Plasma α fetoprotein reference ranges in infancy: effect of prematurity

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SUMMARY The dearth of plasma α fetoprotein reference ranges for preterm infants often impairs the clinical interpretation of plasma α fetoprotein data collected from ill babies. This study tested our hypothesis that meaningful plasma reference ranges could be established for preterm infants by a simple correction of patient age at sampling date for gestational age deficit at birth. Using a modified radioimmunoassay kit method, determinations of α fetoprotein were performed on capillary and venous blood samples collected from 56 babies aged from birth to 5 months with gestational ages ranging from 26 weeks to 43 weeks. Unmodified plasma α fetoprotein values were grouped according to patient age and examined statistically using established normal theory methods, but these yielded excessively wide reference intervals and non-Gaussian distribution parameters. Acceptable reference ranges were derived using logarithmic transformation of plasma α fetoprotein values and rearrangement against patient age corrected for gestational age deficit. These provisional reference ranges for plasma α fetoprotein in preterm (and term) infants are applied to groups of previously meaningless α fetoprotein results and used to test the potential usefulness of plasma α fetoprotein determination as a diagnostic marker in biliary atresia, hepatitis, and yolk sac derived tumours.

Alpha fetoprotein is a single chain α globulin, of molecular weight 68 000, normally unique to the fetus but often found in increased concentrations in plasma of children and non-pregnant adults with liver disease and some forms of malignant tumours. Alpha fetoprotein is produced in the developing fetus in equal amounts by the yolk sac and the fetal liver, but at about 12 weeks after conception the yolk sac degenerates and the fetal liver becomes the main site of synthesis. Fetal plasma α fetoprotein concentration reaches a peak value of 3 000 000 U/ml at a gestation of about 14 weeks.¹ Alpha fetoprotein synthesis almost ceases at parturition and the infant plasma concentration falls exponentially from a mean of 50 000 U/ml to a typical adult concentration of less than 10 U/ml at 6 to 8 months of age.²

The observation that α fetoprotein synthesis does not cease entirely at term^{3–5} may be explained by the presence of fetal hepatocytes, which, though decreasing in number, sustain transient production of α fetoprotein during the early postnatal period. Any clinical condition that interferes with this physiological decline in fetal protein biosynthesis will

disturb the usual fall in plasma α fetoprotein concentration. Such a mechanism has been postulated in neonatal viral hepatitis,⁶ where compensatory proliferation of hepatocytes produces rapid regeneration of liver tissue and restoration of hepatic α fetoprotein synthesis.

Since the onco-fetoprotein α fetoprotein was first identified electrophoretically in the cord blood of human infants,⁷ its merit as a general marker of perinatal disease has been studied intensively. The demonstration that this alpha migrating serum protein (otherwise detected in serum only during pregnancy) was identical in structure to a protein found in mice bearing transplanted hepatocellular carcinoma⁸ was a major stimulus to interest in blood α fetoprotein as a potential tumour marker. This association was confirmed when patients with liver tumours, in particular hepatocellular carcinoma and hepatoblastoma, were shown to have increased serum α fetoprotein concentrations.⁹

Plasma α fetoprotein is now universally established as a reliable biochemical marker of malignancy not only for hepatic tumours but also for yolk sac derived tumours, some tumours of the gastro-

intestinal tract, and rarer teratocarcinomas with yolk sac elements, such as malignant sacrococcygeal teratoma.¹⁰⁻¹³ A valuable feature of plasma α fetoprotein monitoring is its reported specificity¹³ in detecting a small malignant yolk sac focus located in the midst of a 'benign teratoma'.

Clinical interpretation of plasma α fetoprotein concentrations in our term and preterm infants has been based predominantly on the graph of reference ranges published by Tsuchida *et al*, which takes no account of gestational age.¹⁴ We have suspected for some time, however, that correction for gestational age deficit at birth would greatly enhance the clinical value of many plasma α fetoprotein results obtained in early infancy.

