The Significance of Alpha-Fetoprotein in the Serum of Patients with Malignant Teratomas and Related Gonadal Neoplasms

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ABSTRACT

Sera from eleven patients with gonadal germ cell tumors were tested for alpha-fetoprotein (AFP) by counterelectrophoresis. Ovarian neoplasms containing either the endodermal sinus tumor (EST) or dysgerminoma and half of the testicular embryonal carcinomas elaborated the protein into the serum. The presence of AFP in the serum seemed to correlate with early metastasis or a fatal outcome. That the highest levels of AFP were found in cases of EST was in keeping with the theory that the tumor originates in cells of the yolk sac, a principal embryonic site of AFP synthesis.

Introduction

The fetal antigen known as alpha-fetoprotein (AFP) has been the subject of extensive study in recent years since the discovery that it appeared in the serum of patients afflicted with hepatocellular carcinoma. With the report of Masopust et al in 1968, malignant teratomas of the ovaries, testes and extragonadal regions were shown also to elaborate AFP. Through the clinical application of immunoprecipitin analysis by agar gel diffusion and counterelectrophoresis, these two malignant neoplasms have been detected in patients at risk, and post-therapeutic surveillance has been effective in the early discovery of recurrence or metastasis.

In a recent review, Lawrence and Neville suggested that a study of fetal antigens might aid the histopathologist in establishing new functional criteria by which tumors of identical morphology could be sub-classified into categories of etiologic and behavioral relevance. Similarly, they observed that tumors of uncertain histogenesis could be more precisely classified by identifying them as sites of synthesis of known fetal antigens. The endodermal sinus tumor (EST) of Teilum, whose vitel-line origin was proposed on comparative morphological grounds in 1959, was considered by the former authors a likely source of AFP synthesis. Ballas and more recently Wilkinson et al have described single cases of fatal EST associated with high levels of circulating AFP. Both reports cited the work of Gitlin et al who have shown that the human yolk sac in first trimester embryos is an important source of AFP and other plasma proteins.

In this report, a new case of AFP-positive endodermal sinus tumor will be added
to the literature and observations will be made concerning AFP in histogenetically or histologically similar cases.

**Materials and Methods**

The patients who formed the majority of the study group all had histologically proven germ cell gonadal tumors. Cases of benign cystic teratoma and pure seminoma were excluded. Serum from eight of these patients was obtained prior to resection of the primary tumor. The serum from cases 1, 2 and 4 was drawn when abdominal tumor had recurred. Patients 12 and 13 had histologically proven clear cell adenocarcinomas of the vagina and right ovary respectively, the former arising in a young woman whose mother had taken stilbestrol during pregnancy. These cases are included because clear cell carcinomas of the female genital tract have been lumped with the EST under the designation "mesonephric" tumors for many years. The serum from each of these patients was obtained prior to surgery.

Undiluted sera were tested for AFP by the agar counterelectrophoresis technique previously described by the author, a method sensitive to concentrations of AFP of about 100 ng per ml. Slides were examined before and after staining with Amidoschwarz to enhance the detection of weak positives. In order to enhance the sensitivity of the method about three-fold, one ml serum aliquots were concentrated by dehydrating them with 0.14 g of polyacrylamide gel beads* for ten minutes. Native and concentrated sera were run simultaneously with positive and negative controls. Positive sera were graded according to the following criteria:

- **+++** Precipitin lines barely visible on unstained slide, but obvious on stained slide.
- **++** No precipitin lines on unstained slide. Obvious on both sera of stained slide.
- **+** Precipitin line only with concentrated serum of stained slide.

**Results**

As shown in table I, cases 1 and 2, both tested at times of extensive abdominal metastasis, had the highest AFP levels and were high enough to be quantitated by radial immunodiffusion. Both of these tumors were yolk sac carcinomas, endodermal sinus type,—the former admixed with an adult type teratoma, the latter pure.

Of the remaining teratomas in females, only case 3, an embryonal teratoma with a predominating dysgerminoma, was positive. When measured pre-operatively, it was positive only on the stained preparation (+++). However, prior to resection of recurrent tumor and contralateral oophorectomy at a second-look laparotomy, the AFP was positive even on the wet agar slide (+++). The abdominal recurrence proved to be a pure dysgerminoma.

The group of male germ cell tumors consisted of four pure typical embryonal carcinomas, one embryonal carcinoma with teratomatous and trophoblastic components and one adult teratoma containing an embryonal carcinoma. Cases 6, 7 and 10 were weakly positive for AFP (++, +, +). All had early metastasis and two were dead in twelve months. None of the three AFP-negative patients has died, although the follow-up is less than two years in every case. Neither case of clear-cell adenocarcinoma was positive for AFP.

