Review article
Serum tumor markers in testicular cancer
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Abstract
Testicular cancer has become a model for a curable neoplasm, where biochemical markers play a critical role. Serum tumor markers are integral in patient management and contributes to the diagnosis, staging, and risk assessment, as well as evaluation of response to therapy and detection of relapse. We review their biochemistry, biology, and clinical use in the setting of localized and metastatic disease. The integration of tumor markers in prognostic models as well as the significance of marker kinetics during chemotherapy is discussed. © 2013 Elsevier Inc. All rights reserved.

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Introduction
Germ cell tumor (GCT) is a unique neoplasm where biochemical markers play a critical role. Serum tumor markers in patients with testicular cancer are integral in patient management, contributing to diagnosis, staging and risk assessment, evaluation of response to therapy, and detection of relapse [1,2]. This review will detail the biochemistry and biology as well as the clinical usefulness of these markers.

Biochemistry and biology
α-Fetoprotein (AFP)

AFP is single-chain glycoprotein with a molecular weight of 70,000 Da. In the fetus, AFP is a major serum binding protein produced by the fetal yolk sac, liver, and gastrointestinal tract. The highest concentrations approach 3 mg/ml during the 12th to 14th weeks of gestation and decline to <40 ng/ml 1 year after birth [3]. The metabolic half-life of AFP is between 5 and 7 days.

In germ cell tumors AFP is secreted by embryonal cell carcinoma and yolk sac tumor but not by pure choriocarcinoma or pure seminoma. Yolk sac tumors appear to be the primary source of AFP with more than 90% of tumors reacting positively to anti-AFP antibody, and in those tumors not demonstrating reactivity to anti-AFP antibody, serum levels are not elevated [4,5].

Excluding GCT, the most impressive serum AFP levels are seen in patients with hepatocellular carcinoma [6]. AFP can be elevated in other neoplasms, including pancreatic cancer (23%), gastric cancer (20%), colorectal cancer (5%), and bronchial cancer (7%) [7]. Non-neoplastic liver diseases are also associated with a raised serum AFP and include viral hepatitis, cirrhosis, and liver trauma [8]. AFP levels secondary to benign liver disease rarely exceed 500 ng/ml [6,9]. The AFP gene is located on chromosome 4 and a hereditary persistence of an elevated AFP through an autosomal dominant trait has been reported [10].

The carbohydrate moiety of AFP exhibits heterogeneity due to post-translational modifications in which different carbohydrates are added to the polypeptide backbone [11]. Based on the difference in their binding affinity to various lectins and particularly concanavalin-A, it is possible to distinguish with very high sensitivity and specificity AFP of GCT and liver origin [12]. Despite initial promising results [13,14], wide acceptance awaits further clinical trails, as the
Human chorionic gonadotropin (hCG)

Primary production of hCG occurs during pregnancy by the syncytiotrophoblastic cells of the placenta, which maintains the corpus luteum. In GCT, syncytiotrophoblastic cells are responsible for the production of hCG. All patients with choriocarcinoma and 40% to 60% of patients with embryonal cell carcinoma have elevated serum levels of hCG. Approximately 10–20% of patients with pure seminoma have elevated serum hCG though the level is typically below 500 mIU/m. The serum half-life of hCG is between 24 and 36 hours.

hCG is a glycoprotein with a molecular weight of 38,000 Da composed of α and β subunits. The α subunit closely resembles that of other pituitary hormones, including luteinizing hormone (LH), follicular stimulating hormone, and thyroid stimulating hormone. The β subunit contains a 24 amino acid C-terminal extension making it antigenic distinct from the other pituitary hormones allowing production of antibodies used in radioimmunoassay [16,17].

For most assays the upper limit of normal is between 5 and 10 mIU/ml. Some cross reactivity with the β subunit of LH can occur resulting in a false positive test. With the development of more sensitive and specific assays, it became evident that the pituitary gland is capable of producing hCG [18]. Furthermore, hypogonadism can induce LH as well as hCG production by the pituitary gland [19]. Short course of testosterone replacement suppresses pituitary LH and hCG secretion allowing for a “true” measure of serum hCG of potential germ cell origin [20]. Marijuana use has been attributed to a falsely elevated serum hCG, although conflicting data have been reported [21,22]. Various tumors can produce hCG and include liver, pancreas, stomach, breast, kidney, and bladder cancer.

Lactate dehydrogenase (LDH)

LDH is a cytoplasmatic enzyme with a molecular weight of 134,000 Da found in all living cells. LDH catalyzes the reduction of pyruvate to lactate. LDH measured in the serum is a mixture of 5 isoenzymes each as a tetramer formed by a combination of two different subunits encoded by structurally distinct genes, LDHA and LDHB [23]. GCT patients typically express high levels of LDH isoenzyme 1 (LDH-1) [16]. Dying and dead cells leak LDH, which can be measured in the serum. As such, there is a direct relationship between tumor burden and LDH levels.

