

Tumour markers in pediatric solid tumours

A. K. Singal, S. Agarwala

Department of Pediatric Surgery, All India Institute of Medical Sciences, New Delhi, India

Correspondence: Dr. S Agarwala, Department of Pediatric Surgery, AIIMS, New Delhi -110029, India. E-mail: sandpagr@hotmail.com

DEFINITION

Tumour marker as a term generally refers to developmentally regulated proteins which are expressed in or from neoplastic tissues. Traditionally these proteins were protein or carbohydrate molecules, which were expressed in the fetus during the developmental period, thus these were also known as 'oncofetal antigens'. In the present era the term '*tumour marker*' has acquired a broader sense and implies any molecule or marker that aids in diagnosis or treatment of a specific tumour. An ideal tumour marker is a molecule of tumour cell origin released by the tumour cells in quantities easily detectable in the circulation or body fluids of the host and proportional to the tumour burden and is not present in the non-tumour bearing hosts.

Tumour markers are essential tools in the diagnosis and follow up of specific malignancies in childhood. During the last decade several new applications of tumour markers like radio-immunodetection and tagged chemotherapy have further enhanced the scope of tumour markers.

CLASSIFICATION

According to site of isolation

1. Serum tumour markers
2. Urinary markers
3. Genetic markers

According to biochemical nature

1. Oncofetal antigens
 - a. α -feto protein (AFP)
 - b. Carcino- embryonic antigen (CEA)
2. Hormones and their metabolites
 - a. Human Chorionic antigen (hCG)
 - b. Calcitonin
 - c. Catecholamines and metabolites
 - d. Ectopic hormones
 - e. Placental alkaline phosphatase (PLAP)

3. Enzymes
 - a. Neuron specific enolase (NSE)
 - b. Lactate dehydrogenase (LDH)
 - c. Prostatic acid phosphatase
4. Specific proteins
 - a. Neurosecretory proteins - Chromogranin A (CgA), Neuropeptide Y (NpY)
 - b. Ferritin
 - c. Prostate specific antigen (PSA)
 - d. Immunoglobulins
 - e. G_{D2}- disialoganglioside
5. Mucins and other glycopeptides
 - a. CA-125
 - b. CA-19-9
 - c. CA-15-5
6. Genetic markers
 - a. Neuroblastoma - N-myc, TRK-A
 - b. Wilms' tumour- WT1 (11p13), WT2 (11p15).
 - c. Rhabdomyosarcoma – PAX-FKHR, LOH-11p15.
 - d. Ewing's sarcoma- t(11;22)
 - e. Adolescent testicular tumours – Isochromosome 12p or i(12p).

Alpha-feto protein (AFP)

General

AFP is a single-chain glycoprotein produced by the fetal yolk sac, liver and the gut. It is the predominant serum protein in the first trimester and the serum levels continue to rise till the peak is reached at 12-14 weeks of gestation.^[1] After the first trimester, liver becomes the only site of synthesis as the yolk sac regresses. At birth, levels vary between 10,000 ng/ml and 70,000 ng/ml. The gestational age is the prime determinant of serum AFP levels at birth; preterm babies have much higher levels when compared to term infants.^[2] The levels decline with a half life of 5-7 days and the normal adult levels of < 10 ng/ml are reached anywhere between 8 months - 1 year of age.^[3] It is useful to consult a chart of normal ranges in infants if an AFP rise is encountered.^[2]

Functions

1. Fetal counterpart of albumin- contributes to plasma oncotic pressure.
2. Carrier for bilirubin and estrogen.
3. May act as an immunosuppressant or growth stimulator.^[4]

Subfractionation

As the levels may be raised in some benign diseases as well as in normal infants, subfractionation can be done to further identify the source of AFP production. Structural differences in the carbohydrate chain exist according to the site of synthesis and these can be identified by reactivity with lectins on lectin affinity crossed line immunoelectrophoresis. AFP can thus be sub-classified into three subtypes- benign hepatic type, yolk sac type and hepatoblastoma type (accuracy is 93%).^[5] Differentiation between hepatitis and hepatoblastoma in a young infant can still be very difficult as hepatoblastoma type AFP may be found in these subjects.

