Clinical applications of serum carcinoembryonic antigen and alpha-fetoprotein levels in children with solid tumours

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SUMMARY A study was carried out on serum carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) levels, both measured by radioimmunoassay, in 88 children with malignant solid tumours and in 26 children with nonmalignant disorders, who presented during the years 1973-77. Slightly or moderately raised CEA levels were found at presentation in 11 of 66 children with malignant tumours, in 2 others with recurrent tumours, and in 4 children with nonmalignant disorders. Raised CEA levels generally indicated advanced malignant disease, often affecting the liver, or other hepatic disorders, but were not associated with a specific tumour type.

Except in the first months of life, significantly raised AFP levels were detected only in 11 patients with yolk sac-derived tumours, or hepatomas, and in one child with tyrosinosis who later developed a malignant hepatoma. Serial measurements of AFP accurately reflected the clinical response to treatment and in 2 patients indicated recurrence before this could be detected clinically.

Carcinoembryonic antigen (CEA), a glycoprotein, was first found as an apparently specific surface antigen associated with adenocarcinoma of the human colon by Gold and Freedman (1965). However, although about 70% of patients with gastrointestinal malignancy have raised levels, raised CEA has since been found in patients with other adult cancers (Booth *et al.*, 1973; Neville and Laurence, 1974) and certain nonmalignant disorders. Nevertheless, serial studies have shown that in adult patients with CEA-releasing colorectal carcinomas serum levels fall if treatment is successful and rise again when metastases occur (Booth *et al.*, 1974). Studies of serum CEA in children with neuroblastoma (Reynoso *et al.*, 1972; Wang *et al.*, 1974; Frens *et al.*, 1976) showed that serum CEA levels were usually raised when active tumour was present and fell when treatment was successful. That CEA may act as a tumour marker in paediatric malignancy was also suggested by Felberg *et al.* (1976), who found raised levels in retinoblastoma patients.

Alpha-fetoprotein (AFP), a fetal protein chemically similar to albumin, was first identified by

Pedersen in 1944. Raised serum levels were described in association with hepatomas in rodents (Abelev *et al.*, 1963) and humans (Tatarinov, 1964) and in patients with malignant gonadal teratoblastomas (Abelev *et al.*, 1967). Raised levels have also been found in patients with certain nonmalignant disorders (Neville and Cooper, 1976). Early studies (Abelev *et al.*, 1967; Masopust *et al.*, 1968; Mawas *et al.*, 1969) of over 300 children with malignant tumours, using comparatively insensitive bidimensional immunodiffusion methods, showed detectably raised levels in a proportion of children with teratomata, gonadal embryonal carcinomata, hepatoblastoma, and hepatocellular carcinoma, but in none with the commoner tumours such as neuroblastoma and nephroblastoma. Radioimmunoassay has increased the sensitivity of the test by more than 1000-fold, and has permitted the detection of AFP in sera from most children with yolk sac-derived and hepatic malignancies.

Studies in some 30 paediatric cancer patients suggest that AFP monitoring by this sensitive method may be of value during treatment (Hagesawa *et al.*, 1972, 1973; Takahashi, 1973; Itoh *et al.*, 1974; Kohn and Weaver, 1974; Teilum *et al.*, 1974; Pick *et al.*, 1975; Nørgaard-Pedersen *et al.*, 1975,

1976; Tsuchida *et al.*, 1975; Palmer *et al.*, 1976; Urano *et al.*, 1976; Shirai *et al.*, 1976; Sakashita *et al.*, 1976; Schoenfeld *et al.*, 1976; Grigor *et al.*, 1977).

The purpose of our study was to define the frequency of raised serum CEA and AFP levels, both measured by radioimmunoassay, in children with malignant solid tumours, and to assess the clinical value of serial measurements in monitoring response to therapy and detecting tumour recurrence. We also report the findings in 25 children with nonmalignant disorders.

Patients and methods

Eighty-eight children referred during 1973–77 for treatment of malignant solid tumours and 26 children being investigated or treated for what proved to be nonmalignant conditions were studied. In patients found to have raised CEA or AFP levels serial venepunctures were performed to assess the relationship between the serum levels and response to therapy. In other patients blood for CEA and AFP levels was taken during venepuncture for other investigations, or for the administration of cytotoxic drugs. The patients' ages ranged from 5 days to 15 years, 11 being aged less than one year, and their diagnoses are shown in Tables 1 and 2.

