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Combined Measurements of Plasma Aromatic L-Amino Acid Decarboxylase and DOPA as Tumour Markers in Diagnosis and Follow-up of Neuroblastoma

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Abstract—*As neuroblastoma, the most common solid tumour in childhood, may contain all the constituents of the catecholamine biosynthesis cascade, some of these constituents may be produced in excess in a varying mixture reflecting the wide variability in expression of differentiated features of the tumour. We have measured plasma levels of norepinephrine (NE), epinephrine (E), dopamine (DA) and 3,4-dihydroxyphenylalanine (DOPA), and plasma activities of dopamine beta-hydroxylase (DBH) and aromatic L-amino acid decarboxylase (ALAAD) in 18 patients with neuroblastoma, in 13 at various times during the course of their disease. Activities of serum lactic dehydrogenase (LDH), serum levels of ferritin (FER) and neuron-specific enolase (NSE), and urinary vanilmandelic acid (VMA) were also determined.*

NE, E and DBH were found not to reflect tumour activity. In untreated active neuroblastoma DOPA or ALAAD (10 out of 10) or both (six out of 10) were clearly elevated. In all 13 patients where samples were obtained during chemotherapy, ALAAD activities fell within the normal range, while DOPA decreased more slowly. During relapse, DOPA and, especially, ALAAD, rapidly increased; in all six patients who had a relapse both DOPA and ALAAD were elevated. In complete remission (eight patients), ALAAD was normal in all patients, but DOPA remained elevated in the one patient who later experienced a relapse. Our preliminary conclusion is that combined measurements of plasma ALAAD and DOPA may be useful markers for neuroblastoma activity at diagnosis, but even more so in indicating residual disease (DOPA) and in the early detection of relapse (ALAAD).

INTRODUCTION

NEUROBLASTOMA is the most common extracranial malignant solid tumour in childhood. It originates from the sympathetic neural crest and can present in various stages of differentiation. As biochemical markers for diagnosis and for assessment of the course of the disease, urinary vanilmandelic acid (VMA), homovanillic acid (HVA) and other catecholamine metabolites [1, 2], urinary cystathionine [3], and serum ferritin (FER) [4], lactic dehydrogenase (LDH) [5, 6] and neuron-specific enolase (NSE) [7, 8] have been reported to be useful, with varying degrees of specificity and sensitivity.

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Despite this wide range of physical and biochemical techniques for diagnosis and follow-up, residual disease, ultimately giving rise to relapse, often remains undetected. Thus, in spite of the arsenal of chemotherapeutics available, the prognosis of neuroblastoma remains poor, especially in children over 2 years of age [9, 10]. Improvement may be possible when minimal residual disease could be detected reliably at an earlier stage, thereby providing the opportunity to prolong or intensify treatment.

Since neuroblastomas originate from the sympathetic neural crest, they may contain all the constituents of the catecholamine biosynthesis cascade. Some of these constituents, depending on the stage of differentiation of the tumour, may be produced in excess. Elevated plasma values of 3,4-

dihydroxyphenylalanine (DOPA) and of dopamine beta-hydroxylase (DBH) have been reported in some patients [11–14].

We report here the preliminary results of an ongoing study, in which we measured levels of norepinephrine (NE), epinephrine (E), dopamine (DA) and DOPA, and activities of DBH and aromatic L-amino acid decarboxylase (ALAAD) in 76 plasma samples obtained from 18 patients with neuroblastoma. In 13 patients serial samples were obtained at various times during the course of their disease. Urinary VMA and serum LDH, FER and NSE were also determined.

MATERIALS AND METHODS

All materials were obtained from commercial sources, except for alpha-methyl-dopamine (used as internal standard in the DOPA determination), which was a gift from Merck Sharp & Dohme (Rahway, NJ, U.S.A.).

Peripheral venous blood was collected through an indwelling catheter into polystyrene heparinized tubes containing 39 μmol of glutathione. Blood was centrifuged within 15 min (10 min, 4°C, 3000 ***g***), and the plasma stored at −70°C.

Plasma catecholamines (NE, E and DA) were determined, after prior liquid–liquid extraction, by high performance liquid chromatography with electrochemical detection (HPLC-ECD) [15]. DOPA was measured by conversion to dopamine and quantitation of the dopamine by HPLC-ECD [16], while ALAAD was determined by its ability to convert DOPA into dopamine as described previously [17].

