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HEALTH

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Fetal Defect Marker Proficiency Test Mailout[1]
May 2013

Dear Laboratory Director,
Below you will find a summary and critique of the Proficiency Testing mail-out from May 7, 2013, for Fetal Defect Markers, which included samples for first and second trimester screening, as well as amniotic fluids. Your laboratory's results and grades are printed on a separate sheet; also included are the grades from the previous two PT events. These will be mailed to you separately. Please review and sign your evaluation. Retain the signed evaluation in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

I. Graded Results Section: Table 1: Second Trimester Maternal Serum: Summary of All Lab Results

Samples *N = 27	**Sample #**	**MS 296**	**MS 297**	**MS 298**	**MS 299**	**MS 300**
	Gestational Age (weeks)	20.0	17.0	16.0	15.0	18.0
Maternal Race	Ethnic Group	White	Hispanic	Black	White	White
Maternal Weight	Pounds (lbs)	150	140	145	160	155
Maternal Age	Years	25	20	28	30	32
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	187.0 ± 20.8	49.9 ± 4.2	43.5 ± 3.8	96.0 ± 8.3	29.3 ± 2.2
	MOM ± Std. Dev.	3.20 ± 0.36	1.26 ± 0.11	1.15 ± 0.12	3.44 ± 0.28	0.68 ± 0.05
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	1.57 ± 0.13	0.89 ± 0.10	0.72 ± 0.07	0.87 ± 0.06	0.58 ± 0.04
	MOM ± Std. Dev.	0.90 ± 0.12	0.93 ± 0.15	0.98 ± 0.16	1.58 ± 041	0.52 ± 0.10
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	20.2 ± 2.1	27.5 ± 2.9	32.2 ± 3.2	43.8 ± 5.3	51.8 ± 6.2
	MOM ± Std. Dev.	1.19 ± 0.15	1.11 ± 0.12	1. 03 ± 0.11	1.21 ± 0.17	2.59 ± 0.34
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	213.5 ± 21.6	136.3 ± 14.5	125.7 ± 14.3	120.6 ± 14.2	444.2 ± 42.9
	MOM ± Std. Dev.	1.12 ± 0.12	0.80 ± 0.08	0.72 ± 0.08	0.67 ± 0.09	2.68 ± 0.26
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (100%)	(+) (100%)	(-) (100%)
	Recommended Action**	G = 85% U = 93% A = 81% R = 4%	NFA	NFA	G = 89% U = 100% A = 78% R = 7%	NFA
	NTD Risk 1 in	26	5,200	9,125	37	10,000
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 92% U = 69% A = 85%
	Risk Est. 1 in	8,400	5,000	5,000	10,000	21
2. Quad Test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	G = 88% U = 62% A = 81%
	Risk Est. 1 in	20,000	11,899	10,024	22,500	9
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	10,000	10,000	10,000	10,000	4,150

*N = total numbers may vary since some labs do not test all analytes. The values represent the all-lab consensus based on the arithmetic mean ± Std. Dev.
(B) = borderline positive or negative, risk reflects central tendency (Median number for NTD/Down positive or negative/borderline screen). NFA = no further action; FA = further action; G = genetic counseling; U = ultrasound, A = amniocentesis, and R = repeat.**This percentage is normalized to labs requesting further action. ‡ Insulin Dependent Diabetic pregnancy.

[1]The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 27 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 296 Wk 20.0	This specimen was obtained from a 25 year old white woman (Gravida = 3, Parity = 1) in her 20th week gestation with a body weight of 150 lbs. She had a personal history of pregnancy loss. Her specimen, a third pregnancy sample, was a positive screen for NTD (100% consensus; MOM=3.20). Her screen was negative for both Trisomies with all labs in agreement. Recommendations of further action from labs performing the NTD screen were: genetic counseling, 85%, ultrasound, 93%, amniocentesis, 81% and repeat, 4%; The MS296 specimen had an amniotic fluid counterpart that was also elevated (MOM = 2.90).
MS 297 Wk 17.0	This specimen was obtained from a 20 year old Hispanic woman (Gravida = 1, Parity = 0) in her 17th week of gestation with a body weight of 140 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight or an ethnic correction indicated. The labs agreed that both Trisomy screens were negative. Specimen MS297 was not paired with an amniotic fluid specimen.
MS 298 Wk 16.0	This specimen was obtained from a 28 year old Black woman (Gravida = 1, Parity = 0) in her 16th week of gestation with a body weight of 145 lbs. Her family reproductive history was unremarkable. Her sample screened negative for NTD, as was her aneuploidy screen for Trisomies-21 and 18. This sample was not paired to an amniotic fluid specimen.
MS 299 Wk 15.0	This specimen was obtained from a 30 year old White woman (Gravida = 2, Parity = 1) in her 15th week of gestation with a body weight of 160 lbs. She had no family history of reproductive complications. Her sample screened positive for NTD, and her aneuploidy screens were negative for both Trisomy-18 and Trisomy-21. Please see Critique for further discussion of this sample.
MS 300 Wk 18.0	This specimen was obtained from a 32 year old white woman (Gravida = 4, parity = 2) in her 18th week gestation with a body weight of 155 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100%). Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 88%; ultrasound, 62%; and amniocentesis, 81%; while those few labs performing the triple tests were: genetic counseling, 92%; ultrasound 69%, and amniocentesis, 85%. Specimen MS300 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to an amniotic fluid specimen which also had a low AFAFP level (MOM = 0.45).

Notice of Gravida/Parity Clarification for Present and Future Mail outs;

For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of pregnancies including the present one and parity = m indicates the patient already has m children; however, multiple birth is also considered as a single parity.

Example: A woman of gravida = 3, parity = 2 indicates that the pregnant woman has been pregnant twice before, and has two children.

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=20; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 296 Wk 20.0	AFP = 18.5 ± 1.7 µg/ml MOM = 2.90 ± 0.41	The AF296 sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs called AF296 a positive screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen **MS296** whose AFP was also elevated (MOM = 3.20).
AF 297 Wk 15.0	AFP = 10.1 ± 1.8 µg/ml MOM = 0.60 ± 0.09	The AF297 sample was targeted for normal AFAFP value in the routine gestational age range. All labs called AF297 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 298 Wk 16.0	AFP = 8.6 ± 1.4 µg/ml MOM = 0.61 ± 0.09	The AF298 sample was targeted for a screen negative AFAFP value in the routine gestational age screening range. All labs reported this specimen as a screen negative AFAFP value. The AF298 specimen was not paired with a maternal serum sample.
AF 299 Wk 17.0	AFP = 3.3 ± 0.5 µg/ml MOM = 0.28 ± 0.03	The AF299 sample was targeted for a screen negative AFAFP value in the routine gestational age range. All labs reported this specimen as a screen negative AFAFP value. The AF299 specimen was not paired with a maternal serum sample.
AF 300 Wk 18.0	AFP = 4.3 ± 0.6 µg/ml MOM = 0.45 ± 0.05	The AF300 sample was targeted as an NTD negative screen in the routine gestational age screening range. All labs categorized AF300 as a negative NTD screen specimen. This specimen had a maternal serum counterpart, **MS300**, which showed reduced levels of AFP (MOM = 0.68).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 17	**Sample #**	**FT 296**	**FT 297**	**FT 298**	**FT 299**	**FT 300**
	Gestational Age (weeks)	13.0	12.4	11.4	11.1	13.0
Maternal Race	Ethnic Group	Black	Asian	White	White	Hispanic
Maternal Weight	Pounds (lbs)	130	140	145	130	155
Maternal Age	Years	21	28	25	35	23
Fetal Physical Measurements	Crown Rump Length (mm)	67	60	47	44	68
	NT Thickness (mm)	1.50	1.40	2.50	1.19	1.60
	NT – MOM ± Std. Dev.	0.92 ± 0.05	0.95 ± 0.06	2.10 ± 0.11	1.06 ± 0.06	0.97 ± 0.06
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	67.1 ± 8.1	72.9 ± 9.1	161.5 ± 25.5	88.7 ± 13.1	63.1 ± 7.1
	MOM ± Std. Dev.	0.93 ± 0.11	1.04 ± 0.07	2.04 ± 0.17	1.02 ± 0.11	1.01 ± 0.09
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	2955.6 ± 1912.8	2438.1 ± 1770.0	1077.1 ± 793.5	1879.8 ± 1211.0	2890.0 ± 1848.3
	MOM ± Std. Dev.	1.83 ± 1.10	2.49 ± 1.23	1.56 ± 0.91	2.71 ± 1.35	2.49 ± 1.22
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (88%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	G = 93% U = 47% A = 53% C = 67%	NFA	NFA
	Risk Estimate 1 in	15,150	18,000	94	8,150	19,500
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	10,000	5,350	10,000	10,000

*N = total numbers may vary since some labs do not test all analytes. (B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; A = amniocentesis; C = chorionic villus sampling; N = number of labs participating; FT = First Trimester. **This percentage is normalized to labs requesting further action. ***Results from methods that give IU/ml were converted to ng/ml as described in section D.1 below.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

N = 17 all-lab Consensus Values.