CEA levels were measured by the double-antibody technique, the 95% confidence limits of normal healthy adults being 0–15 ng/ml, standard error 1.9 (Booth *et al.*, 1974). In some patients a comparison was made, using the same sera, with CEA levels measured by the Z-gel method (Lo Gerfo *et al.*, 1971), the normal upper limit in healthy adults being 2.5 ng/ml, the reproducibility of the CEA Roche assay being ± 0.5 ng/ml at this level.

AFP levels, normally less than 25 ng/ml in adults (Grigor *et al.*, 1977), were measured by radioimmunoassay. In the first 18 patients AFP

estimations were done in the Department of Cancer Studies, University of Birmingham; in this laboratory the levels in healthy adult males and nonpregnant females were in the range 1–19 ng/ml (mean $\pm$ SD 9.3 ± 8.6). Sera from subsequent patients were tested in the Department of Medical Genetics, Glasgow, using the method of Vince *et al.* (1975), the lower limit of sensitivity varying from <16.7 to <5.0 ng/ml (conversion of these results to units/l $\times 10^{-3}$ using the international reference preparation (first British Standard Human Cord Serum 72/227) can be done by multiplying by 1.5).

Results

CEA. In the majority of patients the CEA levels measured by the double-antibody technique were normal (Fig. 1). Details of the 17 patients with levels >15 ng/ml are given in Table 3. 8 of the 17

Table 2 Details of patients with nonmalignant conditions

Infections (lymphadenopathy 3, toxoplasmosis 1, osteomyelitis 1, hydatid cysts 1)	6
Benign tumours (teratomas 2, liver hamartoma 2, fibroma 1)	5
Inflammatory disorders (rheumatoid arthritis 2, ulcerative colitis 1, sinus histiocytosis 1)	4
Fanconi's anaemia	2
Twin of child with tumour	2
Tyrosinosis	1
Other nonmalignant disorders	6
Total	26

Table 3 Patients with raised serum CEA levels (>15 ng/ml)

Age (years)	Sex	CEA level (ng/ml)	Diagnosis
13.0	M	34.5	Fanconi's anaemia; jaundice; oxymetholone therapy; died 1 year later of leukaemia
7 w	M	29.5	Multiple osteomyelitis
4.4	M	18.0	Tyrosinosis; hepatoma (but included with nonmalignant disorders in Fig. 1)
1.8	M	21.3	Hamartoma of liver
3.9	M	19.0	Histiocytic lymphoma; jaundice
10.2	F	18.0	Histiocytic lymphoma
15.8	F	19.0	Relapsed Hodgkin's disease; hepatic involvement
7.1	M	16.5	Stage I Wilms's tumour
4.7	F	19.8	Stage III Wilms's tumour; hepatic failure
2.5	M	17.3	Stage II Wilms's tumour
7.5	F	15.5	Thoracic mesenchymal tumour
0.9	M	25.3	Stage IV neuroblastoma; hepatic involvement
11.7	F	15.3	Ovarian embryonal carcinoma
1.8	M	21.3	Orchioblastoma (see text)
2.7	F	23.3	Hepatoma (bile duct tumour); jaundice; incomplete resection; CEA 25 before death
1.3	F	31	Hepatoblastoma, recurrent
2.0	M	17.5	Pulmonary blastoma

Table 1 Details of patients with malignant tumours

	No. of patients
Lymphoma (lymphosarcoma 7, histiocytic lymphoma 2, Hodgkin's disease 4)	13
Wilms's tumour	26
Mesenchymal tumours (rhabdomyosarcoma 10, undifferentiated sarcoma 3, leiomyosarcoma 1, fibrosarcoma 1)	15
Neuroblastoma	12
Yolk sac-derived tumours (ovarian embryonal carcinoma 1, orchioblastoma 4, sacrococcygeal teratoma 1)	6
Hepatomas	5
Other tumours (Ewing's sarcoma 2, medulloblastoma 1, pulmonary blastoma 1, optic glioma 1, ependymoma 1, branchial cleft carcinoma 1, gliomus tumour 1, uncertain 3)	11
Total	88

Fig. 1 CEA levels in children with malignant solid tumours and nonmalignant disorders. The number of children studied at presentation (P) and at recurrence (R) of their tumours are shown under the diagnosis. Broken line represents the limit of normal values in adults. NED indicates no evidence of disease.

patients with raised CEA have died and one has progressive malignant disease. In 6 patients the CEA levels have fallen to 15 ng/ml or less (infant with osteomyelitis, all 3 children with Wilms's tumour, the girl with embryonal carcinoma of the ovary, and the boy with orchioblastoma), and in the remaining 2 children the CEA levels have not yet been repeated.

