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HEALTH

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Fetal Defect Marker Proficiency Test Mailout[1]
September 2013

Dear Laboratory Director,
Below you will find a summary and critique of the Proficiency Testing mail-out from September 10, 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 301**	**MS 302**	**MS 303**	**MS 304**	**MS 305**
	Gestational Age (weeks)	19.0	16.0	18.0	17.0	20.0
Maternal Race	Ethnic Group	Black	Asian	White	Hispanic	White
Maternal Weight	Pounds (lbs)	150	140	160	155	145
Maternal Age	Years	25	28	40	30	21
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	29.3 ± 1.50	36.2 ± 2.60	33.4 ± 1.70	41.0 ± 3.0	180.5 ± 13.10
	MOM ± Std. Dev.	0.52 ± 0.04	1.06 ± 0.08	0.79 ± 0.05	1.11 ± 0.10	3.03 ± 0.29
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.57 ± 0.06	0.64 ± 0.08	0.72 ± 0.07	0.80 ± 0.08	1.40 ± 0.11
	MOM ± Std. Dev.	0.40 ± 0.06	0.85 ± 0.18	0.63 ± 0.11	0.86 ± 0.18	0.78 ± 0.12
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	34.0 ± 3.5	26.1 ± 3.3	13.0 ± 1.1	23.5 ± 2.0	17.1 ± 1.2
	MOM ± Std. Dev.	1.80 ± 0.25	0.87 ± 0.08	0.67 ± 0.07	1.01 ± 0.10	0.99 ± 0.11
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	296.7 ± 17.2	138.4 ± 8.4	147.1 ± 10.0	151.9 ± 10.2	175.0 ± 13.1
	MOM ± Std. Dev.	1.71 ± 0.14	0.76 ± 0.07	0.89 ± 0.08	0.92 ± 0.07	0.89 ± 0.11
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 92% U = 88% A = 85%
	NTD Risk 1 in	10,400	6,150	10,000	6,890	38
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (96%)	(-) (100%)	(-) (100%)
	Recommended Action**	G = 85% U = 62% A = 85%	NFA	NFA	NFA	NFA
	Risk Est. 1 in	29	4,773	340	2,800	11,050
2. Quad Test	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	G = 92% U = 68% A = 88%	NFA	NFA	NFA	NFA
	Risk Est. 1 in	37	10,800	528	6,900	20,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (88%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	G = 100% U = 100% A = 100%	NFA	NFA
	Risk Est. 1 in	1,290	10,000	294	10,000	10,000

*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 301 Wk 19.0	This specimen was obtained from a 25 year old Black woman (Gravida = 2, Parity = 1) in her 19th week gestation with a body weight of 150 lbs. She had no family (sibling) history of pregnancy complications. Her specimen 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, 92%; ultrasound, 68%; amniocentesis, 88%; while the triple tests were: genetic counseling, 85%; ultrasound 62%; and amniocentesis, 85%. Specimen MS301 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 = 0.51).
MS 302 Wk 16.0	This specimen was obtained from a 28 year old Asian woman (Gravida = 3, Parity = 2) in her 16th 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 MS302 was not paired with an amniotic fluid specimen.
MS 303 Wk 18.0	This specimen was obtained from a 40 year old White woman (Gravida = 3, Parity = 1) in her 18th week of gestation with a body weight of 160 lbs. She had a family reproductive history of miscarriages. Her sample screened negative for NTD, and her aneuploidy screen was screen negative for Down syndrome. However, her Trisomy-18 risk displayed a borderline negative screen result (see critique). This sample was not paired to an amniotic fluid specimen.
MS 304 Wk 17.0	This specimen was obtained from a 30 year old Hispanic woman (Gravida = 2, Parity = 1) in her 17th week of gestation with a body weight of 155 lbs. She had no family history of reproductive complications. Her sample screened negative for NTD, and her aneuploidy screens were also negative for both Trisomy-18 and Trisomy-21. The MS304 sample was not paired to an amniotic fluid specimen.
MS 305 Wk 20.0	This specimen was obtained from a 21 year old White woman (Gravida = 3, parity = 2) in her 20th week gestation with a body weight of 145 lbs. She had a personal history of pregnancy loss. Her sample was a positive screen for NTD (100% consensus; MOM = 3.03). 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, 92%; ultrasound, 88%; and amniocentesis, 85%. The MS305 specimen had an amniotic fluid counterpart that was also elevated (MOM = 3.24).