As subfractionation is not routinely available, it becomes more important to follow the serial levels in doubtful cases and supplement the information with requisite clinical and radiological information. Falling levels in concordance with the half-life signify normal AFP pattern and should reassure the clinician.

Elevated levels are seen in

1. Liver tumours
 - Serum AFP levels are raised in 90% of cases of hepatoblastoma and in 50-60% of hepatocellular carcinoma.
 - *Supports diagnosis but not diagnostic* - Elevations may be seen in cases of hemangioendotheliomas and mesenchymal hamartomas, benign liver diseases like cirrhosis, hepatitis and cholestasis. Though levels > 10000 ng/ml are rarely seen in these conditions.
 - Levels < 100 ng/ml or > 1,000,000 ng/ml in hepatoblastomas are associated with poor prognosis.⁶ Levels > 1,000,000 g/ml signify a large tumor burden and levels < 100 ng/ml are generally associated with undifferentiated or anaplastic histology.^[6,7]
 - Assessment of response to chemotherapy by monitoring serial levels - The rate of decline of AFP levels has been found to be significantly related to the prognosis and rising levels after an initial decline signify development of drug resistance.^[6]
 - In follow-up for recurrence - AFP levels should be monitored during follow-up every 3 monthly for one year, then 6 monthly for 2 years and annually

thereafter for 5 years to detect recurrence. This is in addition to the radiological follow-up. Levels have not been reported to increase post hepatectomy in spite of the ongoing liver regeneration.

- Certain HCC predisposing syndromes like ataxia telangiectasia and hereditary tyrosinemia have elevated AFP levels, so utility of AFP for screening for HCC is limited in these cases.
 - Radio-immunodetection using ⁹⁹Tc labeled anti-AFP antibodies has been reported in a case of hepatoblastoma and is an area of research.^[8]
 - Future prospects - anti-AFP antibody tagged chemotherapy - so far used in only experimental studies.
2. Germ cell tumours
 - SAFP levels are elevated in patients with yolk sac tumours, embryonal carcinomas and mixed tumours.
 - Use in germ cell tumours includes presumptive diagnosis of a testicular mass, staging refinement, prognostic group allocation, surveillance for recurrence and definition of chemotherapy duration and dose.^[9] Even if the markers were negative at the time of diagnosis, still levels should be checked at the time of follow-up as they may still be the only marker of recurrence.^[9]
 - In patients with germ cell tumours, levels > 10,000 ng/ml are associated with poor prognosis.^[10]
 - Anti-AFP nuclear scans have been used for diagnosis and staging but at best have a 90% sensitivity and 60% specificity, and have been found to be less useful less useful than a combination of CECT and serum AFP.^[11]
 - Abrupt escalation of SAFP can occur post-chemotherapy due to tumor lysis or alteration of hepatic function.^[12]

3. Pancreatoblastoma

As this is an embryonic tumor SAFP levels are often elevated and may help in the diagnosis.^[13] The value in follow-up is not clear.

Human chorionic gonadotrophin (hCG)

General

hCG is a glycoprotein comprised of alpha and beta subunits, synthesized by the syncytiotrophoblasts during pregnancy for the maintenance of corpus luteum. α subunit has antigenic similarities with TSH, LH and FSH, while β subunit is antigenically distinct from these hormones. Hence in oncology practice, α subunit is used for serum assays. Half-life is 24-36 hours and normal levels are less than 5 mIU/ml.^[14]

Elevated in

1. Germ cell tumours - Choriocarcinomas, Germinomas - seminomas or dysgerminomas and embryonal carcinomas.
2. Rarely in other malignancies like hepatoblastomas (3%, may be associated with precocious puberty), and malignancies of pancreas, gut, breast, lung and bladder and even in patients with multiple myeloma.
3. Sudden elevation may occur after chemotherapy induced cell lysis.
4. Conditions leading to increased LH levels like bilateral orchiectomy, oophorectomy may result in false positive results due to immunological cross reactivity.

Neuron specific enolase (NSE)

General

Enolase is a glycolytic enzyme present in many human tissues and cells. The alpha, beta and gamma subunits are called non-neural enolases; alpha and beta are expressed in the glial cells, while gamma subunit is expressed in muscle cells. The δ subunit is present in the neural tissues and is known as the NSE.