Sample#	Summary Comments:
FT 296 Wk 13.0	This specimen was obtained from a 21 year old Black woman with a body weight of 130 lbs. Her gestational age at the time of screening was 13.0 weeks. She had no prior history of pregnancy complications or difficulties. This FT specimen was screen negative and all testing labs were in agreement. The FT296 risk estimate for Trisomy-21 was 1 in 15,150 and the Trisomy-18 risk was 1 in 10,000.
FT 297 Wk 12.4	This specimen was procured from a 28 year old Asian woman of average body weight (140 lbs.). Her gestational age at the time of screening was 12.4 weeks. She had no prior history of any pregnancy complications. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT297 risk estimate for Trisomy-21 was 1 in 18,000, and the Trisomy-18 risk was 1 in 10,000.
FT 298 Wk 11.4	This specimen was obtained from a 25 year old white woman of average body weight (145 lbs.). Her gestational age at the time of screening was 11.4 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen positive with all testing labs in agreement. The FT298 risk estimate for Trisomy-21 was 1 in 94, and the Trisomy-18 risk was also 1 in 5,350. (See critique)
FT 299 Wk 11.1	This specimen was procured from a 35 year old white woman of average body weight (130 lbs.). Her gestational age at the time of screening was 11.1 weeks. She had no prior family history of pregnancy complications and adverse outcomes. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT299 risk estimate for Trisomy-21 was 1 in 8,150, while the Trisomy-18 risk was 1 in 10,000.
FT 300 Wk 13.0	This specimen came from a 23 year old Hispanic woman with a body weight of 155 lbs. Her gestational age at the time of screening was 13.0 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT300 was 1 in 19,500, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results were consistent with the targeted values for the NTD and the Trisomy Screens for risks and outcomes. The Caucasian maternal serum sample **MS296** was targeted as a screen positive specimen for NTD (Figs. 1 and 3) and was matched to the elevated **AF296** sample (Fig. 2). All labs (100%) agreed that specimen **MS296** was screen positive for NTD and negative for both Trisomy screens using the triple and/or quad test (Figs. 4-6). The **MS296** sample was matched with **AF296,** and both exhibited elevated AFP levels strongly suggesting the presence of an NTD. As a follow-up, a polyacrylamide gel electrophoresis is indicated and is performed to show the presence or absence of a diagnostic ACHE band indicating an NTD.

Sample **MS300** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS300** (MSAFP-MOM = 0.68, MSuE3-MOM = 0.58, MShCG-MOM = 2.59, DIA-MOM = 2.68) presented the canonical profile of low MSAFP and low MSuE3, together with elevated MShCG and MSDIA resulting in a T21 positive screen in which all labs were in agreement (100% by both triple and quad test). The T21 risk was 1 in 21 by triple test and 1 in 9 by quad test (Figs. 5, 6). The recommended further actions for the sample **MS300** were genetic counseling, 92%; ultrasound, 69%; and amniocentesis, 85%, from labs performing the triple screen, and genetic counseling, 88%; ultrasound, 62% and amniocentesis, 81%, from labs performing the quad screen.

Two other specimens, **MS297** and **MS298**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS299** specimen at 15 weeks was an interesting case involving high levels of MSAFP with slightly raised MSuE3and normal levels of MSHCG and MSDIA; this resulted in a positive screen for NTD and a negative screen for T21 and T18 (Figs. 4-6). The NTD follow-up actions recommended for specimen **MS299** were genetic counseling, 89%; ultrasound, 100%; amniocentesis, 78%; and repeat testing, 7%. The sample **MS299** was modeled after several literature case studies of pregnant women with fetal Beckwith-Wiedemann Syndrome (BWS) who exhibited elevated levels of MSAFP but not NTD (1-3). The patients from these case studies had experienced a BWS diagnosis and follow-up scans during pregnancy. Prior to their BWS pregnancy, most of the women had completed uncomplicated pregnancies and delivered normal term infants. Following the diagnosis of their BWS pregnancy, the woman decided to continue gestation and underwent diagnostic examinations which included ultrasound, 3-D scans, and subsequent hypermethylation testing of the newborn's H19 gene on chromosome-11.

In the present mock case of sample MS299 involving 2nd trimester specimens, the biomarker test results produced a positive NTD screen of high MSAFP in the 15th week of pregnancy. However, previous literature case studies had demonstrated that elevated levels of MSAFP together with altered MSuE3 profiles often predicted pregnancy complications which could include placental dysfunction, threatened miscarriage, preterm birth, and pre-eclampsia (1, 2, 3, 4). In contrast to these, mock patient MS299 was designed to have no prior history of pregnancy loss or complications. Although an elevated MSAFP analyte in fetal defect testing can generate a NTD positive screen (5- 7), in this case, the elevated MSAFP and slightly raised MSuE3 generated a false positive screen for NTD and did so in 100% of the screening labs.

Mock specimen MS299 was specifically designed to mimic an actual 30 year old woman of gravida 3, parity 2, in her 15th week of pregnancy (1). Similar to the published case report, the maternal serum biomarkers of MS299 revealed an MSAFP MOM of 3.1, MSuE3 Mom of 1.5, MSHCG MOM of 1.2, and MSDIA MOM of 1.0. This pattern was consistent with a canonical positive screen for NTD and a negative screen for T21. However, ultrasound examination in the actual case study patient performed at 20 weeks and again at 30 weeks showed evidence of the fetal overgrowth disorder called BWS. The 3-D scan at 33 and 34 weeks demonstrated a protruding tongue and a fixed open mouth caused by macroglossia (enlarged tongue). In addition, the patient's scans revealed liver (hepatomegaly), kidney (nephromegaly), and heart overgrowth (cardiomegaly), together with an increase in amniotic fluid volume (polyhydramnios). A newborn female was delivered by cesarean section at 37 weeks with a body weight of 3,800 gram (>97 percentile) and a length of 50 cm (>90 percentile). In confirmation of the ultrasound scans, the newborn exhibited macroglossia, diastasis recti, left ear creases, clitoromegaly, and enlarged kidney and liver, with no evidence of tumors. The newborn's karyotype was normal but the molecular analysis of a BWS-related gene critical region at 11p15.5 revealed presence of hypermethylation of the H19 gene, which is consistent with a diagnosis of BWS. The infant's AFP levels were 103,963 ng/ml (MOM = 2.1) at birth, 4,817 ng/ml (MOM = 54) at 3 months, 785 ng/ml (MOM = 62.0) at 6 months, and 59 ng/ml at 12 months (MOM = 9.8). Thus, the pediatric AFP levels were elevated at each time point measured in the first year of life. Postnatal AFP values can be difficult to interpret due to a high variation in serum levels in normal newborns together with constantly decreasing levels during the first year of life (8). However, pediatric AFP levels specific to BWS infants have now been published to aid in this discrimination (34).

This case history was interesting in that the initial clues available to the obstetricians were the NTD positive screen result which subsequently showed no evidence of NTD. Furthermore, the Ache gel electrophoresis bands showed no pattern of ventral wall defects (VWDs) common in BWS fetuses. The ultrasonographic scans showing overgrowth in the patient were not performed until the 20 – 30 week period, thus, post-dating the biochemical screen. Elevated AFP levels have been described in the literature in every case associated with BWS and those elevated levels were often associated with abnormal liver function. BWS is a rare tissue/organ overgrowth disorder, which exhibits a multitude of anatomical abnormalities, including macrosomia (excessive birthweight), macroglossia (enlarged tongue), visceromegaly (enlargement of viscera), omphalocele (ventral body wall defect), pancreatic hypoglycemia (low blood glucose), and unusual linear fissures in the earlobes (9, 10). Malignancies occur in about 10% of the patients and these cancers originate mostly from intra-abdominal embryonal (blastomas) cancers; these can include Wilms tumor, hepatoblastoma, pancreatoblastoma, and adrenal cortical carcinoma (11, 12). Patients can, but are less likely to, present with gonadoblastoma, rhabdomyosarcoma, and neuroblastoma (13). The cause of BWS stems from a mutation or deletion of genes within a region of chromosome 11p15.5, which involves the genes p57, H19, and LIT1 (14).

Serum AFP is elevated in BWS patients in the fetal, prenatal, neonatal, and infant stages similar to patients with ataxia teleangiectasia, hepatobiliary disease, hypothyroidism, and blastoma neoplasms of liver and pancreas. Following birth, parents of BWS patients are urged to seek regular-spaced serial measurements of AFP. In most cases, the diagnosis of BWS is made in the postnatal and not the prenatal period, on the basis of multiple physical abnormalities (1). BWS is diagnosed by the presence of at least three major physical features, namely; a) anterior abdominal wall defect, b) tongue macroglossia, and c) pre-and postnatal structural overgrowths. Other supplemental defects can include several of the following; ear creases, post-auricolor pits (clefts of lower lip and chin), hypoglycemia, nephromegaly (enlarged kidney), and hemihyperplasia (enlargement of one side of body), and polyhydraminos (36). As discussed above, BWS patients have a significantly increased risk of cancer of about 600 times that of the general population. Thus, tumor surveillance is an important part of the clinical management of patients with BWS (39). In a published report of 22 children (newborn to 3 years of age) with confirmed BWS, it was shown that AFP serum levels are elevated in BWS neonates/infants at all age levels measured (34). AFP serum concentrations were shown to decline during the postnatal period at significantly slower rates (longer half-life) than what is reported in healthy children (38). Thus, clinicians suggest that tumor screening in children with BWS should be interpreted with a standard curve specifically established for BWS patients. Most BWS-diagnosed children undergo serial abdominal ultrasonography at 3-month intervals until the age of 7 to 8 years together with serial serum AFP measurements (15). Since elevated serum AFP levels provide a biomarker for hepatoblastoma and pancreatoblastoma, a physician must be aware of distinguishing levels specific to BWS from the characteristics of just embryonic blastomas. Highly elevated serum AFP values detected in BWS patients can initiate the performance of an abdominal computed tomography scan on an urgent basis, since lack of such knowledge can create undue parental and physical anxiety until the presence of a blastoma tumor can be excluded.

