As is known, human \( \alpha \)-fetoprotein (AFP) was the first embryonic and tumour-associated protein that has been accepted as a valuable indicator in the diagnosis and monitoring of cancer. In 1963, AFP was first independently discovered in mouse chemically induced hepatomas by Abelev et al. and in human hepatocellular carcinoma by Tatarinov. Subsequently, the immunodiffusion testing for AFP was suggested for use in the laboratory to aid in the diagnosis of hepatocellular liver carcinoma because AFP was hardly detectable either in cholangiocellular liver carcinoma or in secondary liver carcinomas and other nonmalignant liver diseases. These observations were fully confirmed by several research groups. Furthermore, AFP was found in serum of some patients bearing malignant teratomas of the testis and the ovary.

By radioimmunoassay, serum AFP levels can be measured in normal adult donors at the range of 1–40 ng/ml. The elevated AFP levels of 30–500 ng/ml in normal individuals living in South Africa remain to be clarified. As for the temporary increase of AFP in patients with liver cirrhosis, it might be associated with high risk for the development of hepatocellular carcinoma.

In human early embryogenesis, AFP is synthesized first by the yolk sac and later by the liver, and apparently by embryonic gastrointestinal tract. AFP is the dominating serum protein between the 10th and 18th week of gestation when its concentration increases to 3–4 mg/ml and slowly decreases at birth until 0.01–0.15 mg/ml, so that the serum AFP concentration is approximately 0.3–4% of the peak level in the fetus. After birth, the serum AFP concentration falls rapidly during a few months to the normal adult level, i.e., less than 0.001% of the peak level.

This paper describes recent clinical observations concerning the use of AFP test in malignant tumours with hepatic and nonhepatic origin.

Methods for AFP Determination

At present, several immunochemical methods for the detection and quantitation of AFP in a variety of biological fluids are available (Table I). The reagents commercially available from different laboratories could be completely provided for both clinical and experimental needs. The sensitivity of AFP determination is important, since the evaluation of clinical and experimental investigations depends first upon what method is used, its specificity, and sensitivity. Several modifications of immunodiffusion test for AFP reveal AFP in the range of 1,000–5,000 ng/ml with an absolute specificity. Highly sensitive methods have been developed for the measurement of AFP. Firstly, the indirect immunoaautoradiographic method was used for the determination of AFP in serum of different patients. This method appears to be about 15–30 times more sensitive than
Table I. Sensitivity of Various Methods for Detection and Quantitation of AFP

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (ng/ml)</th>
<th>Some commercial names and companies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single radial immunodiffusion in agar</td>
<td>2,500~5,000</td>
<td>Boehringerwerke, AFP test (W. Germany)</td>
<td>29</td>
</tr>
<tr>
<td>Double immunodiffusion with standard system</td>
<td>1,000~3,000</td>
<td>AFP test, Gamaleya Res. Institute (U.S.S.R.)</td>
<td>21</td>
</tr>
<tr>
<td>Counter-current immunoelectrophoresis</td>
<td>500<del>1,500, 800</del>1,000</td>
<td>Hyland, AFP-test (Belgium), AFP-Rapidophor (Austria)</td>
<td>24</td>
</tr>
<tr>
<td>Hemagglutination test</td>
<td>50~500</td>
<td>FP test, Mochida (Japan)</td>
<td>28</td>
</tr>
<tr>
<td>Immunoautoradiography</td>
<td>50~100</td>
<td>AFP-Radiotest (U.S.S.R.)</td>
<td>12</td>
</tr>
<tr>
<td>Enzyme-immunoassay</td>
<td>1~10</td>
<td>Shino-test, Ltd. (Japan)</td>
<td>10</td>
</tr>
<tr>
<td>Double antibody radioimmunoassay</td>
<td>0.1~1</td>
<td>AFP kit Abbot (U.S.A.), CTS (Belgium)</td>
<td>19, 41</td>
</tr>
</tbody>
</table>

