Current Clinical Applications of Testicular Cancer Biomarkers

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KEYWORDS

• Testicular germ cell tumors • Tumor markers • microRNAs • Serum • Markers • Mitochondrial DNA

KEY POINTS

- Aside from classic serum tumor markers for testicular cancer (human chorionic gonadotropin, alpha fetoprotein, lactate dehydrogenase), limited data on additional molecular biomarkers have been published or validated.
- Larger series with consistent results from independent groups are required to validate new testicular cancer biomarkers.
- microRNA-371-3 has potential utility as a molecular biomarker for germ cell tumor detection and prognosis.

INTRODUCTION

Most germ cell tumors (GCTs) originate in the testes and account for approximately 95% of testicular cancers. Occasionally, GCTs originate in extragonadal sites, such as the mediastinum or retroperitoneum. Clinical and pathologic heterogeneity is an important feature of GCTs. Benign forms demonstrate extensive somatic differentiation (teratoma), whereas malignant GCTs are divided into seminoma and nonseminomatous GCTs (NSGCT).

Serum tumor markers (STMs) are prognostic factors and are important for diagnosis and staging. STM should be determined before and following orchiectomy. The 3 classic STMs for testicular cancer diagnosis and staging are alpha fetoprotein (AFP), which is produced by yolk sac cells; human chorionic gonadotropin (HCG), which is expressed by trophoblasts; and lactate dehydrogenase (LDH).

STMs are increased in approximately 60% of testicular cancer cases. AFP and HCG are increased in 50% to 70% and in 40% to 60% of patients with NSGCTs, respectively. Approximately 90% of NSGCTs present with an increase in one or 2 of these markers. Up to 30% of seminomas can present with or develop an elevated HCG level during the course of the disease.

LDH is a less specific marker with its concentration being proportional to tumor volume. Its level may be elevated in up to 80% of patients with advanced testicular cancer. Negative marker levels do not exclude the diagnosis of a GCT. Placental alkaline phosphatase (PLAP) is an optional marker for monitoring patients with pure seminoma but may have limited value in smokers.

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Traditional STMs are not only specific for testicular cancer. Elevations in HCG are commonly seen in a wide variety of carcinomas (gastric, pancreatic, neuroendocrine, lung, head and neck, lymphoma, leukemia). Similarly, elevations of AFP can be observed in hepatocellular carcinoma and benign liver disease.

A biomarker has been defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" by the World Health Organization. An ideal biomarker for testicular cancer would be an easily detectable molecule that would be unique for GCTs.

Limited contemporary data have been published regarding the use of biomarkers for testicular cancer diagnosis and prognosis in addition to traditional STMs (AFP, HCG, LDH). Cytogenetic and molecular markers based on microRNA (miRNA), cell-circulating mitochondrial DNA, or DNA methylation are available at limited centers but at present are not commonly used in clinical practice (Table 1).

CLINICAL UTILITY OF TRADITIONAL SERUM TUMOR MARKERS

Screening Utility of Serum Tumor Markers

In the context of screening for GCTs, no role of STM has been demonstrated because of the low incidence and mortality of testicular cancer.[1] It is very unlikely that STM as a screening tool would decrease mortality because of the natural history of the disease.

Diagnostic Utility of Serum Tumor Markers

STMs have been shown to assist in determining the origin of GCTs and in some clinical scenarios will dictate treatment. For example, if only seminoma is observed in an orchiectomy specimen, but increased AFP is detected, patients will be treated according to NSGCT protocols. Few conditions other than GCTs cause extreme elevation of STM, but moderate elevations are not as uncommon. See Table 2 for conditions that may cause elevation of STMs.

Ataxia-telangiectasia is a hereditary from of ataxia associated with various skin conditions. More than 95% of affected patients have elevated AFP.[2] Hereditary tyrosinemia is caused by various enzyme deficiencies in the tyrosine degradation pathway. This condition progresses to liver and kidney failure. Because of liver dysfunction, extreme elevations of AFP are present in affected individuals.[3] Similarly, in patients with cirrhotic liver disease and hepatocellular carcinoma, AFP can be elevated but is not always diagnostic of disease (40% of cirrhotic patients have elevation of AFP due to hepatomas).

In primary hypogonadism, a decline in testosterone may cause increased levels of LH.[4] LH is known to have cross reactivity with HCG in some immunoassays. Marijuana use may also result in elevation of HCG.