A second case history of a BWS patient presenting with an elevated triple screen in the second trimester was reported by Aagaard-Tillery et al (32). In that case study, MSAFP was elevated and imaging studies showed the presence of an umbilical cord defect and enlarged ovaries in the mother. The pregnant women developed the HELLP syndrome which is a variant of pre-eclampsia that includes hemalysis, elevated liver enzymes and low platelet count. In a third study, a newborn was diagnosed with BWS at birth (severe physical defects) and a paternal chromosome-11 uniparental disomy (UPD11), which is a condition associated with a high tumor risk (36). Unexpectedly, neonatal serum AFP levels were normal at birth, but rapidly rose within 20 days, leading to the diagnosis of an extremely aggressive hepatoblastoma. In cases of BWS, clinicians have suggested neonatal monitoring of serum AFP every 20 days. In a final study, another newborn with BWS displayed normal serum AFP from day seven to the third month of life, but the infant did not exhibit the anatomical malformations of gigantism and visceromegaly (33).

The serum levels of AFP in BWS infants and children appear to correlate more with individual anatomical defects than just liver dysfunction and hepatoblastomas. In a study of 63 confirmed cases of infant BWS, serum AFP levels above 50,000 ng/ml were present in patients older than 2 months of age and these presented with ultrasound-detected tumors that included Wilms, hepatoblastomas, hemangiomas, and hemagnio-endotheliomas (47). In a further study, a 5-month old girl with BWS displayed a cluster of malformations that encomposed enlarged adrenal and renal glands, and a large liver mass (35). Markedly increased serum AFP levels were determined to be 1,060,000 ng/ml, which is highly suspicious for the presence of hepatoblastoma. However, histological analysis revealed the liver mass was a mesenchymal harmatoma of the liver (benign liver tumor) which mimicked a hepatoblastoma. Regarding pancreatoblastomas, AFP may be elevated in 68% of BWS patients (48); in one 2 month old male infant, AFP was highly elevated in conjunction with bilateral exothalmus (eyeball protrusion), enlargement of the testis, and a large mass at the head of pancreas (32).

The management and treatment of BWS manifestations include well-established standards of care as practiced in the medical community (9). Mild hypoglycemia can be treated by nutritional measures such as: 1) avoid skipping meals, delaying meals, and eating too little; 2) eating carbohydrates and fruit juices; 3) eat small but frequent meals throughout the day coupled with nutritional supplements. Abdominal wall defects such as omphalocele and gastroschisis can be treated with surgical repair at 2-3 years of age. Feeding difficulties due to macroglossia can be minimized through the use of endotracheal intubation, specially modified bottle nipples, and nasogastric tube feedings. Tongue reduction surgery can be performed in infancy and early childhood and speech therapy can be initiated as time permits. Surgery can be performed to equalize differences in leg lengths, while craniofascial surgery can be employed for hemihyperplasia. Neoplasms, such as hepatoblastoma are usually treated with chemodrugs such as doxorubicin, cisplatin, vincristine, and 5-fluorouricil, while pancreatoblastoma patients receive cyclophosamide, etoposide, and cisplatin (19, 20).

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7- 10). As shown in Figs. 7A and 8A, AFP and uE3 mass measurements in serum among the individual kits mostly agreed. In contrast, when the kit specific uE3 MOMs and mass values were compared, MOM values from Siemens DPC Immulite 2000/2500 ranged from 20 to 30% higher than those from Beckman (Fig. 8B) although mass uE3 values were 10% lower; however, preliminary studies in our lab suggest this may derive from a matrix effect in our samples. Regarding the hCG kits (Fig. 10), the Beckman Access 2 instrument results were about 10% higher than those from Beckman UNICEL, while the Siemens Immulite 2000 results were 10% lower than those from the other assay platforms. These differences were somewhat reflected in the MOM values. Finally, the method comparison for Inhibin-A displayed in Fig. 9A shows that the results from the Beckman Access/2 or UNICEL were similar whereas the Diagnostic Systems Lab (DSL) assay platform results were 20-25% lower, which is similarly reflected in the Inhibin MOM values (Fig. 9B).

Interestingly, when the AFP mass measurements in amniotic fluid were compared, the differences among the various methods appeared somewhat variable (Fig. 7C), while AFAFP MOM values (Fig.7D) were mostly the same with only Siemens displaying a 5-10% increased value. In particular, AFAFP-mass value results from the Abbott Axsym and Siemens Immulite 2000 were 5-20% higher than those from the Beckman UNICEL and Access 2 instruments. Since these specimens are derived from actual AF samples, these differences would be comparable to real patient testing**.**

C) Second Trimester Screening Software Utilized:

The alpha and Benetech PRA software packages were each used by 29.6% and 22.2%, of the labs, respectively; Benetch was 3.7%, Robert Maciel (RMA) software was employed by 29.6%; and in-house and "other" softwares comprised 14.8%. Programs classified as "other" are presumably proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples were provided in the present mailout. All laboratories that are **validation-approved** and presently perform first trimester Down syndrome screening are **REQUIRED** to test and report screen results. Those laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT) in millimeters, last menstrual period (LMP), crown-rump length (CRL) in millimeters, race, maternal body weight, and date of blood draw.

The all lab measurement of the gestational 13.0 week 21 year old Black **FT296** specimen for total hCG resulted in a mass mean of 67.1 $\pm$ 8.1 IU/ml, with a MOM of 0.93 $\pm$ 0.11; the all-lab mass mean for PAPP-A was 2955.6 $\pm$ 1912.8 ng/ml with a MOM of 1.83 $\pm$ 1.10. The **FT296** sample displayed a T21 negative screen assessment. As a result, the all-lab T21 risk assessment for **FT296** was 1 in 15,150 (Fig. 13). Further action was not indicated. Finally, 100 % of labs considered the **FT296** specimen screen negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Fig.14).

The **FT297** specimen was obtained from a 28 year old Asian woman with a gestational age of 12.4 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 72.9 $\pm$ 9.1 IU/ml (MOM = 1.04 $\pm$ 0.07); the all-lab PAPP-A mass measurement was 2438.1 ± 1770.0 ng/ml (MOM = 2.49 $\pm$ 1.23). The all-lab T21 screen consensus for **FT297** was negative with a risk assessment of 1 in 18,000 (Fig. 13). No further actions were recommended by the labs. Finally, the **FT297** specimen also screened negative for T18 (1 in 10,000 Fig. 14).

The **FT298** was obtained from a 25 year old White woman whose gestational age all-lab mean was reported as 11.4 weeks. Assay measurements for **FT298** resulted in an all-lab total hCG mass measurement of 161.5 $\pm$ 25.5 IU/ml (MOM = 2.04 $\pm$ 0.17), while the all-lab PAPP-A mass assessment was 1077.1 $\pm$ 793.5 ng/ml (MOM = 1.56 $\pm$ 0.91). Most labs (88%) agreed that the **FT298** sample was screen positive for T21 with a risk of 1 in 94 (Fig.

13) and negative for T18 with a risk assessment of 1 in 5,350 (Fig. 14). Further action was reported as: genetic counseling, 93%; ultrasound, 47%; amniocentesis, 53%; chorionic villi sampling, 67%.

The 11.1 week specimen **FT299** was procured from a 35 year old (130 lbs.) white woman. Total hCG measurement resulted in a mass mean of 88.7 IU/ml $\pm$ 13.1, with a MOM of 1.02 $\pm$ 0.11. In addition, the all-lab mass mean for PAPP-A was 1879.8 $\pm$ 1211.0 ng/ml with a MOM of 2.71 $\pm$ 1.35. This resulted in an all-lab negative T21 risk assessment of 1 in 8,150 (Fig. 13) and a negative T18 risk assessment of 1 in 10,000 (Fig. 14).

Specimen **FT300** was obtained from a 23 year old Hispanic woman of 155 lbs whose gestational age all-lab mean was reported as 13.0 weeks. Assay measurements resulted in an all-lab total hCG concentration of 63.1 $\pm$ 7.1 IU/ml (MOM = 1.01 $\pm$ 0.09), while the all-lab PAPP-A concentration was 2890.0 $\pm$ 1848.3 ng/ml (MOM = 2.49 $\pm$ 1.22). The all-lab FT T21 risk assessment was 1 in 19,500 and all labs agreed that the **FT300** sample was negative for T21 (Fig. 13). Similarly, the **FT300** specimen was also screen negative for T18 with an all-lab risk assessment of 1 in 10,000 (Fig.14).