Serum from 11 patients was stored at -20°C , and the CEA levels were later remeasured by the Z-gel method (Table 4). Of seven samples from neuroblastoma patients only two were marginally raised, to levels which would not be diagnostically helpful. There appears to be no difference in the degree of abnormality between the two methods.

AFP. The results of the serum AFP levels are shown in Fig. 2. The only children with nonmalignant disorders who had levels >25 ng/ml were a 5-day-old baby with a benign sacrococcygeal teratoma (9500 ng/ml), a 7-week-old infant with osteomyelitis (117 ng/ml), a 9-week-old baby with a benign fibroma (120 ng/ml), and a boy with tyrosinosis aged 1.3 years (28 000 ng/ml). The raised values in the 3 infants were normal for their ages, and in the first 2 had fallen to <25 ng/ml when remeasured at ages 3 and 7 months respectively. Although raised AFP levels have been described in tyrosinosis (Bélanger

Table 4 Comparison between double-antibody and Z-gel methods

Patients	Diagnosis	CEA (ng/ml)	
		Double antibody	Z-gel
3.9	Neuroblastoma	10.0	2.2
4.8	"	9.8	3.4
3.8	"	4.5	3.8
4.4	"	9.8	1.5
1.9	"	12.3	1.9
4.6	"	<4.5	2.2
4.9	"	9.8	2.5
7.1	Nephroblastoma before resection	16.5	2.4
	Nephroblastoma (same patient) after resection	7.3	1.4
3.9	Histiocytic lymphoma; jaundice	19.0	1.6
1.8	Hamartoma of liver	21.3	4.0
13.0	Fanconi's anaemia; jaundice	34.5	4.0

et al., 1973), it is notable that our patient was found to have an unsuspected malignant hepatoma at necropsy 3 years after the AFP level was measured. 2 of Bélanger's patients developed hepatocarcinoma terminally, their AFP levels paralleling tumour growth (Bélanger *et al.*, 1976). Among the children with malignant tumours, grossly raised serum AFP

Fig. 2 AFP levels in children with malignant solid tumours and nonmalignant disorders. The number of children studied at presentation (P) and at recurrence (R) of their tumours are shown under the diagnosis. NED indicates no evidence of disease. ● = levels measured in Glasgow; ○ = levels measured in Birmingham.

was found only in children with yolk sac-derived (malignant teratoma, orchioblastoma, ovarian embryonal carcinoma) or hepatic tumours (Table 5).

A slight rise in serum AFP was present in 2 boys aged 5 and 9.5 years with alveolar rhabdomyosarcoma (42 and 45 ng/ml), and in a boy aged 4 years with neuroblastoma (36 ng/ml). The AFP subsequently fell below 25 ng/ml in all 3 boys although only the first child's tumour responded to treatment.

A level of 43 ng/ml was detected in a 9-week-old baby with pelvic fibrosarcoma, and his AFP remained for some time above the adult normal range (37 ng/ml at age 3 and 5.5 months) but was undetectable by 7 months of age, when there was still no evidence of recurrent tumour. None of the other children with malignant tumours had raised AFP levels at presentation, at recurrence, or after successful treatment.

Serial AFP levels. In the children with yolk sac-derived and hepatic malignant tumours serial AFP levels correlated well with disease status, generally falling to <25 ng/ml within a month of successful resection but remaining raised if resection was incomplete. For example, in a boy aged 1.7 years

Table 5 *Children with malignant tumours and grossly raised AFP*

Age (years)	Sex	AFP (ng/ml)	Comment
1.7	M	20 000	Sacroccygeal teratoma (see text)
0.9	M	204	Orchioblastoma, 5 days after resection; disease-free with normal AFP 17 months later
1.8	M	2 300	Orchioblastoma (see text)
5.0	M	5 400	Orchioblastoma
0.5	M	540	Orchioblastoma, 13 days after resection (see text)
11.7	F	17 000	Embryonal carcinoma of ovary, 6 d after incomplete resection (see text)
2.7	F	2 300	Hepatoma (bile duct tumour); incomplete resection; AFP rose to 54 300 before death
1.8	M	2 200	Hepatoblastoma removed; disease-free with normal AFP 2 years later
1.3	F	778 000	Hepatoblastoma (see text)
7.7	F	3 000	Unresectable hepatoblastoma; died after biopsy
26 d	M	330 000	Hepatoblastoma; died after resection