Table 1
Current clinical testicular cancer biomarkers

Molecular Marker	Target	Characteristics	Able to Differentiate GCT from Healthy Controls	Correlation with GCT Stage
miRNA	miRNA367-3p, 371a-3p, 372-3p, 373-3p	Noncoding RNA; very stable; interfere in the translation of mRNA to protein	Y	Y
mtDNA	mtDNA-79; mtDNA-220	Short length, simple structure	Y	N
CircDNA	—	Same methylation pattern as tumor cells	Y	N
CpG island hypermethylation	Gene silencing (APC, GSTP1, p14, p16, PTGS2, RASSF1A)	Easy to detect methylation; techniques already established	Y	N
CTC	—	Easy to detect; molecular techniques	Y	Y

Abbreviations: CircDNA, cell-free circulating plasma DNA; CTC, circulating tumor cell; mRNA, messenger RNA; mtDNA, mitochondrial DNA; N, no; Y, yes.

Table 2
Summary of key information for traditional STMs of GCTs

	AFP	HCG	LDH
Normal limits	1 mg/L to 10–15 mg/L of serum or plasma	1 U/L to 5–10 U/L	Depends on assay method used
Half-life (d)	5–7	1.5–3.0	Not reported
Seminoma GCT	Never elevated in pure seminoma	Yes (15%–20%)	Yes (40%–60%)
Nonseminoma GCT	Yes (10%–20% localized disease; 40%–60% advanced disease)	Yes (10%–20% localized disease; 40%–60% advanced disease)	Yes (40%–60%)
Other malignancies	Hepatocellular carcinoma, gastric, lung, colon, pancreatic	Neuroendocrine, bladder, kidney, lung, head and neck, gastrointestinal, cervix, uterus, vulva, lymphoma, leukemia	Lymphoma, small-cell lung, Ewing sarcoma, osteogenic sarcoma
Nonmalignant conditions	Alcohol abuse, hepatitis, cirrhosis, biliary tract obstruction, hereditary persistence	Marijuana, hypogonadism	Several

Staging Utility of Serum Tumor Markers

STMs cannot only help to establish a diagnosis, but the degree of elevation at diagnosis has prognostic significance.

According to NCCN guidelines, the role of STM in preorchiectomy and postorchiectomy is for staging purposes.[5] Before initiation of any treatment (surgical, chemotherapy, or radiotherapy), STMs should be measured. The magnitude of STM variability (International Germ Cell Cancer Collaborative Group [IGCCCG] classification) is used to determine chemotherapy regimens as well as for evaluation of response to chemotherapy.[6]

Measurement of Response to Treatment by Serum Tumor Markers

The use of STMs to monitor the response to chemotherapy is encouraged as increasing concentrations of markers in seminoma may imply disease progression and the need for salvage therapy.

For patients that undergo radical orchiectomy, the rate of decline of STM should coincide with the half-lives of the STMs. If the STMs remain elevated or decline slower than the expected half-life, this may indicate slowly growing metastatic disease. If STMs are elevated and there is no evidence of retroperitoneal disease on imaging, this is considered clinical stage IS disease. Use of STMs may also allow patients with residual disease to be differentiated from cancer-free patients.[5]

Decline after Treatment of Metastatic Disease

The standard chemotherapy regimen for testicular cancer includes bleomycin, etoposide, and cisplatin (BEP) or etoposide and cisplatin (EP). The number of cycles administered depends on the disease risk classification. Salvage chemotherapy is indicated for men who relapse or progress through primary chemotherapy. Finally, high-dose chemotherapy with autologous bone marrow transplant is indicated in poor-prognosis patients in whom standard chemotherapy regimens and/or salvage therapies have failed.

STMs should be measured the day before starting chemotherapy in order to accurately stratify patients according to IGCCCG classification.[6] Thereafter, STMs should be obtained at the beginning of each cycle. Serial measurements are encouraged as it correlates with the amount of viable tumor tissue remaining. Some studies have demonstrated a correlation between STM decline in the first 2 cycles of chemotherapy and oncologic outcomes (complete response, overall survival).[7] The results of a prospective randomized trial in patients with poor-prognosis according to IGCCCG criteria were recently published.[8] Patients were classified according to their response to the first cycle of BEP chemotherapy. Classification was established by the decline in STMs (normalized after first cycle). The group of patients with unfavorable decline received dose-dense chemotherapy that was associated with an improvement in progression-free and overall survival.

Despite these findings, a return to normal STMs does not always indicate a complete response. Up to 20% of patients who receive systemic chemotherapy for retroperitoneal disease demonstrate viable tumor at pathologic examination of lymph nodes.[9]

AVAILABLE BIOMARKERS FOR TESTICULAR CANCER

The introduction of more sensitive and specific biomarkers for diagnosis, staging, and surveillance of testicular cancer would allow clinicians to better select patients for further treatment. Surgery, chemotherapy, or radiotherapy may be associated with a variety of side effects that have the potential to impact the quality of life in a young patient population. Few studies have examined the clinical applicability of molecular biomarkers (miRNA, circulating mitochondrial DNA, circulating tumor cells) for early detection, staging, and surveillance of testicular cancer.