DBH was measured by its ability to convert tyramine into octopamine. Incubation was carried out for 30 min at 37°C essentially as described by Kato *et al.* [18]. After the incubation the reaction mixture was oxidized *in situ* with sodium periodate. The *p*-hydroxybenzaldehyde formed was extracted with diethylether, back-extracted into 4 M ammonia and quantitated, after acidification with 2 M HCl, by HPLC with spectrophotometric detection at 280 nm on a C18 reversed-phase column with 10 mM acetic acid containing 10% of acetonitrile as the mobile phase. Enzyme activity is expressed as μmol/l/min or U/l. VMA (Pisano method [19]), LDH and FER were routinely measured at the Clinical Chemistry Laboratory of the Sophia Children's Hospital, Rotterdam, while NSE was determined by a commerical radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

A total of 76 blood samples were obtained from 18 patients. From five patients blood could only be taken on one occasion, while serial measurements (blood obtained on at least three occasions) were possible in the other 13 patients. ALAAD could be determined in all 76 samples, DOPA and DBH in 74, catecholamines in 67, LDH in 72, FER in 64 and NSE in 68. VMA was determined in 62 24-h urine collections. Some relevant data on the 18 patients are given in Table 1. State of disease was judged by the usual criteria including sonography, X-ray skeletal survey, CT scanning, bone and MIBG scintigraphy. Staging was according to the system of the St. Jude's Children's Research Hospital (SJCRH), Memphis, Tennessee [20]. Treatment was given in the same way as in the protocol used at the SJCRH with cyclophosphamide and Adriamycin® for stage IIB and for infants with stages IIIA, B and C.

Cyclophosphamide, Adriamycin®, *cis*-platinum and teniposide (VM-26) were used for children over 1 year of age with stages IIIA, B and C. Before chemotherapy was started, surgery was performed, unless the primary tumour seemed unresectable. After induction therapy all patients were reevaluated and underwent a second-look surgical procedure according to the protocol.

Complete remission is defined (SJCRH) as complete resolution of tumour in metastatic sites and more than 90% regression of the primary tumour attained by and maintained through 4 months induction therapy. A patient is also considered to be in complete remission by surgery when the residual primary tumour appears to be completely resected at second-look surgery (or found to be ganglioneuroma only).

For determining normal values for ALAAD, DOPA, DBH and catecholamines, blood was obtained, after informed consent of their parents, from 75 children, who visited the hospital for non-neoplastic ailments, mostly cystitis and urinary reflux problems, and who were in the same age range (0–14 years) as the patients with neuroblastoma. Plasma ALAAD, DOPA and DBH were measured in all controls, and plasma catecholamines in 50.

Because of the skewed nature of the distribution curves for NE, E and DA average values reported are geometric means, while for comparison of these parameters between groups Mann–Whitney's *U*-test was used. For upper limits of normal as indicated in Figs. 1 and 2 (arithmetic) means + 2 standard deviations are used. In the case of DBH, however, which has a strongly skewed distribution, the third-highest value as measured in the 75 controls was arbitrarily taken as upper limit of normal.

RESULTS

For evaluating the result, all measured parameters were grouped into four categories depending on the status of the patient when the blood sample was obtained:

1. active neuroblastoma, untreated
2. active neuroblastoma, during chemotherapy

Table 1. *Clinical data on the patients with neuroblastoma*

No.	Sex	Stage	Primary localization	Age at first diagnosis (months)	Age* (months)	Chemotherapy*	Present state: Tumour free (months)†	Present state: Active tumour (months)‡	Present state: Died (survival in months)‡
1	M	IIB	abd.adr.	12	13	+	23		
2	F	IIIA	abd.symp.tr.	7	8	+	22		
3	M	IIIC	abd.adr.	38	38	−			12
4	F	IIIC	abd.adr.	4	13	+	23		
5	M	IIIC	abd.symp.tr.	72	82	+			12
6	F	IIIA	abd.adr.	38	38	−	25		
7	F	IIIC	abd.adr.	102	130	−		52(6)§	
8	M	IIIA	abd.adr.	20	53	−	49		
9	M	IIIC	abd.adr.	133	133	−			10
10	M	IIIA	thor.+abd.symp.tr.	4	37	−	47		
11	F	IIB	abd.symp.tr.	3	55	−	67		
12	F	IIIC	abd.symp.tr.	27	30	+		20	
13	M	IIIA	abd.symp.tr.	56	56	−		15	
14	M	IIIC	abd.adr.	37	37	−			11
15	F	IIIC	abd.symp.tr.	36	36	−			7
16	M	IIIC	abd.adr.	31	31	−			9
17	M	IIB	pelvic symp.tr.	13	13	−		4	
18	M	IVS	abd.adr.	0.1	0.1	−			<1