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 301 Wk 19.0	AFP = 3.9 ± 0.5 µg/ml MOM = 0.51 ± 0.07	The AF301 sample was targeted as an NTD negative screen in the upper gestational age screening range. All labs categorized AF301 as a negative NTD screen specimen. This specimen had a maternal serum counterpart, MS301, which showed reduced levels of AFP (MOM = 0.52).
AF 302 Wk 19.0	AFP = 3.4 ± 2.4 µg/ml MOM = 0.44 ± 0.29	The AF302 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF302 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 303 Wk 18.0	AFP = 5.8 ± 0.6 µg/ml MOM = 0.62 ± 0.07	The AF303 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 AF303 specimen was not paired with a maternal serum sample.
AF 304 Wk 15.0	AFP = 4.8 ± 0.6 µg/ml MOM = 0.42 ± 0.05	The AF304 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 AF304 specimen was not paired with a maternal serum sample.
AF 305 Wk 20.0	AFP = 20.3 ± 2.6 µg/ml MOM = 3.24 ± 0.52	The AF305 sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs called AF305 a positive screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen MS305 whose AFP was also elevated (MOM = 3.03).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 17	**Sample #**	**FT 301**	**FT 302**	**FT 303**	**FT 304**	**FT 305**
	Gestational Age (weeks)	11.9	11.2	13.0	11.5	12.4
Maternal Race	Ethnic Group	Hispanic	White	Black	White	Asian
Maternal Weight	Pounds (lbs)	135	150	145	130	125
Maternal Age	Years	27	25	30	29	24
Fetal Physical Measurements	Crown Rump Length (mm)	53	45	67	48	59
	NT Thickness (mm)	1.20	2.20	1.60	1.10	1.40
	NT – MOM ± Std. Dev.	0.92 ± 0.05	1.93 ± 0.12	0.98 ± 0.06	0.91 ± 0.05	0.96 ± 0.06
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	79.6 ± 9.5	178.8 ± 30.9	68.2 ± 9.2	76.2 ± 12.0	73.3 ± 9.9
	MOM ± Std. Dev.	1.02 ± 0.09	2.19 ± 0.17	1.00 ± 0.10	0.90 ± 0.08	0.96 ± 0.08
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	1502.9 ± 887.8	549.0 ± 317.8	1996.1 ± 1190.1	1329.6 ± 778.7	1620.4 ± 968.6
	MOM ± Std. Dev.	3.45 ± 2.21	1.89 ± 1.27	2.68 ± 1.69	3.40 ± 2.09	2.90 ± 1.77
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(+) (56%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	G = 60% U = 33% A = 40% C = 33%	NFA	NFA	NFA
	Risk Estimate 1 in	19,500	230	13,000	20,000	20,000
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	10,000	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 301 Wk 11.9	This specimen was obtained from a 27 year old Hispanic woman with a body weight of 135 lbs. Her gestational age at the time of screening was 11.9 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 FT301 risk estimate for Trisomy-21 was 1 in 19,500 and the Trisomy-18 risk was 1 in 10,000.
FT 302 Wk 11.2	This specimen was obtained from a 25 year old White woman of average body weight (150 lbs.). Her gestational age at the time of screening was 11.2 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was borderline screen positive with 56% of labs calling this sample positive for Trisomy-21. The median FT302 risk estimate for Trisomy-21 was 1 in 230 (all lab median cutoff), and the Trisomy-18 risk was 1 in 10,000. (See critique)
FT 303 Wk 13.0	This specimen was procured from a 30 year old Black woman of average body weight (145 lbs.). Her gestational age at the time of screening was 13.0 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 FT303 risk estimate for Trisomy-21 was 1 in 13,000, and the Trisomy-18 risk was 1 in 10,000.
FT 304 Wk 11.5	This specimen was procured from a 29 year old White woman of average body weight (130 lbs.). Her gestational age at the time of screening was 11.5 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 FT304 risk estimate for Trisomy-21 was 1 in 20,000, while the Trisomy-18 risk was 1 in 10,000.
FT 305 Wk 12.4	This specimen came from a 24 year old Asian woman with a body weight of 125 lbs. Her gestational age at the time of screening was 12.4 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 FT305 was 1 in 20,000, 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 **MS305** was targeted as a screen positive specimen for NTD (Figs. 1 and 3) and was matched to the elevated **AF305** sample (Fig. 2). All labs (100%) agreed that specimen **MS305** was screen positive for NTD and negative for both Trisomy screens using the triple and/or quad test (Figs. 4-6). The **AF305** sample, matched to **MS305,** exhibited elevated AFP levels strongly suggesting the presence of an NTD. As a follow-up, a polyacrylamide gel electrophoresis is indicated and should be performed to show the presence of a diagnostic ACHE band, which would indicate an NTD.