Patients and methods

Fifty six babies admitted to the neonatal surgical unit, Royal Hospital for Sick Children, and the paediatric department, Queen Mother's Hospital, from October 1984 to January 1985 were included in this study. At the time of sampling the infants were aged from birth to 5 months, with estimated gestational age at birth ranging from 26 to 43 weeks. Thirty seven preterm infants were studied. Fifty babies were singletons, four others comprised two sets of twins, and the remaining two were from two further twin pregnancies. A detailed list of the range of clinical conditions with disease incidences is available on request from the authors. Babies with conditions associated previously with abnormal plasma α fetoprotein concentrations—for example, six infants with sacrococcygeal teratoma—were excluded. In the case of two infants with gastrointestinal disorders and increasing serial plasma α fetoprotein concentrations only the initial α fetoprotein value was included for statistical analysis.

Residual plasma (typically 50–100 μ l) from capillary and venous blood samples collected for routine biochemical analyses essential to patient care was used for estimation of α fetoprotein. These residual samples provided a single plasma α fetoprotein result for 34 patients; plasma was obtained twice from further blood samples in 13 others, and three to six serial (weekly intervals) α fetoprotein plasma samples were obtained for the remaining nine babies. Heparinised blood was spun, separated, and stored at 4°C until the primary analyses were completed and the plasma stored at -20°C, pending α fetoprotein analysis.¹⁵ Study samples were included with batches of routine α fetoprotein determinations and handled in exactly the same manner as α fetoprotein requests for general diagnosis.

Assay method. Plasma α fetoprotein was measured using a routine 'tumour marker' assay based on the Amersham International AFP kit IM.984,¹⁶ with the following modifications:

- (1) Preparation of six additional standards by dilution of kit standards with the 'zero' standard to give 10 standards in the range 0–110 U/ml.
- (2) Preincubation of standards and patient samples with anti-AFP serum for three hours at room temperature before addition of tracer.
- (3) Aspiration of supernatants under mild vacuum (Venturi pump).
- (4) All results with an α fetoprotein concentration of 3.4 U/ml or less reported as less than 4 U/ml.
- (5) Appropriate dilution with the 'zero' standard and re-analysis of all specimens with α fetoprotein values greater than 100 U/ml.

Alpha fetoprotein results were reported in U/ml based on the International Agency for Research on Cancer Standard 72/225 (1 ng α fetoprotein=1.09 International Units).

Interassay imprecision expressed as percentage coefficient of variation was 3.9% at a mean α fetoprotein concentration of 50 U/ml and 3.5% at a mean concentration of 25 U/ml.

Statistical methods. Data reduction was achieved with the aid of a desk top minicomputer, using established normal theory methods on age grouped α fetoprotein results, which yielded the following statistics together with their standard errors and 95% confidence limits¹⁷: mean, median, standard deviation, and percentage coefficient of variation.

Previous studies on paediatric and adult α fetoprotein reference ranges prompted a simple logarithmic transformation of uncorrected α fetoprotein results and α fetoprotein results corrected for gestational age with re-application of the data reduction procedure. Linear ($Y=mX+c$) and exponential ($\ln(Y)=mX+1n(c)$) least squares regression analyses were performed on ungrouped α fetoprotein data paired with both uncorrected and corrected patient ages.

Results

An essential prerequisite to the lengthy derivation of age related plasma α fetoprotein reference ranges was the verification of underlying relations between plasma α fetoprotein concentration and patient age. Linear and exponential least squares regression analyses were performed on the entire set of 97 plasma α fetoprotein values matched in the first instance with patient age at sampling and then with

patient age corrected for gestational age deficit at birth (Table 1).

Results of linear regression analysis quickly confirmed earlier assumptions of a significant negative correlation ($r=-0.42$, $n=97$, $p<0.01$) between plasma α fetoprotein concentrations and uncorrected age. As expected the introduction of correction for gestational age produced a beneficial effect on the degree of correlation ($r=-0.58$, $n=94$, $p<0.01$) measured by linear regression. A remarkable doubling of the correlation coefficient, yielding an identical r value of -0.85 for both uncorrected data and data corrected for gestational age deficit, followed the application of exponential least squares regression. The latter findings were not attributable entirely to chance but could reasonably have been deduced from previous observations of the exponential fall in plasma α fetoprotein concentration with increasing age in term^{2 14 18} and preterm¹⁹ infants.

