National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Testicular Cancer

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Abbreviations: AFP, α-fetoprotein; CIS, carcinoma *in situ;* c-KIT, stem cell factor receptor; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; hCGα, free α-subunit of human chorionic gonadotropin; IGCCCG, International Germ Cell Cancer Collaborative Group; IS, International Standard; U, International Unit; ITGCNU, intratubular germ cell neoplasia unclassified; LDH, lactic dehydrogenase; LH, luteinizing hormone; NSGCT, nonseminomatous germ cell tumors; PLAP, placental (germ cell) alkaline phosphatase; RPLND, retroperitoneal lymph node dissection; TIN, testicular intratubular neoplasia; TSH, thyroid stimulating hormone.

INTRODUCTION

About 95% of all malignant testicular tumors are of germ cell origin, most of the rest being lymphomas, Leydig or Sertoli cell tumors and mesotheliomas. Germ cell tumors of adolescents and adults are classified into two main types, seminomas and nonseminomatous germ cell cancers of the testis (NSGCT). Testicular cancers represent about 1% of all malignancies in males, but they are the most common tumors in men aged 15-35 years. They represent a significant cause of death in this age group in spite of the fact that presently more than 90% of the cases are cured (1). Germ cell tumors may also originate in extragonadal sites, e.g., the sacrococcygeal region, mediastinum and the pineal gland (2). Those of the sacrum are predominantly found in males of a young age. Based on the histology, age of the patient at diagnosis, clinical behavior and chromosomal constitution, these tumors can be subdivided into three distinct entities with different clinical and biological characteristics (3) (4) (5): a) teratomas and yolk sac tumors of newborns and infants, b) seminomas and nonseminomas of adolescents and young adults, and c) spermatocytic seminoma of the elderly. This review focuses on seminomas and nonseminomas in adolescence and adulthood.

The incidence of testicular cancers varies considerably in different countries. In the USA about 7200 new cases are diagnosed each year (1) and the age-adjusted incidence is 5.2/100,000. The incidence is about 4-fold higher in white than in black men. In Europe, the age-adjusted incidence is lowest in Lithuania (0.9/100,000), intermediate in Finland (2.5/100,000) and highest in Denmark (9.2/100 000) (6). The incidence in various European countries has increased by 2-5% per year. In the USA the incidence increased by 52% from the mid-1970s to the mid-1990s (7). The cause of germ cell tumors is unknown, but familial clustering has been observed and cryptorchidism and Klinefelter's syndrome are predisposing factors (1).

At presentation most patients have diffuse testicular swelling, hardness and pain. At an early stage a painless testicular mass is a pathognomonic finding but a testicular mass is most often caused by infectious epididymitis or orchitis. If a trial of antibiotic treatment is performed and this does not reduce symptoms, ultrasonography is indicated. If the findings indicate testicular cancer but serum concentrations of α-fetoprotein (AFP), human chorionic gonadotropin (hCG) and lactate dehydrogenase (LDH) are normal, the diagnosis is confirmed by testicular biopsy. As a rule, orchiectomy is performed prior to any further treatment, but may be delayed until after chemotherapy in individuals with life-threatening metastatic disease. After orchiectomy, additional therapy depends on the type and stage of the disease. Seminoma patients with Stage I, IIa and IIb disease are treated with radiation to the retroperitoneal and

ipsilateral pelvic lymph nodes. About 4-10% relapse and more than 90% of those are cured by chemotherapy. Surveillance is an option for Stage I seminoma but about 15-20% relapse and need to be treated by chemotherapy. Because the morbidity of radiotherapy is low, surveillance is not recommended in the United States. Patients with Stage I nonseminomatous tumors are treated by orchiectomy. After this, surveillance and nerve-sparing retroperitoneal lymph-node dissection are accepted treatment options. About 20% of patients under surveillance will have a relapse and require chemotherapy. Patients with Stage II nonseminomatous tumors are treated with either chemotherapy or retroperitoneal lymph node dissection. Testicular cancer patients with advanced disease are treated with chemotherapy (1).

Serum tumour markers have an important role in the management of patients with testicular cancer, contributing to diagnosis, staging and risk assessment, evaluation of response to therapy and early detection of relapse. Increasing marker concentrations alone are sufficient to initiate treatment. AFP, hCG, and LDH are established serum markers. Most cases of non-seminomatous germ cell tumors (NSGCT) have elevated serum levels of one or more of these markers while LDH, and hCG are useful in seminomas. Other markers have been evaluated but provide limited additional clinical information.