Serum levels are elevated in

1. Neuroendocrine tumours like neuroblastoma, pheochromocytoma, medullary thyroid carcinoma, pancreatic islet cell malignancies and carcinoids.^[15]
2. Rarely may be elevated in Ewing's sarcoma, Wilms' tumour or dysgerminomas.^[16]

Role in neuroblastoma (NB)

- Sensitive for detection but can rise with other tumours as seen above, so specificity is less.
- Serum levels correlate with tumour burden; levels > 100 ng/ml at diagnosis correlate larger tumour burden and poor prognosis, while levels < 30 ng/ml portend a good response to therapy.^[17]
- No differences in levels are seen with different disease sites.
- Serial levels can be used for monitoring response to chemotherapy. Rising levels post therapy signify recurrence/ relapse. Levels may rise before clinical or radiological recurrence.^[17, 18]

Neurosecretory proteins

Chromogranin A (CgA) and Neuropeptide Y (NpY) are markers of neural differentiation potentially associated with neuroblastomas. Both are developmentally regulated proteins located in the neurosecretory granules of the neuroendocrine cells.

CgA

CgA is an acidic monomeric protein that is stored and co-released with catecholamines by exocytosis from neurosecretory vesicles. The physiologic role of this protein has not been fully elucidated, but it has been hypothesized that it may serve as a precursor to other biologically active peptides or may serve a function in the intracellular production of hormones and neuropeptides. Plasma CgA has been found to be elevated in patients with variety of endocrine neoplasia which liberate peptide hormones such as pheochromocytoma, parathyroid adenoma, thyroid C cell tumours, carcinoids, oat cell carcinoma of the lung and pancreatic islet cell tumours.^[19] Serum CgA levels are often elevated in patients with neuroblastoma and serve as a reliable tumour marker with a sensitivity of 91% and a specificity of 100%.^[20] Moreover the levels have been found to correlate with stage and the prognosis. Levels greater than 190ng/ml were found to have a poor prognostic value.^[20]

NpY

NpY is another developmentally regulated neurosecretory protein that has been found to have value as tumour marker in neuroblastoma and other neuroectodermal tumours. In the children with neuroblastoma the serum levels are often elevated and correlate with disease stage.^[21] NpY expression in tumour tissue has been found to be of value in differentiating stage IVS from stage IV, as NpY expression is seen in tissue from stage IVS and not in stage IV.^[22] This suggests the different origin of these tumours.

Plasma Catecholamines and their urinary metabolites

Most of the neuroblastomas and other neural crest derived tumours are hormonally active tumours which overproduce catecholamines epinephrine (E), norepinephrine (NE) and dopamine (DA); and their metabolites like vanillyl-mandellic acid (VMA), homovanillic acid (HVA), methoxydopamine (MDA) and methylated catecholamines namely metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3MT). Neuroblastoma cells lack the enzyme phenylethanolamine N-methyltransferase, which converts norepinephrine into epinephrine. The metabolic scheme is depicted in figure 1, it can be seen that NE and E are primarily metabolized into VMA while Dopamine is metabolized into HVA.

Neuroblastoma cells lack the storage vesicles for catecholamines unlike normal neural crest cells, and these catecholamines upon release into the circulation are rap-

idly degraded into VMA and HVA. This also explains why the analysis of metabolites is considerably more sensitive and useful as tumour markers than the catecholamines themselves. New high performance liquid chromatography assays permit separate estimation of various fractions of metabolites and total catecholamines with high degree of accuracy. The urinary samples are stored in 10% HCl to prevent auto degradation.