Increased expression of embryonic miRNA clusters (miR-371–3 and miR-302–367) can be detected in the serum of patients with GCT at higher rates compared with controls.[10–12] These findings hold promise for the clinical management of GCT, especially for seminoma because traditional STMs have limited utility for diagnosis or surveillance.

miRNAs are a new class of noncoding RNA. They are not only involved in physiologic processes (cell differentiation) but they are also involved in pathologic responses (carcinogenesis). miRNAs interfere with the translation of a given messenger RNA to protein; thus, they can act as a tumor-suppressor gene or oncogene. miRNAs are characterized by strong stability in body fluids once released from tumor cells.[13,14] In testicular cancer, miRNAs have shown to mimic the effects of mutated p53. miRNA expression has also been studied in other genitourinary malignancies.[15]

Testicular GCTs arise from carcinoma in situ cells that resemble malignant (pluripotent) primordial germ cells. They persist in the testis during puberty and early adulthood and then progress to seminoma or NSGCT. The miRNA profile of a cell may change during the course of malignant transformation.

Elevated levels of miR-371-3 have been observed in patients with GCTs compared with controls with a significant decrease in the level of miR-371-3 following orchiectomy. The rapid decline following surgery and the correlation of miRNA levels with tumor aggressiveness hold promise for the clinical utility of this biomarker.[10] This miRNA has also been shown to be elevated in thyroid cancer, and it is not exclusively specific for GCT.[16] The level of miR-371a-3p in blood and other body fluids has also been investigated.[17] The study included 25 patients with GCT, 6 with testicular intraepithelial neoplasia, 20 healthy men, and 24 patients with nontesticular malignancies. Moreover, 5 patients with GCT and 5 healthy controls had testicular vein blood examined for miR-371a-3p in an effort to demonstrate local release of miRNA. Increased levels of miR-371a-3p were observed in GCTs with rapid decay after orchiectomy. No correlation was found between the miRNA levels in testicular intraepithelial neoplasia (TIN) cases and controls. Although these results are promising, larger series with standardized results (miRNA technique for measurement seems to be controversial among investigators at normalization) need to be reported in order to become the standard of care.

Serum miR-367-3p, miR-371a-3p, miR-372-3p, and miR-373-3p have also been found to be significantly increased in patients with GCT compared with healthy controls.[18] The sensitivity and specificity of miR-371a-3p for the detection of GCT was 84.7% and 99.0%, respectively. These results were consistent with what has been reported in other previously published series.[19]

The unique characteristics of the mitochondrial genome, such as short length, simple molecular structure, and high copy number, have made monitoring aberrant changes of mitochondrial DNA (mtDNA) quantity a promising molecular marker for early tumor detection with advantages over nuclear genome-based methods. Recently, circulating cell-free (ccf) mtDNA in blood has emerged as a noninvasive diagnostic and prognostic biomarker for solid tumors.[20] Accumulating evidence suggests that plasma or serum ccf mtDNA levels are significantly different between patients with cancer and healthy individuals. Furthermore, quantification of ccf mtDNA levels in blood may assist in differentiating affected individuals from cancer-free patients.

A significant increase in short (79 bp) and large (220 bp) mtDNA fragments in patients with seminoma and NSGCT were detected compared with healthy controls.[21] No correlation with clinicopathological variables (clinical stage, pathologic stage, or lymph node invasion) was observed. mtDNA-79 showed an improved capacity (against traditional STMs) to distinguish between patients and healthy controls (mtDNA sensitivity 60%, specificity 94%).

Cell-free circulating plasma DNA (circDNA) is DNA found in blood plasma that is not associated with any cell fraction. circDNA is generally shed from normal cells, including. Among individuals with cancer, a proportion of circDNA is derived

from tumor cells and contains the same mutations and methylation patterns as the primary tumor.[22] Furthermore, studies have demonstrated that circDNA can be detected in most patients harboring solid tumors with advanced disease as well as in a lower fraction of patients with localized disease.[23] Thus, tumor-specific methylation in circDNA is a potential target for the development of noninvasive, blood-based assays for cancer diagnosis. A 9-fold increase in the level of circDNA among patients with testicular cancer compared with healthy controls has been described. No correlation between circDNA and STM, age, or histologic subtype was observed.[24]

One of the surprising aspects of cancer biology that has emerged from The Cancer Genome Atlas (TCGA) sequencing projects was the wide diversity of mutations associated with cancer.[25] Even within a single tumor type, mutational profiles may be very different between patients. It is not unusual for even the most commonly altered genes to be mutated in less than half of cases. The TCGA ovarian cancer sequencing project identified 7 significantly mutated genes, but these were only present in 2% to 6% of samples.[26] Limited data are available regarding GCT. This mutational heterogeneity provides a challenge for the development of cancer diagnostic tests based on DNA sequence changes, because large proportions of the genome would need to be interrogated to provide a test of adequate sensitivity.

