*Indranil Ghosh, MD and Sameer Bakhshi, MD**

Tumor markers in Pediatric Uro-malignancies

**Department of Medical Oncology, Dr. B. R. A. Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India*

A tumor marker represents a qualitative or quantitative alteration of a molecule, substance or process, associated with a tumor that can be detected by a special assay performed on a body tissue or fluid, other than routine histopathologic or laboratory evaluations.[1] Traditionally it denoted assays in body fluids for a molecule used to detect or monitor a particular tumor. In recent times the term 'biomarker' has come to practice, which denotes, "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[2] The following discussion focuses on some of the common biomarkers in use in pediatric uro-malignancies.

A) Germ cell tumors

Germ cell tumors, at all sites, constitute 3% of all pediatric malignancies, of which around two-thirds are extra-gonadal.[3] They are rare tumors and age-adjusted incidence is 5.8 per million in age-group < 15 years.[4] Alfa-fetoprotein (AFP), beta-subunit of human chorionic gonadotrophin (ß-hCG) and lactate dehydrogenase (LDH) are useful serum tumor markers for pediatric germ cell tumors (Table 1).

Table 1. Levels of AFP and ß-hCG in various germ cell tumors

Tumor type	AFP	ß-hCG
Seminoma/dysgerminoma	Normal	Normal to mild elevation
Yolk sac tumor	Elevated	Normal
Embryonal carcinoma	Elevated	Elevated
Teratoma	Normal	Normal
Choriocarcinoma	Normal	Elevated

AFP is a 69,000 kD single chain polypeptide which is similar in size and structure to human serum albumin. In human embryos, AFP is first made in the yolk sac and later in the fetal liver. As the fetal liver matures, it gradually switches to albumin synthesis. AFP levels decrease gradually after birth, reaching adult levels by 8 to 12 months.[5,6] In one study, mean serum AFP levels at birth were 41,687 ng/ml in 256 term babies and 158,125 ng/ml in 90 premature babies born before the 37th gestational week.[7] Normal adult AFP levels are low, but detectable (<5 ng/ml).[8] Half life of AFP also varies considerably in the first 2 years of life (5.5 days between birth and 2 wk, 11 days between 2 wk to 2 months, and 33 days between 2 and 4 months of age).[9] Age-dependant normal values have been estimated for clinical interpretation of absolute values.[9,10] The principal GCT associated with raised AFP is yolk sac tumor, while hepatoblastoma, hepatocellular carcinoma and neuroblastoma are the other pediatric tumors causing elevated AFP.[11] Benign conditions with a raised AFP include viral hepatitis and cirrhosis.[12] Methods have been suggested to distinguish between AFP of tumor or hepatic origin. A high AFP to concanavalin binding ratio (>10%) may favor a germ cell tumor origin rather than hepatic.[13,14] Subfractionation of AFP has also been suggested as a measure to identify the source and also to predict the occurrence of malignant component in a tumor.[15,16]

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced in pregnancy that is made by the developing embryo soon after conception and later by the syncytiotrophoblast. It is heterodimeric, with an á (alpha) subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and â (beta) subunit that is unique to hCG. The normal range for men and non-pregnant women is between 0-5 IU/ml. Choriocarcinoma and seminoma/germinoma with syncytiotrophoblastic cells may give rise to elevated ß-hCG in serum. Serum half-life is 18-36 hours. False-positivity can result from

cross-reactivity with LH (hypogonadism) or from ß-hCG released from host of other tumors like hepatic, renal, adrenal, and leukemia, lymphoma, as well as benign causes like hepatic necrosis, skeletal muscle disease and renal disease.[17] Pregnancy and gestational trophoblastic neoplasia should also be considered in adolescent females. Serum LDH is a non-specific marker of tumor burden and may be elevated in any histology. Serum LDH levels and its isoenzyme pattern have been suggested as useful tumor markers for diagnosis and post-therapy surveillance in patients with ovarian dysgerminomas.[18]

The role of monitoring of serum tumor markers in GCTs is summarized in (Table 2).