D. 1.) <u>First Trimester Assay Kit Performance</u>:

In order to compare the Beckman UNICEL assays (53% users) for PAPP-A with those of the older Siemens Immulite and DSL assay platforms, a conversion factor was calculated from participating labs using data from the last seven PT mailouts (Note: this conversion factor may not be applicable to real patient samples because of potential matrix effects in the PT samples). Hence, Beckman UNICEL (y-axis) data for PAPP-A in MOM were plotted versus Siemens Immulite 2000 (x-axis) data in MOM yielding a linear correlation with an R^2 value of 0.9858, a slope of 0.4234 and a Y intercept of -0.0354 (Fig. 15). Using the respective correlation equation allowed us to convert mIU/ml values into ng/ml and to directly compare Beckman UNICEL PAPP-A mass units of ng/ml to the mIU/mL mass units generated by Siemens Immulite and DSL (Fig. 12A). However, for grading purposes, each lab's results were compared to their own peer group without conversion.

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11, and 12 for the five FT samples. As shown in Fig 11A, FT hCG measurements by Beckman Access/2 were ~10-15% higher than those by Beckman UNICEL, while the Siemens Immulite DPC instruments measured approximately 20% below the Beckman Access 2/UNICEL instruments. Overall, the hCG MoM values reflected the mass values but the differences were somewhat diminished (Fig. 11B). The results from the three PAPP-A kits, even when converted to the same mass units, were not consistent among one other (Fig. 12A). The Beckman UNICEL PAPP-A was about one third that of Siemens Immulite 2000, while Anshlite was 25% lower than Beckman UNICEL. In comparison, when the PAPP-A kit MOMs were compared, Siemens Immulite 2000 were about double those from Anshlite and Beckman (Fig. 12B).

E) <u>First Trimester Screening Software Utilized</u>:

The alpha and Benetech software packages were each used by 31% and 19% of the labs, respectively; Robert Maciel (RMA) software was employed by 31%; and in-house software comprised 19%. None of the labs used programs classified as "other" which are proprietary software packages.

G.J. Mizejewski, Ph.D.

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Teachings on Alpha-fetoprotein

Vol. 6, Part 1

By: G.J. Mizejewski, Ph.D.

Title: Alpha-fetoprotein – Derived Peptides as Epitopes for Hepatoma Immunotherapy: A Commentary.

Abstract

The various immunological roles of human alpha-fetoprotein (HAFP), and its correlation with hepatomas, i.e., hepatocellular carcinomas (HCCs), are not often addressed together in biomedical reports considering that HAFP is an established biomarker for hepatomas. Studies reporting measurement of HAFP serum levels in hepatoma patients in basic/clinical research settings has greatly increased over the years. Recent reports have now expanded our base knowledge in the mounting of an immune response against AFP, a self-antigen, during hepatoma tumorigenesis. Advances in the detection and identification of AFP-derived peptide epitopes are opening new vistas of knowledge regarding the immunological role of AFP-peptides as T-cell stimulating antigens in the course of hepatoma growth and progression. The present commentary addresses HAFP-derived peptides as immunologic responders in HCC and their use in the study and generation of AFP-peptide sensitized T-cells directed against hepatoma cells. Attempts were further made to relate the AFP-derived peptide epitopes to T-cell activities during the course of hepatoma immunotherapies and to profile the traits and properties of the peptides themselves. Hence, the present commentary was divided into two sections; 1) the characterization, properties, and traits of AFP peptide epitopes, and 2) the use of AFP-derived peptides in the therapeutic induction of T-cells primed against hepatoma cells using both in vivo and in vitro models.

I. A. Introduction:

a. Human Alpha-fetoprotein (HAFP) is a tumor-associated fetal glycoprotein that functions in the regulation of both ontogenic and oncogenic growth [1, 28]. HAFP is classified as a three-domain cysteine-rich translated protein of the albuminoid gene family consisting of four members: albumin, vitamin-D binding protein, AFP, and alpha-albumin [29, 32]. HAFP, a 69-kDa single-chain polypeptide, is synthesized in the yolk sac, fetal liver, and gastrointestinal tract during pregnancy and in hepatoblastomas, mesenchymal hamartomas, and hemangiomas during infancy; it is reexpressed in multiple adult tumors of endodermal and mixed

mesodermal/endodermal origin including hepatoma and germ cell tumors.[20, 30]. Thus, HAFP has long been employed in the clinical laboratory as a serum biomarker for hepatomas and liver disorders [38] (Table-1). At present, a major biological role of HAFP during pregnancy, infancy, and adult stages has not yet been established; however, studies in AFP gene knockout mice have demonstrated that rodent AFP serves to determine fertility in female offspring [12, 14].

A vast biomedical literature has been amassed concerning the clinical use of HAFP as a biomarker in adults for hepatomas i.e., hepatocellular carcinomas (HCC). These studies have largely addressed the monitoring of AFP levels in the sera of adult patients which display HCCs and other hepatic disorders. The first liver tumors to be associated with abnormal AFP serum levels were hepatoblastomas and hepatomas [20, 38]. Later, other types of liver disorders found to exhibit elevated AFP levels included cirrhosis and various forms of hepatitis (Table-1). As in the hepatic distress disorders, serum AFP is also elevated in immune deficiency and neurological wasting diseases which include ataxia telangectasias, oculomator appraxias, and related disorders. [31, 34]. However, there exists a paucity of reports that have attempted to correlate the serum AFP elevations with the recent discoveries of AFP peptides as T-cell sensitizing antigens.

b. Objectives: The present commentary has attempted to link the immunobiological activities of AFP-peptides to growth and progression of hepatomas in the adult patient. The physiological roles attributed to AFP-derived peptides in the induction of the immune response during growth and progression of hepatomas have not kept pace with the increase of investigational reports regarding AFP peptides as epitopic antigens. Thus, the objectives of the present treatise are two-fold. First, this commentary will address the recent reports of AFP peptides in conjunction with their therapeutic utility as an MHC-bound class I peptide antigen directed against hepatomas. Secondly, the use of AFP-derived peptide antigens as inducers of T-cell cytokine responses in hepatomas will be denoted. For detailed accounts of the physiology, biochemistry and genetics of AFP, the reader is directed to earlier reviews on these topics [11, 17, 21, 22]. For a recent update of the use of recombinant AFP in the treatment of autoimmune diseases, a report by Dudich [13] should be of interest.

Thus, the present commentary was divided into two sections. These sections will include: (I) the properties and characterization of AFP-derived peptide epitopes; and (II) the use of AFP MHC bound peptides as therapeutic agents directed against hepatomas.

Table 1: Alpha-fetoprotein (AFP) as a Biomarker for both hepatic disorders and malignant tumors. The AFP was measured in serum and reported as nanogram (ng)/mL concentrations.

I. Hepatic Disorders:

Liver Condition	Number of Patients	Range of AFP Concentrations (ng/ml)	Median AFP Concentration (ng/ml)
A. Subacute Hepatic Necrosis	75	30 to 3,300	900
B. Virus B Hepatitis	101	40 to 405	350
C. Chronic Active Hepatitis	35	10 to 400	225
D. Generalized Liver Distress	37	10 to 350	82
E. Alcoholic Hepatitis Without Cirrhosis	40	30 to 440	75
F. Extra hepatic Biliary Obstruction	26	30 to 500	65
G. Choledocholithiasis	22	40 to 230	61
H. Primary Biliary Cirrhosis	35	10 to 250	50
I. Hepatocellular Carcinoma	35	2,000 to 11,000	5,000

* Median serum values.
Data were extracted and compiled from the following references #30 and #38.

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ABSTRACTS

A) Screening Abstract "Picks-of-the-Month":

(1) Source: Taiwan J Obstet Gynecol 52:125-128, 2013.

Title: Prenatal diagnosis and molecular cytogenetic characterization of a de novo interstitial duplication of 14q (14q31.3-->q32.12) associated with abnormal maternal serum biochemistry.

Authors: Chen CP, Hwang KS, Su HY, Lin SP, Su YN, Chern SR, Su JW, Chen YT, Chen WL, Wang W.