Table II. First Steps of Cancer Testing by Immunodiffusion Test on AFP

<table>
<thead>
<tr>
<th>Area and country</th>
<th>Year and reference</th>
<th>Hepatocellular carcinomas</th>
<th>Cholangiocellular carcinomas</th>
<th>Other than liver cancer</th>
<th>Non-cancer liver diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrakhan, U.S.S.R.</td>
<td>1963 (46)</td>
<td>2/2</td>
<td>0/1</td>
<td>0/8</td>
<td>0/38</td>
</tr>
<tr>
<td>Astrakhan, U.S.S.R.</td>
<td>1964 (48)</td>
<td>3/3</td>
<td>0/2</td>
<td>0/13</td>
<td>18/12</td>
</tr>
<tr>
<td>Astrakhan, U.S.S.R.</td>
<td>1966 (49)</td>
<td>5/5</td>
<td>0/3</td>
<td>0/19</td>
<td>0/79</td>
</tr>
<tr>
<td>Moscow, U.S.S.R.</td>
<td>1967 (2)</td>
<td>19/24</td>
<td>0/6</td>
<td>134/226</td>
<td>0/68</td>
</tr>
<tr>
<td>Paris, France</td>
<td>1967 (59)</td>
<td>37/47</td>
<td>—</td>
<td>0/60</td>
<td>0/284</td>
</tr>
<tr>
<td>Astrakhan, U.S.S.R.</td>
<td>1967 (51)</td>
<td>12/14</td>
<td>0/5</td>
<td>0/21</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>5 years</td>
<td>78/95</td>
<td>0/15</td>
<td>13/347</td>
<td>1/481</td>
</tr>
</tbody>
</table>

a) Acute yellow liver atrophy after viral hepatitis.
b) Testicular tumors

an ordinary gel precipitation, but maintains the absolute specificity of an immunochemical reaction. Secondly, the radioimmunoassay or enzyme-immunoassay was also applied recently to AFP determination. The minimum detectable amounts of AFP were 0.1 and 1.0 ng/ml, respectively.

By the use of AFP tests in a variety of tumours, new information has come to light which is allowing more extensive clinical application of AFP testing. Although AFP elevation is still seen chiefly in hepatocellular carcinoma, there are, at present, very important applications of AFP testing for the diagnosis and monitoring of germ cell carcinoma and for the diagnosis of some gastrointestinal tumours.

AFP in Cancer Testing

Primary Cancer of the Liver

In 1963, the presence of AFP in serum of two patients was first associated with hepatocellular carcinoma by Tatarinov who suggested subsequently immunodiffusion AFP test for differential diagnosis of primary cancer of the liver and secondary liver cancer. Since AFP could not be found normally in any pathological and cancerous conditions, including secondary liver cancer and cholangiocellular liver carcinoma, its finding in adult patients was considered as diagnostic of hepatocellular carcinoma.

In 1967, the first confirmations of above-mentioned clinical results were reported by three laboratories in Astrakhan, Moscow, and Paris.
and Paris. As shown in Table II, during the first 5 years of AFP application, 110 patients suffering from primary cancer of the liver, 450 patients with a variety of tumours, and approximately 500 patients who had different nonmalignant diseases were studied. Of 95 patients with biopsy-documented diagnosis of hepatocellular carcinoma, 78 patients had initial serum elevated AFP levels which were detectable by immunodiffusion, while control serum samples from normal adults and from the patients with malignant and benign diseases were normally AFP-negative. Only one of the 481 nonmalignant cases had positive AFP and that patient had acute yellow atrophy of the liver. Among the 347 cases of non-hepatic tumors, the so-called false-positives were found only in 13 cases and all cases had embryonal teratoblastomas of the testis. In principle, analogous results were obtained in a variety of geographical areas with the percentage of AFP-positive cases with hepatoma ranging from 42% to 87% (Table III). Although there were differences in the percentage of AFP-positive hepatomas between Asia-African and Caucasian countries, it was obvious that the worldwide application of AFP test became absolutely necessary for the diagnosis of hepatocellular carcinoma. The specificity of AFP test for hepatocellular carcinoma appears to be very high and not affected by the racial, geographic, or enviromental factors. The geographical and ethnic differences of positive test on AFP in hepatocellular carcinoma could be explained by differences in age, sex, cancer size, and sensitivity of AFP detection.

Immunofluorescence study has shown that AFP is produced by only a part of tumour cells, and serum AFP level can be correlated with the number of AFP-producing cells in the tumour. No correlation was found with any of the clinical parameters investigated nor with the histological subtype or degree of differentiation of liver cancer.

Immunodiffusion test on AFP was also used in mass survey for primary cancer of the liver by two groups from Senegal and China (Table IV).