Serum tumor markers, especially AFP and hCG, are valuable in monitoring response to therapy. Since LDH does not arise from a single source and increased concentrations often reflect other disease entities, it must be incorporated with other clinical signs when making management decisions [24,25]. Venkitaraman et al. estimated that 47% of all patient-visits where serum LDH was elevated were actually false positive results [25].

Placental alkaline phosphatase (PLAP)

PLAP is fetal isoenzyme that is frequently elevated in patients with seminoma (60%–70%). Serum concentrations of PLAP are increased up to 10-fold in smokers [26]. With this, as well as the limited commercial assays, it is not routinely utilized in the management of GCT.

Clinical application of tumor markers

Diagnosis

Serum tumor markers should be obtained prior to radical orchectomy. The classification of GCT is based on histologic examination. However, if AFP is elevated in a tumor diagnosed as seminoma based on histology, it is reclassified as nonseminomatous germ cell tumor (NSGCT) and treated accordingly [1]. As seminoma does not produce AFP, nonseminomatous histology is presumed to be in a metastatic lesion or occult in the testis primary.

At presentation, approximately 50% to 70% of patients with NSGCT have an elevated serum AFP and in 40% to 60% hCG is raised. Measured together, up to 90% have an elevation of one or both serum tumor markers. In clinical stage 1 (confined to the testis), fewer patients present with elevated serum tumor markers. Forty-five percent of patients with clinical stage 1 NSGCT have normal pre-orchectomy levels of AFP or hCG [27,28], and in 33% both markers are negative [29].

Marker concentration is dependent on histologic subtype and tumor burden. The Spanish Germ-Cell Cancer Group [30] reported on the clinical presentation of 1,490 patients with GCT. Seventy-seven percent of the patients with seminoma, and 45% of the patients with nonseminoma, had clinical stage 1 disease. hCG was elevated in 21% of seminoma patients, and AFP and/or hCG were elevated in 79% of nonseminoma cases. Abnormal LDH levels were identified in 8% and 14% for seminoma and nonseminoma patients, respectively.

Serum tumor markers in clinical stage 1

The absence of yolk sac tumor in the orchietomy specimen was shown to predict relapse in patients who underwent surveillance for clinical stage 1 NSGCT [31]. Furthermore, normal AFP at presentation (pre-orchietomy) was associated with the absence of yolk sac histology and an increased risk for occult retroperitoneal metastasis [28,32]. A recent study [28] evaluated patients with clinical stage 1 NSGCT with normal post-orchietomy serum tumor markers who underwent primary retroperitoneal lymph node dissection (RPLND). The risk of occult retroperitoneal metas-
tasis was 18.5% vs. 33% in patients presenting with elevated or normal pre-orchiectomy AFP, respectively \( (P = 0.001) \). Similarly, 73% of patients with elevated pre-orchiectomy level had yolk sac elements in the orchiectomy specimen compared with only 39% when pre-orchiectomy AFP was normal. However, conflicting results \([29,33]\) limit the use of pre-orchiectomy marker status as a predictor of occult disease, and further management of clinical stage 1 testicular cancer is dependent on the response of the serum tumor markers to the removal of the primary tumor.

Patients whose serum tumor markers normalize after orchiectomy are clinically disease free and therefore surveillance is a reasonable treatment option \([34]\). Sites of recurrence for patients failing surveillance include the retroperitoneum and lung most commonly \([31]\). Of patients failing surveillance, 16% to 45% have only radiological evidence of disease without marker elevation, while 13% to 29% have only marker elevation at time of relapse \([29,31–36]\). The pattern of markers at recurrence cannot be predicted by their pre-orchiectomy status. In one study, 30% of patients who initially had positive markers relapsed with undetectable markers, while 60% who initially had negative marker levels presented with elevated markers at first recurrence \([37]\).

Patients with clinical stage 1 disease who fail to normalize their serum tumor markers following removal of the primary tumor are categorized as clinical stage 1S. Saxman et al. \([38]\) reported on the outcomes of 30 patients with clinical stage 1S NSGCT who underwent primary RPLND. Of the 6 patients with an elevated AFP with or without concurrent hCG elevation, 5 (83%) relapsed and required chemotherapy as did 6 of 24 (25%) with only hCG elevation, while 13% to 29% have only radiological evidence of disease without marker elevation, while 16% to 45% have only radiological evidence of disease without marker elevation, while 13% to 29% have only marker elevation at time of relapse \([29,31–36]\). The pattern of markers at recurrence cannot be predicted by their pre-orchiectomy status. In one study, 30% of patients who initially had positive markers relapsed with undetectable markers, while 60% who initially had negative marker levels presented with elevated markers at first recurrence \([37]\).