Uses in neuroblastoma

1. Diagnosis

- Urinary estimations of catecholamines and their metabolites are diagnostic in upto 90% of the cases with NB.^[23,24] Initially 24 hour urinary values were used but now random assays are used and these have been found to be equally sensitive and the cumbersome exercise of urine collection in children can be avoided. In random estimations, the quantities are expressed as mg/g of creatinine to take into account the urine production rate and make the results more reproducible.^[25] Among all the metabolites as shown in figure 1, VMA and HVA are the ones that are most com-

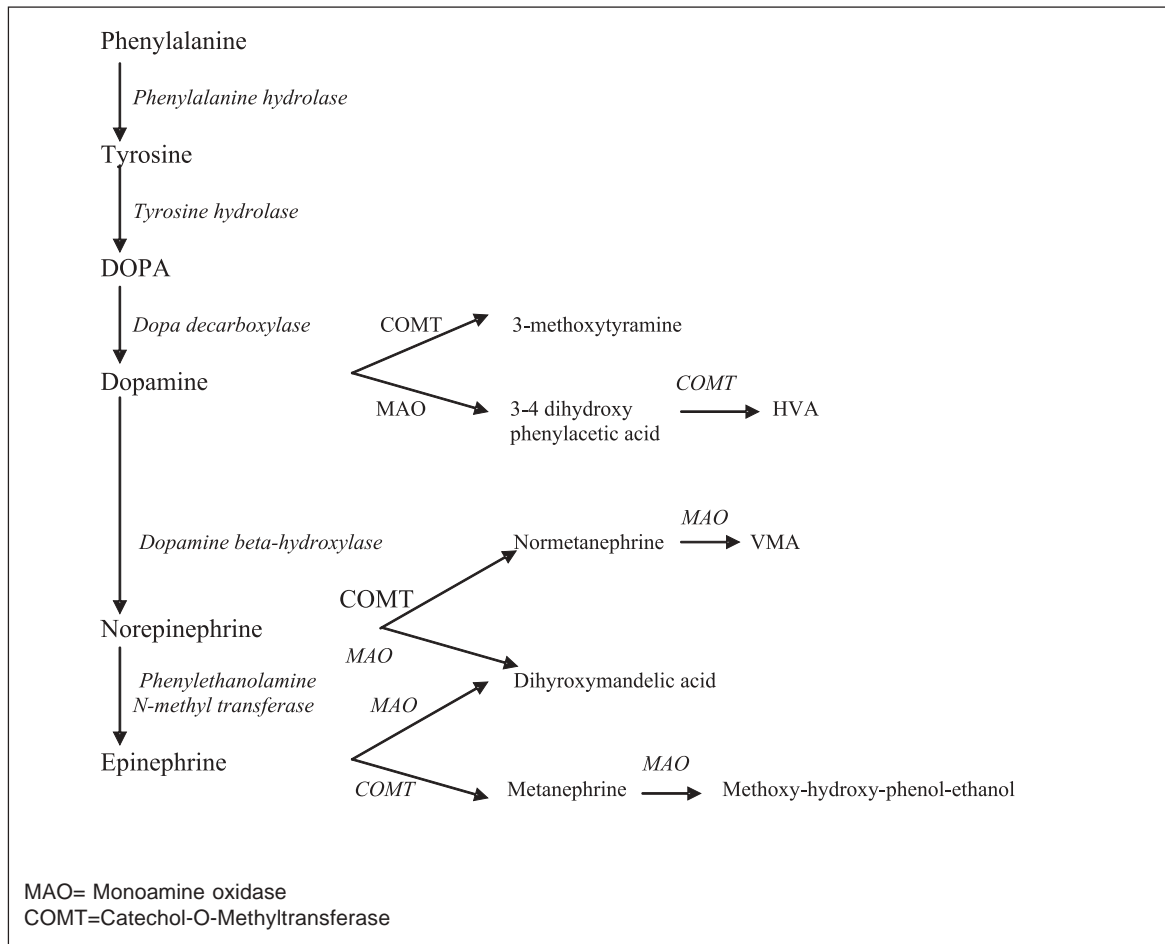


Figure 1: Catecholamine synthesis and metabolism. MAO, monoamine oxidase, COMT, catechol-O-methyltransferase

