*The Turkish Journal of Pediatrics*
*Volume 26, Nos 1-4, 1984*

# URO-ONCOLOGY: Testis Tumors

## TUMOR MARKERS IN TESTIS TUMORS*

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*Key words: AFP, monoclonal antibody, tumor markers*

The concept of tumor markers has widened over the years and the term itself is much more recent than the basic idea. As we all remember, in the early 1930's Zondek clearly thought of detecting human chorionic gonadotropin (hCG), a placental hormone secreted by the syncytiotrophoblast, in body fluids, to diagnose trophoblastic tumors of gestational and germ cell origin [1].

The term "tumor marker" by definition is any means, usually of a chemical nature, which helps to discriminate between one type of tumor and other normal or disease states. Tumor markers have applications in screening for cancer, monitoring the course of malignant disease, detecting relapse at an early phase and recently, targeting isotopes for localization of or directing therapeutic agents. In trying to present an overview of this subject, we shall confine ourselves to the major and important markers in testicular tumors, new advances, namely application of monoclonal antibodies in the detection of tumor markers, and lastly studies done in our laboratory related to Hbf production in these tumors.

The tumor cell membrane has quite a heterogeneous mixture of molecules, mainly chemical moieties, which usually reside on the cell surface. Alternatively, some molecules of tumor cell antigens may be localized intracellularly. The tumor cell antigens may be localized intracellularly. The tumor cell antigens represent the cell of origin of the tumor, related to the function, differentiation and virus-induced membrane changes[2].

Tumor antigens are mostly either membrane-bound or membrane-shed molecules, and they may cause an immune response. We may have four distinct classes of tumor-specific antigens: 1) antigens unique to a particular tumor (certain transformed cell lines, sarcomas, etc.); 2) antigens which are expressed in a class or specific type of tumor (teratocarcinomas, CEA, AFP, etc.); 3) antigens which are specific for the transforming agent employed to produce the tumor, such as DNA and RNA tumor viruses; and 4) tumor antigens common to many different tumors, e.g. p 53 tumor antigen[2]. At the present time, enough is known about the

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p 53 protein to suggest that this cellular protein may play a central role in transformation as well as normal cellular growth processes.

As was pointed out recently, by Levine[2], there are at least six possible explanations for the expression of tumor antigens on tumor cells: 1) alteration of a normal molecule on the surface of the cell; 2) expression of proteins or glycoproteins that are predominantly found in fetal tissues such as CEA, AFP, etc.; 3) increased concentrations of normal cellular protein in transformed tumor cells; 4) viral-cellular protein complex presenting as new antigenic sites; 5) tumor cells possibly containing new or "re-arranged" genetic information, peculiar to the tumor cell; and 6) the acquisition of foreign genetic information like a DNA virus in tumor cells leading to the expression of foreign antigens.

During the last several years it has become clear that various types of neoplastic diseases produce certain proteins which circulate in the serum and may be useful in the detection of tumors. Several methods described to quantitate these proteins in the serum make it feasible to utilise these products as tumor markers.

The testis is a complicated organ having multiple cell types. At the present time, various testicular cell lines have been established and four are non-tumorigenic. The fifth is a transformed cell line derived from a testicular tumor (TR - ST cell line). The availability of this series of cell lines and the ability to grow them will provide valuable information on testicular physiology and hormone action[3]. Classification of testis tumors on the basis of morphologic criteria has improved our understanding of the natural course of these tumors. In addition, specific tumor markers which are secreted by distinct clones of cells may also help to identify certain biologically distinct categories of morphologically similar tumor types. Testis tumors are, in general divided into "seminoma" and "nonseminomatous" types. Several studies reported to date have investigated the value of serum tumor markers in testicular tumors with responses to chemotherapy and surgery as well[4-6].

We shall briefly review some important tumor markers in testis tumors:

*1. Alpha-fetoprotein (AFP).* AFP is the major serum protein found in the circulation of early human embryonic and fetal life. It is secreted by the fetal liver and yolk sac and reaches a peak at about 12 to 15 weeks of gestation (3 mg/ml). Its concentration decreases when the child is 1 year old (less than 30 ng/ml). It is a glycoprotein with a molecular weight of 70.000 daltons, and its biologic role still remains obscure. The recent development of sensitive radioimmunoassay has revealed that in the normal fetus, the serum AFP level is in the range of 67 - 2000 $\mu$g/ml and after one year of age the serum AFP level is less than 3 ng/ml. Serum AFP levels in normal man range from 5 to 30 ng/ml and a level of more than 40 ng/ml is accepted as elevated[7,8]. It has been shown that human AFP and albumin genes are linked on the long arm of chromosome 4. Recently, the AFP coding sequence has been

defined and the primary structure of AFP has been described[9]. Elevated levels were found in the serum of patients with primary hepatocellular carcinoma, various non-malignant liver diseases, ataxia-telangiectasia, hereditary tyrosinemia, and neoplasia of germ cells[10,11]. AFP levels are usually (75% of the cases) elevated in advanced teratocarcinoma of the testis. The half-life of AFP in the circulation is 3.5 to 5 days.