B) Neuroblastoma

Neuroblastoma is the second most common solid malignancy in childhood and the most common malignancy in infants. Incidence is 9.8 per million in age group < 15 year and 52.1 per million in infants.[4] Neuroblastoma falls into the group of small round blue cell tumors of childhood, along with lymphomas, primitive neuroectodermal tumors and rhabdomyosarcoma. Distinguishing among them sometimes may not be possible with light microscopy alone and require immunohistochemistry, electron microscopy or demonstration of elevated serum catecholamine (dopamine and norepinephrine) or urinary catecholamine metabolites, like vanillylmandelic acid (VMA) or homovanillic acid (HVA).[19] Majority of neuroblastoms possess enzymes necessary for synthesis of catecholamines (dopamine, norepinephrine and epinephrine) and their degradation, which gives rise to metabolites like VMA and HVA. The major pathways of degradation of catecholamines have been depicted in Fig. 1. HVA is the major metabolite of dopamine, while VMA is the major metabolite of epinephrine and norepinephrine. Reference ranges for the various metabolites have been created for this purpose.[20,21] Urine random sample values are reported usually as ratios to urine creatinine. Using combination of two or more metabolites in urine, sensitivity approaches 90% at diagnosis while it decreases to ~50% for detection of recurrent disease.[22]

Table 2. Role of monitoring serum tumor markers in germ cell tumors

To predict the histology (*e.g.* an elevated AFP in seminoma may denote presence of unidentified non-seminomatous histology)

Persistent elevated markers post surgery signifies presence of residual disease

Serial measurement can help in assessing response to chemotherapy

Rising markers on follow-up may herald onset of relapse

A higher AFP level at presentation may also signify poor prognosis

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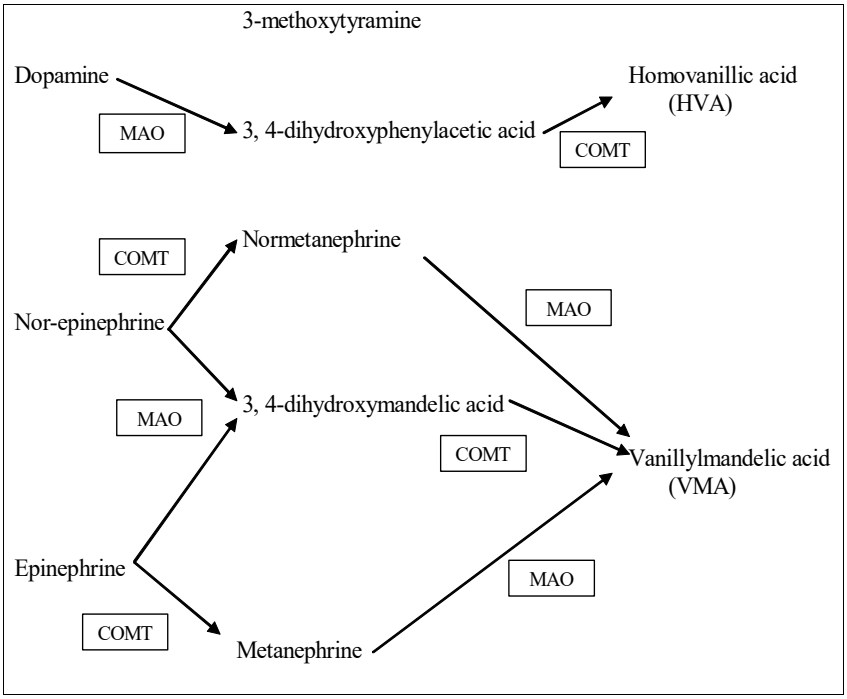
Relative proportion of various metabolites has also been correlated with prognosis. In one study, high VMA levels were associated with favorable biological features, high dopamine levels were associated with biologically unfavorable disease, and normal HVA levels were correlated with a better outcome.[23] A meta-analysis has confirmed that low urinary ratio (usually < 1) of VMA: HVA was associated with a worse overall and disease-free survival.[24] Normal VMA levels have also been correlated with MYCN amplification and poor prognosis in infants with disseminated neuroblastoma.[25,26] This occurs because poorly differentiated tumors produce more dopamine than norepinephrine or epinephrine. Caution should be exercised while interpreting these results as catecholamines from diet (*e.g*. banana and vanilla) may give rise to false-positive results.[27] Apart from their role in diagnosis and monitoring of neuroblastoma, another area where urinary catecholamines can be used is screening. The results of a mass screening program in Japan had shown that the survival for infants detected in this fashion was 97%.[28] But subsequent large population-based studies from North America, France, Germany and England have established that neuroblastoma screening does not reduce mortality and may detect more of diseases with favorable prognosis, which have a high rate of spontaneous regression and do not warrant treatment.[29,30,31,32] Currently, there is not enough evidence to recommend neuroblastoma screening.