CURRENTLY AVAILABLE MARKERS FOR TESTICULAR CANCER

Table 1 lists the most widely investigated tissue-based and serum-based tumor markers for testicular cancer. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use.

TUMOR MARKERS IN TESTICULAR CANCER: NACB RECOMMENDATIONS

Table 2 presents a summary of recommendations from representative guidelines published on the use of tumor markers in testicular cancer. This table also summarizes the National Academy of Clinical Biochemistry (NACB) guidelines for the use of markers in this malignancy. A number of groups have made detailed recommendations regarding the management of testicular cancer (8) (9) (10) (11), and those relating to tumour marker use are summarized in Table 3. Table 4 summarizes the prognostic significance of serum tumor markers in metastatic testicular cancer, according to the consensus statement of the International Germ Cell Consensus Classification (IGCCC), which remains the cornerstone for diagnosis and treatment of testicular germ cell tumors. Below, we briefly review the histological types of testicular cancer and present a more detailed discussion on the markers listed in these tables.

HISTOLOGICAL TYPES OF TESTICULAR CANCER

In the most recent WHO-Mostofi classification (12) (5), testicular cancers are subdivided into two major types, seminomas and nonseminomatous germ cell tumors (NSGCT) which differ with respect to both marker expression and treatment . The incidence of seminoma peaks in the fourth decade of life and that of NSGCT in the third. Seminomas can be either pure seminomas or the rare spermatocytic seminomas that occur in older age groups. Most NSGCTs are a mixture of histological types, i.e., embryonal carcinomas, choriocarcinomas, teratomas, and yolk sac tumors. About 10-20% of the nonseminomas also contain a seminoma component. These are classified as combined tumors according to the British classification (13), but as nonseminomas according to the WHO-classification system (12). Teratomas are further subdivided as mature or immature. Somatic cancers of various types occasionally develop from a teratoma and are classified as non-germ cell malignancies. Metastases may contain any component occurring in the primary tumor and occasionally components not detected in the primary tumor (12). Fewer than 10% of NSGCT contain a single tissue type and all histological types of tissue should be described (14).

The precursor lesion of testicular seminomas and nonseminomas is carcinoma *in situ* (CIS) (15), also referred to as intratubular germ cell neoplasia unclassified (ITGCNU) and testicular intratubular neoplasia (TIN). CIS cells are found within the spermatogonial niche of the seminiferous tubule in the adult testis in close connection to the Sertoli cells, the nursing cells of spermatogenesis (16). The CIS cells can be detected in the adjacent parenchyma of most invasive tumors, and are more frequently associated with NSGCTs than with seminomas (17). ITGCNU is considered to represent the pre-malignant counterpart of an embryonic germ cell, most likely a primordial germ cell or gonocyte. This theory is supported by multiple findings, including epidemiology, morphology, immunohistochemistry and molecular characterization (18) (19).

Recent data indicate that infertile men with bilateral microlithiasis have an increased risk (up to 20%) of developing testicular seminomas and NSGCTs (20). Surgical biopsy to assess the presence of ITGCNU (21) is indicated in this condition.

Marker expression and tumor type

Certain markers have been found to be informative for the classification of seminomas and NSGCTs. Placental/germ cell alkaline phosphatase (PLAP) is detected in most seminomas and embryonal carcinomas, in 50% of yolk sac tumors and choriocarcinomas but only rarely in teratomas. HCG is expressed by syncytiotrophoblasts, choriocarcinoma and approximately 30%

of seminomas. Of the other tissue markers, the stem cell factor receptor (c-KIT) has been used mainly to detect ITGCNU and seminoma, CD30 to detect embryonal carcinoma, and AFP to detect yolk sac tumors and a 10-20% subset of embryonal carcinomas and teratomas. Recently, a potentially valuable marker OCT3/4, also known as POU5F1, has been identified (22) (23) (24) (25).

Established Markers for Testicular Cancer

Although a large number of serum markers have been studied, only hCG, AFP and LDH have thus far been shown to have independent diagnostic and prognostic value (Tables 2 and 3). The clinical value of other markers remains to be established. Table 5 summarizes analytical limitations of the assays available for some of the most important established and experimental tumor markers. The implications of these limitations for tumor marker use in routine clinical practice are discussed in greater detail below.

α-Fetoprotein (AFP)

Biochemistry and biology. AFP is a homolog of albumin and is thought to act as a carrier protein in the fetus. During pregnancy, AFP is initially produced by the yolk sac and later by the fetal liver (26). Concentrations in fetal plasma reach levels of 3 g/L in the 12-14th week of pregnancy and decrease thereafter to 10-200 mg/L at term (27). After birth, circulating concentrations decrease with a half life of 5 days, falling to adult levels at 8-10 months of age (28) (29). The high values in early childhood must be remembered when using AFP as a marker for testicular yolk sac tumors, which is the most common testicular neoplasm in infants (30) (31). In adults with testicular cancer AFP is a sensitive marker for NSGCT and some embryonal cancers (32).