*2. Human chorionic gonadotropin (HCG).* HCG is a glycoprotein consisting of two dissimilar, non-covalently linked polypeptide subunits (alpha and beta). The alpha subunit has 89 - 92 amino acid residues in identical sequences. It is identical with the other alpha subunits present in LH, FSH and TSH. The intact molecule, free alpha and beta subunits, and natural fragments of HCG are all immunogenic and antigenic. The beta subunit is the hormone-specific unit. It has distinct differences among the AA sequences from other hormones. The beta subunit, which determines the unique biologic and immunologic properties of HCG is the most important part[12]. Dissociated alpha and beta subunits do not have biologic activity. HCG is found only in the presence of trophoblastic tissue. A peak level in the serum is observed between 10 and 12 weeks of gestation, and it is normally present in elevated levels only during pregnancy. The sensitive radioimmunoassay developed by Vaitukaitis *et al* specific for $\beta$-subunit in serum was an important step, and it is sensitive to less than 1 ng/ml, with levels greater than 1 ng/ml accepted as abnormal for men[13]. The presence of choriocarcinoma cells in the testicular tumor will cause significant elevations of HCG levels in the serum. The half - life of HCG in the circulation is approximately 18 hours.

*3. Carcinoembryonic antigen (CEA).* Another marker found to be elevated in patients with a variety of cancers is CEA, a broadly distributed marker that has been useful in monitoring the response of certain cancer patients to therapy. CEA is a glycoprotein with a molecular weight of 180,000 daltons. It is synthesized during normal human development mainly in the fetal gut. In adults the CEA gene is normally not expressed CEA levels can be elevated in carcinoma of the colon, prostate, breast and also in heavy smokers without neoplastic disease. CEA can also be elevated in seminomatous and non-seminomatous testis tumors.

In most of the clinical studies related to the value of serum tumor markers in the staging and prognosis of tumors of the testis, three important points were usually evaluated: 1) How reliable are these three tumor markers (AFP, HCG, CEA) in detecting the presence of metastatic testicular tumor? 2) Do marker levels indicate the stage of the tumor more accurately than the clinical tests? 3) Do the levels of tumor markers after treatment identify tumor recurrence before it is clinically demonstrable?

Several prospective studies were designed to answer these questions. HCG or AFP produced by primary tumor of the testis will frequently result in elevated levels of

markers before orchiectomy. It has been suggested that the levels should be drawn in the pre-orchiectomy, post-orchiectomy, pre-treatment (irradiation, chemotherapy or combined) and post-treatment periods routinely for at least 4 to 8 weeks[14]. A study done by Scardino *et al*[14] revealed that either HCG or AFP levels were elevated in 91% of the patients with clinically demonstrable non-seminomatous tumors. In 24 patients who had recurrent disease 67% had elevated AFP or HCG at a time when recurrence was still clinically undetectable. No false positive values were obtained. CEA, on the other hand was found to be elevated in 33% of patients with seminoma and in 7% of patients with non-seminomatous tumor. Determinations of HCG and AFP before lymph node dissection decreased the error in clinical staging. HCG and AFP markers appear to be quite sensitive in detecting minimal tumor burden, and they can be used to identify the presence of metastases earlier than the clinical findings. A similar study done by Lange *et al* confirmed the findings, and elevated levels of AFP and HCG were only found in non-seminomatous germ cell testicular tumors[15]. Both studies indicated the importance of determining two markers (AFP and HCG) together in order to have a more accurate indication of the metastases. These serum tumor markers had limited value in the differential diagnosis of scrotal masses[15]. When the remission rates induced by chemotherapy alone or by combined chemotherapy and surgery were analyzed[16] in relation to specific serum tumor marker elevations in the pre-treatment phase in patients with stage III or stage II non-seminomatous germ-cell tumors, complete remission occurred in 92% of patients with normal levels of AFP and HCG and in 39% with abnormal levels of both AFP and HCG. Patients with very high levels of serum AFP and HCG (>1000 ng/ml) respond poorly to chernotherapy[16]. Progressive disease is frequently accompanied by a rise in both serum tumor markers. The study done by Vugrin *et al* suggested that normal serum tumor markers at the time of surgery for residual disease are a favorable prognostic feature. In addition, when the tumor markers were analyzed according to the histology of the primary tumor, 87% of the patients with choriocarcinoma elements had an elevated HCG, and 92% of the patients with yolk sac elements had elevated levels of AFP[16].