abd. = abdomen; adr. = adrenal; symp.tr. = sympathetic trunk; thor. = thorax.
*When first blood sample of patient was obtained.
†Months after complete remission.
‡Months after diagnosis.
§52 months after diagnosis, 6 months after second relapse.

3. complete remission
4. relapse

Values for the catecholamines NE, E and DA vary widely, irrespective of the status of the patient. In the patient with neuroblastoma, plasma levels of NE, E and DA are higher than in controls (Table 2). In four samples from patients with neuroblastoma, DA was strongly elevated (>2.5 nmol/l); in the same four samples DOPA was found to be exceedingly high (>500 nmol/l).

Values for the other components of the catecholamine biosynthesis cascade (ALAAD, DOPA and DBH) are given in Fig. 1. Values of ALAAD and DOPA (Fig. 1A and B) were increased in the active, untreated state. During therapy most ALAAD, but not DOPA, values were normal, while in complete remission all ALAAD and most DOPA values fell within the normal range. Increasing neuroblastoma activity coincided with increased DOPA and ALAAD values.

Table 2. *(Geometric) mean values and ranges of plasma norepinephrine, epinephrine and dopamine* (nmol/l) *in patients with neuroblastoma and in controls. Also indicated is the* P*-value for the difference between the two groups*

	Neuroblastoma $n = 67$ Mean	Neuroblastoma $n = 67$ Range	Controls $n = 50$ Mean	Controls $n = 50$ Range	*P*-value
NE	2.23	0.21–9.38	1.78	0.49–6.43	0.047
E	0.62	0.09–2.22	0.47	0.05–2.22	0.015
DA	0.53	0.01–4.04	0.17	0.01–0.85	0.001

Figure 3 shows the results of serial measurements of DOPA and ALAAD in six of the 13 patients who were followed longitudinally during the course of their disease. Examination of the data from the serial measurements of all 13 patients confirmed the pattern seen in Fig. 1. In all but one patient (pt. 16) ALAAD activities at first responded rapidly to chemotherapy with decreases, while increasing again rapidly during renewed tumour growth. It is worth mentioning that in one patient ALAAD had risen considerably before clinical manifestation of relapse was apparent. In patient 16 (who had very low ALAAD at first) levels rose continuously during therapy. The effect of chemotherapy on DOPA levels was more variable. Usually a slow decline in plasma levels was seen, but sometimes levels increased. DOPA values did not always normalize, and increased less rapidly than ALAAD during relapse.

Figure 1C shows that some patients had elevated DBH activities, but that values were not different between the four categories. In the serial measurements of the individual patients also no significant changes were found during therapy, remission or