Assay methods, standardization and reference values. AFP is quantified by two-site immunometric assays employing monoclonal antibodies or combinations of monoclonal and polyclonal antibodies. Results are generally comparable to those obtained with the competitive RIA format used previously. The WHO standard 72/225, in which one International Unit (U) of AFP corresponds to 1.21 ng, is used for calibration. Laboratories report values in mass units (ng/ml or µg/l) or kU/L. Reference values should be established for each assay to reflect differences in assay bias. Most centers quote an upper reference limit for AFP in the range of 10-15 µg/L. Circulating concentrations increase slightly with age: in one study the upper reference limit increased from 9.3 kU/L in subjects below 40 years old to 12.6 kU/L in those above 40 (33).

False positive results. Rising levels of serum AFP indicate persistent germ cell tumor, even in the absence of radiographic evidence of disease, provided other possible causes can be excluded (see below) (1). Moderately elevated AFP levels may persist even after chemotherapy, particularly when persistent disease has a large cystic component, serving as a reservoir leaking AFP into the circulation (34). Elevated serum concentrations of AFP occur in most hepatocellular carcinomas and 10-30% of other gastrointestinal cancers, but these diseases are rare in patients with testicular cancer. Elevated AFP values may not reflect cancer, and it is therefore important to identify positive results caused by other diseases and by non-specific interference. Benign liver disease, in particular hepatitis, and liver damage induced by chemotherapy are often associated with moderately elevated serum AFP levels, and may result in misinterpretation especially if levels are rising (35) (36).

The carbohydrate composition of AFP derived from the liver and the yolk sac are different (37) and lectin binding can differentiate increased levels caused by testicular cancer and liver disease (38), but such methods are not routinely used. Patients who initially have elevated AFP levels may have normal levels during a relapse if therapy has eliminated AFP-producing elements but not all other components (39). Moderately elevated values that do not increase do not usually indicate relapse (36).

HCG and hCGβ

Biochemistry and biology. HCG is a member of the glycoprotein hormone family, which includes luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). All four contain a common α-subunit. The distinct β-subunits confer biological activity and display various degrees of homology, with that between LHβ and hCGß being about 80%. The beta-subunit of hCG (hCGβ) contains a 24 amino acid C-terminal extension not present in LHβ so antibodies to this part of the molecule are specific for hCG,. While the subunits lack hCG activity, hCGβ has been shown to enhance the growth of tumor cells in culture by preventing apoptosis (40). HCG is expressed at very high concentrations by the placenta and trophoblastic tumors including choriocarcinoma of the testis. HCG is heavily glycosylated, hCGβ containing 6 and hCGα 4 carbohydrate chains**.** The glycosylation of hCG secreted by tumors is often different from that of pregnancy hCG. An antibody, B152, detects only a hyperglycosylated variant of hCG. This form predominates in early pregnancy and is possibly more cancer specific than “normal” hCG (41).

Nomenclature, assay methods, standardization and reference values. Specific determination of hCG is based on antibodies reacting with hCGβ (42). This has caused confusion of the nomenclature of hCG assays: the expressions "β-hCG" or "hCG-beta assay" may denote assays measuring both hCG and hCGβ or only hCGβ. According to the nomenclature recommended by the International Federation of Clinical Chemistry (IFCC) hCG denotes the intact αß heterodimer, hCGß the free β-subunit and hCGα the free α-subunit (43). Assays should be defined according to what they measure, i.e., hCG and hCGβ separately or hCG + hCGβ together (32).

Assays for hCG are calibrated against the Third International Standard (IS 75/537) in which concentrations are expressed in international units (U) based on bioactivity. However it is difficult to compare concentrations of hCG with those of hCGβ and hCGα which are also expressed in arbitrary units of the relevant International Standards (IS 75/551 and IRP 75/569, respectively. Recently established WHO Reference Reagents have values assigned in molar concentrations, which should facilitate direct comparison of hCG and hCGβ concentrations in the future (43) (44).

As seminomas may produce solely hCGß and not intact hCG, assays measuring hCG and hCGβ together are recommended to monitor testicular cancer (45) (9) Such assays often utilize antibodies to epitopes on the C-terminal peptides of hCGβ. While specific for hCG and hCGβ, the relatively low affinities of these antibodies limits assay sensitivity (46). Theoretically it should be possible to improve detection of testicular cancer by using separate assays for hCG and hCGβ (45) (32) but this remains to be confirmed.