As there was an increase in the sensitivity of AFP measurement by different methods (Table I), it became necessary to consider if the specificity of an elevated AFP serum concentration was still diagnostic for hepatocellular carcinoma.

### Table III. Presence of AFP in Serum of Patients with Hepatocellular Carcinoma in Different Countries (Immunodiffusion Test on AFP)

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of patients examined</th>
<th>Rate of positive cases (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia</td>
<td>100</td>
<td>87</td>
<td>26</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>42</td>
<td>64</td>
<td>43</td>
</tr>
<tr>
<td>Singapore</td>
<td>29</td>
<td>72</td>
<td>35</td>
</tr>
<tr>
<td>Japan</td>
<td>227</td>
<td>78</td>
<td>19</td>
</tr>
<tr>
<td>South Africa</td>
<td>130</td>
<td>78</td>
<td>38</td>
</tr>
<tr>
<td>Senegal</td>
<td>44</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td>Mozambique</td>
<td>37</td>
<td>68</td>
<td>36</td>
</tr>
<tr>
<td>Uganda</td>
<td>40</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>France</td>
<td>30</td>
<td>60</td>
<td>11</td>
</tr>
<tr>
<td>Greece</td>
<td>35</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>Spain</td>
<td>17</td>
<td>42</td>
<td>55</td>
</tr>
<tr>
<td>England</td>
<td>41</td>
<td>43</td>
<td>25</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>56</td>
<td>70</td>
<td>42</td>
</tr>
<tr>
<td>U.S.S.R.</td>
<td>112</td>
<td>77</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table IV. AFP Test in Screening for Hepatocellular Carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Senegal (27)</th>
<th>China (58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number screened</td>
<td>9,864</td>
<td>343,999</td>
</tr>
<tr>
<td>Total number with elevated AFP</td>
<td>6</td>
<td>147</td>
</tr>
<tr>
<td>Number with elevated AFP/100,000</td>
<td>60</td>
<td>43</td>
</tr>
<tr>
<td>Number with apparent false-positives</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Number of false-negatives/total evaluable</td>
<td>3/9,864</td>
<td>7/11,004</td>
</tr>
<tr>
<td>Number + AFP before clinical evidence/total evaluable primary liver cancer patients</td>
<td>3/9</td>
<td>33/53</td>
</tr>
</tbody>
</table>
cellular carcinoma. By indirect immunoauto-
radiography, an elevated AFP levels could
be found in serum of patients with infections
and serum hepatitis,\(^5\) as well as in HB-anti-
gen-positive sera,\(^4\) although the association
of HB-antigen and AFP is not completely
clarified. By radioimmunoassay, the increase
of positive tests on AFP was observed both
in primary cancer of the liver and noncancer
liver diseases, as well as in other noncancer
diseases. In spite of this decrease in the
specificity of the AFP radioimmunoassay,
quantitative determination of AFP by radio-
immunoassay is valuable for the monitoring
of effective treatment of hepatocellular car-
cinoma by surgical resection and/or system-
atic chemotherapy.\(^3\) It is important that
in such cases the increased AFP level
indicates residual liver cancer cells either in
primary tumour site or in metastatic organs.

Thus, less sensitivity AFP tests may be
important for the immunodiagnosis and the
screening of hepatocellular carcinoma by surgical resection and/or system-
atic chemotherapy.\(^3\) It is important that
in such cases the increased AFP level
indicates residual liver cancer cells either in
primary tumour site or in metastatic organs.

Germ Cell Tumours

The relationship between AFP and the
so-called embryonal teratocarcinomas of the
testis was first found by Abelev et al.\(^2\) and
by Masopust et al.\(^3\) These results are listed
in Table V. Subsequently, these observa-
tions were confirmed and extended by many
authors\(^9, 20, 34, 45, 54, 56, 57, 60\) It has been shown
that an elevated AFP concentrations are
always associated with endodermal sinus tu-
mours, both gonadal and extragonadal sites.
In accordance with histological classification
suggested by Teilum,\(^53\) the differentiated
forms of embryonal carcinoma may be di-
vided into three histologic types of (1) endo-
dermal sinus carcinoma or yolk sac tumour,
(2) choriocarcinoma, and (3) teratoma. In
embryonal carcinomas, probably, there is a
variety of combinations of the three different

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Number of patients examined</th>
<th>Number of positive reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teratoblastoma with features of embryonal carcinoma</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Teratoblastoma with features of seminoma and choriocarcinoma</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Teratoblastoma</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Seminoma</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Tumour of the stroma cells</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>10</td>
</tr>
</tbody>
</table>

Since all embryonal carcinomas which
have endodermal sinus elements are associat-
ed with an initial increased serum AFP
levels, the measurement of AFP may be
used for clinical diagnosis and for the estima-
tion of prognosis. In addition, the AFP
level may also serve in the monitoring of surgical, chemotherapeutic, and radiothera-
peutic treatments of germ cell tumours.