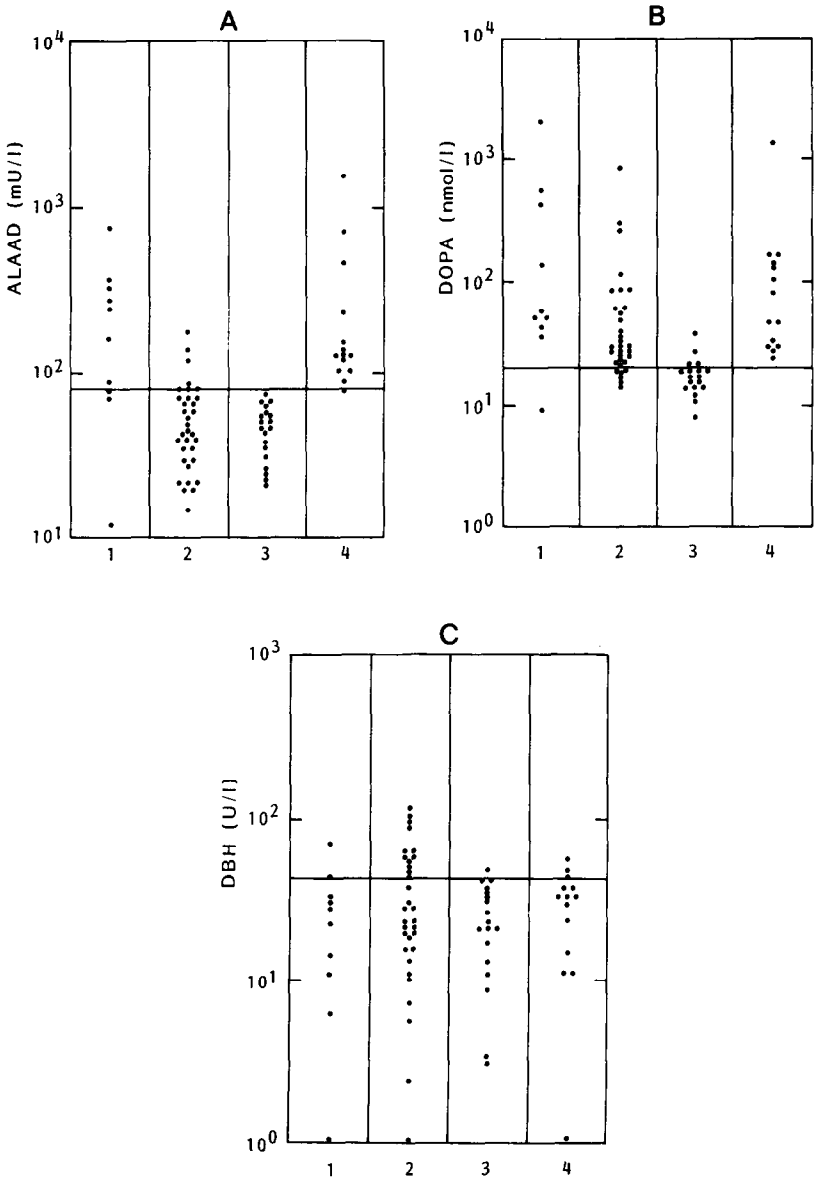

Fig. 1. Plasma values of ALAAD (A), DOPA (B) and DBH (C) in patients with neuroblastoma. Categories 1: active tumour, untreated; 2: active tumour, during chemotherapy; 3: complete remission; 4: relapse. Horizontal lines indicate upper limits of normal.

relapse. In general, a similar pattern was seen with serum LDH and NSE and urinary VMA (Fig. 2A, C and D), while serum FER levels behaved somewhat differently in not falling during therapy (Fig. 2B).

The usefulness of the biochemical parameters at first diagnosis, at complete remission and at relapse in our group of patients can be judged from Fig. 3 and Table 3. At first diagnosis DOPA, LDH and NSE were clearly the best markers. In complete remission nearly all parameters had normalized, but VMA was elevated in two patients. In patient No. 7 who had a subsequent relapse DOPA remained elevated, as did FER, which however was normal when relapse actually occurred. ALAAD and DOPA were by far the best markers for relapse.

In the last sample taken during partial or complete remission before relapse occurred DOPA was the only parameter which was elevated in all six cases (data not shown).

DISCUSSION

Neuroblastomas seem to produce excessive amounts of intermediate constituents of the catecholamine biosynthesis cascade, i.e. DOPA and ALAAD. The ratio between plasma levels of these two products varied widely in this study, possibly as a reflection of differences in maturation of the tumour cells and in extent of tumour necrosis. In one patient (pt. 16) plasma DOPA, but not ALAAD, was highly elevated, in another (pt. 9)

