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Development of the Serum α-Fetoprotein Reference Range in Patients Beckwith-Wiedemann Spectrum

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Abstract

Objective: To establish reference ranges for serum α-fetoprotein (AFP) at various ages in patients with Beckwith-Wiedemann spectrum (BWSp), in order to better predict the risk for hepatoblastoma in this population.

Study design: A retrospective analysis of AFP measurements collected from patients with BWSp was performed. Factors including sex, prematurity, molecular diagnosis of patients, and performing laboratory were evaluated for significant differences. 1372 AFP values were collected from 147 patients and the predictive AFP values at various ages were calculated to establish reference ranges. Mixed-effects polynomial regression models were used to study various potential factors affecting log(AFP) values.

Results: Overall, predicted AFP values declined to normal range for age (<10 ng/mL) by 14 months old. Patient sex and performing laboratory were found not to influence values. A significant difference was demonstrated between premature and non-premature patients, and separate reference values were established. Significant differences in the predicted AFP value were not broadly apparent between molecular subtypes; however, interpretation was limited due to the small sample size of some of these subtypes.

Conclusion: Predictive AFP values were created for premature and non-premature patients with BWSp to aid with interpretation and monitoring of the risk for hepatoblastoma. Further analysis is needed to determine whether AFP values differ within the less common molecular subtypes of patients with BWSsp.

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Keywords

tumor screening; hepatoblastoma

Beckwith-Wiedemann syndrome (BWS, OMIM #130650) is characterized by fetal and childhood overgrowth, macroglossia, organomegaly, and an increased risk for embryonal tumors(1). Isolated lateralized overgrowth or hemihyperplasia (ILO, OMIM #235000) is characterized by asymmetric overgrowth of part(s) of the body and also an increased risk of tumor development(2, 3). Patients with BWS, ILO, and 11p15.5 molecular anomalies are now recognized as part of the Beckwith-Wiedemann spectrum (BWSp)(1). Hepatoblastoma is the second most common tumor to develop in patients with BWS, with a relative risk 2280 times greater than the general population(1, 4).

Serum α-fetoprotein (AFP) is a liver marker produced at increased rates by HB cells, and is increased in >95% of patients with HB(5). Elevated AFP serum levels can also be seen in benign liver disorders; however, these AFP levels are typically not as high as those seen with HB (6). Additionally, hyperbilirubinemia and hypothyroidism can cause AFP elevations in neonates and infants (7). In the absence of these conditions, AFP can signal the development of a liver tumor or more rarely, a yolk sac tumor. Monitoring AFP values allows for detection of HB tumors at an earlier stage, which minimizes surgery and treatment required and may improve prognosis for patients(8–11). The interpretation of AFP values in the general pediatric population is challenging, as a variety of factors may influence values including prematurity, comorbidities, and sex, along with the wide variation that exists between individuals(6, 7, 12–26). AFP values can be even more difficult to interpret in the BWSp population, as patients tend to have higher values that decline more slowly compared with an unaffected population(27).. As a result, the use of AFP screening in BWSp tumor surveillance protocols is debated(28–31), primarily due to the complexity of its interpretation.

In this study, we aimed to establish AFP reference ranges for the BWSp population to aid in the interpretation. We also evaluated whether sex, performing laboratory, prematurity, and molecular subtype affected AFP values.

Methods

Patients enrolled in an international BWS registry with a clinical or molecular diagnosis of BWS, ILO, or an 11p15.5 molecular anomaly who previously received or were currently receiving AFP screening as part of their clinical care were eligible to participate. The BWS Registry enrolls patients with proven or suspected genetic/epigenetic and growth disorders. Enrolled patients include those recruited at Children's Hospital of Philadelphia, referred from outside institutions, as well as self-referrals from patients and/or families. Medical records are collected and reviewed at regular intervals. Medical records were reviewed for the patient's sex, gestational age, BWSp molecular subtype, age at each AFP collection, performing laboratory of each AFP, and AFP values (ng/mL). BWSp molecular subtypes were defined as loss of methylation at KCNQ1OT1:TSS-DMR (IC2 LOM), paternal uniparental isodisomy of chromosome 11 (pUPD), gain of methylation at H19/IGF2:IG-

DMR (IC1 GOM), *CDKN1C* mutations, 11p15.5 anomaly (i.e. duplications, deletions, chromosome rearrangements), clinical diagnosis of BWS, and clinical diagnosis of ILO. Data were abstracted prior to July 2017. Values that were collected from patients until the age of 4 years were included in the analysis. As patients are recruited from across the United States and from multiple countries, prior to the American Association for Cancer Research guidelines(10) there was no consistent protocol for AFP frequency being followed. Most patients were having AFPs at either 6 week or 3 month intervals. Patients known to develop a HB, or those known to have liver disease, hyperbilirubinemia, or hypothyroidism were excluded from analysis. For the analysis, the age at each AFP collection was corrected in patients who were premature (<37 weeks' gestation) to a gestational age of 38 weeks. Institutional Review Board approval and patient/guardian consent was obtained for this study (IRB 13–010658).

