N, Akiyama A, Ochi H, Matsumoto K, Satoh T. Pretreatment thrombocytosis as an independent predictive factor for chemoresistance and poor survival in epithelial ovarian cancer. J Ovarian Res. 2020 May 6;13(1):55. doi: 10.1186/s13048-020-00651-6. PMID: 32375852; PMCID: PMC7201937.
12) Murukesh N, Dive C, Jayson GC. Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. Br J Cancer. 2010 Jan 5;102(1):8-18. doi: 10.1038/sj.bjc.6605483. Epub 2009 Dec 15. PMID: 20010945; PMCID: PMC2813747.
13) Lan T., Chen L, Wei X. Inflammatory Cytokines in Cancer: Comprehensive Understanding and Clinical Progress in Gene Therapy. Cells. 2021 Jan 8;10(1):100. doi: 10.3390/cells10010100. PMID: 33429846; PMCID: PMC7827947.

14) Samuel PJ. Cytokine Analysis: A Fresh Perspective. J Interferon Cytokine Res. 2020 Nov;40(11):511-514. doi: 10.1089/jir.2020.0089. PMID: 33201764.

15) D'Esposito V, Liguoro D, Ambrosio MR, Collina F, Cantile M, Spinelli R, Raciti GA, Miele C, Valentino R, Campiglia P, De Laurentiis M, Di Bonito M, Botti G, Franco R, Beguinot F, Formisano P. Adipose microenvironment promotes triple negative breast cancer cell invasiveness and dissemination by producing CCL5. Oncotarget. 2016 Apr 26;7(17):24495-509. doi: 10.18632/oncotarget.8336. PMID: 27027351; PMCID: PMC5029717.

16) Ambrosio MR, D'Esposito V, Costa V, Liguoro D, Collina F, Cantile M, Prevete N, Passaro C, Mosca G, De Laurentiis M, Di Bonito M, Botti G, Franco R, Beguinot F, Ciccodicola A, Formisano P. Glucose impairs tamoxifen responsiveness modulating connective tissue growth factor in breast cancer cells. Oncotarget. 2017 Nov 20;8(65):109000-109017. doi: 10.18632/oncotarget.22552. PMID: 29312586; PMCID: PMC5752499.

17) Cavaillon JM. Pro- versus anti-inflammatory cytokines: mith or reality. Cell Mol Biol (Noisy-Le-grand) 2001 Jun; 47(4):695-702. PMID: 11502077

18) Besse A, Trimoreau F, Praloran V, Denizot Y. Effect of cytokines and growth factors on the macrophage colony-stimulating factor secretion by human bone marrow stromal cells. Cytokine. 2000 May;12(5):522-5. doi: 10.1006/cyto.1999.0580. PMID: 10857771

19) Qiu Y, Su M, Liu L, Tang Y, Pan Y, Sun J. Clinical Application of Cytokines in Cancer Immunotherapy. Drug Des Devel Ther. 2021 May 27;15:2269-2287. doi: 10.2147/DDDT.S308578. PMID: 34079226; PMCID: PMC8166316.

20) Deets KA, Vance RE. Inflammasomes and adaptive immune responses. Nat Immunol. 2021 Apr;22(4):412-422. doi: 10.1038/s41590-021-00869-6. Epub 2021 Feb 18. PMID: 33603227.

21) Cho H, Seo Y, Loke KM, Kim SW, Oh SM, Kim JH, Soh J, Kim HS, Lee H, Kim J, Min JJ, Jung DW, Williams DR. Can-cer-Stimulated CAFs Enhance Monocyte Differentiation and Protumoral TAM Activation via IL6 and GM-CSF Secretion. Clin Cancer Res. 2018 Nov 1;24(21):5407-5421. doi: 10.1158/1078-0432.CCR-18-0125. Epub 2018 Jun 29. PMID: 29959142.

22) Liao Z, Tan ZW, Zhu P, Tan NS. Cancer-associated fibroblasts in tumor microenvironment - Accomplices in tumor ma-lignancy. Cell Immunol. 2019

Sep;343:103729. doi: 10.1016/j.cellimm.2017.12.003. Epub 2018 Feb 13. PMID: 29397066.

23) Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. Nat Rev Cancer. 2021 Aug;21(8):481-499. doi: 10.1038/s41568-021-00363-z. Epub 2021 Jun 3. PMID: 34083781; PMCID: PMC8173513.

24) Murata M. Inflammation and cancer. Environ Health Prev Med. 2018 Oct 20;23(1):50. doi: 10.1186/s12199-018-0740-1. PMID: 30340457; PMCID: PMC6195709.

25) Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. Semin Im-munol. 2014 Feb;26(1):54-74. doi: 10.1016/j.smim.2014.01.001. Epub 2014 Feb 16. PMID: 24552665.

26) Clara JA, Monge C, Yang Y, Takebe N. Targeting signalling pathways and the immune microenvironment of cancer stem cells - a clinical update. Nat Rev Clin Oncol. 2020 Apr;17(4):204-232. doi: 10.1038/s41571-019-0293-2. Epub 2019 Dec 2. PMID: 31792354.

27) Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol. 2013 May;14(6):e218-28. doi: 10.1016/S1470-2045(12)70582-X. PMID: 23639322.

28) Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 2003 Aug 15;374(Pt 1):1-20. doi: 10.1042/BJ20030407. PMID: 12773095; PMCID: PMC1223585.

29) Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res. 2014;2014:149185. doi: 10.1155/2014/149185. Epub 2014 May 13. PMID: 24901008; PMCID: PMC4036716.

30) Visconti L, Nelissen K, Deckx L, van den Akker M, Adriaensen W, Daniels L, Matheï C, Linsen L, Hellings N, Stinissen P, Buntinx F. Prognostic value of circulating cytokines on overall survival and disease-free survival in cancer patients. Biomark Med. 2014;8(2):297-306. doi: 10.2217/bmm.13.122. PMID: 24521026

31) Yazdi AS, Ghoreschi K. The Interleukin-1 Family. Adv Exp Med Biol. 2016;941:21-29. doi: 10.1007/978-94-024-0921-5_2. PMID: 27734407

32) Zhang W, Borcherding N, Kolb R. IL-1 Signaling in Tumor Microenvironment. Adv Exp Med Biol. 2020;1240:1-23. doi: 10.1007/978-3-030-38315-2_1. PMID: 32060884.

33) Morikawa M, Derynck R, Miyazono K. TGF-β and the TGF-β Family: Context-Dependent Roles in Cell and Tissue Physi-ology. Cold Spring Harb Perspect Biol. 2016 May 2;8(5):a021873. doi: 10.1101/cshperspect.a021873. PMID: 27141051; PMCID: PMC4852809.

34) Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014 Sep 4;6(10):a016295. doi: 10.1101/cshperspect.a016295. PMID: 25190079; PMCID: PMC4176007.

35) Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. Semin Immunol. 2014 Feb;26(1):54-74. doi: 10.1016/j.smim.2014.01.001. Epub 2014 Feb 16. PMID: 24552665.

36) Wang X, Wong K, Ouyang W, Rutz S. Targeting IL-10 Family Cytokines for the Treatment of Human Diseases. Cold Spring Harb Perspect Biol. 2019 Feb 1;11(2):a028548. doi: 10.1101/cshperspect.a028548. PMID: 29038121; PMCID: PMC6360861.

37) Ho IC, Miaw SC. Regulation of IL-4 Expression in Immunity and Diseases. Adv Exp Med Biol. 2016;941:31-77. doi: 10.1007/978-94-024-0921-5_3. PMID: 27734408.

38) Barata JT, Durum SK, Seddon B. Flip the coin: IL-7 and IL-7R in health and disease. Nat Immunol. 2019 Dec;20(12):1584-1593. doi: 10.1038/s41590-019-0479-x. Epub 2019 Nov 19. PMID: 31745336.

39) Kim JH. Interleukin-8 in the Tumor Immune Niche: Lessons from Comparative Oncology. Adv Exp Med Biol. 2020;1240:25-33. doi: 10.1007/978-3-030-38315-2_2. PMID: 32060885.