Fig. 3 Boy aged 1.7 years with malignant sacroccygeal teratoma.

who had incomplete resection of a malignant sacroccygeal teratoma, AFP levels fell but never returned to normal, and rose again before death from metastases (Fig. 3).

In a boy aged 1.8 years with orchioblastoma, serum levels fell rapidly to normal after successful resection (Fig. 4). His CEA levels also fell, but less promptly. Another boy aged 6 months with orchioblastoma had AFP levels of 540, 46, and 10 ng/ml, 13, 28, and 41 days after apparently successful resection. However, the level 4.5 months after resection rose to 3060 ng/ml; despite the absence of abnormal clinical findings when this blood specimen was taken, 16 days later he developed a pathological fracture of the femur and the presence of multiple skeletal metastases was confirmed by x-rays and needle aspirate.

An 11-year-old girl with embryonal carcinoma of the ovary had the primary tumour resected, but metastatic nodules were present in the peritoneal cavity. These were treated by whole abdominal irradiation and chemotherapy with vincristine, actinomycin D, and cyclophosphamide. Her AFP levels fell to normal (Fig. 5) and she remains clinically free of disease 33 months from the start of

Figs. 4-6 AFP levels are shown as solid lines and CEA levels as broken lines.

Fig. 4 Boy aged 1.8 years with orchioblastoma.

Fig. 5 Girl aged 11 years with embryonal carcinoma of ovary.

treatment, chemotherapy having been stopped after 24 months.

Apparently complete resection of a hepatoma in a girl aged 1.3 years was followed by a sharp fall in serum AFP levels (Fig. 6). Postoperatively right phrenic palsy occurred but thoracotomy revealed no cause. Her AFP levels did not return to normal and then rose again. Extensive investigations, including chest and skeletal radiography, bone marrow aspirate, liver scan, hepatic angiography, and another laparotomy failed to demonstrate recurrence. However, 2 months later metastases were visible in both lungs on chest x-rays. Chemotherapy with actinomycin D and adriamycin led to temporary disappearance of the metastases and a fall in AFP level, but she also became jaundiced. As the AFP levels rose the pulmonary metastases again became visible. The jaundice faded after actinomycin and adriamycin therapy were stopped but AFP continued to rise and the pulmonary metastases slowly enlarged, despite treatment with cyclophosphamide, and she died. Her CEA levels also reflected the clinical course, though less closely than did the AFP.

Discussion

The best established biochemical marker of paediatric malignancy is the raised urinary catecholamine excretion in patients with neuroblastoma (Marsden and Steward, 1976). The usefulness of measuring

Fig. 6 Girl aged 1.3 years with hepatoblastoma.

CEA levels in paediatric malignancy has not been well assessed, although 16 of 19 children with active neuroblastoma were reported to have raised levels which fell to normal if treatment was successful (Reynoso *et al.*, 1972; Wang *et al.*, 1974; Frens *et al.*, 1976). 4 of 5 retinoblastoma patients had raised CEA levels which fell after enucleation, and raised levels were found in 29.7% of their nonaffected family members (Felberg *et al.*, 1976).

In our study we have found raised CEA levels in 13 of 68 children with malignant solid tumours. In general the rise has been small and no patient had levels in the range (>50 ng/ml) commonly found in adults with gastrointestinal cancer (Booth *et al.*, 1973). There was no specificity for any tumour type, a small proportion of children with lymphomas, Wilms's tumour, mesenchymal tumours, neuroblastoma, yolk sac tumours, hepatomas, and other tumours having raised levels.