Statistical Analyses

Data were collected and summarized using descriptive measures, including means with standard deviations (SD) and medians with ranges and 1st and 3rd quartiles for continuous variables, and frequency counts and percentages for categorical variables.

Logarithmic transformation was applied to skewed AFP [log(AFP)] before analyses were performed. Cross-sectional analyses of longitudinal data were conducted to examine the association of age with log(AFP). Mixed-effects polynomial regression models with random coefficients (intercepts and slopes) and unstructured covariance structures for the random effects were applied to determine whether age had an association with log(AFP). These models posit that subjects vary not only in their baseline level of response but also in terms of their change in response by age. Mixed-effects models implemented via maximum likelihood account for correlations arising from the repeated measures and used all measurements obtained from each subject. Nonlinear effects of age (ie, quadratic and cubic terms) were investigated and included in the models if they were significant. Interaction effects of age (linear and non-linear terms) with prematurity, sex, molecular diagnosis and performing laboratory were separately investigated to determine whether the effects of age on log(AFP) differed by each of these factors. Predicted log(AFP) values at each age were calculated from the fitted models and expressed as predicted AFP values (anti-log of predicted log(AFP) values) with 95% confidence intervals and p values comparing the differences in AFP values between the groups. Sensitivity analyses were performed to examine the potential outliers and influential points. These measurements were assessed by visual examination of histograms and normal probability plots of residuals from each model. Stata 15.1 (Stata Corporation, College Station, TX, USA) was used to conduct statistical analyses and the significance level was set at 0.05.

Results

We collected 1372 AFP values from 147 patients for analysis; Table I summarizes the characteristics of the patients. There was an even distribution of males and females and approximately one-third of the patients were premature. The most common molecular

diagnoses were IC2 LOM and pUPD. The average number of AFP values collected from each patient was 9.3 (SD=8.5) with a range of 1 to 34 values.

Overall, predicted AFP values declined to normal range for age (<10 ng/mL) by 14 months old (Table 2). Prematurity was found to influence values significantly, with the largest difference noted before 6 months of age (Table 2). This difference became non-significant after 10 months of age. Patients who were premature had higher AFP values compared with patients who were full-term, and more variation in the trend of decline was observed in the patients who were premature (Figure). No significant differences were found between patients in regard to sex or performing laboratory. A wide range of actual AFP values was observed for the group (Table 3).

The predicted AFP values within the molecular subtypes of BWSp were also evaluated (Table 4; available at www.jpeds.com). Similar values were observed between patients with IC2 LOM and pUPD, and between patients with a clinical BWS diagnosis and ILO diagnosis. Predicted AFP value declined to normal range by 21 months in patients with IC1 GOM, by 16 months in patients with a clinical BWS/ILO diagnosis, by 15 months in patients with 11p15.5 anomalies, by 13 months in patients with IC2 LOM and pUPD, and by 9 months in patients with *CDKN1C* mutations. The trend of decline between the subtypes is demonstrated in Figure, B.

Discussion

Although rare, HB can be an extremely aggressive liver tumor; the prognosis is dependent on the stage of the tumor and ability to resect surgically (8, 9, 11). Patients with a lower stage, local tumor usually undergo complete resection and achieve survival rates reported between 80–100%(8, 11). Tumors detected at later stages have often already metastasized, leading to more extensive treatment; prognosis is therefore poorer compared with lower stages, and survival rates are between 40–65%(8, 9, 11, 32). AFP is synthesized by the liver during the neonatal and infant period, with initially high levels at birth, which decline with age until normal adult levels are reached(33). Elevated serum AFP has been demonstrated to be the best indicator of HB, elevated in more than 95% of patients with HB(5). Patients with BWSp are at increased risk for developing HB compared with the general pediatric population(1, 4) and some experts recommend that AFP levels be monitored in an effort to detect these tumors early(10).