Although shortage of α fetoprotein data precluded the subsequent derivation of separate male and female reference ranges in this study, an evaluation of the possible sex dependency of plasma α fetoprotein concentration was attempted. Exponential and linear regression analyses were undertaken on age related α fetoprotein data segregated according to patient sex (Table 1). The almost identical values obtained for slope (-0.3695 for males versus -0.3761 for females) and $\ln$ [intercept] (23.4434 for males versus 23.6554 for females) parameters, derived from exponential regression of gestationally corrected α fetoprotein data for male and female infants, did not support the presence of a significant sex dependency. This was further confirmed by an even scatter of α fetoprotein results for boys and girls as shown in Figures 1 and 2.

Due to limited time and analytical resources it was

impossible to gather the desired total of 20 plasma α fetoprotein values for each week of life from birth to 6 months hoped for at the outset of this study. No further action was taken to correct this disappointing shortfall in weekly age group totals. We had decided earlier that, for ethical reasons, the study infants would not be exposed to additional non-essential blood sampling simply to supplement incomplete age group quotas. Furthermore, the main incentive for this update of plasma α fetoprotein reference ranges was to improve diagnostic procedures for ill preterm and term infants admitted to neonatal surgical or medical units. It seemed inappropriate, therefore, to collect supplementary blood samples from 'well' baby populations, particularly when relevant but limited data could be gathered in the course of essential patient care. Thus α fetoprotein values were arranged almost entirely in age related groups of two weeks. Some small age related data sets with fewer than five plasma α fetoprotein values, although unusable for subsequent reference range computation, were retained for scattergram plotting in Figures 1 and 2.

Major anomalies were revealed during the first statistical analysis of age related α fetoprotein values, indicating that data were not disturbed in Gaussian form. With a range of five orders of magnitude, many individual values remained inexplicably outwith published plasma α fetoprotein reference ranges¹⁴ and highlighted the need to establish revised ranges that took account of prematurity. These initial findings confirmed our earlier misgivings on the suitability of normal theory statistical analyses applied to unmodified plasma α fetoprotein frequency distributions.

Immediate improvement in distribution parameters was obtained by statistical evaluation of

Table 1 Least squares regression of plasma α fetoprotein (U/ml) concentration versus patient age (weeks)

	<i>n</i>	Linear regression ($Y=mX+c$)		Exponential regression ($\ln(Y)=mX+\ln(c)$)	
		Correlation coefficient <i>r</i>	<i>p</i> Value	Correlation coefficient <i>r</i>	<i>p</i> Value
Uncorrected age:					
All patients	97	-0.42	<0.01	-0.85	<0.01
Male infants	54	-0.38	<0.01	-0.82	<0.01
Female infants	43	-0.48	<0.01	-0.91	<0.01
Corrected age:*					
All patients	94	-0.58	<0.01	-0.85	<0.01
Male infants	54	-0.55	<0.01	-0.81	<0.01
Female infants	40	-0.67	<0.01	-0.90	<0.01

*Definition of correction:

Corrected age (weeks) for preterm infants=Chronological age (weeks) at time of blood sampling minus gestational age deficit (weeks).

Gestational age deficit=Number of weeks preterm at birth (term=40 weeks)—for example, infant X with an estimated gestational age at birth of 28 weeks was 6 weeks old at the time blood was collected for α fetoprotein determination. Gestational age deficit=40-28—that is, 12 weeks. Corrected age (weeks)=6-12—that is, -6 weeks.

Fig. 1 Plasma α fetoprotein results and Glasgow (Yorkhill) reference range for both preterm and term infants with no correction for gestational age deficit.

Fig. 2 Plasma α fetoprotein results and Glasgow (Yorkhill) reference range for both preterm and term infants with correction for gestational age deficit.

plasma α fetoprotein values ranked by patient age corrected for gestational age deficit (Table 2).

Previous work on maternal serum α fetoprotein reference ranges suggested that logarithmic transformation could be applied usefully to infant plasma α fetoprotein values.^{16 20} The benefits of transformation were immediately evident from analysis of logarithmic α fetoprotein values versus uncorrected patient age with virtually coincident group means and medians and progressively decreasing lower ($-2SD$) limits, which were all above zero for the first time. Logarithmic transformation of plasma α fetoprotein data and ranking of log α fetoprotein

versus corrected patient age were combined in Table 3, on which the definitive plasma α fetoprotein reference ranges were based. Due mainly to the limited number of α fetoprotein results, the inconsistent values evident in Table 3 rendered it unsuitable for use as a table of reference ranges.