**Discussion**

In the most widely accepted classification of germ cell tumor histogenesis, embryonal carcinoma occupies a pivotal posi-

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* Lyphogel®, Gelman Instrument Co., Ann Arbor, MI.
**TABLE I**

**Serum Alpha-Fetoprotein (AFP) in Patients with Gondal Germ Cell Tumors or with Clear Cell Carcinomas of Female Genital Tract**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Pathologic Diagnosis</th>
<th>Serum AFP</th>
<th>Therapy</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before Surgery</td>
<td>After Surgery</td>
<td>During Recurrence</td>
</tr>
<tr>
<td>1*</td>
<td>20</td>
<td>F</td>
<td>Adult teratoma with yolk sac carcinoma, endodermal sinus type, right ovary</td>
<td>N.D.</td>
<td>N.D.</td>
<td>++++</td>
</tr>
<tr>
<td>2**</td>
<td>18</td>
<td>F</td>
<td>Embryonal adenocarcinoma with features of mesonephric carcinoma, probably yolk sac carcinoma, endodermal sinus type, left ovary</td>
<td>N.D.</td>
<td>N.D.</td>
<td>++++</td>
</tr>
<tr>
<td>3***</td>
<td>8</td>
<td>F</td>
<td>Embryonal teratoma with dysgerminoma, right ovary</td>
<td>++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>F</td>
<td>Embryonal teratoma with predominance of neural elements, right ovary</td>
<td>N.D.</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>F</td>
<td>Solid adult teratoma with glial and other mature neural elements, left ovary</td>
<td>-</td>
<td>N.D.</td>
<td>N.R.</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>M</td>
<td>Typical embryonal carcinoma with teratoma and choriocarcinoma, left testis</td>
<td>++</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>M</td>
<td>Typical embryonal carcinoma, right testis</td>
<td>+</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>M</td>
<td>Typical embryonal carcinoma, left testis</td>
<td>-</td>
<td>N.D.</td>
<td>N.R.</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>M</td>
<td>Typical embryonal carcinoma with seminoma, left testis</td>
<td>-</td>
<td>N.D.</td>
<td>N.R.</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>M</td>
<td>Typical embryonal carcinoma, right testis</td>
<td>+</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>F</td>
<td>Papillary clear cell adenocarcinoma of vagina (maternal stilbestrol ingestion)</td>
<td>-</td>
<td>N.D.</td>
<td>N.R.</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>F</td>
<td>Clear cell adenocarcinoma, right ovary</td>
<td>-</td>
<td>N.D.</td>
<td>N.R.</td>
</tr>
</tbody>
</table>

+++ to ++++ = Positive for AFP (see text); — = Negative for AFP; D.O.D. = Died of disease; N.E.D. = No evidence of disease; N.D. = Not done; N.R. = No recurrence; C = Chemotherapy; S = Surgery; R = Radiotherapy; * Previously reported in reference 1; ** Slides not available for review. Cytologic smears and cell block of ascitic fluid identical to Case 1; *** Mentioned briefly in reference 1.

The primitive cells of these tumors are regarded as multipotential, capable of differentiation along embryonic lines (teratoma) or in extra embryonic directions (endodermal sinus tumor and choriocarcinoma). The totipotential germ cells,
precursors of the embryonal cells and themselves of yolk sac origin, may also differentiate into cells which eventuate in seminomas or dysgerminomas, tumors that exhibit neither embryonic nor extra-embryonic structures.

In cases 1 and 2, the major or exclusive malignant element was the EST (figure 1). Both tumors were associated with very high levels of AFP, findings consistent with Teilum's hypothesis of their vitelline origin. It remains to demonstrate AFP levels in small localized tumors, since both of these were measured when there was ascites owing to widespread abdominal metastasis. Since endodermal sinus tumors comprise over 90 percent of so-called embryonal carcinomas in girls under 20 years of age, it is expected that this population would yield the highest number positive for AFP. None of these tumors has been subjected to in vitro studies of AFP synthesis, nor has immunofluorescence been employed to demonstrate AFP in the PAS-positive glob-
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Figure 3. Case 3. An elongated cystic space containing several embryonal epithelial elements which are difficult to classify. The vesicular bodies at left may be yolk-sac derivatives, capable of producing AFP. The major malignant element was a dysgerminoma (not shown). (H & E ×40)

ules encountered in the cytoplasm of human tumor cells (figure 2). Of equally great interest would be the demonstration of AFP in the serum and tumor cells of patients with polyvesicular vitelline tumors of the ovary and the testicular adenocarcinoma of infancy, both characterized as yolk sac tumors.19

If yolk sac tissue is the principal site of AFP synthesis in teratoid neoplasms, how can positive tests be explained in case 3 in which the predominant malignant element was dysgerminoma? The homologous male seminoma has been associated with negative AFP assays by extremely sensitive techniques.7 Nevertheless, large primary and metastatic dysgerminomas, as were present in case 3, may synthesize small amounts of the protein. On review of the original slides primitive embryonal structures are seen which are difficult to classify, but which may represent small vitelline foci or perhaps male-type embryonal carcinoma (figure 3).