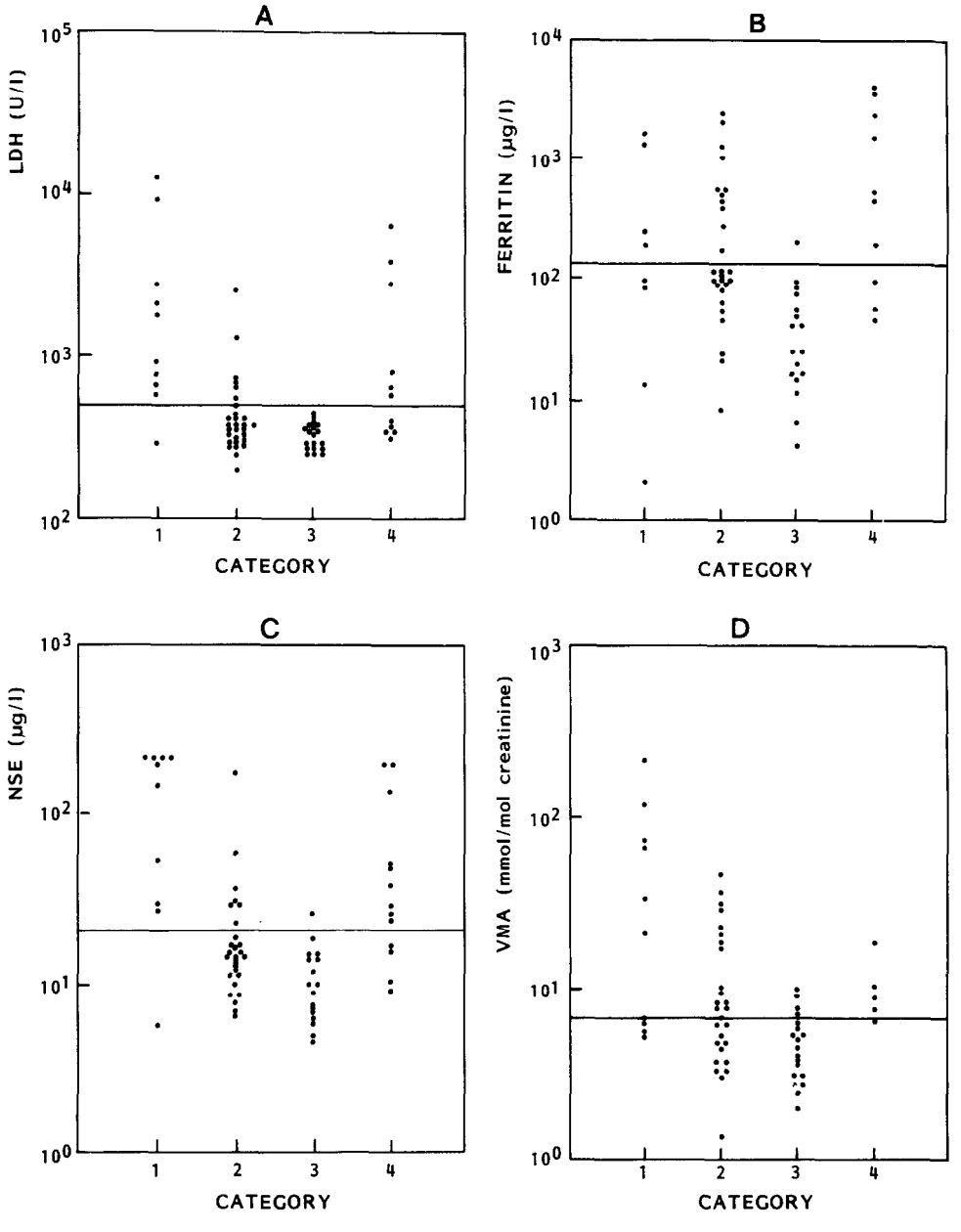

Fig. 2. Plasma values of LDH (A), FER (B) and NSE (C) and urinary levels of VMA (D) in patients with neuroblastoma. For categories, see legend to Fig. 1. Horizontal lines indicate upper limits of normal.

plasma ALAAD, but not DOPA, was elevated, while in most patients both plasma ALAAD and DOPA were increased.

The high percentage of elevated DOPA in patients with active untreated neuroblastoma (nine out of 10) agrees with previous reports [13, 14]. It is interesting that the one patient where DOPA was not increased had highly elevated ALAAD activities. All 11 patients who died or who still have active tumour had elevated DOPA levels when last sampled, and all patients in complete remission reached normal DOPA levels, with the exception of patient 7 who subsequently had a relapse. Persistently high DOPA may thus be indicative of the presence of residual tumour and of poor prognosis. Actual occurrence of relapse may be better indicated by a sharp rise in plasma ALAAD.