HCG is secreted at low levels by the pituitary, producing plasma levels that are measurable by sensitive methods. The serum concentrations may increase with age, particularly in women after the menopause (47) (48). For most assays, the upper reference limit of hCG is stated to be 5-10 U/L. When determined by ultra sensitive methods, the upper limit for postmenopausal women is 5 U/L while it is 3 U/L in menstruating women The upper reference limit for men below 50 years of age is 0.7 U/L and for men above this age 2.1 U/L (48). Cut-off values lower than the commonly used 5-10 U/L can be used to diagnose patients with testicular cancer. However, although most men with testicular cancer are young, their hCG levels may be increased due to testicular malfunction. Therefore diagnosis of active disease in a patient with a history of a germ cell tumor requires sequential determinations and rising values. The detection limit of most commercial assays does not allow reliable measurement of levels below 5 U/L and the utility of ultra sensitive assays and lower cut-off values needs to be determined (32). When

expressed in molar concentrations, 5 U/L of hCG corresponds to 15 pmol/L. The upper reference limit for hCGß is 2 pmol/L and is independent of age and gender (48).

Specificity and confounding factors. It is important to note that chemotherapy often causes gonadal suppression that increases the hCG levels. Therefore, levels increasing from below 2 up to 5-8 U/L during chemotherapy are often iatrogenic and do not necessarily indicate relapse. Moderately elevated levels of hCG may be of pituitary origin, especially if accompanying serum levels of LH and FSH exceed 30–50 U/L and are attributed to interrupted feedback inhibition from the gonads. This can be confirmed by short-term testosterone treatment, which suppresses pituitary secretion of hCG (49) (50).

Non-trophoblastic tumors may in extremely rare cases produce hCG, whereas hCGß is often expressed at moderate levels by a large variety of tumors, including ovarian, gastrointestinal, bladder, lung, and head and neck cancers (50). Some patients with such tumors will have elevated hCG levels when measurement is carried out by an assay recognizing both hCG and hCGβ.

Falsely elevated results for serum hCG can be caused by heterophilic antibodies. This has so far only been reported in women (51) but there is no reason to expect it cannot occur in men as well. False positive results can be identified by analysis of hCG in urine or by repeating the assay after adding mouse IgG to the sample to block the interference (51) (32).

Apparently false negative results will be obtained with assays measuring only hCG if the tumor produces hCGβ but not hCG. While more common in seminoma (52) it may also occur in NSGCT patients (53).

Lactate dehydrogenase (LDH)

Biochemistry and biology. LDH in the circulation exists as a tetramer that may contain various combinations of two subunits, LDH-A, LDH-B. The various subunits can combine in five isoenzymes, LDH-1 [consisting of four B subunits (B_4)], LDH-2 [B_3A_1], LDH-3 [B_2A_2], LDH-4 [B_1A_3] and LDH-5 [A_4]. The gene encoding LDH-A is located on chromosome 11 while the gene for LDH-B is located on the short arm of chromosome 12 (54). Interestingly, all invasive seminomas and NSGCTs show additional copies of this chromosomal arm (55), suggesting that it may play a role in disease progression. No gain of 12p is detected in ITGCNU (56) (57). A correlation between copy number of 12p, tumor invasiveness, and the serum level of LDH-1 has been reported, but thus far the relevant 12p-genes have not been identified (58). While theoretically interesting, these findings need to be confirmed.

Specificity and confounding factors. Serum concentrations of LDH are measured enzymatically and the values are method-dependent. The degree of elevation is therefore most conveniently expressed relative to the upper reference limit. LDH-1 can be determined by zymography or by immunoprecipitation of the other isoenzymes and determination of residual catalytic activity. LDH is expressed in many tissues and elevated levels may be caused by a wide variety of diseases. Despite its lack of specificity, LDH is a useful marker, especially for staging of seminoma and NSGCT (57). Hemolysis may cause falsely elevated values and should be avoided.

Placental alkaline phosphatase (PLAP)

Biochemistry and biology. A tumor-associated isoenzyme of alkaline phosphatase was first described in a patient with lung cancer and later detected in serum of patients with other cancers and identified as placental alkaline phosphatase (PLAP) (59). In fact, two genes encode the proteins detected as PLAP activity, i.e., placental (PLAP) and germ cell alkaline phosphatase (GCAP). Both genes map to chromosome 2 and the proteins cannot be distinguished from each other using routine enzymatic or immunohistochemical methods (60). PLAP is elevated most frequently in those with seminoma (60-70%) (61) (62), and less frequently in those with other germ cell tumors, including ITGCNU (14). An enzymatic method can be used to detect ITGCNU cells in frozen tissue sections (63).

