Testicular tumor markers: Corner-stones in the management of malignant germ cell tumors

Hoden-Tumormarker: Eckpfeiler in der Behandlung maligner Keimzelltumoren

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Abstract

Management of testicular tumor patients without marker assessment is unacceptable in these days. Markers are included in the TNM scheme since 1997 in form of the S classification. The combination of AFP, hCG, LDH and PLAP shows high specificity and sensitivity, in contrast to most tumor markers in other malignancies.

While preoperative markers are usually available, it is less common to monitor the immediate postoperative course. Still there are patients with the first control of tumor markers four weeks after surgery. In patients showing normal markers after four weeks, one may have missed a prolonged marker decline, such overlooking remaining tumor tissue which could have been treated more easily if detected in time, and not as late as metastases can be identified by imaging alone. If markers have not normalized after four weeks, there is a substantial delay in starting adjuvant treatment with a probable decreased chance for cure of the disease.

Furthermore, tumor markers are important for classifying the risk of primary metastatic disease as well as for evaluation of response to chemotherapy. Marker normalization is a prerequisite for successful post-chemotherapy surgery. During follow-up, rising markers are able to identify relapsing tumors at an earlier stage than imaging techniques alone.

Keywords: testicular cancer; tumor marker; AFP; hCG; LDH; PLAP.

Zusammenfassung

Die Behandlung maligner Hodentumoren ohne die Bestimmung der Tumormarker ist heute nicht mehr akzeptabel. Tumormarker sind seit 1997 als S Klassifikation Bestandteil des TNM Schemas. Die Kombination von AFP, hCG, LDH und PLAP zeichnet sich durch eine hohe Spezifität und Sensitivität aus, die von den Tumormarkern anderer Karzinome meist nicht erreicht wird.

Während üblicherweise Marker präoperativ bestimmt werden, ist es leider weniger üblich, den unmittelbaren postoperativen Verlauf mittels Markern zu überwachen. Nach wie vor gibt es Fälle, bei denen die erste Tumormarker-Kontrolle erst nach vier Wochen durchgeführt wurde. Bei Patienten mit normalen Markern nach dieser Zeit kann es sein, dass eine verlängerte Halbwerts-Zeit und damit Mikrometastasen übersehen wurden, die, rechtzeitig erkannt, leichter therapierbar gewesen wären als erst durch die bildgebenden Verfahren erfassbare Metastasen. Falls die Marker nach diesen vier Wochen nicht normalisiert sind, wurde der Beginn einer adjuvanten Chemotherapie verzögert, was möglicherweise zu einer Verschlechterung der Heilungschancen führt.

Marker sind weiters wichtig zur Risikoeinschätzung primär metastasierter Hodentumoren und zur Beurteilung des Therapie-Ergebnisses. Normalisierte Marker sind die Voraussetzung für eine erfolgreiche Operation nach Chemotherapie. In der Nachsorge können Marker ein Rezidiv in einem früheren Stadium anzeigen, als das mit bildgebenden Techniken alleine möglich wäre.

Schlüsselwörter: Maligner Hodentumor; Tumormarker; AFP; HCG; LDH; PLAP.

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Introduction

Malignant Germ Cell tumors are the most frequent male neoplasms between 15 and 34 years of age with wide geographic varieties. The incidence of germ cell tumors in men has doubled within the last 40 years and affects now 6.34/100 000 in the European Union. The peak incidence is seen at the ages 30 to 34 at 17/100.000. The death rate dropped from 1.5 in the early 70s to 0.4/100.000 today, giving an overall cure rate of more than 95% mainly by the development of cisplatinum-based chemotherapy and strict follow-up procedures with tumor markers and imaging techniques.

Male germ cell tumors are the sole tumor entity not only characterized by the TNM-system, but since the 5th edition UICC 1997 [1] are also defined by "S"-staging which has been exclusively developed only for this malignancy. Introduction of the "S"-staging, being the abbreviation for the assessment of serum tumor markers alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and lactat-dehydrogenase (LDH), highlights the importance of tumor markers for diagnosis, prognosis and monitoring of therapy as well as for follow-up in this group of patients (Table 1).