Despite the increased risk of HB in the BWSp population, the use of AFP screening in tumor surveillance protocols is debated, most often due to the complexity of its interpretation (1, 10, 28–31, 34–37). As a result, the international BWS consensus recommendations do not include AFP screening in their tumor surveillance recommendations(1), whereas the North American-based guidelines do(10). The invasiveness of AFP testing via phlebotomy, and the burden on families have also been cited as factors leading to poor screening adherence(37). In contrast, tumor screening has also been considered to have beneficial effects on parental anxiety by offering a sense of control and continued reassurance(38–41).

Despite the controversy surrounding the use of AFP in tumor screening, evidence exists to support the role of AFP in detecting HB early among patients with BWS and ILO(37, 42, 43). In more than half of these reported patients, the rise in AFP preceded evidence of tumor by imaging. In the general pediatric population, AFP has also been shown to be beneficial in monitoring HB recurrence, with increasing AFP values often presenting months before imaging detection(44). Without the rise in AFP values to prompt clinical suspicion and more frequent imaging studies, HB in these patients may have remained undiagnosed longer and presented at later stages. Additionally, it has been demonstrated that patients with BWS and tumors that were diagnosed through screening experience an improved survival rate compared with patients with BWS and tumors who did not receive screening(45).

To establish reference intervals it is recommended that a minimum of 120 reference subjects be selected and the majority of clinical laboratories refer to the central 95% of the studied population(46). In our study, we included 147 patients and utilized 95% confidence intervals when establishing our reference intervals. We additionally evaluated whether the performing laboratory affected values in order to account for potential technique differences biasing the results and found no difference.

Contrary to some previous reports suggesting sex-related differences in AFP values (16, 18, 20), we did not find a difference in values between males and females, consistent with other previous reports(7, 12–14, 26). It has been recommended that serial AFP measurements be performed in the same laboratory due to differing assay methods(24), however, we did not find differences in AFP values between performing laboratories. After correcting for gestational age, there was still a significant difference in AFP values between premature patients and non-premature patients, consistent with previous studies in the general pediatric population(7, 12, 14, 15, 17, 19). As a result of our findings, we established reference intervals for premature and non-premature patients, and suggest interpreting values based on a patient's corrected gestational age.

In the general pediatric population, AFP values typically reach normal levels between 6 months and 1 year of age(19, 22, 25, 26). Everman et al previously reported that compared with the general pediatric population, patients with BWS have higher AFP values that decline at a slower rate.(27) The results of our study support this finding, as patients in our cohort did not have levels in the normal range until 14 months of age based on the predictive model. Some patients reached normal values by 6 months and others did not reach normal values even by 4 years old. Although AFP values tend to be higher in BWSp patients, elevated AFP levels can persist until the age of 2 or 3 years in the general pediatric population(6, 13, 15, 18). As a result, an elevated AFP value in a patient should not be used to diagnose BWSp or considered indicative of a possible diagnosis. A diagnosis of BWSp should be made through careful clinical examination and molecular analysis(1). In addition, an initially elevated AFP value may not indicate HB and the value should be correlated with ultrasound or other imaging findings. The AFP trend should be followed with the expectation that values will decline(10). AFP values normally decline over time and therefore, it is most important to evaluate the trend rather than an individual value when interpreting AFPs. Although transient spikes in AFP values were observed in some patients, all patients with persistent spikes were subsequently diagnosed with hepatoblastoma. As

patients with BWS undergo routine AFP measurements and abdominal ultrasounds, a spike in AFP value compared with previous values should be correlated with imaging findings. This study provides a detailed standard for physicians in interpretation of AFP values in BWSp.

Patients with BWSp due to paternal uniparental isodisomy (pUPD) or genome-wide paternal uniparental isodisomy are at highest risk for HB(1). Additionally, HB represents the most common tumor to develop in patients with the most common epigenetic cause of BWSp (IC2 LOM) and these patients may have additional risk factors for HB unique to their subtype(28). In this study, similar AFP values were found among patients with pUPD and IC2 LOM, suggesting that these established norms may be useful for detecting elevated AFP values among the molecular subtypes with the highest HB risk.

Although Everman et al (27) provided evidence of AFP norms in the BWS population, their sample size was small (22 patients with 128 AFP values) and the majority of the values were collected from patients over the age of 1 year, which did not allow for careful interpretation of values in the first year of life, when the risk of HB is greatest(47). In our study, we observed 557 values (41% of all values analyzed) from 121 patients under the age of one year, providing strength in our results. Our results suggest that AFP values may differ for the less common molecular subtypes, but the sample size of patients with IC1 GOM, *CDKN1C* mutations, and 11p15.5 anomalies was small, limiting those findings. Further analysis using a larger sample size of patients with these rarer molecular subtypes is needed to determine whether AFP values truly differ among the less common molecular subtypes. Additionally, evaluating the influence of nonmalignant ultrasound findings (such as hepatomegaly, hemangiomas, cysts, etc) and racial/ethnic differences may be useful to distinguish other factors that could affect AFP values. Our data, regardless, provide normative values for the 2 most common BWSp molecular subtypes (IC2 LOM and pUPD), which are also the subtypes of patients who are most likely to develop HB.