Gastrointestinal Tumours

The first finding of AFP in serum of patients
with other than hepatocellular carcinoma or
embryonal teratomas was considered
false-positive reactions for AFP.\(^4, 35\) However,
further investigations\(^7, 18, 52\) have shown that
several tumours having nonhepatic or non-
endodermal sinus origin are associated with
elevated serum AFP levels. Most often, the
elevated AFP levels are found in the patients
with gastric, pancreatic, and biliary tract
tumours (Table VI). It is very important
that AFP could be detectable not only by a
Table VI. Occurrence of Elevated AFP Levels in Patients with Gastrointestinal Tumours

<table>
<thead>
<tr>
<th>Primary tumour site</th>
<th>Number of patients examined</th>
<th>Immunodiffusion</th>
<th>Radioimmunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Stomach</td>
<td>95</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>45</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>Esophagus</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Small bowel</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colon-rectum</td>
<td>191</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>353</td>
<td>4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Values are in thousands.

Table VII. Cancer Tests for Different Areas in 1976

<table>
<thead>
<tr>
<th>Test</th>
<th>U.S.A.</th>
<th>Europe</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>3,000</td>
<td>2,000</td>
<td>1,000</td>
<td>6,000</td>
</tr>
<tr>
<td>CEA</td>
<td>1,300</td>
<td>80</td>
<td>50</td>
<td>1,430</td>
</tr>
<tr>
<td>AFP</td>
<td>50</td>
<td>75</td>
<td>250</td>
<td>375</td>
</tr>
<tr>
<td>Human chorionic gonadotropin</td>
<td>200</td>
<td>50</td>
<td>25</td>
<td>275</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>4,570</td>
<td>2,215</td>
<td>1,325</td>
<td>8,115</td>
</tr>
</tbody>
</table>

Values are in thousands.

Comparative immunodiffusion studies show that AFP from patients with gastric and pancreatic as well as with colon carcinomas is identical to AFP from fetal serum and from the serum of patients with hepatocellular or embryonal cell tumours.

It could be postulated that tumour cells from these AFP-producing malignancies themselves possess the capacity to produce AFP. Apparently, AFP is a product of an activated embryonic gene which is suppressed in differentiated adult tissues. However, the origin and mechanism of AFP synthesis in nonhepatic and non-embryonal cell tumours remain to be studied.

It is not possible to use the AFP for the early diagnosis of gastrointestinal tract malignancies, excluding hepatocellular carcinoma, but the serial quantitations of AFP in the AFP-producing tumours is important for evaluating the effectiveness of surgery and/or chemotherapy, as the AFP concentration is an indication of the amount of tumour present.

Future Outlook in Cancer Testing by AFP

During the last 15 years, AFP test was extensively being investigated both in primary cancer of the liver, in embryonal carcinomas, and in other malignant and benign diseases. At present the testing for AFP is being performed worldwide for both cancer testing and for the diagnosis of maternal and fetal disorders. By comparison, in 1976 the AFP test was being performed in Europe and other areas excluding U.S.A., at the same time when acid phosphatase and carcinoembryonic antigen (CEA) tests were being more frequently used in U.S.A. than in Europe and Asia (Table VII). According to a projection made by King, the testing for AFP should show the most dramatic growth by 1980, primarily because of the potential for fetal
neural tube defects. This material is summarized in Table VIII.

In the near future, AFP will be combined with a variety of immunochemical tests such as CEA, acid phosphatase, human chorionic gonadotropin, and other placental proteins which will be used to diagnose primary and recurrent diseases. This will hopefully help us to define better approaches to chemotherapy and to the immunotherapy of tumours.

**REFERENCES**

REVIEW ON \(\alpha\)-FETOPROTEIN