40) Ge Y, Huang M, Yao YM. Autophagy and proinflammatory cytokines: Interactions and clinical implications. Cytokine Growth Factor Rev. 2018 Oct;43:38-46. doi: 10.1016/j.cytogfr.2018.07.001. Epub 2018 Jul 19. PMID: 30031632.

41) Candido J, Hagemann T. Cancer-related inflammation. J Clin Immunol. 2013 Jan;33 Suppl 1:S79-84. doi: 10.1007/s10875-012-9847-0. Epub 2012 Dec 9. PMID: 23225204.

42) Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. Biomark Res. 2020 Sep 29;8:49. doi: 10.1186/s40364-020-00228-x. PMID: 33005420; PMCID: PMC7526126.

43) Lu X. Impact of IL-12 in Cancer. Curr Cancer Drug Targets. 2017;17(8):682-697. doi: 10.2174/1568009617666170427102729. PMID: 28460617.

44) Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. Cell. 2019 Mar 7;176(6):1248-1264. doi: 10.1016/j.cell.2019.01.021. PMID: 30849371; PMCID: PMC6410740.

45) Becher B, Tugues S, Greter M. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. Immunity. 2016 Nov 15;45(5):963-973. doi: 10.1016/j.immuni.2016.10.026. PMID: 27851925.

46) Morein D, Erlichman N, Ben-Baruch A. Beyond Cell Motility: The Expanding Roles of Chemokines and Their Receptors in Malignancy. Front Immunol. 2020 Jun 4;11:952. doi: 10.3389/fimmu.2020.00952. PMID: 32582148; PMCID: PMC7287041.

47) Baird DC, Meyers GJ, Hu JS. Testicular Cancer: Diagnosis and Treatment. Am Fam Physician. 2018 Feb 15;97(4):261-268. PMID: 29671528.

48) Al-Obaidy KI, Idrees MT. Testicular Tumors: A Contemporary Update on Morphologic, Immunohistochemical and Mo-lecular Features. Adv Anat Pathol. 2021 Jul 1;28(4):258-275. doi: 10.1097/PAP.0000000000000302. PMID: 33871428

49) 38) Kalavska K, Kucerova L, Schmidtova S, Chovanec M, Mego M. Cancer Stem Cell Niche and Immune-Active Tumor Mi-croenvironment in Testicular Germ Cell Tumors. Adv Exp Med Biol. 2020;1226:111-121. doi: 10.1007/978-3-030-36214-0_9. PMID: 32030680.

50) Moody SC, Wakitani S, Young JC, Western PS, Loveland KL. Evidence that activin A directly modulates early human male germline differentiation status. Reproduction. 2020 Jul;160(1):141-154. doi: 10.1530/REP-20-0095. PMID: 32484162.

51) Harpelunde Poulsen K, Nielsen JE, Grønkær Toft B, Joensen UN, Rasmussen LJ, Blomberg Jensen M, Mitchell RT, Juul A, Rajpert-De Meyts E, Jørgensen A. Influence of Nodal signalling on pluripotency factor expression, tumour cell proliferation and cisplatin-sensitivity in testicular germ cell tumours. BMC Cancer. 2020 Apr 23;20(1):349. doi: 10.1186/s12885-020-06820-6. PMID: 32326899; PMCID: PMC7181506

52) Boguslawska J, Kryst P, Poletajew S, Piekielko-Witkowska A. TGF-β and microRNA Interplay in Genitourinary Cancers. Cells. 2019 Dec 12;8(12):1619. doi: 10.3390/cells8121619. PMID: 31842336; PMCID: PMC6952810.

53) Chia VM, Quraishi SM, Graubard BI, Rubertone MV, Erickson RL, Stanczyk FZ, McGlynn KA. Insulin-like growth factor 1, insulin-like growth factor-binding protein 3, and testicular germ-cell tumor risk. Am J Epidemiol. 2008 Jun 15;167(12):1438-45. doi: 10.1093/aje/kwn080. Epub 2008 Apr 11. PMID: 18408226.