Although our number of patients is still rather small, it thus seems possible that the combination of ALAAD and DOPA measurements can be a highly sensitive marker for neuroblastoma activity, in primary diagnosis as well as in indicating complete remission, residual tumour and relapse. Both parameters can be measured concurrently with the same HPLC system, as both rely on the measurement of dopamine formed. A 1-ml blood sample is

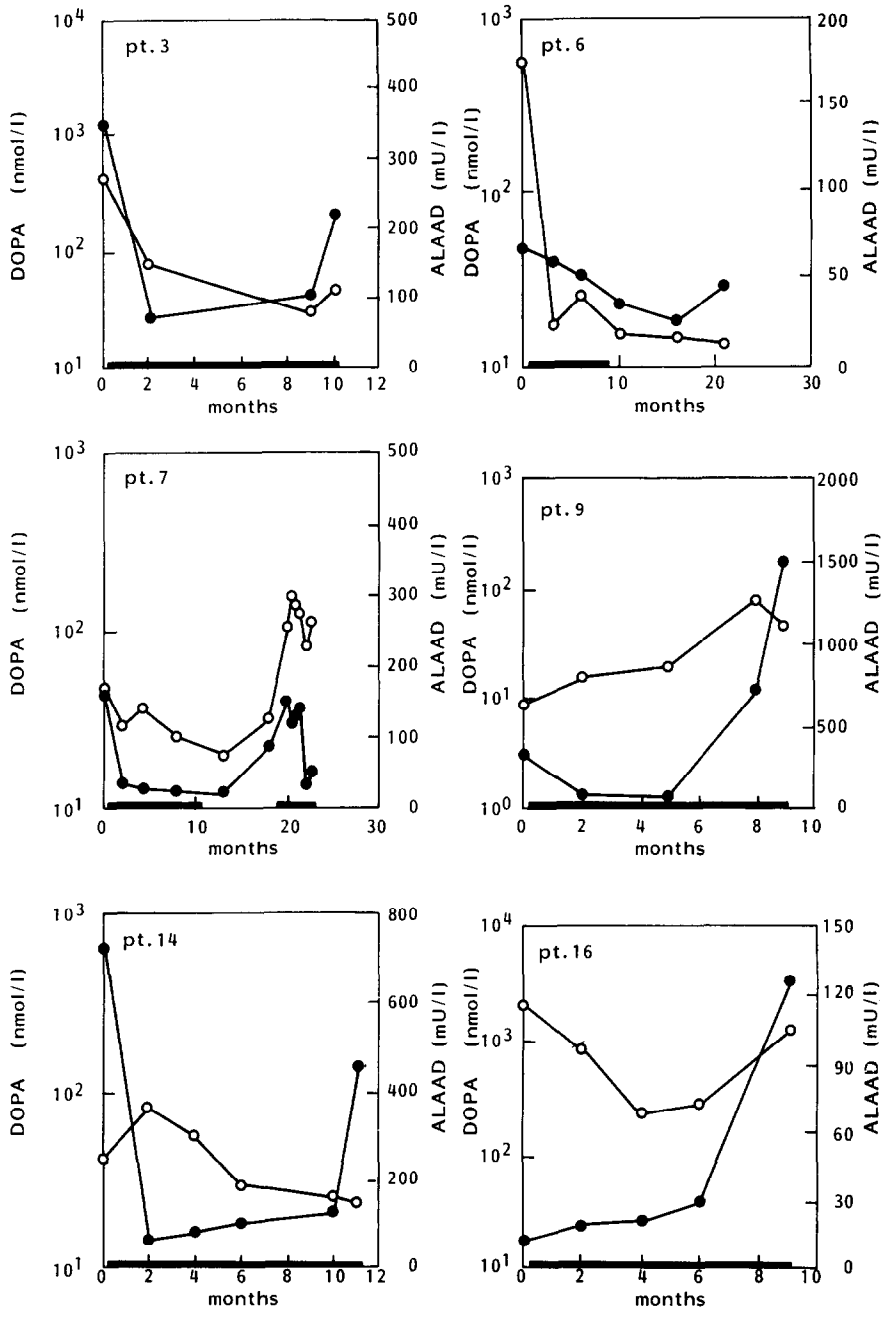

Fig. 3. Serial measurements of DOPA (open symbols, left ordinate) and ALAAD (closed symbols, right ordinate) in six patients. Abscissa indicates months elapsed since first blood sample was obtained. Periods when patients were treated with chemotherapy are indicated in black at the lower side.

sufficient, as routine determinations of ALAAD and DOPA require respectively 50 and 400 µl of plasma. ALAAD and DOPA values are not influenced by sympathetic activity or stress [16, 21].