For the purpose of model simplification, marker levels were not included in the seminoma IGCCCG risk model. However, other studies emphasized the importance of LDH in patients with metastatic seminoma. In a multi-institutional study, an elevated level of LDH (\( \geq 2 \times \) upper limit) and the presence of non-pulmonary visceral metastases were independent adverse prognostic factors in patients with advanced seminoma \([41]\). Others investigators have identified potential prognostic significance in LDH isoenzyme 1 \([42]\). A prospective multicenter trial evaluated the significance of hCG elevation in 726 patients with metastatic pure seminoma \([43]\). While hCG elevation was asso-

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Table 1
International Germ Cell Consensus Classification prognostic groups in patients with metastatic disease treated with first line chemotherapy

<table>
<thead>
<tr>
<th>Prognostic Group</th>
<th>NSGCT</th>
<th>Seminoma</th>
<th>5-year survival*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Good prognosis</strong></td>
<td>Primary site: testis or RP and metastases: nodal or pulmonary and marker level: S1</td>
<td>Primary site: all and metastases: nodal or pulmonary and marker level: any LDH, any hCG</td>
<td>Seminoma</td>
</tr>
<tr>
<td><strong>Intermediate prognosis</strong></td>
<td>Primary site: testis or RP and metastases: nodal or pulmonary and marker level: S2</td>
<td>Primary site: all and metastases: non-pulmonary visceral and marker level: any LDH, any hCG</td>
<td>NSGCT</td>
</tr>
<tr>
<td><strong>Poor prognosis</strong></td>
<td>Primary site: mediastinal or metastases: non-pulmonary visceral or marker level: S3</td>
<td>No patients classified as poor prognosis</td>
<td>NSGCT</td>
</tr>
</tbody>
</table>

NSGCT = nonseminomatous germ cell tumor; RP = retroperitoneum.

S1: a-fetoprotein (AFP) <1000 ng/mL, and human chorionic gonadotrophin (hCG) <5000 mIU/mL, and lactic dehydrogenase (LDH) <1.5 upper limit of normal.

S2: AFP = 1000–10,000 ng/mL, or hCG = 5000–50,000 mIU/mL, or LDH = 1.5–10 upper limit of normal.

S3: AFP >10,000 ng/mL, or hCG >50,000 mIU/mL, or LDH >10 upper limit of normal.

* Survival data for Seminoma patients based on IGCCCG \([1]\). Survival for NSGCT patients is based on a more recent meta-analysis \([77]\).
associated with a larger tumor mass (primary tumor and/or metastases), predictors of recurrence included stage of disease, and elevation of LDH and not hCG elevation.

**Monitoring response to therapy for NSGCT**

The treatment of metastatic NSGCT consists of cisplatin-based chemotherapy. Responses include (1) complete remission, defined by normalization of serum tumor markers and resolution of radiographic disease, (2) normalization of serum tumor markers with persistent radiographic tumor (partial remission marker negative), (3) partial remission marker positive, and (4) disease progression. The majority of patients normalize serum tumor markers with induction chemotherapy. The Southeastern Cancer Study Group reported marker normalization in 183 of 184 patients with minimal or moderate extent of disease according to the Indiana classification system [44]. Approximately 90% of this study cohort would have been categorized as good risk according to the IGCCCG. Of the patients who achieve markers normalization, about 70% will also have complete radiographic response, thereby obtaining complete remission. As the relapse rate is about 5%, we observe these patients without post chemotherapy RPLND [45]. In the remaining 25%–40% of good risk patients a residual mass will persist after chemotherapy despite normalization of serum tumor markers [44,46,47]. Post-chemotherapy surgery is endorsed due to high incidence of residual teratoma or active cancer [48]. Slightly elevated AFP or HCG after induction chemotherapy, with no increasing levels, is not a contraindication to post-chemotherapy surgery [49,50]. Furthermore, a few patients have been described in whom markers plateau at a modestly elevated level despite no evidence of disease [51]. Typically, these patients present with hCG >50,000 mIU/ml. When followed over time, hCG levels eventually normalize [52].

In poor-risk disease, approximately 40% fail to normalize their markers after first line chemotherapy [53]. Regardless of risk classification, failure of serum tumor normalization typically indicates residual or refractory malignancy (unless another cause is identified). No histologic confirmation is required. The brain and the remaining testicle are considered sanctuary sites and should be evaluated radiographically. These patients are candidates for salvage chemotherapy or surgery. Prognostic factors have been identified for patients failing or relapsing after conventional-dose chemotherapy. Within this group, high levels of hCG (>1,000 U/L) before initiation of high dose chemotherapy was found to be an independent adverse prognostic variable [54]. In a more contemporary series from the Indiana University no difference in disease-free survival was found on the basis of hCG levels [55].