Diagnosis and preoperative staging investigations

Patients present to the urologist because of an enlarged, hard and sometimes painful testicle. Mandatory preoperative investigation consists of clinical evaluation, sonography of both testes, contrast medium-enhanced CT scan of chest and abdomen and determination of serum tumor markers.

Preoperative marker levels alone do not have an impact on staging and prognosis, but they may prove the diagnosis of a malignant testis tumor and may give a hint at the tumor compounds [3]. In case of a retroperitoneal mass with normal testicles, marker examination is important to discriminate an extragonadal germ cell tumor from tumors of other origin, especially mandatory in young patients to avoid open biopsies.

The lowest ever achieved postoperative level (nadir) and the marker decrease according to the typical half-lifetime characterize the tumor stage. Therefore it should be clear that one single postoperative marker assessment is insufficient [4].

Markers in male germ cell tumors

80% of all germ cell tumors present with elevated markers at some point during the course of the disease. The commonly used combination of AFP, hCG, LDH and PLAP shows high specificity and sensitivity, in contrast to most tumor markers in other malignancies. Nevertheless one should be aware of other associated causes of elevation of the markers mentioned, that are not germ cell tumor related (Table 2).

AFP

Under normal circumstances, alpha-fetoprotein is produced by the yolk sac and fetal liver cells. A natural increase is seen during fetal time. Later on, until the 10th year, AFP decreases to levels below the normal upper value of 10 μg/l.

50–70% of the germ cell carcinomas show increased AFP levels (embryonal carcinomas 70%, yolk sac tumors

Table 1 S category of TNM system (UICC (2002) for testicular germ cell tumors [2]. RR = reference range.

	LDH (multiples of reference range)	hCG (U/l)	AFP (μg/l)
S x	Markers not available		
S 0	Normal markers		
S 1	< 1,5 × RR	< 5000	< 1000
S 2	1,5 × RR–10 × RR	5000–50.000	1000–10.000
S 3	> 10 × RR	> 50.000	> 10.000

75%). Pure seminomas and chorioncarcinomas never produce AFP. Elevated AFP after therapy at a stable low level may be caused by cystic teratomatous elements, only turning negative after their operative removal [5]. Half-lifetime is not very well defined and varies between three to six days.

HCG

Human chorionic gonadotropin (HCG) is produced by trophoblastic structures or syncytiotrophoblastic giant cells [7, 8]. It consists of an alpha and a beta subunit. The alpha subunit is homologous with the alpha subunits of LH, FSH and TSH. The beta subunit is 70% homologous with the beta chain of LH. HCG shows elevated values in 15% of seminomas, 80% of embryonal carcinomas and 100% of chorioncarcinomas.

High levels of HCG may cause gynecomastia and testicular atrophy, very high levels may even cause hyperthyreosis due to cross reactions with the TSH receptors in the thyroid gland [9].

The short half-lifetime of only 16 to 36 hours facilitates follow-up during and after therapy.

LDH

Lactat-dehydrogenase (LDH) is an enzyme expressed in response to tumor cell regression as well as damage, cytolytic and inflammatory processes in many tissues including smooth, cardiac and skeletal muscle, as well as kidney, liver and erythrocytes. There are five isoforms of LDH, LDH-1 being the most frequently elevated isoenzyme in testis cancer [10]. The LDH-1 level also correlates with the number of chromosomal copies of 12p, the gene locus of LDH-1 isoenzyme [11].

LDH is mainly important for risk calculation in metastatic disease (S-stage and IGCCCG, Table 3) similar to lymphomas and non-Hodgkin lymphomas. In case of AFP and HCG negative bulky disease, it is the only parameter for the evaluation of response to therapy.

PLAP

Human placental alkaline phosphatase consists of two isoenzymes: placental alkaline phosphatase (PLAP) and the 98% identical germ cell alkaline phosphatase

(GCAP), both originating from syncytiotrophoblast cells. It is detectable in up to 90% of seminomas and 15 to 35% of nonseminomas and intratubular neoplasia (TIN). The upper value is 100 mU/l, half-lifetime is not well defined, but should be shorter than three days in nonsmokers [12].