Abstract: OBJECTIVE: To present prenatal diagnosis and molecular cytogenetic characterization of a de novo interstitial duplication of 14q (14q31.3-->q32.12) in a pregnancy associated with abnormal maternal serum biochemistry. CASE REPORT: A 19-year-old woman underwent amniocentesis in the second trimester because of abnormal maternal serum biochemistry. Her husband was 33 years old. At 16 weeks of gestation, the levels of alpha-fetoprotein, unconjugated estriol, total beta-human chorionic gonadotropin, and inhibin A were 0.8 multiples of median (MoM), 0.84 MoM, 3.06 MoM, and 1.14 MoM, respectively, consistent with a positive trisomy 21 risk of 1/269. Results of an amniocentesis revealed a small de novo interstitial duplication of 14q encompassing 14q31-q32.1. An array comparative genomic hybridization analysis detected a 6.6-Mb duplication at chromosome 14q31.3-q32.12. Results of a fluorescence in situ hybridization analysis showed a direct duplication of interstitial 14q. The karyotype was 46,XY,dup(14) (q31.3q32.12). Level II ultrasound was unremarkable. The parents decided to continue the pregnancy. A 3805-g healthy male baby was delivered at 39 weeks of gestation. When examined at 6 months of age, the neonate was normal in growth and psychomotor development with no apparent phenotypic abnormalities, although long-term follow-ups are required. CONCLUSION: Abnormal maternal serum biochemistry in the second trimester may be a distinctive prenatal feature in pregnancy associated with fetal chromosome 14q duplication.

(2) Source: Am J Obstet Gynecol, 2013.

Title: A proposed method to predict preterm birth using clinical data, standard maternal serum screening, and cholesterol.

Authors: Alleman BW, Smith AR, Byers HM, Bedell B, Ryckman KK, Murray JC, Borowski KS.

Abstract: OBJECTIVE: The objective of the study was to create a predictive model for preterm birth (PTB) from available clinical data and serum analytes. STUDY DESIGN: Serum analytes and routine pregnancy screening plus cholesterol and corresponding health information were linked to birth certificate data for a cohort of 2699 Iowa women with serum sampled in the first and second trimester. Stepwise logistic regression was used to select the best predictive model for PTB. RESULTS: Serum screening markers remained significant predictors of PTB, even after controlling for maternal characteristics. The best predictive model included maternal characteristics, first-trimester total cholesterol (TC), TC change between trimesters, and second-trimester alpha-fetoprotein and inhibin A. The model showed better discriminatory ability than PTB history alone and performed similarly in subgroups of women without past PTB. CONCLUSION: Using clinical and serum screening data, a potentially useful predictor of PTB was constructed. Validation and replication in other populations, and incorporation of other measures that identify PTB risk, like cervical length, can be a step toward identifying additional women who may benefit from new or currently available interventions.

(3) Source: Conn Med 77:43-45, 2013.

Title: Hereditary persistence of alpha-fetoprotein: a rare cause for unexplained alpha-fetoprotein elevations in pregnancy.

Authors: Rood K, Stiller R.

Abstract: Markedly elevated maternal serum alpha-fetoprotein (MSAFP) levels were found in a 26 year old healthy, nulliparous Polish woman during pregnancy. No fetal abnormalities were identified on targeted ultrasound and amniocentesis revealed normal amniotic fluid alpha-fetoprotein (AFP) values. Maternal ultrasound screening for liver and ovarian germ cell malignancies were also negative. The mother delivered a live, healthy female at term and a repeat maternal serum AFP postpartum remained markedly elevated, suggestive of hereditary persistence of alpha-fetoprotein.

(4) Source: Am J Obstet Gynecol, 2013.

Title: Down syndrome maternal serum marker screening after 18 weeks of gestation: a countrywide study.

Authors: Dreux S, Nguyen C, Czerkiewicz I, Schmitz T, Azria E, Foure MA, Muller F.

Abstract: OBJECTIVE: The objective of the study was to evaluate the efficacy of maternal serum markers in detecting Down syndrome after 18 weeks of gestation in women who book late for maternity care in a large national retrospective study. STUDY DESIGN: During the period 2007-2012, 27,648 women, regardless of maternal age (17.4% were 35 years old and over), were included in a late Down syndrome screening program (18(+0) to 35(+6) weeks) using the maternal serum markers alpha-fetoprotein and human chorionic gonadotrophin-beta. Samples were assayed in a single laboratory. A dataset of median markers previously established in our laboratory was used for risk calculation. The control group consisted of 27,648 women (14(+0) to 17(+6) weeks) randomly selected from the routine database. RESULTS: When the later screening group was compared with the standard second-trimester control group, the median multiples of medians (1.01 vs 0.98 for alpha-fetoprotein, 1.03 vs 0.98 for human chorionic gonadotrophin-beta), median risks (1 of 2414 vs 1 of 2720), false-positive rates (11.1% vs 11.6%), and trisomy 21 detection rates (83.3% vs 85.7%) did not differ significantly. CONCLUSION: Late Down syndrome maternal serum screening is feasible with a good sensitivity/specificity compromise throughout gestation and is of clinical value in late-booking women.

B) Case History Screening "Picks-of-the-Month":

(1) Source: Tumori 98:179e-182e, 2012.

Title: Familial synchronous bilateral teratoid Wilms tumor with elevated alpha-fetoprotein level.

Authors: Okur A, Pinarli FG, Karadeniz C, Poyraz A, Fidan K, Basaklar C, Oguz A.

Abstract: Familial Wilms tumor is a rare entity that accounts for only 1-2% of all Wilms tumor cases, with an earlier age of onset and an increased frequency of bilateral tumors. Teratoid Wilms tumor is a variant of nephroblastoma with a predominance of heterologous tissues comprising more than 50% of the tumor volume. Wilms tumor does not usually secrete any specific tumor marker and all teratoid Wilms tumor cases previously reported were sporadic non-secreting neoplasms. Here we describe an infant with familial synchronous bilateral teratoid Wilms tumor whose serum alpha-fetoprotein level was elevated. To our knowledge, this extremely rare type of case is reported for the first time in the literature.

(2) Source: Ann Clin Biochem 50:162-165, 2013.

Title: Extensive bowel resection in infancy is another cause of increasing plasma alphafetoprotein concentration.

Authors: Wojtulewicz J, Coakley J.

Abstract: BACKGROUND: Plasma alphafetoprotein (AFP) concentrations are high at birth and decline progressively to reach adult levels between eight and 24 months of age. AFP is an important tumour marker for hepatoblastoma but may also increase following hepatic resection. The liver and intestine have similar embryonic origins from endoderm, so we postulated that plasma AFP may also rise after extensive bowel resection. METHODS: AFP was measured postoperatively in plasma in four infants who had undergone resections of large amounts of intestine. AFP was measured twice in two babies and multiple times in the other two. RESULTS: In three of the four infants, the AFP concentrations were markedly above the expected levels for age, and in all four babies, AFP concentrations rose when they may have been expected to fall. CONCLUSION: Rising plasma AFP concentrations post extensive bowel resection in infants is a new finding which is possibly due to intestinal regeneration.

(3) Source: J Neurol Surg A Cent Eur Neurosurg, 2013.

Title: Endoscopic Approach for the Treatment of Pineal Region Tumors.

Authors: Herrada-Pineda T, Revilla-Pacheco F, Manrique-Guzman S.

Abstract: Tumors of the pineal region account for 3 to 8% of the tumors involving the central nervous system. The most common are germ cell tumors (39%). Less common examples include teratomas, primitive neuroectodermic tumors, astrocytomas, and choriocarcinomas. Clinical presentation in pediatric patients is in direct relation to the anatomical structures surrounding the pineal region and to the associated hydrocephalus that is present in almost 90% of cases. The diagnosis of a tumor in the pineal region is based on radiological findings and the presence of tumor markers such as alpha-fetoprotein and human chorionic gonadotrophic hormone subfraction beta. Neuroendoscopy is considered to be one of the minimally invasive techniques useful for the management of such patients. This minimally invasive approach to pineal region tumors allows the treatment of hydrocephalus in a safe and effective way, avoiding the complications derived from other procedures such as external ventricular drainage or shunt surgery.

(4) Source: J Prenat Med 6:7-9, 2012.

Title: Elevated maternal serum alpha-fetoprotein level in a fetus with Beckwith-Wiedemann syndrome in the second trimester of pregnancy.

Authors: Guanciali-Franchi P, Di Luzio L, Iezzi I, Celentano C, Matarrelli B, Liberati M, Palka G.

Abstract: BACKGROUND: Beckwith-Wiedemann syndrome (BWS) is a rare disorder characterized by macrosomia, macroglossia, visceromegaly, and omphalocele and an increased risk of growing tumors. Prenatal and postnatal high levels of serum alpha-fetoprotein are associated with several diseases and neoplasms including hepatoblastomas and other hepatic tumors. The diagnosis of BWS is usually made in the postnatal period on the basis of physical exam features and hypermethylation of the H19 gene. CASE: A 30-year-old woman gravida 3, para 2, underwent maternal serum screening at 15 weeks' gestation. The screening was negative for Down's syn drome (risk 1/6085), but positive for NTDs. Further ultrasound examination at 20 and 30 weeks'

evidenced a fetal overgrowth and a 3-D scan at 33 weeks' gestation presented a protruding tongue, and a fixed opened mouth caused by macroglossia. CONCLUSIONS: BWS was suspected on the basis of clinical features, and molecular analysis of critical region 11p15.5 revealing the hypermethylation of H19 gene supported the diagnosis.

C) News of Note: Abstracts of New Markers:

(1) Source: J Obstet Gynaecol 33:269-271, 2013.

Title: Can quadruple test parameters predict SGA infants?

Authors: Guducu N, Gonenc G, Isci H, Yigiter AB, Dunder I.