Table 1: Data of selected tumour markers

S. No.	Marker	Normal levels	Raised in	Importance
1	SAFP	< 10 ng/ml	Infants up to 8 months of age Liver tumours – Hepatoblastoma, hepatocellular carcinoma Germ cell tumours – yolk sac, immature teratoma, mixed, and embryonal carcinomas Pancreatoblastoma	Consult chart Diagnosis, prognosis, follow up Diagnosis, prognosis, follow up
2	hCG	< 5 ng/ml	Germ cell tumours	May help in diagnosis
3	NSE	0 – 12.5 ng/ml	Neuroblastoma, neuroendocrine tumours	Diagnosis, follow up
4	CgA	0 – 5 nmol/ml	Neuroblastoma, neuroendocrine tumours	Diagnosis, prognosis, follow up
5	NpY	0 – 8 pmol/ml	Neuroblastoma	Diagnosis prognosis
6	VMA (urine)	0 – 35 mmol/l/24 h	Neuroblastoma, neuroendocrine tumours	Diagnosis, diff. stage IV from IVS
7	HVA (urine)	0 – 40 mmol/l/24 h	Neuroblastoma, neuroendocrine tumours	Diagnosis

monly used and have a sensitivity which exceeds 90% in combination, though their individual sensitivities are below 80% each.^[24] Other metabolites with good sensitivity and specificity are NME and DA. Combination of NME with HVA or VMA has recently been reported to be 100% sensitive and specific. DA is not very sensitive and specific in infants but its importance increases with the age of the child.^[25] Serum VMA and HVA have been studied in patients with neuroblastoma but the levels are less frequently elevated than the urinary levels.^[26]

- Only 10% of the tumours are truly non-secretory.^[25,27]
- Repeated urine sampling does not enhance the detection rate with these tests as intermittent secretion has not been reported.^[25]
- The levels of these metabolites are rarely raised in ganglioneuromas; and when raised careful clinical and pathological evaluation is a must to rule out a focus of NB.^[25,28]

2. Prognosis

Gitlow et al identified a group of patients with NB who had high urinary HVA and whose prognosis turned out to be particularly poor.^[29] Subsequent reports have associated high HVA/VMA ratios as an indicator of poor prognosis.^[30] As HVA is a metabolite of DA and VMA is a metabolite of NE and E, high HVA/VMA ratio reflects lack of enzyme dopamine- α -hydroxylase and an immature histology and associated poor prognosis.^[29,30]

3. Recurrence

The catecholamine and their metabolites are not very useful for detecting recurrence during follow-up of patients who have been treated for NB previously. In cases with diagnosed recurrence, the levels of these metabolites was found to be raised in only 55% of the patients, this contrasts with more than 90% sensitivity at the time of presentation. Thus relapse or progression cannot be reliably detected or excluded by monitoring tumour markers alone; MIBG scan and radiological investigations are required.^[31]

4. Screening

Mass-screening programs run in Quebec (Canada), Japan and Germany for early detection of children with NB utilize analysis of spot sample of urine absorbed onto a filter paper strip for HVA and VMA. These are based on the premise that earlier detection of NB may offer a survival advantage. The results have not been encouraging as most of the tumours detected in this way have been found to be biologically favorable anyway and have a high tendency for spontaneous regression.^[32] Though the pro-

gram has led to an increase in the number of cases diagnosed but this has not led to a decrease in the mortality attributable to NB.^[33] The consensus from the results of these programs is that there are two different subsets of NB, and the more favorable type presents earlier and is the one that is detected by screening. The poorer prognosis group is not detected by screening, hence the mortality attributable to NB has not decreased.^[34,35]

Another issue related to screening is the possibility of unnecessary treatment of biologically favorable tumours destined for regression. In this regard, Yamamoto et al have defined criteria for observation of tumours detected on routine screening – small masses on radiography, no invasion of spinal cord or vascular structures, relatively moderate catecholamine excretion and parental consent.^[36]

Ferritin

In patients with actively growing NB, serum ferritin levels are frequently raised and can be of value as a tumour marker. Though not very specific, serum ferritin has been found to have a prognostic significance, which is independent of the age, stage and histology.^[37-39] The serum levels have been found to correlate with the tumour burden, higher levels portend an overall poor outcome.^[37] The raised levels have been reported to return to normal after a clinical remission has been achieved.^[37] Further, serum ferritin has been found to be of value in differentiating between stage IV and stage IVS as in stage IV serum ferritin is raised in upto 85% of the patients and levels are subnormal in stage IVS.^[40] Ferritin is also non-specifically raised in acute lymphocytic leukemia.