Elevated values may also be seen in other cancer entities (liver, pancreas, stomach, ovary and lung). The main disadvantage of this marker is its up to tenfold elevation in more than 80% of cigarette smokers [13, 14]. In patients who smoke, an elevation over the individual level may be a hint for recurrent disease. A long stay in smoky rooms without sufficient ventilation may cause transient PLAP-elevation also in nonsmokers.

To date, PLAP is the most frequent elevated marker in seminomas and a reliable tool for their monitoring. The clinical usefulness in nonseminomas is not yet clear. Due to the work intensity for laboratory-evaluation it may be routinely used in non-smoking seminoma patients only.

Other possible causes of marker elevation

Although rare at the affected age, tumor marker elevation can also be found without evidence of a germ cell tumor (Table 2) [8, 15–19]. The origin of AFP (malignant yolk sac or liver) could be discriminated because of differences in glycosylation of AFP-proteins but so far no routine-test is available [20, 21].

Table 2 AFP, HCG and LDH elevation in men without germ cell tumors [8, 15–19].

	Neoplasms	Non malignant diseases
AFP	Hepatocellular, gastric, colon, pancreatic, biliary and lung carcinoma, liver metastases	Virus hepatitis, liver cirrhosis, fatty liver hepatitis Ataxia teleangiectatica hereditary tyrosinaemia hereditary persistent AFP
hCG	Pancreatic, islet cell, gastric, intestinal, stomach, lung, ovarian, renal, urothelial and mamma carcinoma, hepatoma, retroperitoneal sarcoma, melanoma, Non-Hodgkin lymphoma, M. Hodgkin, myeloma, leukemia	Cannabis consumption, hytadidiform mole, hypergonadotropic hypogonadism
LDH	Leukemia, all cancers, mainly in metastatic stage of disease	Skeletal muscle disease, myocardial infarction, pulmonary embolism, hemolysis, pernicious anemia, thalassemia

Preanalytical handling of blood samples

To avoid serum-level amplification by releasing of tumor markers into the circulation, clipping of the vena spermatica should be performed before operative manipulation of the testis.

No data are available on circadian changes of the reviewed tumor marker levels, hence these changes cannot be ruled out, blood sampling should always be performed at the same time of the day. Conditions that may elevate the markers should be reported to the laboratory.

For the en bloc determinations of AFP, hCG and PLAP serum is aliquoted, frozen and stored at $-20°$ C for a maximum period of two weeks. Storage for a longer period should be at $-70°$ C.

Laboratory procedures

Blood specimen should be centrifuged for 10 minutes at $3000 \times$ g to avoid thrombocyte contamination causing artefactual increase of LDH. Furthermore, special attention has to be given to hemolysis. Hemolytic samples may not be used for LDH analyses. Although there is less interference with hemolysis for the determination of AFP, hCG and PLAP, for all tests the manufacturers' recommendations for interference with hemolysis, icterus and lipemia have to be taken into account.

The half-lifetime of a marker can only be calculated after repeated measurements if the tumor marker is determined with the same method to avoid analytical variations attributable to different test kits. This is of special importance for the determination of hCG. There are more than 100 commercial tests available using different antibody combinations such resulting in more or less clear interassay discordance [22]. However, tumors are capable of secreting various hCG-related molecules like nicked hCG and especially free hCGβ [6]. Therefore it is of fundamental importance, when analyzing testicular tumors, that a combination of antibodies is used that detects not only hCG but also nicked hCG as well as free hCGβ. These nicked isoforms are not recognized by some RIAs which may give false-negative values or an underestimation of the hCG levels [23]. False-negative values may also occur due to interference with one-step immunoassays [24]. In spreading tumors with high hCG concentrations, determination of hCG with immunometric methods may result in false low values (high dose hook effect [25]). Sample dilution and re-measurement should therefore be performed if hCG values do not rationally correspond to the tumor mass.