The high levels of dopamine occasionally found probably resulted from the very high DOPA levels in these patients as they were similar to those found in patients with parkinsonism who, during treatment with L-DOPA, had similar plasma DOPA levels [21].

NSE is reported to be secreted by tumours arising from amine-precursor-uptake-and-decarboxylation (APUD) cells, just as is ALAAD [22]. In agreement with others we found high levels of NSE in most patients with neuroblastoma in the active untreated stage [7, 8, 23]. Levels decreased rapidly during therapy and remission, but did not always increase sharply during relapse. NSE is not a specific marker for neuroblastoma activity [23]. Like LDH, its use in following the course of the disease is also hampered by the unreliability of measurements in

Table 3. Biochemical markers for neuroblastoma at first diagnosis (untreated), at complete remission and at relapse

Patient No.	First diagnosis										Complete remission								Relapse					
	3	6	7	9	13	14	15	16	17	18	1	2	4	6	7*	8	10	11	3	7	9	14	15	16
ALAAD	++	−	++	++	+	++	−	−	++	++	−	−	−	−	−	−	−	−	+	+	++	+	+	+
DOPA	++	++	++	−	++	++	++	++	+	++	−	−	−	−	+	−	−	−	+	+	++	+	++	++
LDH	+	−	+	++	++	++	++	++	+	+	−	−	−	−	−	−	−	−	−	−	++	−	+	++
FER	+	−	−	++	+	++	−	nd	−	nd	−	−	−	−	+	nd	−	−	−	−	nd	++	+	++
NSE	++	−	++	++	++	++	++	++	+	+	−	−	−	−	−	−	−	−	−	−	nd	−	+	++
VMA	++	++	−	−	−	++	−	++	++	++	−	−	+	−	−	−	+	−	+	−	nd	+	−	nd

++ > twice upper limit of normal; + > upper limit of normal; − < upper limit of normal; nd = not determined.
*Subsequent relapse.

haemolytic serum, a situation which occurs regularly when sampling blood from young children.

Results with serum FER and LDH and urinary VMA agree with most previous reports [1, 2, 4–6, 9, 24]. LDH was mostly, but not always, elevated in active untreated neuroblastoma, and decreased during treatment and remission. Residual tumour and relapse were, however, not adequately detected. FER was occasionally elevated at primary diagnosis. Two patients, in whom FER was very high and remained so despite therapy, died. Overall, however, FER did not seem to reflect tumour activity accurately. VMA was not elevated in four out of 10 patients at primary diagnosis, and was elevated in two patients in complete remission with no subsequent relapse. VMA thus does not seem to be a very reliable indicator of tumour activity.

Homovanillic acid (HVA) and vanillactic acid (VLA) are sometimes reported to be useful for diagnosis and follow-up of neuroblastoma [1, 2, 9, 24]. We have not quantitated these parameters, but, insofar as both are metabolites from DOPA and DA, they may indeed be expected to be elevated when DOPA levels are.

Normal values of many biochemical parameters for children are different from those in adults. We have used in this study normal values as determined in children of the same age-group as in patients with neuroblastoma. Even within this age-group, however, differences are found. This is most notable for plasma DBH which rises from nearly zero at birth until stabilizing at 6–8 years of age in a wide range [25]. Plasma ALAAD and, to a lesser extent, DOPA levels seem to be somewhat higher in the first year of life than thereafter, as is also the case with plasma FER and urinary VMA.

More work needs to be done to determine the specificity, sensitivity and prognostic value of plasma DOPA and ALAAD as compared to other tumour markers in neuroblastoma. Apart from more follow-up studies of patients with neuroblastoma, more data on ALAAD and DOPA plasma values in age-matched controls and in children with various other neoplastic and non-neoplastic diseases have to be collected.

Conclusion: our preliminary results suggest that combined determination of plasma levels of ALAAD and DOPA is a useful tool for the diagnosis, and even more so for the detection of residual disease and relapse of neuroblastoma. Serial determinations of ALAAD and DOPA are good monitors of the course of the disease and may give early warning of relapse. Sufficient data on the specificity are as yet lacking.

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