Abbreviations: MAO – monoamine oxidase, COMT – catechol-O-methyl transferase

Fig. 1. Catecholamine degradation pathway

Neuroblastoma is one of the pediatric malignancies where numerous molecular alterations have been studied as prognostic markers (Table 3). *MYCN* gene amplification, loss of heterozygosity (LOH) of 11q and LOH of 1p have commonly been used for treatment decisions. In the recent International Neuroblastoma Risk Group classification, *MYCN* oncogene amplification, LOH of chromosome 11q, and DNA ploidy (= 1.0) were the most highly statistically significant and clinically relevant molecular markers predicting poor prognosis.[33] Other biomarkers that are being investigated are summarized in Table 3. Likewise, gene-expression profiling using cDNA microarrays and genome-wide copy number analyses has identified distinct prognostic groups.[34,35]

Table 3. Molecular prognostic markers in neuroblastoma

MYCN gene amplification	Gain of 17q
Ploidy	Tumor cell telomere length
LOH 11q	Telomerase activity
LOH 1p	CD44 expression
	TrkA expression
	High-level expression of drug-resistance gene *MRP1*
	Profile of GABA-ergic receptors
	Gene expression profiling

C) Wilms' tumor

Wilms' tumor is the most common renal neoplasm in children. Though current treatment achieves a long term disease-free survival of 80-85%, rest of the patients relapse, and efforts are being made to identify novel prognostic markers so that this subset of patients may be targeted for more aggressive treatment. One such

factor is LOH at chromosomes 1p and 16q. Tumor-specific LOH for both chromosomes 1p and 16q could identify a subset of favorable histology Wilms' tumor patients with increased risk of relapse and death.[36] In an ongoing phase III trial by the Children's Oncology Group, combined loss of 1p and 16q are used to select favorable histology Wilms' tumor patients for more aggressive therapy.[37] Other markers shown to be of prognostic value are increase in gene copy number or expression at chromosome 1q and telomerase expression level.[38,39] *CACNA1E* is located at 1q25.3 and it encodes the ion-conducting á1 subunit of R-type voltage-dependent calcium channels. Overexpression of *CACNA1E* at molecular and protein level is associated with poor relapse-free survival in favorable histology Wilms' tumor.[40] Gene-expression profiling has led to discovery of many other novel molecular prognostic factors, most of these are not yet incorporated in clinical decision making.[41]

D) Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is usually divided into three histological subsets– embryonal, alveolar and pleomorphic, with distinct biologic and molecular markers. Alveolar RMS is associated in 70% cases with a translocation, t (2;13) (q35-37; q14), while a variant translocation, t (1;13) (p36;q14) is found in a smaller proportion.[42] These translocations generate the fusion transcripts, *PAX3-FKHR* and *PAX7-FKHR* respectively, which can act as sensitive and specific markers for alveolar RMS. Presence of *PAX3-FKHR*, as compared to *PAX7-FKHR* has been shown to confer a worse prognosis in metastatic alveolar RMS.[43] On the other hand, embryonal RMS is associated with LOH of 11p15.5.[44] Recently, a new classification scheme has been proposed based upon gene-expression profiling and LOH analysis.[45]

Conclusion

Serum tumor markers have played an important part in management of many tumors. Tumor markers like AFP, ß-hCG and LDH are indispensable in the management of GCTs but meaningful clinical usage requires understanding of the biology and temporal variations in the levels. With the advent of molecular techniques, more and more diverse molecular markers are being identified in malignant tumors, and pediatric malignancies like neuroblastoma are no exception. Though some markers like MYCN gene amplification have resulted in a paradigm shift in the management, many other promising markers will have to be examined in large prospective studies before being incorporated in clinical decision-making.

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