Several enzyme immunoassays and fluorescence immunoassays are available which detect AFP down to 0.1 μg/l. In contrast to the determination of hCG, the use of different kits with varying antibody specificities does not result in relevant analytical variations [7]. Nevertheless, to achieve comparable results in tumor markers for the calculation of the individual tumor marker half-lifetime, en

bloc measurement is recommended and change of reagent types have to be avoided.

For the determination of PLAP, only the INNOTEST hPLAP (Innogenetics N.V., Gent, Belgium) microplate enzyme immunoassay is available which shows cross-reactivity with other alkaline phosphatase isoenzymes below 0.01%. As this immunoassay cannot be performed automatically, special attention has to be given to the intra– and inter-assay variability (the general recommendation is $< 5\%$ and $< 10\%$) [25].

It is very helpful to follow the EGTM Consensus Recommendations on quality requirements [25], especially in case of changing methods which have to be reported and interpreted to the treating physician.

Calculation of half-lifetime

Half-lifetime of tumor markers is calculated according to the following formula, using two levels of two consecutive days [26]:

$$t/2 = \frac{-0,693 \times \Delta T}{\ln (concT/concT_0)}$$

t/2: half-lifetime; ΔT: Time between marker determinations in days, ln: logarithmus naturalis, conc T: current marker level, $concT_0$: first level

In case of prolonged half-life (AFP $>$ 5 days, HCG $>$ 2 days) without radiological signs of disseminated disease, the tumor is classified as stage I S. The same is true for PLAP in seminomas ($>$ 3 days) although not yet established.

Optimal frequency of pre- and postoperative marker-assessment

The optimal timing of perioperative marker assessment is not well defined. Therefore it is important to have two marker-pairs for half-lifetime calculation. The two values should be either equal or the second should be lower than the first, even within "normal" ranges. If the second one is higher than the first one, this is proof for clinically undetected tumor tissue and staging has to be corrected into stage I S with consequent systemic treatment [27]. Two pairs of markers for half-lifetime calculation will also help to define patient's individual marker half-lifetime.

Monitoring marker dynamics is most important for staging and estimation of prognosis. Not a single value but half-lifetime, postoperative nadir and the course over time will define the current stage of the disease such making sufficient therapy decisions possible.

Taking into account the different half-lifetime of the markers discussed, the following management is recommended for daily practice:

Assessment of AFP, HCG, PLAP and LDH preoperatively (day **–1**) and three postoperative samples. Common time points are day 1, 5 and 10 or better day 1, 3 and 7. In the rare case of non-normalization, thereafter in weekly intervals until the nadir level is reached. This is important to have sufficient information for starting adjuvant therapy within a short time of three to four weeks after operation.

Markers in localized disease

Nonseminomas Stage I und I S

If the primary tumor has been removed and there is no radiological sign of metastasis, and tumor markers in serum decrease to normal ranges corresponding to half-lifetime, the patient is in stage I. Stage I is discriminated into I A (without vascular invasion) and I B (venous or lymphatic invasion of vessels). I B tumors should be adjuvantly treated by two courses of BEP chemotherapy because of its approximately 50% risk for relapse [28].

The case is different if, without radiological correlation of metastasis, serum markers postoperatively do not or only for a short while decrease and then increase again. If excluded that the rise of markers is due to some other origin, one has to assume a stage I S. Even micrometastasis, radiologically undetectable, can cause very high marker levels. Depending on the level the risk will be evaluated identically to patients with metastasis and chemotherapy will be induced [15, 27, 29].

Seminomas Stage I

Pure seminomas are expressing pathological values of PLAP in 45–60% [14], LDH in 30% and HCG in up to 20%, but all of them are AFP-negative. Every germ cell tumor which seems to be a pure seminoma but demonstrates pathological AFP levels must be re-evaluated for non-seminomatous elements. Anyhow the tumor has to be classified and treated as a nonseminoma.