Assay methods, standardization and reference values. PLAP has usually been determined by zymography but it can be also be measured by immunoassay or enzymatically after immunocapture (62). The result should be compared with locally determined reference values. Because of homology with other alkaline phosphatase isoenzymes, antibody selection is critical. However, the antibodies available so far cannot distinguish between the PLAP and GCAP isozymes. Therefore, PLAP denotes both of these isozymes.

Specificity and confounding factors. Serum concentrations of PLAP are increased up to 10-fold in smokers and is therefore of little value in this group (62). This and the paucity of commercial assays limit its clinical application and serum assays for PLAP are not routinely included in the diagnostic work up of testicular cancer patients.

Other markers

Although pregnancy-specific beta-1 glycoprotein (or SP1) and hCG are both expressed in trophoblastic cells, hCG is the superior marker (64). Consequently, SP1 is not routinely measured. Neuron-specific enolase (NSE) is elevated in about 30-50% of patients with seminomas and less often in NSGCT patients (65) (66), but in spite of these promising results the use of NSE is limited.

TISSUE MARKERS

Genetic aberrations

A gain of 12p is observed in germ cell tumors both of testicular and extragonadal origin. This indicates that gain of 12p-sequences may be of crucial importance for the development of this cancer and, indeed, this finding is used to diagnose germ cell tumors at extragonadal sites (67). However, the expression level of 12p sequences does not correlate with stage of the disease and treatment sensitivity/resistance (68) (69) (70). The crucial determinant of response to cis-platin-based compounds appears to occur downstream of DNA binding in the intrinsic or extrinsic pathways of apoptosis or DNA repair (71) (72) (73).

While the majority of germ cell tumors show an intact DNA mismatch repair pathway, a defect leading to microsatellite instability has been observed in tumors refractory to cisplatin (74) (75) (76). Other potentially relevant findings in the context of treatment sensitivity and resistance relate to a possible defect in caspase 9 function (77). All these factors might be important and it is unlikely that a single factor determines treatment sensitivity or resistance. This is illustrated by the finding that mature teratomas are resistant to various DNA-damaging treatment protocols (73), possibly due to epigenetic changes occurring during somatic differentiation.

The majority of invasive seminomas and nonseminomas contain additional copies of the X chromosome (78). Interestingly, as during normal (female) development, X-inactivation can occur in these tumors, in which *XIST* is the regulatory gene (79). Detection of unmethylated *XIST* DNA in plasma has been suggested to be useful for molecular diagnosis and the monitoring of testicular GCT-patients (80). This observation merits further investigation.

A number of studies have linked the development of germ cell tumors to a deregulated G_1/S checkpoint, possibly related to the lack of a functional retinoblastoma (gene) cell cycle regulator (81) and consequently no up-regulation of p21 after induction of DNA damage. Interestingly, cells without p21 show reduced cisplatin-induced DNA damage repair capacity and increased sensitivity to cisplatin (82). The treatment resistant mature teratomas show, in

contrast to other invasive components, positive staining for multiple proteins potentially related to treatment resistance. In addition, they are positive for retinoblastoma (gene) and p21 allowing them to go into G_1/S cycle arrest (83) (84). This might explain the observation that residual mature teratoma is found in about 30-40% in remnants of initial metastases after chemotherapy. A predictive model for the histology of a residual retroperitoneal mass, based on primary tumor histology, pre chemotherapy markers, mass size, and size reduction under chemotherapy, has been developed (85). Absence of teratoma elements or viable cancer cells in the primary tumor has been identified as the most powerful predictor for benign residual tissue (86). Caution is however warranted, because small teratoma areas may be missed in the primary tumor, and absence of teratoma elements does not exclude occurrence of malignant cells in residual masses. These findings may again be related to the origin of these tumors (87), because RB expression is not found in human fetal gonocytes and ITGCNU (88) (89).