Lactate dehydrogenase (LDH)

LDH is a non-specific tumour marker and raised levels signify a rapid cell turnover rate and a large tumour burden. Useful in NB, All and NHL and Ewing's sarcoma.^[41]

Disialoganglioside (GD₂)

GD₂ is a ganglioside present on the cell surface of the NB cells and thus was used mainly for immunohistochemistry. It has been shown to be shed by the NB cells into circulation and can be used as a tumour marker.^[42-44] Circulating GD₂ is a very specific tumor marker for NB and due to its immunosuppressive effects also plays a part in the tumour progression. High circulating GD₂ levels have been found to bear a strong correlation with a rapid progression and a poor prognosis.^[44]

Future prospects using antibody against NB cells are an active area of research.

Glycoprotein markers

1. CA125: CA125 is a tumour marker primarily for gastrointestinal and genitourinary malignancies in adults. Elevated CA125 levels have been reported in some children with malignant yolk sac tumours and immature teratomas, while they were normal in patients harboring mature teratomas.^[45] CA 125 has some value in the diagnosis of hepatocellular carcinoma, where it has been found to be more sensitive than AFP but less specific.^[46] In children with non-Hodgkin's lymphomas, upto 60% have elevated CA 125 levels which correlate with the occurrence of pleural effusion or ascites and these return to normal after a remission is achieved.^[46]
2. CA 19-9: Raised levels of Ca 19-9 are seen in adult gastro-intestinal tract malignancies but have not been of any value in pediatric colorectal carcinomas.^[47] CA 19-9 has also been found to be raised in children with immature teratomas and malignant germ cell tumours but not in mature teratomas.^[45]
3. Tissue polypeptide specific antigen (TPS): TPS has got a limited attention as tumour marker in pediatric oncology owing to its non-specificity. It has been shown to be of some value in distinguishing benign abdominal masses from malignancies such as Wilms' tumour and NB where it is invariably raised. Clinical use is limited as levels rise non-specifically with infections also.^[48]

GENETIC MARKERS

Certain genetic alterations are specific to tumour types and these may aid in diagnosis, planning the therapy and help in prognosticating the outcome. As such they can be discussed under the broader umbrella of tumour markers.

Neuroblastoma

1. N-myc amplification: 25% of primary NB's show amplification of proto-oncogene N-myc, the locus for which lies on the short arm of chromosome 2. Amplification almost always is present at the outset if it is going to occur, so it appears to be an intrinsic biological property.^[49,50]

Significance

- Associated with higher stage -5-10% of stage1, 2 & 4S have N-myc amplification while 30-40% of stage4 tumours show N-myc amplification.^[49,50]
- Correlates with rapid tumour progression and poor prognosis.^[49-52]
- More copies i.e. more the amplification, poorer the outcome.
- Unfavorable effect is most apparent in infants with

stage4S disease.^[53]

2. Hyperdiploid karyotype: Expressed as DI (DNA Index). DI >1 signifying hyperdiploid state is associated with a better outcome. The favorable impact is more pronounced in infants with advanced stage at presentation.^[52]
3. 1p deletion is a poor prognostic factor and an independent risk factor for relapse. Strong association has been noted between 1p deletion and N-myc amplification.^[54]
4. TRKA (gene for nerve growth factor receptor) expression is inversely related to N-myc amplification and is a favorable marker.^[55]

Rhabdomyosarcoma

RMS is classified into two major histologic subtypes with differing clinical behavior – embryonal (ERMS) and alveolar (ARMS). The genetic alterations encountered in both of these are distinct and can permit differentiation if histopathology is questionable.

ERMS: Characteristic alteration is loss of heterozygosity at 11p15 locus or 11p15 LOH which is also the location for the IGF-2 gene. LOH leads to an over expression of IGF-2 gene and this event is proposed to play a part in the pathogenesis of ERMS.^[56]

ARMS: Characteristic translocation between long arm of chromosome 2 and 13 designated as t (2; 13)(q35; q14) leading to a fusion gene called PAX3-FKHR gene. This fusion gene is expressed in the fibroblasts and specifically turns on an array of myogenic growth factors and thus plays a part in the pathogenesis.^[57]

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