Staging and prognosis of metastatic disease

An exemplary international collaboration within the International Germ Cell Cancer Collaborative Group (IGCCCG) defined prognostic groups in metastatic germ cell tumors in men [29]. This classification is based on the site of the primary tumor (gonadal or extragonadal), the localization of metastases (pulmonary or non-pulmonary visceral), as well as the extent of elevation of the markers AFP, HCG and LDH (Table 3). The "intermediate prognosis" group in nonseminomas is exclusively based on the extent of tumormarkers!

Evaluation of response to chemotherapy

The role of velocity of marker-decline during the first chemotherapy course in metastatic disease is not yet clearly

Table 3 IGCCCG classification for metastatic disease [29].

Nonseminomatous Tumors			
Good Prognosis [56%]	Primary tumor testis or retroperitoneum **and** good markers: S1 **and** no non-pulmonary visceral metastases	PFS: OS:	89% 92%
Intermediate Prognosis [28%]	Primary tumor testis or retroperitoneum **and** intermediate markers: S2 **and** no non-pulmonary viseral metastases	PFS: OS:	75% 80%
Poor Prognosis [16%]	Primary tumor testis or retroperitoneum **and** non-pulmonary visceral metastases **or** poor markers: S3 **or** primary mediastinal tumor	PFS: OS:	41% 48%
Seminomas			
Good Prognosis [90%]	Any primary tumor and any marker levels **and** no non-pulmonary visceral metastases	PFS: OS:	82% 86%
Intermediate Prognosis [10%]	Any primary tumor and any marker levels **and** non-pulmonary visceral metastases	PFS: OS:	67% 72%

PFS:% Progression free survival
OS:% Overall survival
S1, S2, S3: Serum tumor marker levels according to UICC [2],(Table 1).

defined on account of conflicting results [30–33]. A transient elevation of AFP during the first course may be an unfavorable prognostic sign [34].

For definition of complete remission (CR), the tumor markers have to be in normal range after six weeks of standard chemotherapy. To characterize persistent residual masses in advanced tumors stages, the terms "partial remission marker-negative" (PRm-) and "partial remission marker-positive" (PRm+) have been introduced. Resection of residual masses in PRm- nonseminomatous germ cell tumors will show necrosis or mature teratoma only in 80 to 90% of cases, not requiring further therapy, while in only 10 to 20% persistent vital tumor tissue will be found [35]. PRm+ represents therapy failure of first line chemotherapy with poor prognosis requiring salvage treatment. One should be aware of patients with initially very high tumor marker levels, which after chemotherapy may show the phenomenon of hCG plateau, which does not necessarily correlate with residual tumor tissue [36]. In the rare case of patients not showing any decrease of tumor markers under chemotherapy (absolute refractory disease), the prognosis is even worse [37].

On the contrary, if there is the tendency to normalizing marker levels and increase of metastatic tumors, one has to be aware of the so-called "growing mature teratoma syndrom", which requires completion of chemotherapy with subsequent resection of the teratoma [36].

Follow-up

Approximately two thirds of relapsing malignant germ cell tumors will present with pathological tumor marker levels [38]. In more than one third tumor marker elevation will be the first detectable sign of recurrent disease [39]. Therefore marker determination is an inevitable must during follow-up together with chest X-ray and abdominal CT scans. Follow-up without markers will cause at least unnecessary harm to the patients if not result in some patients' death. Furthermore avoidable additional costs may araise from a management without markers.

70% of relapsing tumors will be seen within the first year, 96% within the first and the second year after primary therapy. Recommendations of the European Group on Tumor Markers (EGTM) for follow-up examinations in non-seminomas are marker measurement, chest X-ray and clinical examination monthly in the first year, every second month in the second year and every three months thereafter [3]. Seminomas may be followed every three months. Routine investigation of tumor markers is also mandatory in patients with primary normal marker levels, because metastasis may be able to produce elevated markers [39].

If during follow-up positive markers seem to be the only proof of a relapse, markers should be repeated to exclude laboratory failures. Other possible causes for marker elevation (Table 2) and a tumor in the contralateral testis should be excluded. On the other hand in the rare case of a suspicious relapse found by radiological imaging without elevated markers, histological proof is mandatory. Such masses may be of different origin or mature or malignant teratomas which only may be cured by surgery.

Acknowledgements

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