Vascular invasion

Particular attention must be paid to the presence or absence of vascular invasion as a predictor of metastatic spread and occult metastases (90). Distinguishing venous from lymphatic invasion does not add information as to the risk of occult metastasis. Besides vascular invasion, high proliferative activity (assessed with the monoclonal antibody MIB-1), and to a lesser extent the presence of embryonal carcinoma in the primary tumor and a high pathologic stage, have been reported to be predictors of systemic spread in clinical Stage I NSGCT (for review, see (91)). However, the predictive value of this model is limited, as the group defined as high risk in fact has a 50% risk of occult metastasis, and the low risk group a 16% risk. Prospective assessment of risk factors for relapse in clinical Stage I NSGCT also showed that vascular invasion was the strongest predictive factor (92). With the addition of two other risk parameters (MIB-1 score >70% and embryonal carcinoma $\geq$50%) the positive predictive value increased to 63.6%. Thus, even with an optimal combination of prognostic factors and reference pathology, more than one third of patients predicted to have pathologic Stage II or a relapse during follow-up will not have metastatic disease and will be over-treated with adjuvant therapy. On the other hand, patients at low risk can be predicted with better accuracy (86.5%), suggesting that surveillance may be an option for highly compliant patients. Recently, cluster analysis has been used to identify prognostic subgroups in patients with embryonal carcinoma (93).

CLINICAL APPLICATION OF TUMOUR MARKERS IN TESTICULAR CANCER

Diagnosis

Patients with a testicular germ cell tumor may present with a painless testicular mass, while others also have symptoms caused by metastatic disease. The clinical workup comprises physical examination, ultrasound of the testis, and computerized tomography (CT) scan of the pelvis, abdomen, and chest. Determination of hCG, AFP and LDH in serum before therapy is mandatory in all patients. The marker concentration in serum is dependent on histological type and tumor load, i.e., stage. In a recent large collaborative study 64% of the tumors were NSGCT and 36% seminomas (94). Of the latter 77% presented with Stage I disease, i.e., tumor localized to the testis, and 21% had elevated serum levels of hCG. Of those with NSGCT 52% had Stage I disease and 79% had elevated marker levels (both hCG and AFP elevated in 44%, only AFP in 26% and only hCG in 9% (94). In seminoma patients hCG concentrations are usually below 300 U/L. Levels >1000 U/L are mostly associated with NSGCT. Levels >10000 U/L are mainly seen in patients with pure choriocarcinoma but occasionally may occur in seminoma. LDH is elevated in 40-60% of patients with seminoma or NSGCT (32). The classification of a tumor is based on histological examination, but if serum AFP is elevated, a tumor classified as a seminoma is reclassified as NSGCT and treated accordingly (1).

Staging, risk stratification and selection of therapy

Elevated serum concentrations of AFP, hCG, and LDH are associated with adverse prognosis (95) (96). A high serum hCG concentration is a strong prognostic factor, and the risk increases with increasing concentration (97). The International Germ Cell Cancer Collaborative Group (IGCCCG) has incorporated serum concentrations of hCG, AFP and LDH in a scheme for classification of metastatic germ cell tumors (Table 4). Tumors are classified as having good, intermediate or poor prognosis based on marker levels, primary site of the tumor, and presence or absence of non-pulmonary visceral metastases (96).

The selection of treatment is based on tumor type and prognostic group. Stage I seminomas may be treated by orchiectomy alone, which leads to cure in 80-85% of the cases. Orchiectomy in combination with radiotherapy of the abdominal lymph nodes leads to cure in 97-99% of the cases, and this approach is routinely used in many centers. Without radiotherapy 15-20% of the patients relapse, but most of these are cured by second line therapy. Therefore surveillance at increased frequency is an alternative to radiotherapy.

When treated by orchiectomy only, Stage I NSGCT have a 30% risk of relapse. The risk is higher (50%) if perivascular infiltration is present than if it is absent (risk 15-20%). The relapse risk if very low if retroperitoneal lymph node dissection (RPLND) is performed. This procedure is associated with morbidity and therefore surveillance is used as an alternative to RPLND. Chemotherapy is another alternative to RPLND, but residual retroperitoneal tumors consisting of teratomas, which need to be treated by surgery, are often observed. If serum marker levels do not normalize or increase after RPLND, positive retroperitoneal lymph nodes or systemic disease requiring chemotherapy are most likely present (98) (99).

After primary therapy elevated marker levels that are not explained by decreasing marker levels deriving from the primary tumor indicate metastatic disease, but normal marker levels do not exclude the presence of metastases (95).

Further risk stratification. Embryonal carcinoma is the most common cell type in NSGCT. It is totipotential and tumors with pure embryonal carcinoma are associated with early metastatic disease. There is therefore a need to estimate prognosis of tumors containing this cell type more accurately. Cluster analysis of the serum markers AFP and hCG in combination with the tissue markers p53, Ki67 and apoptosis index suggest that a pattern with high Ki67, low apoptosis, and low p53 is associated with better survival than other patterns. Classification with this algorithm has been reported to be independent of the IGCCCG classification (97). If these results can be confirmed, this could provide a tool for more precise tailoring of therapy.