54) Devouassoux-Shisheboran M, Mauduit C, Tabone E, Droz JP, Benahmed M. Growth regulatory factors and signalling proteins in testicular germ cell tumours. APMIS. 2003 Jan;111(1):212-24; discussion 224. doi: 10.1034/j.1600-0463.2003.11101251.x. PMID: 12752264.

55) Hauser S, Kaminski A, Syring I, Holdenrieder S, Dieckmann KP, Muller SC, Ellinger J. Evaluation of Serum Biomarkers (FGF-2, HGF, MIF and PTN) in Patients With Testicular Germ Cell Cancer. In Vivo. 2019 Nov-Dec;33(6):1935-1940. doi: 10.21873/invivo.11688. PMID: 31662522; PMCID: PMC6899099.

56) Aigner A, Brachmann P, Beyer J, Jäger R, Raulais D, Vigny M, Neubauer A, Heidenreich A, Weinknecht S, Czubayko F, Zugmaier G. Marked increase of the growth factors pleiotrophin and fibroblast growth factor-2 in serum of testicular cancer patients. Ann Oncol. 2003 Oct;14(10):1525-9. doi: 10.1093/annonc/mdg416. PMID: 14504053.

57) Lobo J, Gillis AJM, van den Berg A, Looijenga LHJ. Prediction of relapse in stage I testicular germ cell tumor patients on surveillance: investigation of biomarkers. BMC Cancer. 2020 Aug 5;20(1):728. doi: 10.1186/s12885-020-07220-6. PMID: 32758242; PMCID: PMC7405370.

58) Fankhauser CD, Roth L, Grossmann NC, Kranzbühler B, Eberli D, Sulser T, Moch H, Bode PK, Beyer J, Hermanns T. CXCL12 expression is an adverse predictor for disease recurrence in patients with metastatic non-seminomatous testicular germ cell tumors. BMC Cancer. 2019 Aug 14;19(1):802. doi: 10.1186/s12885-019-5961-1. PMID: 31412792; PMCID: PMC6693197.

59) Fukuda S, Shirahama T, Imazono Y, Tsushima T, Ohmori H, Kayajima T, Take S, Nishiyama K, Yonezawa S, Akiba S, Akiyama S, Ohi Y. Expression of vascular endothelial growth factor in patients with testicular germ cell tumors as an indicator of metastatic disease. Cancer. 1999 Mar 15;85(6):1323-30. PMID: 10189138.

60) Svetlovska D, Miskovska V, Cholujova D, Gronesova P, Cingelova S, Chovanec M, Sycova-Mila Z, Obertova J, Palacka P, Rajec J, Kalavska K, Usakova V, Luha J, Ondrus D, Spanik S, Mardiak J, Mego M. Plasma Cytokines Correlated With Disease Characteristics, Progression-Free Survival, and Overall Survival in Testicular Germ-Cell Tumor Patients. Clin Genitourin Cancer. 2017 Jun;15(3):411-416.e2. doi: 10.1016/j.clgc.2017.01.027. Epub 2017 Feb 8. PMID: 28237180

61) Fustino N, Rakheja D, Ateek CS, Neumann JC, Amatruda JF. Bone morphogenetic protein signalling activity distinguishes histological subsets of paediatric germ cell tumours. Int J Androl. 2011 Aug;34(4 Pt 2):e218-33. doi: 10.1111/j.1365-2605.2011.01186.x. Epub 2011 Jun 22. PMID: 21696393; PMCID: PMC4129369.

62) Loveland KL, Klein B, Pueschl D, Indumathy S, Bergmann M, Loveland BE, Hedger MP, Schuppe HC. Cytokines in Male Fertility and Reproductive Pathologies: Immunoregulation and Beyond. Front Endocrinol (Lausanne). 2017 Nov 20;8:307. doi: 10.3389/fendo.2017.00307. PMID: 29250030; PMCID: PMC5715375.

63) Wijayarathna R, de Kretser DM. Activins in reproductive biology and beyond. Hum Reprod Update. 2016 Apr;22(3):342-57. doi: 10.1093/humupd/dmv058. Epub 2016 Feb 15. PMID: 26884470.