Various attempts have been made to identify those patients in the high risk group destined to fail first line chemotherapy. Retrospective studies evaluated the rate of marker decline during chemotherapy as a predictor of treatment failure. Marker levels should decrease with their appropriate half life, providing all markers producing cells were removed by surgery or eliminated by chemotherapy. On the other hand, if viable tumor persists, the rate of marker decline during chemotherapy will be the sum of new markers production and the amount still present from the original tumor. During successful treatment, the vast majority of cancer cells would be eliminated by the first cycle of chemotherapy, so by the second cycle the amount of new marker production should be negligible. In this case, AFP and HCG level should halve every 5 to 7 days and 2 to 3 days, respectively [56]. It is generally agreed that patients whose markers normalize by the second chemotherapy cycle have a good prognosis, whereas failure to normalize marker levels after chemotherapy indicates residual refractory cancer. In-between, there are patients with a slow marker decline who eventually normalize their serum tumor markers. The prognostic significance of slow marker decline remains controversial [56].

Determination of marker decline is typically based on a marker value measured before and after the first or second cycle of chemotherapy. Marker levels between day 1 and day 10 of the first cycle of chemotherapy are excluded due to the possibility of an increase in serum marker levels during the induction of chemotherapy [57]. The expected time to reach normal marker levels is predicted, assuming an exponential decline. Fizazi et al. suggested that patients treated with first line chemotherapy who had a prolonged predicted time to normal marker values (AFP >9 weeks or HCG >6 weeks) had inferior progression-free and overall survival compared with those patients with appropriate serum tumor marker decline [58]. These differences were predominantly seen in patients with a poor prognosis as classified by the IGCCCG. However, other studies have failed to confirm this observation [59,60].

Phase II studies from Memorial Sloan-Kettering Cancer Center and the Swedish Norwegian Testicular Cancer Group have used these criteria to predict resistance to standard cisplatin-based therapy, thereby selecting patients for a treatment intensification [61–63]. A recent phase III trial randomized intermediate and poor risk patients to 4 cycles of cisplatin-based chemotherapy or high dose chemotherapy and hematopoietic stem-cell rescue [64]. In a subset analysis, patients with slow marker decline assigned for high dose chemotherapy had an improved survival compared with patients with slow markers decline treated with standard chemotherapy. As the study did not randomize patients based on the rate of marker decline, this result was not considered definitive by the authors. Currently, treatment decision-making regarding chemotherapeutic regimens based on serum marker decline is not routinely performed [56].

**Tumor markers in late relapse of GCT**

Most GCT relapses occur within 2 years of initial treatment. A relapse after 2 years is considered late relapse and
occurs in 2% to 4% of patients. These tumors are highly resistant to chemotherapy, therefore, surgical resection is the preferred treatment [65]. Most patients with late relapse of NSGCT have elevated levels of tumor markers. AFP is the predominant marker in this subgroup (76%–52% of patients), followed by hCG (28%–10% of patients). In 10% of patients, hCG is the only elevated marker at late relapse [66–70].

Yolk sac tumor is the most common germ cell subtype in the patients with late recurrences [71]. This may explain the predominance of AFP as a marker of late relapse. The increase in AFP may precede radiologic detection by 2 to 44 months, reflecting the slow-growing nature of some tumors. Given the unfavorable results of chemotherapy at late relapse, it is reasonable to withhold any treatment until the disease site is apparent and amenable to surgical excision [67].

**Elevated serum tumor markers and surgery**

Persistent serum tumor marker after chemotherapy has historically been considered a relative contraindication to surgery due to supposed systemic disease and low chance of cure with local therapy alone. These patients have typically been treated with subsequent salvage chemotherapy. Over the last 15 years, however, several centers have experienced surgical cures in this population. In selected patients with progressive disease that is suitable for resection, “desperation RPLND” may be performed.

Published studies evaluating the therapeutic benefit of post-chemotherapy surgery in patients with elevated markers have reported survival rates ranging from 33% to 75% [49,72–75]. More favorable outcomes are reported in patients with an elevated serum AFP (as opposed to hCG), and stable serum tumor markers at time of surgery.

Indiana University recently reviewed its experience of desperation RPLND [76]. This study included 114 patients with elevated serum tumor markers after either induction chemotherapy (50 patients) or salvage chemotherapy (64 patients). The 5-year overall survival was 54%. Sixty-one patients (53.5%) are alive with a median follow-up of 5 years. Retroperitoneal pathology revealed germ-cell cancer in 53.5%, teratoma in 34.2%, and fibrosis in 12.2%, with 5-year survival rates of 31%, 77%, and 86%, respectively. Despite poor prognostic features, patients resistant to chemotherapy with persistent cancer can be cured a third of the time with aggressive surgery. As such, it is our approach to proceed with surgery in selected patients with elevated serum tumor markers and resectable disease, and forgo either second or third line chemotherapy.

**References**


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