It is interesting that only one of our 12 patients with active neuroblastoma had a raised level, in view of the much higher proportion in the American series (Reynoso *et al.*, 1972; Wang *et al.*, 1974; Frens *et al.*, 1976). This difference cannot be explained by a smaller tumour load in our patients, since 8 had stage IV disease (7 have died) and of the 4 who did not have detectable metastases at presentation, 2 have since died of recurrence. Our findings may differ from the Americans' by chance, but alternatively may be related to our assay technique, as the Z-gel method was used for the American work. We therefore remeasured by the Z-gel technique all samples still available from neuroblastoma patients and a few others. The finding of normal or only slightly raised values confirmed the findings from the double-antibody assay, thus eliminating a methodological explanation.

In 5 of the 13 children with malignant tumours and raised CEA, the levels returned to normal after successful treatment; one of these was a girl with Wilms's tumour who was in liver failure due to interference by the tumour with the hepatic blood supply. However, raised CEA was associated in 7 children with advanced disease which could not be cured; 5 children in this group had primary or secondary hepatic tumours.

The 4 children with 'benign' disorders who had raised CEA are of special interest. One had successful removal of a hamartoma of the liver; however, the other child in the series with liver hamartoma had a normal CEA level. Another boy had Fanconi's anaemia and jaundice associated with oxymetholone therapy, which may also have induced the growth of a benign hepatoma; he died later of leukaemia and is reported elsewhere (Obeid *et al.*, 1978). The third child who had tyrosinosis died from portal hypertension shortly after the CEA estimation and was found at necropsy to have an unsuspected malignant hepatoma. The fourth was an infant aged 7 weeks with multiple staphylococcal osteomyelitis whose level subsequently fell to 8.5 ng/ml.

In summary, therefore, children do not usually have raised CEA levels when they are first seen with malignant disease. The detection of raised CEA levels generally indicates advanced malignant disease, often involving the liver, or other hepatic disorders.

AFP is a product of the fetal liver, gastrointestinal tract, and yolk sac. Its synthesis reaches a peak around the 13th week of gestation, when serum levels of 3–4 mg/ml are found. The level then decreases progressively to about 30 µg/ml at birth. By the end of the sixth month the level is usually less than 50 ng/ml, and from the age of 2 years through adulthood the normal serum levels are 2.6 ± 1.6 (SD) ng/ml (Neville and Cooper, 1976;

Elgort *et al.*, 1976). The half-life of the protein in the circulation has been calculated to be between 4 and 9 days, and levels generally fall to the normal range within a month of successful resection of an AFP-producing tumour (Mawas *et al.*, 1969; Hagesawa *et al.*, 1973; Grigor *et al.*, 1977). Caution is required in the interpretation of AFP levels during the first year of life since, using a gel precipitation method, Elgort *et al.* (1976) found high levels in 79% of infants with viral hepatitis and slightly raised levels (15–250 ng/ml) in some children aged less than one year with nonhepatic, nonmalignant disorders and with nonyolk sac, nonhepatic tumours. Even after one year of age 13% of their patients with Wilms's tumour and 5.8% of their patients with neuroblastoma had AFP levels of between 15 and 250 ng/ml. Other disorders which are associated with high serum AFP levels in childhood are Indian childhood cirrhosis (Nayak *et al.*, 1972), hepatitis (Chandra, 1973), certain other non-neoplastic liver and inflammatory bowel diseases (Neville and Cooper, 1976), ataxia telangiectasia (Waldmann and McIntire, 1972), and tyrosinosis (Bélanger *et al.*, 1973).

However, in practice, after the first month of life we have found that it is usually possible to exclude on clinical grounds the other conditions which cause raised AFP when a yolk sac or hepatic tumour is suspected. Moreover, these malignancies generally produce very high serum AFP levels, well outside the physiological range, thus permitting prediction of the histological type of a tumour before surgery.

All our 11 patients with yolk sac and hepatic malignancies had raised AFP levels at presentation which fell to normal if treatment was successful. It is clear that serial levels, especially if measured by radioimmunoassay, may be useful in the detection of recurrence before this is evident clinically, as shown in 2 of our patients and by others (Hagesawa *et al.*, 1972; Kohn and Weaver, 1974; Nørgaard-Pedersen *et al.*, 1975; Grigor *et al.*, 1977). This is important because recurrent or residual disease detected initially by raised serum AFP may occasionally be curable (Grigor *et al.*, 1977). However these authors also point out that in adult males with germ cell tumours, recurrent or progressive disease may be present in the absence of raised AFP levels, presumably because non-yolk sac elements of teratomas are responsible for recurrence in some patients. Further serial studies are required to determine whether this is also true in children.

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