Sample **MS301** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS301** (MSAFP-MOM = 0.52, MSuE3-MOM = 0.40, MShCG-MOM = 1.80, DIA-MOM = 1.71) presented the canonical profile of low MSAFP and MSuE3 and elevated MShCG and MSDIA, resulting in a T21 positive screen with which all labs agreed. In addition, the matched **AF301** specimen had low AFP levels (MOM value = 0.51). The T21 risk was 1 in 29 by triple test and 1 in 37 by quad test (Figs. 5, 6). The recommended further actions for the sample **MS301** were genetic counseling, 92%; ultrasound, 68%; and amniocentesis, 88%, from labs performing the quad screen, and genetic counseling, 85%; ultrasound, 62% and amniocentesis, 85%, from labs performing the triple screen.

Two other specimens, **MS302** and **MS304**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS303** specimen from a 18 weeks old pregnancy represented an interesting case involving low levels of MSAFP, MSuE3, MSHCG and MSDIA; this profile resulted in a negative screen for NTD and T21, but yielded a borderline screen negative risk for T18 (median risk 1 in 294; positive risk cutoff 1 in 100) (Fig. 4), with 3 labs (12%) reporting a positive result. The T18 follow-up actions recommended for specimen **MS303** by these 3 labs were genetic counseling, 100%; ultrasound, 100%; amniocentesis, 100%; and no repeat testing. Sample **MS303** was modeled after several literature case studies of pregnant women with hereditary Thrombophilia (TBP) that exhibited low levels of DS biomarkers (1-3). Literature reports have described significant associations of hereditary TBP with both early and late fetal loss, preterm delivery, and small-for-gestational-age births (4, 5). Prior to their diagnosis of TBP, most of the case study women had had miscarriages and complicated pregnancies, although some had delivered normal term infants. Despite the diagnosis of TBP, most women continued with their pregnancy but underwent diagnostic examinations that included ultrasound, 3-D scans, and subsequent thrombophilia-related testing including blood factor mutation analysis. Combined thrombophilic defects in such patients can include mutations in blood Factor-V Leiden, prothrombin, and methylenetetrahydrofolate reductase, as well as activated anti-thrombin, protein-C resistance, and Protein-S deficiency. Patients with inherited TBP usually receive prophylactic anti-coagulant low molecular weight heparin injections throughout their pregnancy together with folate supplementation, while others are given low dose aspirin.

For a possible explanation of the lower DS analyte levels in TBP during pregnancy, one must consider the analytes themselves. hCG is produced by the syncytiotrophoblast of the placenta mainly in the first trimester and early second trimester and low levels are associated with a higher incidence of low birthweight below the 10th percentile and with miscarriage (6-7). It appears that low levels of hCG may also be attributed to impaired placentation and a smaller, less mature placental mass (8). Unconjugated estriol during pregnancy is produced by conversion of 16-hydroxy-dehydroepiandrosterone (DHEA) sulfate derived from the fetal adrenal glands (9). It has been proposed that impaired or reduced activity of placental sulfatase may lower the mid-trimester maternal uE3 levels (10). Finally, AFP is a fetal protein synthesized first in the yolk sac and later in the fetal liver and gastrointestinal tract (11). However, because small amounts of AFP are also produced by the placenta in the first trimester (12), one could propose that low AFP levels might also result from the size, volume, and maturity of the placenta.

Alternatively, the treatment of the TBP patient with low molecular weight heparin may have influenced the analyte levels, resulting in lowered mass values, and hence, lowered MOM values (13). Recent reports have suggested that heparin can act by different mechanisms (14-17). For example, it has been demonstrated that heparin significantly suppresses first trimester trophoblast invasion (18, 19), which may negatively affect the production of AFP and hCG. Furthermore, since heparin-bound affinity columns have been used in the binding and subsequent purification of a number of blood proteins, receptors, and glycoproteins (20), it is conceivable that heparin bound to blood and placental proteins could reduce or mask biomarker detection in some immunoassays (21, 22). Indeed, a recent study showed that women with hereditary TBP that were treated with low molecular weight heparin had lower levels of DS analytes that raised the calculated Trisomy-18 risk (1). In conclusion, the presence of hereditary TBP during pregnancy may potentially confound the Trisomy risk calculations and result in false positive risk assessments for T18.