Abstract: The aim of this study was to investigate the role of quadruple test parameters in predicting the risk of delivering a small for gestational age (SGA) infant. The quadruple test results of patients performed at 16-18 weeks of pregnancy were searched retrospectively. Alpha-fetoprotein (AFP) levels higher than 2 MoM were associated with a risk of delivering an SGA infant ($p = 0.025$), sensitivity 8%, specificity 98%, positive predictive value 25%, negative predictive value 91%. Other quadruple test parameters were not related to delivery of an SGA infant. The introduction of AFP as a screening test for the detection of SGA infants does not seem feasible, but we suggest clinicians take it into account when counselling the patients about the results of Down syndrome screening tests.

(2) Source: Hong Kong Med J 19:101-108, 2013.

Title: Prospective assessment of the Hong Kong Hospital Authority universal Down syndrome screening programme.

Authors: Sahota DS, Leung WC, Chan WP, To WW, Lau ET, Leung TY.

Abstract: OBJECTIVE. To evaluate the performance of the locally developed universal Down syndrome screening programme. DESIGN. Population-based cohort study in the period July 2010 to June 2011 inclusive. SETTING. Four Hong Kong Hospital Authority Departments of Obstetrics and Gynaecology and a central university-based laboratory for maternal serum processing and risk determination. PARTICIPANTS. Women were offered either a first-trimester combined test (nuchal translucency, free beta human chorionic gonadotropin, and pregnancy-associated plasma protein-A) or nuchal-translucency-only test, or a second-trimester double test (alpha-fetoprotein and total human chorionic gonadotropin) for detection of Down syndrome according to their gestational age. Those with a trisomy 21 term risk of 1:250 or higher were offered a diagnostic test. RESULTS. A total of 16 205 pregnancies were screened of which 13 331 (82.3%) had a first-trimester combined test, 125 (0.8%) had a nuchal-translucency test only, and 2749 (17.0%) had a second-trimester double test. There were 38 pregnancies affected by Down syndrome. The first-trimester screening tests had a 91.2% (31/34) detection rate with a screen-positive rate of 5.1% (690/13 456). The second-trimester test had a 100% (4/4) detection rate with a screen-positive rate of 6.3% (172/2749). There were seven (0.9%) pregnancies that miscarried following an invasive diagnostic test. There were two Down syndrome-affected live births, both with an estimated first-trimester trisomy 21 term risk lower than 1:250. CONCLUSION. The universal screening programme offered at the four units was effective and achieved the expected detection rates and low false-positive rates, and to maintain these, the current emphasis on training, quality control, and regular auditing must continue.

(3) Source: Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29:693-696, 2012.

Title: [Establishment of median values for second trimester maternal serum biomarkers in Weihai region].

Authors: Lan XQ, Wang SH, Deng YZ.

Abstract: OBJECTIVE: To establish the median values for second trimester biomarkers in Weihai region, and to assess its value for improving the performance and efficiency of prenatal screening. METHODS: Maternal serum alpha-fetoprotein (AFP) and free beta human chorionic gonadotropin (Free beta-hCG) were determined for 24 400 pregnant women at 105 to 146 gestational days. A regression equation was derived after adjusting for different gestational ages. The median values were further adjusted with body weight. RESULTS: The median values of AFP and Free beta-hCG were respectively 6% and 24% higher than those embedded in a 2T software. After adjusting with gestational age and weight, there is a significant difference in multiple of the median (MoM) of serum biomarkers between local population and that embedded in the 2T model. CONCLUSION: To establish the median values for different gestational ages for local region may help to improve the efficiency of prenatal screening.

(4) Source: J Med Screen 19:164-170, 2012.

Title: Feasibility of using plasma rather than serum in first and second trimester multiple marker Down's syndrome screening.

Authors: Lambert-Messerlian GM, Palomaki GE, Eklund EE, Kloza EM, Neveux LM, Phipps MG, Canick JA.

Abstract: OBJECTIVE: To compare maternal plasma with serum for measuring markers currently used in first and second trimester screening for Down's syndrome. SETTING: A laboratory-based investigation of two sample types in assays used in prenatal screening for Down's syndrome. METHODS: A paired data-set included both plasma and serum from 101 pregnant women. A nested case/control data-set included only plasma samples from 34 first and 23 second trimester Down's syndrome pregnancies, each matched with six euploid controls. Analyte levels were measured and converted to multiples of the median (MoM). RESULTS: In the paired data-set, each of the five analytes (alphafetoprotein, unconjugated estriol, human chorionic gonadotropin, inhibin-A and pregnancy-associated plasma protein A) in serum and plasma was highly correlated ($r > 0.970$) and after conversion to MoM, the resulting distributions were equivalent ($P > 0.7$). In the matched data-set, plasma-based median MoM levels in cases were consistent with the published serum counterparts for all markers. CONCLUSIONS: This study provides strong evidence that current serum-based prenatal screening can be performed equally well using plasma samples. This may prove useful, especially if secondary screening using a DNA-based test requires maternal plasma.

D) News of Note: Abstracts of New Testing Agents/Methods:

(1) Source: Am J Obstet Gynecol, 2013.

Title: Association of early-preterm birth with abnormal levels of routinely collected first- and second-trimester biomarkers.

Authors: Jelliffe-Pawlowski LL, Shaw GM, Currier RJ, Stevenson DK, Baer RJ, O'Brodovich HM, Gould JB.

Abstract: OBJECTIVE: The purpose of this study was to examine the relationship between typically measured prenatal screening biomarkers and early-preterm birth in euploid pregnancies. STUDY DESIGN: The study included 345 early-preterm cases (<30 weeks of gestation) and 1725 control subjects who were drawn from a population-based sample of California pregnancies who had both first- and second-trimester screening results. Logistic regression analyses were used to compare patterns of biomarkers in cases and control subjects and to develop predictive models. Replicability of the biomarker early-preterm relationships that was revealed by the models was evaluated by examination of the frequency and associated adjusted relative risks (RRs) for early-preterm birth and for preterm birth in general (<37 weeks of gestation) in pregnancies with identified abnormal markers compared with pregnancies without these markers in a subsequent independent California cohort of screened pregnancies (n = 76,588). RESULTS: The final model for early-preterm birth included first-trimester pregnancy-associated plasma protein A in the </=5th percentile, second-trimester alpha-fetoprotein in the >/=95th percentile, and second-trimester inhibin in the >/=95th percentile (odds ratios, 2.3-3.6). In general, pregnancies in the subsequent cohort with a biomarker pattern that were found to be associated with early-preterm delivery in the first sample were at an increased risk for early-preterm birth and preterm birth in general (<37 weeks of gestation; adjusted RR, 1.6-27.4). Pregnancies with >/=2 biomarker abnormalities were at particularly increased risk (adjusted RR, 3.6-27.4). CONCLUSION: When considered across cohorts and in combination, abnormalities in routinely collected biomarkers reveal predictable risks for early-preterm birth.

(2) Source: Ann Clin Biochem 48:438-440, 2011.

Title: Interference in the autoDELFIA(R) hAFP immunoassay and effect on second-trimester Down's syndrome screening.

Authors: Mannings L, Trow S, Newman J, Nix B, Evans C.

Abstract: BACKGROUND: Falsely decreased serum alphafetoprotein (AFP) concentrations are reported in the autoDELFIA((R)) hAFP immunoassay due to interference by complement. AFP is measured, using this assay, as part of second-trimester and integrated Down's syndrome screening tests. Decreased AFP concentrations increase the calculated risk of Down's syndrome; therefore falsely low AFP, due to assay interference, may artificially increase a patient's risk, and have the potential to cause false screen positive results. It was our aim to assess whether negative interference in the autoDELFIA((R)) hAFP assay was a cause of very low AFP concentrations, and to examine the effect of falsely decreased concentrations on the calculated risk of Down's syndrome. METHODS: Three hundred and twenty-three sequential Down's screening serum samples with very low serum AFP concentration (<15 KU/L) using the autoDELFIA((R)) hAFP immunoassay were selected and AFP re-measured using the E170 AFP immunoassay. RESULTS: Interference was detected in nine samples (from eight patients) on the basis of discordant AFP concentrations. The interference decreased following storage of samples at 4 degrees C to deplete complement. Use of the falsely low AFP concentrations to calculate risk of Down's syndrome resulted in significantly increased calculated risk compared with complement depleted results. CONCLUSIONS: Laboratories should be aware that falsely low AFP concentrations due to complement interference may be obtained using the autoDELFIA((R)) hAFP immunoassay. We have shown that falsely low AFP concentrations increase the calculated risk of Down's syndrome. This is a potential cause of false Down's syndrome screen positive results.

(3) Source: Biosens Bioelectron 44:101-107, 2013.

Title: Multiplex electrochemiluminescence immunoassay of two tumor markers using multicolor quantum dots as labels and graphene asconductingbridge.

Authors: Guo Z, Hao T, Du S, Chen B, Wang Z, Li X, Wang S.