In summary, we established AFP norms for use in patients with BWSp, thereby providing a useful tool for interpretation of AFP as a tumor screening marker in this patient population, specifically as it applies to the (epi)genetic types of BWSp most commonly affected by HB. This work demonstrated that gestational age, but none of the other analyzed factors, affected AFP values.. Use of these norms can aid in the continued discussion of the utility of AFP screening in BWSp in the context of cost effectiveness, family anxiety, and health care environment and resource utilization.

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List of Abbreviations

AFP	α-fetoprotein
BWS	Beckwith-Wiedemann Syndrome
BWSp	Beckwith-Wiedemann Spectrum
IC1 GOM	gain of methylation at H19/IGF2:IG-DMR
IC2 LOM	loss of methylation at KCNQ1OT1:TSS-DMR
ILO	Isolated Lateralized Overgrowth/Hemihyperplasia
pUPD	paternal uniparental isodisomy of chromosome 11

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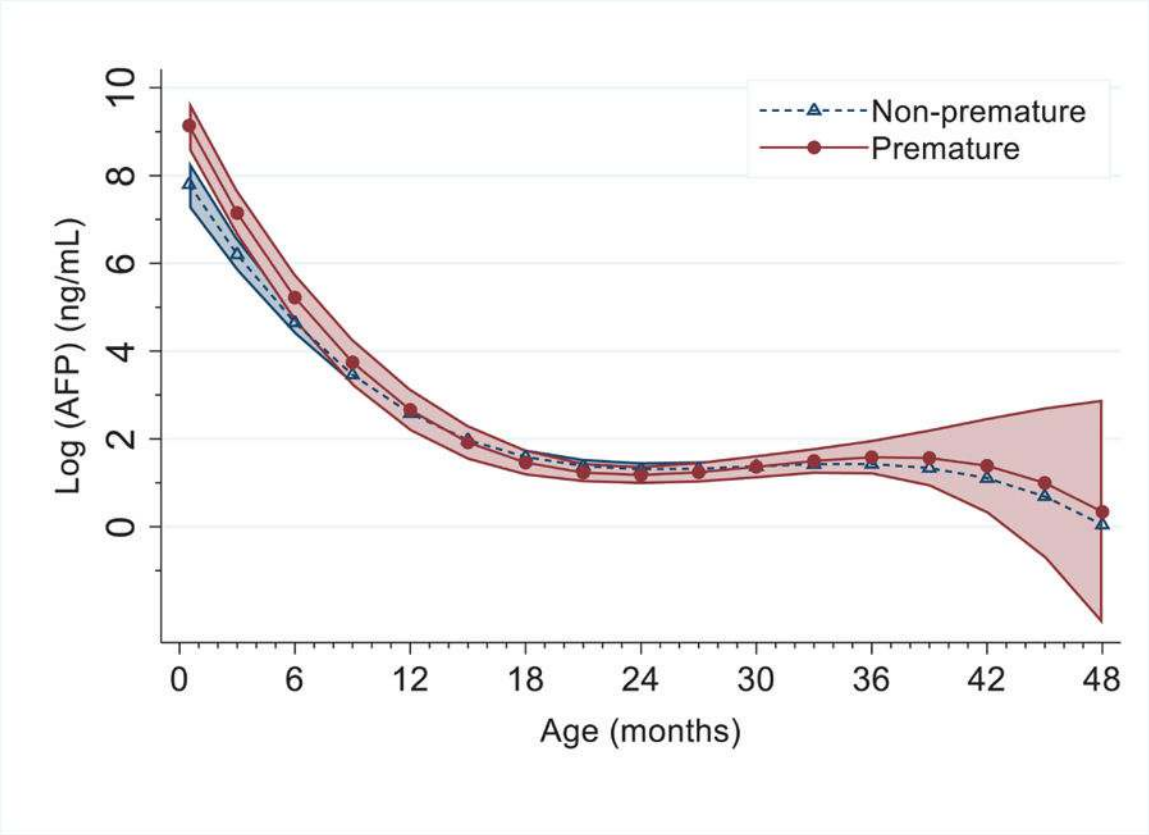

FIGURE 1A.