Monitoring of Response to Therapy

If AFP or hCG in serum is elevated before therapy, the rate of marker decline reflects the response to therapy. Persistent marker elevation after chemotherapy indicates residual disease and the need for further therapy (100) (101). Chemotherapy may induce a transient increase or surge in marker concentrations during the first week of treatment (102).

The half-life of hCG is 1.5 days and that of AFP 5 days (103) (104). Half-lives >3.5 days for hCG and >7 days for AFP during chemotherapy predict recurrence and adverse prognosis (105). Marker half-life is calculated from the slope of the logarithm of the marker concentration versus time. It is preferable to use marker concentrations from several time points and to calculate the half-life from the slope of the regression line (32). The half-life should be determined after the initial marker surge during two cycles of chemotherapy between days 7 and 56. A slow rate of marker decline is of potential use in poor-risk patients and may imply a need for more aggressive therapy (105).

Surveillance

After successful primary therapy, all patients are monitored with physical examination, tumor marker determinations and CT scan. With such surveillance relapse is in most cases detected before clinical symptoms appear. Most relapses occur within the first year and relapses after two years are rare but some cases may relapse even after 10 years. The surveillance is tailored to take into account tumor type, Stage, treatment and likelihood of relapse (Table 3). Patients with good risk disease treated with surgery alone are monitored most frequently, e.g., every two weeks during the first 6 months. Some centers recommend weekly monitoring in order to detect a relapse before tumor grows to a size associated with adverse prognosis, as estimated by serum concentrations of AFP >500 U/L and of hCG >1000 U/L (106). In all patients monitoring is continued for five years (11).

CONCLUSIONS

Tumor markers are of central importance in the diagnosis, staging, risk assessment and monitoring of patients with testicular cancer. Several serum markers have been described but only AFP, hCG and LDH have been thoroughly validated and shown to have independent prognostic value. Several tissue markers may prove to be clinically important in the diagnosis and classification of testicular germ cell tumors. Germ cell tumors also display typical chromosomal abnormalities and amplification of 12p is sufficiently characteristic to be useful in the clinic to identify extratesticular germ cell tumors. Developments in DNA-based diagnostics have revealed a number of changes that may in the future enable more accurate stratification of prognosis.

Table 1. Currently available serum and tissue markers.

Marker	Proposed use	Level of evidence	Phase of development	References
Established serum markers				
AFP	Diagnosis Prognosis Staging Risk stratification Monitoring of disease	IA	Generally available	(1) (39) (95) (103)
hCG	Diagnosis Prognosis Staging Risk stratification Monitoring of disease	IA	Generally available	(1) (39) (52)
LDH	Diagnosis Prognosis Staging Risk stratification Monitoring of disease	IA	Generally available	(58) (94)
Potentially useful experimental serum markers				
hCGß	Diagnosis Monitoring	IV	Experimental	(45) (52)
LD-1	Diagnosis Risk stratification	IV	Experimental	(107)
PLAP	Diagnosis	IV	Experimental	(61) (60)
NSE	Diagnosis	IV	Experimental	(65) (66)
Established tissue markers				
PLAP	Histological typing ITGCNU	IIA	Antibodies for immunohistochemistry generally available	(14)
c-KIT, stem cell factor rec.	Typing of seminoma and ITGCNU	IIA	Antibodies for immunohistochemistry available	(18)
CD30	Embryonal carcinoma	IV	Antibodies for immunohistochemistry generally available	(108) (24)
AFP	Typing of yolk sac tumors and embryonal carcinoma	IIA	Antibodies for immunohistochemistry generally available	(14)
HCG	Typing of seminoma and choriocarcinoma	II	Antibodies for immunohistochemistry generally available	(14)
Amplification of 12p	Diagnosis of extragonadal tumors	II	Limited availability	(56) (57)
Vascular invasion	Risk stratification	II	Limited availability	(90)
Experimental tissue markers				
OCT3/4, POUF1	Risk stratification	IV	Experimental	(22)

AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; NSE, neuron specific enolase NSGCT, nonseminomatous germ cell tumors; PLAP, placental (germ cell) alkaline phosphatase.

Table 2. Recommendations for use of markers in testicular cancer by different Expert Groups.