Sera from the two ovarian teratomas (cases 4 and 5) in which nervous system components predominated were both negative for AFP, despite pathologic evidence of widespread omental and peritoneal metastasis in the former patient eight months after primary surgery (figure 4). Neither of these patients harbored germ cell foci in their teratomas. In Gitlin's experiments AFP was not synthesized by human or rat embryonic brain tissue cultures,6 suggesting that neither benign nor malignant teratomas in which nervous tissue flourished would produce AFP. However, in a recent report,4 a 17 year old girl with a recurrent embryonal teratoma containing primitive neural tissue was found to have a serum AFP of 1,500 ng per ml. Following extirpation of the recurrent tumor and chemotherapy the AFP fell to normal levels, although it failed to rise when another recurrence developed which consisted only of mature glial implants. The authors suggested that the source of AFP might have been immature neural components, although the solid cell mass in one illustration is not a convincing neuroectodermal derivative.

Embryonal carcinoma of the classical type seen in the testis3 is known to give positive tests for AFP in from 27 percent to 50 percent of cases14 depending on the analytical method employed. Weakly positive results in cases 6, 7 and 10 lead to several speculations: (1) Is the low level of serum AFP merely a reflection of the
small size of the tumors (3.5, 7 and 4 cm maximal diameter each) when compared to the EST cases? (2) Do the neoplastic embryonal cells actually produce AFP or are occult foci of EST responsible? (3) If the embryonal cells do produce AFP, do they synthesize smaller amounts than EST cells? (4) Why is the classical embryonal carcinoma almost exclusively a tumor of the testis and EST mainly an ovarian expression? The answers to all of these questions await further investigation. Of clinical and histogenetic interest is the recent observation that in patients with mixed testicular teratomas containing trophoblastic and embryonal elements, the levels of serum AFP and human chorionic gonadotropin varied discordantly during chemotherapy, suggesting that the two proteins were being synthesized by distinct cellular populations.\textsuperscript{2}

Cases 12 and 13 (figure 5), clear cell carcinomas of the vagina and ovary, would have been called mesonephromas in the
TABLE II
PROBABILITY OF GERM CELL TUMOR BEING ASSOCIATED WITH POSITIVE
SERUM ALPHA-FETOPROTEIN (AFP)

I. Most probable
   A. Teratoma with endodermal sinus tumor
   B. Pure endodermal sinus tumor
   C. Embryonal carcinoma, pure or mixed
   D. Gonocytoma, type II
   E. (Yolk sac carcinoma, polyvesicular vitelline type)*
   F. (Yolk sac carcinoma, infantile testicular type)*

II. Least probable
   A. Benign cystic teratoma
   B. Pure choriocarcinoma
   C. Teratomas with epidermoid carcinoma, carcinoid tumor, or struma ovarii
   D. Solid adult teratoma

III. Probability indeterminate
   A. Embryonal teratoma with predominant nervous tissue elements (see case 4)
   B. Embryonal teratoma with predominant dysgerminoma (see case 3)
   C. Pure seminoma or dysgerminoma

* No cases yet reported. AFP is expected on morphologic grounds.

past. Recent authors have rejected such designations because of the ultrastructural similarity of the tumor cells to Arias-Stella cells in endometrial glands or because they are frequently associated with ovarian endometriosis, vaginal adenosis, or typical endometrial carcinoma, each an alteration of Müllerian derivatives. That pre-operative sera from both of these women were negative for AFP is further support for exclusion of these tumors from the category of "mesonephromas" in which, regrettably, some pathologists still include the EST.

The relative probability of germ cell tumors yielding a positive serum AFP by immunoprecipitin techniques is compiled in table II from this discussion and from a review of the literature. Strict histologic criteria are the basis for inclusion in the table. The following conclusions seem justified:

1. Accurate histologic diagnosis of these teratoid neoplasms should include reference to the major tissue components with special emphasis on germ cell elements. Future reports of AFP in teratomas should subclassify the tumors according to recognized morphologic criteria so that the data becomes more intelligible.
2. The association of endodermal sinus tumors with high serum levels of AFP is compatible with the hypothesis of a vitelline origin.

3. A strongly positive AFP test by counter-electrophoresis indicates a poor prognosis.

4. A positive serum AFP in embryonal carcinoma may be correlated with metastasis or a fatal outcome.

5. Clear cell carcinomas of the female genital tract are histologically distinct from EST and do not give a positive serum AFP.

Acknowledgments

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References


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