Predictive Log(AFP) of age with 95% confidence intervals (CIs) by prematurity. Age corrected in premature patients (<37 weeks gestation) to gestational age of 38 weeks.

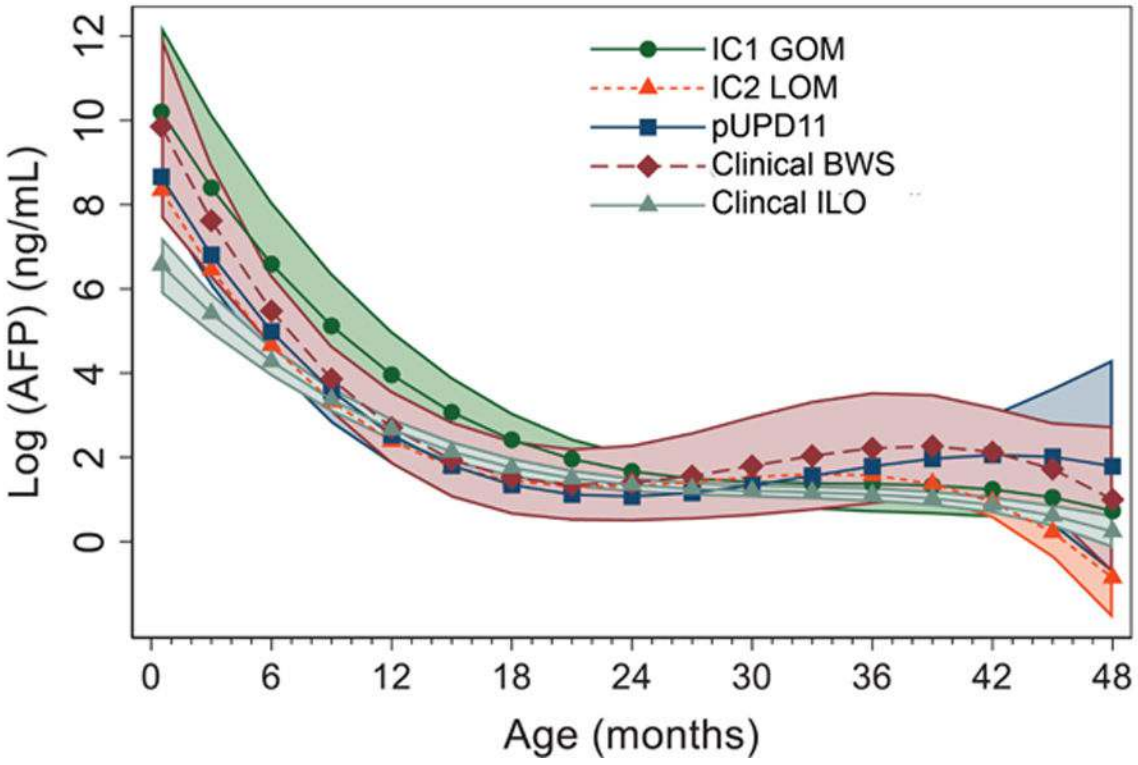

FIGURE 1B.

Predictive Log(AFP) of age with 95% confidence intervals (CIs) by molecular diagnosis. Abbreviations: IC1 GOM (imprinting center 1 gain of methylation); IC2 LOM (imprinting center 2 loss of methylation); pUPD11 (paternal uniparental disomy of chromosome 11).

TABLE 1

Characteristics of Patients

Characteristic	Patients (n=147) n (%)
Sex, Male	79 (53.7%)
Premature (<37 weeks)	52 (35.4%)
Molecular Subtype	
IC1 GOM	9 (6.1%)
IC2 LOM	45 (30.6%)
pUPD	35 (23.8%)
CDKN1C mutation	2(1.4%)
11p15.5 anomaly	8 (5.4%)
clinical BWS diagnosis	16 (10.9%)
clinical ILO diagnosis	32(21.8%)

Abbreviations: IC1 GOM (Gain of methylation at H19/IGF2:IG-DMR); IC2 LOM (loss of methylation at KCNQ1OT1:TSS-DMR); pUPD (paternal uniparental isodisomy of chromosome 11); BWS (Beckwith-Wiedemann syndrome); ILO (isolated lateralized overgrowth)