	EGTM 1999 (8)	EAU 2001 (9)	NACB 2002 (10)	NACB 2005
AFP and hCG for				
Screening	N	N	N	N
Diagnosis / case-finding	Y	Y	Y	Y
Staging /prognosis	Y	Y	Y	Y
Detecting recurrence	Y	Y	Y	Y
Monitoring therapy	Y	Y	Y	Y
AFP for				
Differential diagnosis of NSGCT	Y	Y	Y	Y
LDH for				
Diagnosis / case-finding	Y	Y	Y	Y
Staging / prognosis	Y	Y	Y	Y
Detecting recurrence	Y	Y	Y	Y
Monitoring therapy	Y	Y	Y	Y

AFP, α-fetoprotein; hCG, human chorionic gonadotropin; LDH, lactic dehydrogenase; EGTM, European Group on Tumour Markers; EAU, European Association of Urologists; NACB, National Academy of Clinical Biochemistry

Table 3. Recommended frequency of tumor marker measurements in the follow-up of testicular cancer patients (11).

	Frequency of tumour marker measurements (no of times per year)					
	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6-10**
Stage 1 seminoma after radiotherapy	4	3	3	2	2	
Stage I seminoma surveillance after chemotherapy	6	4	3	2	2	1
Stage I NSGCT surveillance	6[a]	4[b]	2	2	2	[c]
Stage I NSGCT after RPLND or adjuvant chemotherapy	6	3	2	2	2	[c]
Stage IIa-IIb seminoma after radiotherapy	6	4	3	2	2	1
Stage IIa-IIB NSGCT after RPLND and chemotherapy or primary chemotherapy	4	2	2	2	2	1
Seminoma and NSGCT of advanced stage	12	6	4	3	2	1

[a] Measurements every two months recommended; measurements every month for the first six months advisable.
[b] Measurements every three months recommended; measurements every two months advisable.
[c] Measurement once a year advisable.

Table 4. Classification of metastatic germ cell tumors into various risk groups according to the International Germ Cell Consensus Classification (8).

GOOD PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Testis/retroperitoneal primary **and** No non-pulmonary visceral metastases **and** Good markers - all of AFP < 1000 µg/L and hCG < 5000 U/L (1000 µg/L) and LDH < 1.5 x N (upper limit of normal) **56% of non-seminomas** **5 year PFS 89%** **5 year Survival 92%**	Any primary site **and** No non-pulmonary visceral metastases **and** Normal AFP, any hCG, any LDH **90% of seminomas** **5 year PFS 82%** **5 year Survival 86%**
INTERMEDIATE PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Testis/retroperitoneal primary **And** No non-pulmonary visceral metastases **And** Intermediate markers - any of: AFP $\geq$ 1000 and $\leq$ 10,000 µg/L or hCG $\geq$ 5000 U/L and $\leq$ 50,000 U/L or LDH $\geq$ 1.5 x N and $\leq$ 10 x N **28% of non-seminomas** **5 year PFS 75%** **5 year Survival 80%**	Any primary site **and** No non-pulmonary visceral metastases **and** Normal AFP, any hCG, any LDH **10% of seminomas** **5 year PFS 67%** **5 year Survival 72%**
POOR PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Mediastinal primary **Or** Non-pulmonary visceral metastases **Or** Poor markers - any of: AFP > 10,000 µg/L or hCG > 50,000 U/L (10000 µg/L or LDH > 10 x N **16% of non-seminomas** **5 year PFS 41%** **5 year Survival 48%**	**No patients classified as poor prognosis**

AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ,; LDH, lactic dehydrogenase; N, upper limit of normal; PFS, progression free survival.

Table 5. Analytical requirements and potential interfering factors for established and experimental serum markers

Established markers			
Marker	Sample type	Analytical requirements	Confounding factors
AFP	Serum or plasma	Detection limit <2 µg/L	Hepatitis Heterophilic antibodies Drug-induced hepatic damage Hepatocellular cancer
HCG	Serum or plasma Urine to confirm false results	Detection limit <5 U/L Cross-reaction with LH <2% Equimolar recognition of hCGβ (or use of separate assay for hCGβ)	Chemotherapy-induced elevation of hCG to >10 U/L Heterophilic antibodies Nontrophoblastic cancers producing hCGβ
LDH	Serum	Reference values are method-dependent Clinical decision limits based on upper reference limit.	Elevated values also caused by - Hemolysis - Liver disease - Muscle disease - Myocardial infarction
Experimental markers			
hCGβ	Serum or plasma	Detection limit 0.5 pmol/L	Nontrophoblastic cancers
LD-1	Serum	Reference values method-dependent	Hemolysis, muscle disease, heart disease
PLAP	Serum	Reference values method-dependent	Smokers may have 10-fold increased values
NSE	Serum	Reference values method-dependent	Hemolysis causes falsely elevated values

AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; hCGα, free α-subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; NSE, neuron specific enolase; PLAP, placental (germ cell) alkaline phosphatase.

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