TBP is a disorder of hemostasis that predisposes an individual to a thrombotic event. Pregnancy is considered a hypercoagulable state due to a vast variety of physiologic changes. Such changes can include increases in blood coagulation proteins, including fibrinogen, Von Willebrand factor, and blood clotting factors II, V, VII, VIII, IX, and X (23, 24). There can also occur decreases in physiologic anticoagulants manifested by a decrease in protein-S, an increase in protein-C resistance, and a decrease in fibrinolytic activity (25). Overall, pregnancy is associated with increased clotting potential, decreased anti-coagulant activity, and decreased fibrinolysis. Inherited TBPs display deficiencies in components of the coagulation system, such as blood Factor-V Leiden mutation, prothrombin gene mutation, anti-thrombin deficiency, protein-C and protein-S deficiency, and hyper-homocystinemia (HCE). HCE is associated with deficiencies in vitamins B6, B12, folic acid, and methylenetetrahydrofolate reductase (26, 27).

The most common coagulation conditions associated with TBP are: a) deep vein thrombosis and pulmonary embolism, collectively called venous thromboembolism. These result in pain, swelling, limb redness, venous valve damage, and break-away clots that migrate to pulmonary arteries. Such lung damage could result in sudden shortness of breath, severe chest pain, and palpitations complicated by pulmonary collapse, shock, and cardiac arrest (28, 29). The first major form of TBP was an anti-thrombin deficiency described in 1965, while the most common form of TBP is a blood Factor-V Leiden mutation discovered in the 1990s (30-33). A third form, called Protein-C deficiency, is a clotting disorder of the newborn leading to tissue death and skin/organ bleeding.

Studies suggest that 50% of cases of thromboembolism in pregnant women are associated with either an inherited or acquired TBP. Both inherited and acquired TBPs can lead to an increased risk of maternal thromboembolism and adverse pregnancy outcomes including perinatal morbidity and mortality. In this regard, diagnosis of the inherited and acquired TBP is sometimes used interchangeably, which is a misconception since each requires different tests. It is deemed important that the clinician identify which patients are at risk, the laboratory tests that should be performed, and which patients need to receive treatment. In women with a history of TBP it has been recommended that clinicians perform a workup periconceptually because TBP manifestations can present early in pregnancy. It is likewise important to select specialized laboratory tests to discriminate between hereditary and acquired TBP since the hereditary form of TBP shows decreases in levels of AFP, uE3 and hCG, while the acquired form shows normal AFP and uE3 levels and slightly raised hCG levels.

Hereditary TBPs are genetic conditions that cause thrombosis, while acquired TBPs refer to disorders that arise later in life. Hereditary TBPs are the most common and the more mild conditions often resulting from aberrant coagulation factors (34). One such factor is prothrombin with a gene mutation at position 20,210 in the 3' untranslated region of the gene (35). The more severe forms of hereditary TBPs are caused by deficiencies in Anti-thrombin-III, Protein-C, and Protein-S anti-coagulants in addition to a blood Factor-VII mutation and an abnormal form of fibrinogen in a condition called familial dysfibrinogenemia. Blood groups other than type-O also contribute to a 2-4 fold increased relative thrombosis risk; however, individuals with type-O blood group exhibit lower levels of Blood Factor-VIII and Von Willebrand factor (35).

The acquired form of TBP comprises a number of conditions which increase thrombotic events. A major example of acquired TBP is the antiphospholipid (APL) antibody syndrome (35). APL is regarded as an autoimmune disease caused by antibodies directed against cell membrane constituents such as lupus coagulant, cardiolipin, and anti-B2-glycoproteins. In some cases, APL syndrome can cause arterial as well as venous thrombosis, and is associated with miscarriage, skin disorders, and migraine (36). Other examples of acquired TBP can include a heparin-induced thrombocytopenia (HIT), which is an immune reaction against the anti-coagulant drug, heparin, and is associated with low platelet counts (37). HIT also is strongly linked to the risk of venous/arterial thrombosis. Hematologic conditions with slow blood flow can increase thrombotic risk and these conditions can encompass Sickle Cell disease, bone marrow proliferative disorders, polycythemia vera (excess red blood cells), metastatic cancer, Nephrotic (kidney) syndrome, and obesity (38). The obese population is at risk for TBP and exhibits high levels of coagulation proteins such as fibrinogen, Blood Factors VII, and VII, and Von Willebrand's Factor.

B) <u>Assay Kit Performance</u>

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format in Figs. 7-10. As shown in Figs. 7-10A and B, mass measurements for all four analytes in serum among the individual kits mostly agreed as did the associated MOM values. Similarly, when the AFP mass measurements in amniotic fluid were compared (Figs 7C, D), the differences between the different kits were unremarkable, except for sample **AF302**. In this sample, the results from the Siemens Immulite 2000 were 3.6 times higher than those from the Beckman UNICEL and Access 2 instruments. As shown in Figure 2, this resulted in two distinct populations of MOMs, with all the higher MOMs derived from mass values determined with the Siemens DPC instrument. Although the reason for this discrepancy is not known, the fact that specimens are derived from actual AF samples suggests that this apparent assay interference may also occur in real patient samples.