TABLE 2

Predicted α-Fetoprotein (AFP) Values by Age and Prematurity

Age Corrected	Overall	Premature Patients[a]	Non-premature Patients	P-value
	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	
1 Day	6,286 (4,128 – 9,572)	14,068 (7,868 – 25,154)	3,398 (1,934 – 5,971)	0.001 **
1 Week	5,364 (3,554 – 8,095)	11,767 (6,634 – 20,870)	2,944 (1,700 – 5,100)	0.001 **
2 Weeks	4,471 (2,992 – 6,681)	9,586 (5,449 – 16,861)	2,497 (1,465 – 4,257)	0.001 **
3 Weeks	3,737 (2,525 – 5,533)	7,835 (4,486 – 13,684)	2,124 (1,266 – 3,564)	0.001 **
4 Weeks	3,134 (2,136 – 4,597)	6,425 (3,701 – 11,155)	1,811 (1,096 – 2,993)	0.001 **
5 Weeks	2,635 (1,812 – 3,833)	5,286 (3,060 – 9,132)	1,548 (951 – 2,521)	0.001 **
6 Weeks	2,222 (1,540 – 3,206)	4,364 (2,536 – 7,508)	1,327 (827 – 2,130)	0.001 **
7 Weeks	1,880 (1,313 – 2,691)	3,614 (2,107 – 6,198)	1,141 (721 – 1,805)	0.001 **
8 Weeks	1,594 (1,122 – 2,266)	3,002 (1,754 – 5,137)	983 (630 – 1,534)	0.002 **
9 Weeks	1,356 (961 – 1,914)	2,502 (1,465 – 4,274)	849 (551 – 1,308)	0.002 **
10 Weeks	1,157 (825 – 1,622)	2,092 (1,226 – 3,570)	735 (484 – 1,118)	0.003 **
11 Weeks	989 (710 – 1,379)	1,754 (1,28 – 2,992)	638 (425 – 959)	0.003 **
12 Weeks	849 (613 – 1,176)	1,476 (865 – 2,517)	556 (374 – 824)	0.004 **
4 Months	401 (297 – 541)	634 (370 – 1,087)	282 (201 – 396)	0.013 *
5 Months	227 (170 – 302)	334 (193 – 576)	168 (124 – 227)	0.031 *
6 Months	134 (102 – 177)	185 (107 – 321)	105 (79.9 – 137)	0.068
7 Months	83.2 (63.9 – 108)	108 (62.1 – 187)	67.8 (53.0 – 86.6)	0.131
8 Months	53.7 (41.6 – 69.4)	66.0 (38.1 – 114)	45.6 (36.4 – 57.2)	0.222
9 Months	36.2 (28.2 – 46.3)	42.2 (24.6 – 72.5)	31.8 (25.7 – 39.3)	0.339
10 Months	25.3 (19.92 – 32.2)	28.3 (16.68 – 47.9)	23.0 (18.78 – 28.1)	0.473
11 Months	18.37 (14.58 – 23.2)	19.72 (11.85 – 32.8)	17.17 (14.13 – 20.9)	0.619
12 Months	13.82 (11.06 – 17.25)	14.32 (8.80 – 23.3)	13.24 (10.95 – 16.00)	0.769
13 Months	10.74 (8.68 – 13.28)	10.79 (6.81 – 17.10)	10.51 (8.73 – 12.66)	0.917
14 Months	8.62 (7.04 – 10.55)	8.43 (5.48 – 12.97)	8.59 (7.15 – 10.31)	0.939
15 Months	7.12 (5.88 – 8.62)	6.81 (4.58 – 10.15)	7.20 (6.02 – 8.62)	0.803
16 Months	6.05 (5.05 – 7.24)	5.68 (3.95 – 8.18)	6.20 (5.19 – 7.39)	0.676
17 Months	5.27 (4.45 – 6.25)	4.88 (3.51 – 6.79)	5.45 (4.59 – 6.48)	0.562
18 Months	4.71 (4.02 – 5.52)	4.31 (3.20 – 5.80)	4.90 (4.14 – 5.81)	0.462
19 Months	4.30 (3.70 – 4.99)	3.90 (2.99 – 5.10)	4.49 (3.80 – 5.31)	0.380
20 Months	4.00 (3.48 – 4.61)	3.62 (2.84 – 4.60)	4.20 (3.56 – 4.95)	0.319
21 Months	3.80 (3.32 – 4.35)	3.43 (2.75 – 4.27)	3.98 (3.39 – 4.69)	0.281
22 Months	3.66 (3.21 – 4.18)	3.31 (2.69 – 4.08)	3.84 (3.27 – 4.51)	0.269
23 Months	3.59 (3.15 – 4.08)	3.25 (2.65 – 3.98)	3.75 (3.19 – 4.40)	0.284