In a recent study done in Denmark by the Danish Testicular Cancer study group, Pedersen *et al* also tried to investigate the correlation of the HCG and AFP levels with histology and stage[6]. This study revealed that the greatest value of postoperative AFP and HCG determinations in testicular cancer is in monitoring patients, both during treatment and in follow-up. In patients with clinically and radiologically determined stage I disease, increasing or stationary postoperative marker values or prolonged half - life indicated metastatic disease[6]. This study also emphasized the allowances which have to be made for the half - life of AFP and HCG in interpreting abnormal levels.

In summary, 1) these tumor markers (AFP and HCG) probably have limited value in the differential diagnosis of scrotal masses; 2) the marker levels are extremely sensitive indicators of metastatic disease before lymphadenectomy; 3) measuring both (AFP and HCG) is more helpful than the determination of either alone; 4) these markers have greatest clinical value in monitoring the effectiveness of radiation or chemotherapy in patients with stage II or stage III disease; and finally, 5) the measurements usually affect the management and survival of patients with nonseminomatous germ cell testicular tumors significantly.

In a search for new markers, placental alkaline phosphatase (PLAP) has also been investigated[6], but the exact clinical relevance of this marker has not been established.[6] We have studied hemoglobin-F levels and F-cell numbers in different urogenital cancers and have measured AFP and $\beta$-HCG levels[17]. Hb-F levels were found to be elevated in patients with testicular and prostatic cancer. No significant correlation was observed between Hb-F, AFP and HCG levels.

The recent development of monoclonal antibody (hybridoma) technology has made it possible to obtain unlimited quantities of highly specific and pure antibodies against different antigens[18]. Monoclonal antibody studies of testicular tumors have dealt with cell surface antigens. Among the oncodevelopmental antigens, alpha-fetoprotein and beta human chorionic gonadotropin have been studied. Monoclonal antibodies to AFP have been used to monitor patients with malignant hepatomas and germ cell tumors[19], but the usefulness of monoclonal Ab to AFP over conventional antibodies remains to be determined. The use of monoclonal antibody to CEA for the serologic detection of tumor antigens has been reported[20].

Finally, the advance in the monoclonal antibody production has heightened interest in the "in vivo" localization and imaging of tumors. Monoclonal antibodies labeled with radioactive iodine ($I^{125}$ or $I^{131}$) or Indium ($In^{111}$) have been used for tumor imaging, and it appears that monoclonal anti-tumor antibodies have great promise for radioimmunodetection and possibly therapy of tumors[21,22].

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## DISCUSSION

**Hargreave:** Can I just ask you one thing. I worry slightly about labeling someone "hydrocele". In my hospital, we've got a waiting list, and if you put the patient down on the waiting list as hydrocele, there's a real risk that that patient could wait for three to six months before being called into hospital and for that reason, I teach the medical students (and maybe you'll criticize me for this) that if they have a young man with a hydrocele and they cannot feel the testis, they should aspirate that hydrocele in the outpatient clinic, so that they can feel the testis. Now would you criticize me for that, or not?

**Blandy:** That is, I know, the traditional method. In my department young men with tense hydroceles are very rare, and they get treated as testicular cancers.

**Hargreave:** So you really bring them in the same day or the next day and get on with it?

**Blandy:** The delay between finding and exploring a funny testicle should, I think, be just long enough for me to get the markers done. I think it's reasonable and correct to have pre-operative markers. And sometimes it's worth waiting a day or two in order to make sure that it is benign.

**Hargreave:** So you tend to rename them, with a label like, "possibly a testicular tumor, and needs exploration". But if for some reason you cannot get them in instantly, would you then say it was reasonable to aspirate?

**Blandy:** Well, I just don't like sticking needles into the scrotum. Still I know the Scandinavians routinely do fine needle aspiration in their testicular tumors, and they're probably quite safe.