Unequivocal evidence for an exponential relation between plasma α fetoprotein concentration and infant age (regardless of prematurity) directed us to perform exponential least squares analyses on each column of derived data (lower/upper limits, mean, and median) versus respective mid-interval ages contained in Table 3. This latest statistical elaboration

Table 2 Range of observed plasma α fetoprotein values ranked by patient age corrected for gestational age deficit at birth

Corrected age interval (weeks)	Mid-range point (weeks)	No of results	Male:female ratio	Lowest α fetoprotein result (U/ml)	Highest α fetoprotein result (U/ml)
-10.0 - -8.1	-9.0	5	2:3	34572	196617
-8.0 - -6.1	-7.0	12	6:6	12012	391072
-6.0 - -4.1	-5.0	8	2:6	7293	161620
-4.0 - -2.1	-3.0	8	3:5	1549	67771
-2.0 - -0.1	-1.0	10	4:6	1932	40723
0.0 - 1.9	1.0	17	10:7	891	69720
2.0 - 3.9	3.0	16	13:3	105	20477
4.0 - 5.9	5.0	6	6:0	128	651
6.0 - 7.9	7.0	3	3:0	30	191
10.0 - 11.9	11.0	1	0:1	32	32
12.0 - 13.9	13.0	1	0:1	25	25
14.0 - 15.9	15.0	3	1:2	17	53
16.0 - 17.9	17.0	2	2:0	55	77
18.0 - 19.9	19.0	1	1:0	50	50

Table 3 Plasma α fetoprotein ranked by patient age corrected for gestational age deficit at birth

Corrected age interval (weeks)	Mid-range point (weeks)	No of results	Derived from logarithmic α fetoprotein data transformation (U/ml)			
			Mean	Median	Mean -2SD	Mean +2SD
-10.0 - -8.1	-9.0	5	90461	136106	18871	433644
-8.0 - -6.1	-7.0	12	105952	100897	19593	572936
-6.0 - -4.1	-5.0	8	44760	62135	4137	484258
-4.0 - -2.1	-3.0	8	13865	14864	893	215166
-2.0 - -0.1	-1.0	10	13641	23988	980	189915
0.0 - 1.9	1.0	17	9253	12777	411	208446
2.0 - 3.9	3.0	16	1167	766	45.9	29694
4.0 - 5.9	5.0	6	281	251	86.1	916

Table 4 Plasma α fetoprotein reference ranges for preterm infants ranked by patient age uncorrected for gestational age deficit at birth

Uncorrected postnatal age (weeks)	Derived from exponential regressions of logarithmic α fetoprotein data (U/ml)			
	Mean -2SD limit	Mean +2SD limit	Mean	Median
0	2146	473000	31864	29186
2	895	189300	13024	12208
4	374	75780	5323	5106
6	156	30330	2176	2136
8	65	12140	889	893
10	27	4860	363	374
12	11	1945	149	156
14	5	779	60.7	65.4
16	<4	312	24.8	27.3
18	<4	125	10.1	11.4
20	<4	50	4.1	4.8

tion provided the plasma α fetoprotein reference ranges in terms of unmodified infant age (Table 4) and of infant age adjusted for gestational age deficit (Table 5). Despite the presence of gaps related to a shortage of α fetoprotein data, visual corroboration of the validity of our entire statistical approach is clear from inspection of Figures 1 and 2 based on

Tables 4 and 5. These show almost coincident mean and median regression lines with near parallel and equidistant lower (-2SD) and upper (+2SD) limit regression lines. The presence of some five α fetoprotein values out of a total of 93 collected from our ostensibly tumour free population sample, located at the margins of the mean (2SD) limits of

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Corrected postnatal age (weeks)	Derived from exponential regressions of logarithmic α fetoprotein data (U/ml)			
	Mean -2SD limit	Mean +2SD limit	Mean	Median
-10	38910	2034000	281298	410815
-8	15960	988900	125633	172801
-6	6548	480800	56109	72684
-4	2686	233800	25060	30573
-2	1102	113700	11192	12860
0	452	55270	4999	5409
2	185	26870	2232	2275
4	76	13070	997	957
6	31	6353	445	403
8	13	3089	199	169
10	5	1502	88.8	71.2
12	<4	730	39.7	30.0
14	<4	355	17.7	12.6
16	<4	173	7.9	5.3
18	<4	84	3.5	2.2
20	<4	41	1.6	0.9

Figure 2, is encouraging evidence that the theoretical characteristics of a normal distribution are present.