Age Corrected	Overall	Premature Patients[a]	Non-premature Patients	P-value
	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	
24 Months	3.56 (3.12 – 4.05)	3.24 (2.64 – 3.98)	3.70 (3.15 – 4.34)	0.325
27 Months	3.68 (3.19 – 4.24)	3.45 (2.72 – 4.37)	3.74 (3.16 – 4.44)	0.584
30 Months	4.00 (3.41 – 4.68)	3.91 (3.00 – 5.11)	3.95 (3.29 – 4.76)	0.95
33 Months	4.33 (3.65 – 5.15)	4.47 (3.32 – 6.01)	4.16 (3.41 – 5.08)	0.695
36 Months	4.47 (3.66 – 5.45)	4.87 (3.28 – 7.23)	4.17 (3.38 – 5.15)	0.500
39 Months	4.16 (3.19 – 5.42)	4.78 (2.49 – 9.18)	3.81 (3.06 – 4.74)	0.516
42 Months	3.33 (2.24 – 4.95)	4.01 (1.35 – 11.93)	3.02 (2.37 – 3.85)	0.620
45 Months	2.18 (1.19 – 3.99)	2.71 (0.48 – 15.17)	1.99 (1.46 – 2.71)	0.730
48 Months	1.11 (0.45 – 2.72)	1.40 (0.11 – 18.12)	1.04 (0.67 – 1.62)	0.824

** significant at p<0.01

* significant at p<0.05

[a] Prematurity defined as gestational age <37 weeks. Ages reported were corrected to full-term gestational age (38 weeks)

TABLE 3

Minimum and Maximum α-Fetoprotein (AFP) values Observed by Age

Age[a]	Minimum AFP (ng/mL)	Maximum AFP (ng/mL)
1 day	7,240	476,000
1 month	2,540	396,570
2 months	196	395,420
3 months	36.9	286,000
4 months	12.8	247,451
5 months	18.1	125,000
6 months	2.03	52,411
7 months	6.75	10,669
8 months	5.97	14,543
9 months	4.20	6,668
10 months	3.99	6,168
11 months	4.70	3,504
12 months	1.50	1,498
13 months	2.20	2,245
14 months	2.20	727
15 months	1.10	117.8
16 months	1.56	305.0
17 months	1.20	85.0
18 months	1.50	36.2
19 months	1.70	48
20 months	1.60	39
21 months	1.40	38
22 months	2.01	19.1
23 months	1.30	34.0
24 months	1.50	10.5
27 months	0.80	16.0
30 months	1.15	18.0
33 months	1.20	39.5
36 months	0.70	19.0
39 months	0.60	39.0
42 months	0.60	10.0
45 months	0.80	37.0
48 months	0.60	104.0

[a]Ages were not corrected for gestational age

Table 4; online.