Abstract: A multiplex electrochemiluminescence (ECL) immunoassay for simultaneous determination of two different tumor markers, alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA), using multicolor quantum dots as labels and graphene as conducting bridge was developed. Herein, a typical sandwich immune complex was constructed on the glass carbon electrode, with QDs and QDs labeled on secondary anti-AFP and anti-CEA antibodies, respectively, thus to obtain distinguishable ECL signals. Because most of those QDs labeled on secondary antibodies were beyond the space domain of the ECL reaction, graphene was used as a conducting bridge to promote the electron transfer between QDs and the electrode, leading to about 30-fold enhancement of the ECL intensity. Experimental results revealed that the multiplex electrochemiluminescence immunoassay enabled the simultaneous monitoring of AFP and CEA in a single run with a working range of 0.001-0.1pg/mL. The detection limit (LOD) for both analytes at 0.4fg/mL was very low. No obvious cross-reactivity was found. Precision, recovery and stability were satisfactory. This novel multiplex ECL immunoassay provided a simple, sensitive, specific and reliable alternative for the simultaneous detection of tumor markers in clinical laboratory.

(4) Source: World J Surg, 2013.

Title: Total Tumor Volume Is a Better Marker of Tumor Burden in Hepatocellular Carcinoma Defined by the Milan Criteria.

Authors: Lee YH, Hsia CY, Hsu CY, Huang YH, Lin HC, Huo TI.

Abstract: BACKGROUND: The Milan criteria are used to define small hepatocellular carcinoma (HCC) and to select patients for curative treatments. Total tumor volume (TTV) is an alternative parameter for tumor burden. We aimed to evaluate whether TTV is a feasible prognostic marker in HCC patients with upper boundary TTV of 65.5 cm3, which is equivalent to a single 5 cm tumor nodule defined by the Milan criteria. METHODS: A total of 774 HCC patients with TTV <65.5 cm3 receiving surgical resection, liver transplantation, or radiofrequency ablation (RFA) as the primary treatment were retrospectively analyzed. RESULTS: Of these patients, 50 (6.5 %) did not fulfill the Milan criteria. Patients beyond the Milan criteria more often had larger tumor size and TTV, as well as more tumor nodules (p values all <0.01). There was no significant survival difference between patients within and beyond the Milan criteria (p = 0.205). Patients with TTV >15 cm3 had a significantly poorer survival than patients with TTV <15 cm3 (p = 0.007). There was no survival difference between patients receiving surgical treatments versus RFA (p = 0.932). In the Cox proportional hazards model, TTV >15 cm3 [risk ratio (RR): 1.474, p = 0.005], serum bilirubin >/=1.5 mg/dL (RR: 1.663, p = 0.003), serum sodium <135 mmol/L (RR: 2.016, p = 0.01), and alpha-fetoprotein (AFP) >/=100 ng/mL (RR: 1.37, p = 0.033) were independent predictors of poor prognosis. CONCLUSIONS: Total tumor volume, is an independent and better prognostic marker than the Milan criteria to indicate tumor burden in HCC patients who had tumor volume defined by the Milan criteria and underwent curative therapies.

E) Abstracts of New Assay Methodologies:

(1) Source: Biosens Bioelectron 45:195-200, 2013.

Title: Signal amplification for electrochemical immunosensing by in situ assembly of host-guest linked gold nanorod superstructure on immunocomplex.

Authors: Lin D, Wu J, Ju H, Yan F.

Abstract: An amplification strategy for signal tracing was developed by introducing a host-guest binding reaction into the assembly process of gold nanorods (AuNRs) superstructure. The amplification pathway firstly used a thio-beta-cyclodextrin (SH-beta-CD) functionalized gold nanoparticles to label signal antibody, and then in situ assembled multi-layer SH-beta-CD end-functionalized AuNRs to sandwich immunocomplex on immunosensor surface by using 4,4,4,4-(21H, 23H-porphine-5,10,15,20-tetrayl) tetrakis (benzoic acid) as a bridge to achieve simple and convenient host-guest reaction. The built end-to-end AuNRs superstructure showed excellent performance for the signal amplification in connection with the electrochemical biosensing by preoxidation and then voltammetric analysis of gold element. Using alpha-fetoprotein as an analyte, the immunosensor was constructed by covalently binding capture antibody to chitosan-carbon nanotubes-poly(diallyldimethylammonium chloride) modified electrode. The superstructure rich in AuNRs brought an enhanced detection sensitivity of protein, which could detect alpha-fetoprotein in a linear range from 0.5pg mL(-1) to 0.5ng mL(-1) with a detection limit down to 0.032pg mL(-1). The immunoassay exhibited good stability and acceptable reproducibility and accuracy. The in situ superstructure assembly could be extended to other labeled recognition systems, providing a promising novel avenue for signal amplification and potential applications in bioanalysis and clinical diagnostics.

(2) Source: Lab Chip 13:1797-1802, 2013.

Title: A portable flow-through fluorescent immunoassay lab-on-a-chip device using ZnO nanorod-decorated glass capillaries.

Authors: Hu W, Lu Z, Liu Y, Chen T, Zhou X, Li CM.

Abstract: We report a portable flow-through fluorescent immunoassay lab-on-a-chip device using inexpensive disposable glass capillaries for medical diagnostics. The device is made up of a number of serially connected glass capillaries, of which each interior surface is grown using zinc oxide (ZnO) nanorods, which different probe antibodies are attached to. The ZnO nanorods not only provide a large surface area for high density probe attachment, but also enhance the fluorescent signals to significantly improve the detection signal responses. The glass capillary also allows for convenient flow-through detection. Coupled with a homemade handheld analyzer integrated with an automatic pump system and a fluorescence readout module, a portable immunoassay capillary device enables quantitative detection of multiple biomarkers in 30 min with detection limits of 1-5 ng mL(-1) and wide dynamic ranges for prostate specific antigen (PSA), alpha-Fetoprotein (AFP) and carcinoembryonic antigen (CEA) in serum. This new conceptual lab-on-a-chip device eliminates the need for expensive micro-fabrication processes, while offering inexpensive and disposable, but replaceable tube-type "microchannels" for multiplexed detection in portable clinical diagnostics.

(3) Source: Biosens Bioelectron 46C:155-161, 2013.

Title: Detection of cancer biomarkers by piezoelectric biosensor using PZT ceramic resonator as the transducer.

Authors: Su L, Zou L, Fong CC, Wong WL, Wei F, Wong KY, Wu RS, Yang M.

Abstract: A novel piezoelectric biosensor using lead titanate zirconate (PZT) ceramic resonator as transducer was developed for label-free, cost-effective, and direct detection of cancer biomarkers. We designed a dual sensing scheme where two ceramic resonators were connected in parallel, in which one resonator was used as the sensing unit and the other as the control unit, in order to minimize environment influences including temperature fluctuation and to achieve the required frequency stability for biosensing applications. Detection of selected cancer biomarkers, such as

prostate specific antigen (PSA) and alpha-fetoprotein (AFP) was carried out to evaluate the performance of the biosensor. The device showed high sensitivity (0.25ng/ml) and fast detection (within 30min) with small amount of sample (1mul), which is compatible to that required by clinical measurements. The results also showed that the ceramic resonator-based piezoelectric biosensor platform could be utilized with different chemical interfaces, and the miniaturized size of the ceramic resonators makes it suitable for fabricating sensor arrays for multiplex detection.

(4) Source: Anticancer Res 33:997-1001, 2013.

Title: Fucosylated Fraction of Alpha-fetoprotein as a Serological Marker of Early Hepatocellular Carcinoma.

Authors: Moriya S, Morimoto M, Numata K, Nozaki A, Shimoyama Y, Kondo M, Nakano M, Maeda S, Tanaka K.

Abstract: Aim: This study aimed to evaluate the fucosylated fraction of alpha-fetoprotein (AFP-L3) as a marker of early hepatocellular carcinoma (HCC). PATIENTS AND METHODS: We diagnosed early HCC in 15 patients (15 tumors) by gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid-enhanced magnetic resonance imaging and confirmed the diagnoses using new criteria of the International Consensus Group for Hepatocellular Neoplasia. We measured the AFP-L3%, simultaneously, using a liquid-phase binding assay-electrokinetic analyte transport assay. We compared the AFP-L3% levels between patients with early HCC and a control cohort with benign liver disease. RESULTS: The AFP-L3% levels were higher in patients with early HCC than in the controls (4.1%+/-4.0% vs. 2.0%+/-3.5%, p=0.024). The sensitivity and specificity with AFP-L3% were 33.3% and 78.7% at a cut-off value of 5%, and 20.0% and 88.0% at a cut-off value of 7%, respectively. CONCLUSION: AFP-L3% is a suitable serological marker for evaluating early HCC.

F) Special Abstract Selection:

(1) Source: Tumour Biol, 2013.

Title: Review of the adenocarcinoma cell surface receptor for human alpha-fetoprotein; proposed identification of a widespread mucin as the tumor cell receptor.

Authors: Mizejewski GJ.