The variability of cancer mutational profiles contrasts with the stability of DNA methylation changes that are a hallmark of oncogenic transformation. Given the greater consistency of DNA methylation changes in cancer compared with mutations, methylation is a promising target for biomarker development. CpG island hypermethylation of promoter regions is associated with gene silencing and has been reported for several genes in testicular cancer tissues (APC, GSTP1, p14 [ARF], p16 [INK], PTGS2, RASSF1A).[27] Detection of methylation changes is feasible in blood samples and has potential utility as a specific biomarker for testicular cancer.

Various levels of aberrant DNA methylation have been detected in up to 50% of patients with testicular cancer (APC, p14 [ARF], p16 [INK], PTGS2, RASSF1A).[28] Although the potential feasibility of hypermethylation as a testicular cancer biomarker has been investigated, limited data exist that examines the correlation between methylation, stage of disease, or tumor aggressiveness.

Finally, circulating tumor cells (CTCs) have been investigated as a potential biomarker for detection of testicular cancer. CTCs are cells that have shed into the vasculature from a primary tumor and circulate in the bloodstream. Several studies suggest that very small tumors shed cells at less than 1.0% per day.[29] CTCs are derived from clones in the primary tumor. A correlation between the incidence of CTCs in the peripheral blood of patients with testicular cancer and stage of disease/recurrence after chemotherapy has been reported.[30] A higher concentration of CTCs was described when testicular vein blood was analyzed. Other investigators have previously reported detection of CTCs in patients with GCTs.[31]

FUTURE BIOMARKERS FOR TESTICULAR CANCER UNDER INVESTIGATION

Recently, newly discovered biomarkers have been reported to differentiate between histologic subtypes of testicular cancer. These biomarkers include High Mobility Group A (HMGA), POZ-AT hook-zinc finger protein (PATZ), Aurora-B, Nek-2, Octamer Binding Transcription Factor 3/4 (OCT3/4), c-kit, PLAP, NANOG, SOX2, and CDK10.

HMGA1 and HMGA2 are proteins that are expressed depending on the state of differentiation of GCT. HMGA1 is overexpressed in seminoma. HMGA1 and HGMA2 are overexpressed in pluripotential embryonal carcinoma cells. HMGA1 expression is lost in yolk sac tumors, and expression of both proteins is lost in adult teratoma tissue.[32]

PATZ functions as a nuclear transcriptional repressor. PATZ and HMGA1 cytoplasmic delocalization associates with estrogen receptor downregulation in seminomas. Moreover, the PATZ interacting protein RNF4 is overexpressed in spermatocytes. RNF4 is not expressed in dedifferentiated tumors (embryonal carcinoma, yolk sac), suggesting a role in progression of GCT. Aurora-B is expressed in carcinoma in situ (CIS), seminoma, and embryonal carcinomas but not in teratoma and yolk sac carcinomas.[33,34]

OCT3/4 is another marker that has been reported in testicular cancer. OCT is a transcription factor of the family of octamer-binding proteins known as the key regulators of pluripotency.[35] Its expression has been reported in carcinoma in situ, seminoma, and embryonal carcinoma. Although OCT is a potential biomarker for testicular cancer, OCT has also been expressed in normal testicular tissue in some studies. However, there may be technical challenges related to the consistency of the antibody used for OCT detection.[36,37]

SOX2 is a transcription factor that has been reported in embryonal carcinomas, the undifferentiated part of nonseminomas, but absent in seminomas, yolk sac tumors, and normal spermatogenesis. SOX17 has also been shown to discriminate carcinoma in situ and seminoma from

embryonal carcinoma.[38] No clinical applicability of the use of the aforementioned biomarkers has been reported to date. CDK10 is a nuclear structural protein that has been expressed in seminoma.[39]

In summary, new testicular cancer biomarkers for diagnosis, staging, or follow-up remain promising but still lack evidence from large clinical studies to determine if traditional STMs can be replaced. A better understanding of the molecular mechanisms underlying the development of GCT may provide new insight into more effective diagnosis and treatment of GCT.

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