Predicted Serum α-fetoprotein (AFP) by Molecular Subtype

Age-corrected[a]	IC1 GOM (n=9)	IC2 LOM (n=45)	pUPD11 (n=35)
	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL
1 Day	38,921 (4,671 – 324,327)	6,249 (3,686 – 10,593)	8,486 (4,21 – 17,909)
1 Week	33,215 (4,095 – 269,418)	5,265 (3,154 – 8,791)	7,188 (3,417 – 15,122)
2 Weeks	27,678 (3,519 – 217,710)	4,327 (2,637 – 7,102)	5,941 (2,832 – 12,465)
3 Weeks	23,122 (3,29 – 176,487)	3,569 (2,211 – 5,761)	4,925 (2,351 – 10,317)
4 Weeks	19,363 (2,612 – 143,520)	2,953 (1,858 – 4,692)	4,095 (1,956 – 8,571)
5 Weeks	16,255 (2,257 – 117,075)	2,452 (1,567 – 3,838)	3,415 (1,631 – 7,148)
6 Weeks	13,679 (1,953 – 95,797)	2,043 (1,324 – 3,151)	2,856 (1,363 – 5,982)
7 Weeks	11,539 (1,694 – 78,624)	1,708 (1,122 – 2,598)	2,395 (1,142 – 5,24)
8 Weeks	9,758 (1,471 – 64,723)	1,432 (954 – 2,151)	2,014 (958 – 4,234)
9 Weeks	8,271 (1,280 – 53,438)	1,205 (812 – 1,787)	1,699 (806 – 3,580)
10 Weeks	7,027 (1,116 – 44,250)	1,017 (694 – 1,491)	1,437 (680 – 3,36)
11 Weeks	5,984 (975 – 36,747)	861 (594 – 1,249)	1,219 (575 – 2,583)
12 Weeks	5,108 (853 – 30,604)	732 (510 – 1,050)	1,037 (488 – 2,204)
4 Months	2,338 (440 – 12,426)	331 (241 – 455)	469 (217 – 1015)
5 Months	1,279 (264 – 6,201)	182 (136 – 244)	256 (117 – 561)
6 Months	727 (163 – 3,238)	106 (80.4 – 139)	147 (66.5 – 324)
7 Months	430 (105 – 1,766)	64.7 (49.6 – 84.3)	87.9 (39.7 – 194)
8 Months	264 (69.3 – 1,002)	41.5 (32.0 – 53.8)	55.0 (25.0 – 121)
9 Months	168 (47.5 – 591)	27.8 (21.5 – 36.0)	35.9 (16.51 – 77.9)
10 Months	110 (33.6 – 362)	19.53 (15.11 – 25.2)	24.4 (11.43 – 51.9)
11 Months	74.9 (24.5 – 229)	14.28 (11.05 – 18.46)	17.20 (8.28 – 35.7)
12 Months	52.5 (18.42 – 150)	10.86 (8.41 – 14.04)	12.60 (6.26 – 25.4)
13 Months	38.0 (14.28 – 101)	8.57 (6.64 – 11.08)	9.56 (4.92 – 18.57)
14 Months	28.2 (11.38 – 70.1)	7.00 (5.42 – 9.05)	7.51 (4.03 – 14.00)
15 Months	21.6 (9.31 – 50.1)	5.91 (4.57 – 7.64)	6.08 (3.41 – 10.85)
16 Months	16.94 (7.81 – 36.7)	5.14 (3.97 – 6.65)	5.08 (2.98 – 8.65)
17 Months	13.63 (6.70 – 27.7)	4.59 (3.54 – 5.96)	4.36 (2.68 – 7.09)
18 Months	11.22 (5.87 – 21.4)	4.21 (3.23 – 5.49)	3.85 (2.48 – 5.98)
19 Months	9.45 (5.24 – 17.02)	3.95 (3.02 – 5.18)	3.49 (2.35 – 5.18)
20 Months	8.12 (4.75 – 13.87)	3.79 (2.87 – 5.00)	3.23 (2.27 – 4.61)
21 Months	7.11 (4.36 – 11.60)	3.69 (2.77 – 4.92)	3.06 (2.22 – 4.21)
22 Months	6.34 (4.03 – 9.97)	3.66 (2.72 – 4.93)	2.96 (2.21 – 3.97)
23 Months	5.75 (3.75 – 8.81)	3.68 (2.71 – 5.01)	2.91 (2.21 – 3.84)
24 Months	5.30 (3.50 – 8.01)	3.74 (2.72 – 5.16)	2.92 (2.23 – 3.82)
27 Months	4.47 (2.86 – 6.98)	4.12 (2.89 – 5.88)	3.19 (2.39 – 4.24)

Age-corrected[a]	IC1 GOM (n=9)	IC2 LOM (n=45)	pUPD11 (n=35)
	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL	AFP (95% CI), ng/mL
30 Months	4.12 (2.41 – 7.04)	4.63 (3.17 – 6.75)	3.81 (2.79 – 5.20)
33 Months	4.00 (2.14 – 7.48)	4.98 (3.41 – 7.27)	4.77 (3.51 – 6.49)
36 Months	3.94 (1.99 – 7.78)	4.82 (3.38 – 6.88)	5.98 (4.31 – 8.28)
39 Months	3.80 (1.90 – 7.57)	3.95 (2.83 – 5.52)	7.16 (4.23 – 12.13)
42 Months	3.46 (1.76 – 6.78)	2.57 (1.73 – 3.83)	7.84 (2.98 – 20.6)
45 Months	2.86 (1.40 – 5.82)	1.25 (0.68 – 2.30)	7.50 (1.46 – 38.4)
48 Months	2.08 (0.81 – 5.29)	0.43 (0.16 – 1.13)	5.99 (0.47 – 75.7)

[a]Age corrected in premature patients (<37 weeks gestation) to gestational age of 38 weeks

Abbreviations: IC1 GOM (imprinting center 1 gain of methylation); IC2 LOM (imprinting center 2 loss of methylation); pUPD11 (paternal uniparental disomy of chromosome 11); BWS (Beckwith-Wiedemann syndrome); ILO (isolated lateralized overgrowth)