Abstract: The identification of a tumor cell receptor for alpha-fetoprotein (AFP) has long been sought in the field of medicine. The uptake and endocytosis of AFP by rat tumor cells in 1983 sparked a series of confirmatory reports which were extended to include multiple tumor types in rats, mice, and humans. The following year, French investigators characterized the binding properties of the AFP receptor but they did not purify and characterize the receptor. It was not until 1991-1992 that an AFP receptor was partially purified and characterized from both human monocytes and breast cancer cells. By 1993, monoclonal antibodies had been raised against the "AFP receptor" derived from breast cancer extracts with claims that the receptor was a widespread oncoprotein biomarker for cancer. To date, that receptor has yet to be identified due to its complex multimeric structure and carbohydrate composition. The present report will review the literature of

the multiple AFP receptors previously including their cellular uptake, transmembrane passage, and partial biochemical characterization. . In addition, evidence derived from computer modeling, proteolytic/fragmentation cleavage patterns, domain structure analysis, and protein binding software analysis will be presented in a proposed identification of a widespread protein/gene family of transmembrane proteins which fits many, if not most, of the criteria attributed to the AFP receptor. The proposed receptor protein family is tentatively identified as an epithelial cell surface mucin constituting one (or more) of many classes of single-pass transmembrane proteins. Present data do not support the concept that the AFP receptor is a "universal" tumor receptor and/or biomarker, but rather a widespread mucin protein that functions primarily in protecting and lubricating epithelial mucosal layers, and engaging in signal transduction; the mucin only binds AFP as a molecule serving in a subordinate or ancillary function.

(2) Source: Fetal Diagn Ther 29:135-138, 2011.

Title: First and second trimester markers of fetal aneuploidy in pregnant women with HIV infection.

Authors: Spencer K.

Abstract: OBJECTIVE: To evaluate if levels of pregnancy-associated plasma protein-A (PAPP-A), free beta-hCG and nuchal translucency (NT) used in first trimester aneuploidy screening and alpha-fetoprotein (AFP), unconjugated oestriol (UE3) and free beta-hCG in the second trimester are altered in pregnancies with human immunodeficiency virus (HIV) infection. METHODS: Median MoM values of biochemical markers and delta-NT in 92 women with HIV infection in the first trimester were compared with those from 912 HIV-seronegative controls. In the second trimester, biochemical marker levels were compared in 52 women with HIV infection and 378 HIV-seronegative controls. RESULTS: First trimester free beta-hCG median MoM levels in HIV-infected pregnancies were not different to controls (0.978 vs. 0.981, p = 1.000), as were PAPP-A levels (1.190 vs. 1.102, p = 0.099) and delta-NT (0.1374 vs. 0.0445, p = 0.0631). Second trimester levels of free beta-hCG were not significantly different (1.0575 vs. 0.9619, p = 0.1827), as was AFP (0.9734 vs. 0.9350, p = 0.6576), although UE3 was significantly lower (0.970 vs. 1.110, p = 0.0005). CONCLUSIONS: In the first trimester, marker levels are not affected by the presence of HIV infection, and risks for aneuploidy are likely to be accurate in this group. Further studies are required to evaluate if UE3 levels continue to be low in HIV-infected women since this may have an impact on screening in the second trimester.

(3) Source: PLoS One 7:e40199, 2012.

Title: Birth weight, breast cancer and the potential mediating hormonal environment.

Authors: Bukowski R, Chlebowski RT, Thune I, Furberg AS, Hankins GD, Malone FD, D'Alton ME.

Abstract: BACKGROUND: Previous studies have shown that woman's risk of breast cancer in later life is associated with her infants birth weights. The objective of this study was to determine if this association is independent of breast cancer risk factors, mother's own birth weight and to evaluate association between infants birth weight and hormonal environment during pregnancy. Independent association would have implications for understanding the mechanism, but also for prediction and prevention of breast cancer. METHODS AND FINDINGS: Risk of breast cancer in relation to a first infant's birth weight, mother's own birth weight and breast cancer risk factors were evaluated in a prospective cohort of 410 women in the Framingham Study. Serum concentrations of estriol (E3), anti-estrogen alpha-fetoprotein (AFP), and pregnancy-associated plasma protein-A (PAPP-A) were measured in 23,824 pregnant women from a separate prospective cohort, the FASTER trial. During follow-up (median, 14 years) 31 women (7.6%) were diagnosed with breast cancer. Women with large birth weight infants (in the top quintile) had a higher breast cancer risk compared to other women (hazard ratio (HR), 2.5; 95% confidence interval (CI), 1.2-5.2; P = 0.012). The finding was not affected by adjustment for birth weight of the mother and traditional breast cancer risk factors (adjusted HR, 2.5; 95% CI, 1.2-5.6; P = 0.021). An infant's birth weight had a strong positive relationship with the mother's serum E3/AFP ratio and PAPP-A concentration during pregnancy. Adjustment for breast cancer risk factors did not have a material effect on these relationships. CONCLUSIONS: Giving birth to an infant with high birth weight was associated with increased breast cancer risk in later life, independently of mother's own birth weight and breast cancer risk factors and was also associated with a hormonal environment during pregnancy favoring future breast cancer development and progression.

(4) Source: Taiwan J Obstet Gynecol 52:120-124, 2013.

Title: Prenatal diagnosis and molecular cytogenetic characterization of a de novo interstitial duplication of 11q (11q22.3-->q23.3) associated with abnormal maternal serum biochemistry.

Authors: Chen CP, Su YN, Lin SP, Chern SR, Su JW, Chen YT, Lee MS, Wang W.

Abstract: OBJECTIVE: To present prenatal diagnosis and molecular cytogenetic characterization of a de novo interstitial duplication of 11q (11q22.3-->q23.3) in a pregnancy associated with abnormal maternal serum biochemistry. CASE REPORT: A 33-year-old woman underwent amniocentesis in the second trimester because of abnormal maternal serum biochemistry. Her husband was 33 years old. At 17 weeks of gestation, the levels of alpha-fetoprotein (AFP), unconjugated estriol (uE3), total beta-human chorionic gonadotropin (beta-hCG), and inhibin A were 0.65 multiples of median (MoM), 0.61 MoM, 0.32 MoM, and 0.55 MoM, respectively, consistent with a positive trisomy 18 risk of 1/128. Results of amniocentesis revealed a small de novo interstitial duplication of 11q encompassing 11q23. An array comparative genomic hybridization analysis detected a 9.04-Mb duplication at chromosome 11q22.3-q23.3. A polymorphic DNA marker analysis was carried out, which determined a paternal origin of the duplication. Results of fluorescence in situ hybridization analysis showed a direct duplication of interstitial 11q. The karyotype was 46,XX,dup(11)(q22.3q23.3). Level II ultrasound was unremarkable. The parents opted to continue the pregnancy. A 2792-g female baby was delivered at 38

weeks of gestation. When examined at 10 months of age, the neonate was small for age and was abnormal in psychomotor development with apparent facial dysmorphisms, and small hands and feet. CONCLUSION: Low levels of AFP, uE3, beta-hCG, and inhibin A in the second trimester maternal serum biochemistry may be a distinctive prenatal feature in pregnancy with fetal chromosome 11q duplication.

(4) Source: Hepatol Int 7:59-64, 2013.

Title: Recently introduced biomarkers for screening of hepatocellular carcinoma: a systematic review and meta-analysis.

Authors: Witjes CD, van Aalten SM, Steyerberg EW, Borsboom GJ, de Man RA, Verhoef C, Ijzermans JN.

Abstract: Early detection of hepatocellular carcinoma (HCC) is essential for improved prognosis and long-term survival. To date, screening for HCC depends on serological testing (alpha-fetoprotein, AFP) and imaging (ultrasonography), both of which are not highly sensitive. A meta-analysis was performed to discuss recent developments in biomarkers that may be effective in screening for HCC. A systematic search of PubMed, Embase, and Web of Science was performed for articles published between January 2005 and October 2010, and focusing on biomarkers for HCC in urine, serum, or saliva. Data on sensitivity and specificity of tests were extracted from each included article and displayed with a summary ROC. A meta-analysis was carried out in which the area under the curve for each biomarker was used to compare the accuracy of different tests. In seven well-defined studies, three biomarkers were identified for potential use, namely, Golgi protein 73 (GP73), interleukin-6 (IL-6), and squamous cell carcinoma antigen (SCCA). Comparison with AFP showed that GP73 was superior (p = 0.006; 95 % CL -0.23, -0.12), IL-6 was similar (p = 0.66; 95 % CL -0.31, 0.25), and SCCA was inferior to AFP (p = 0.001; 95 % CL 0.12, 0.23). GP73 is a valuable serum marker that seems to be superior to AFP and can be useful in the diagnosis and screening of HCC. Although GP73 may improve the detection and treatment of one of the most common malignancies worldwide, additional research is required.

VI. Potentially helpful website connections/locations:

1) http://health.allrefer.com/health/alpha-fetoprotein-info.html

2) www.healthopedia.com/alpha-fetoprotein

3) http://pregnancy.about.com/cs/afp/a/afptesting.htm

4) http://www.webmd.com/baby/alpha-fetoprotein-afp-in-blood

5) http://pregnancy.about.com/od/afp/Alphafetoprotein_Testing.htm

6) http://www.americanpregnancy.org/prenataltesting/afpplus.html