Discussion

Clinical interpretation of plasma α fetoprotein concentrations obtained during the first few weeks of life can be rendered difficult due to frequent misclassification of high plasma α fetoprotein concentrations observed in preterm infants. This study has determined a provisional range of plasma α fetoprotein reference values for ill term and preterm infants that, although sufficiently wide to enclose almost all results from the 'normal' subjects in this study, seemed to detect pathological increases in plasma α fetoprotein.

The predictive capability of these ranges was tested first with plasma α fetoprotein results collected from five infants (three term and two pre-term) excluded at the outset of the data treatment due to the presence of sacrococcygeal teratoma, which, in retrospect, proved clinically and histologically benign. Serial plasma α fetoprotein results (mainly post-excision) from two preterm babies (with gestational ages of 31 and 32 weeks) with benign sacrococcygeal teratoma were plotted on Tsuchida's graph¹⁴ (Fig. 3(a)). Several values were located either at or above the upper limit of the '95% prediction band' without discernible clinical cause. When the patient ages were adjusted for gestational age deficit and the same plasma AFP data replotted on our revised reference range graph (Fig. 3(b)) all α fetoprotein values for benign teratoma, including results for the three term

infants, remained comfortably within mean (2SD) limits.

Due to the rare incidence of yolk sac derived and hepatic malignant tumours in early infancy, no plasma α fetoprotein results were available for evaluation during the study from babies with suspected or proven α fetoprotein producing tumours. None the less we attempted a minimal evaluation of the ranges in relation to malignancy by plotting serum α fetoprotein results obtained by radioimmunoassay from two published cases of malignant tumour in infancy (pre-resection hepatoblastoma and post-resection orchidoblastoma).¹³ Both serum α fetoprotein values were located well above the (+2SD) upper limit (Figure 3(b)).

Assessment of the diagnostic potential of our α fetoprotein ranges in relation to non-malignant paediatric liver disease was impossible without resorting to previously published α fetoprotein data, there being no established cases in the group studied. Maximal serum α fetoprotein results collected by Zeltzer *et al* from 19 term infants with an established diagnosis of biliary atresia or hepatitis⁶ were plotted on the graph (Figure 3(b)). Results of this retrospective comparison plot tended to suggest that earlier biochemical differentiation between neonatal hepatitis and biliary atresia may be realised by plasma α fetoprotein monitoring, although not without further study. Clearly, these preliminary findings must be treated with caution in view of the substantial methodological differences between Zeltzer's radial immunodiffusion technique and modern α fetoprotein radioimmunoassays and perhaps more importantly the assumed patient ages at sampling.

Fig. 3(a) Serial plasma α fetoprotein results from two preterm infants (●—● and ●—●—●) in our study with benign sacrococcygeal teratoma plotted on the reference range graph of Tsuchida et al.¹⁴ (b) Serial plasma α fetoprotein results from two preterm and three term infants (represented by black dots) in our study with benign sacrococcygeal teratoma plotted on the Glasgow (Yorkhill) reference range graph together with serum α fetoprotein data from two published cases of malignant tumour in infancy (pre-resection hepatoblastoma (▲) and post-resection orchioblastoma (■)¹³) and maximal serum α fetoprotein results published for 19 term infants with biliary atresia (◆) or hepatitis (○).⁶

In conclusion, we have shown, despite the limited data available, the relative simplicity of deriving paediatric reference ranges for plasma α fetoprotein with the aid of gestational deficit adjustment and a single step data transformation. Our findings may encourage others to derive similar laboratory reference ranges for plasma α fetoprotein based on paediatric results obtained by local assay methods and thereby confirm or refute the value of plasma α

fetoprotein as a marker for non-malignant disease in early infancy.

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