C) Second Trimester Screening Software Utilized

The alpha and Benetech PRA software packages were each used by 33.3% and 25.9%, of the labs, respectively; Robert Maciel (RMA) software was employed by 25.9%; 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. **Started with the mail-out in May 2013, first trimester results are graded.** 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 11.9 week 27 year old Hispanic **FT301** specimen for total hCG resulted in a mass mean of 79.6 ± 9.5 IU/ml, with a MOM of 1.02 ± 0.09; the all-lab mass mean for PAPP-A was 1502.9 ± 887.8 ng/ml with a MOM of 3.45 ± 2.21. The **FT301** sample displayed a T21 negative screen assessment. As a result, the all-lab T21 risk assessment for **FT301** was 1 in 19,500 (Fig. 13). Further action was not indicated. Finally, 100 % of labs considered the **FT301** specimen screen negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Fig. 14).

For the **FT302** (25 year old) White specimen, the gestational age all-lab mean was reported as 11.2 weeks. Assay measurements for **FT302** resulted in an all-lab total hCG mass measurement of 178.8 ± 30.9 IU/ml (MOM = 2.19 ± 0.17), while the all-lab PAPP-A mass assessment was 549.0 ± 317.8 ng/ml (MOM = 1.89 ± 1.27). A small majority of labs (56%) considered the **FT302** sample screen positive for T21 with a risk of 1 in 230 (Fig. 13), but an all-lab consensus was not achieved and thus this sample is considered borderline positive. Further action from those labs that considered this sample screen positive was reported as: genetic counseling, 60%; ultrasound, 33%; amniocentesis, 40%; chorionic villi sampling, 33%. A negative screen was achieved for T18 with a risk assessment of 1 in 10,000 (Fig. 14).

The **FT303** specimen was obtained from a 30 year old Black woman with a gestational age of 13.0 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 68.2 ± 9.2 IU/ml (MOM = 1.00 ± 0.10); the all-lab PAPP-A mass measurement was 1996.1 ± 1190.1 ng/ml (MOM = 2.68 ± 1.69). The all-lab T21 screen consensus for **FT303** was negative with a risk assessment of 1 in 13,000 (Fig. 13). No further actions were recommended by the labs. Finally, the **FT303** specimen also screened negative for T18 (1 in 10,000, Fig. 14).

The 11.5 week specimen **FT304** was obtained from a 29 year old (130 lbs.) White woman. Total hCG measurement resulted in a mass mean of 76.2 IU/ml ± 12.0, with a MOM of 0.90 ± 0.08. In addition, the all-lab mass mean for PAPP-A was 1329.6 ± 778.7 ng/ml with a MOM of 3.40 ± 2.09. This resulted in an all-lab negative T21 risk assessment of 1 in 20,000 (Fig. 13) and a negative screen for T18 of 1 in 10,000 (Fig. 14).

For the 24 year old (125 lbs) Asian **FT305** specimen, the gestational age all-lab mean was reported as 12.4 weeks. Assay measurements resulted in an all-lab total hCG concentration of 73.3 ± 9.9 IU/ml (MOM = 0.96 ± 0.08), while the all-lab PAPP-A concentration was 1620.4 ± 968.6 ng/ml (MOM = 2.90 ± 1.77). The all-lab FT T21 risk assessment was 1 in 20,000 with all labs in agreement (Fig. 13). Similarly, the **FT305** specimen was also screen negative for T18 with an all-lab risk assessment of 1 in 10,000 (Fig. 14).

E) First Trimester Assay Kit Performance

In order to compare the Beckman UNICEL assays (59% users) for PAPP-A with those of the older Siemens Immulite and EIA/ELISA 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 0.9761, a slope of 0.414 and a Y intercept of -0.0387 (Fig. 15). Using the 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. However, for grading purposes, each lab's results were compared to their own peer group without conversion. Despite this conversion, and even when comparing MOM values instead of mass values, the results from the Siemens Immulite method were three to four times higher than those from Beckman or AnshLite (Figs. 12A, B).

For hCG, as shown in Fig 11A, 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/UNICEL instrument. When converted to MOM values, however, these differences essentially disappeared (Fig. 11B).

F) First Trimester Screening Software Utilized

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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AFP peptide epitopes and the MHC proteins

AFP peptide sequences

Recent studies have indicated that not all AFP-specific T cell clones are deleted during ontogeny, and that AFP derived peptide antigenic sites persist and are recognized by both murine and human T cells. During the last decade, multiple research teams have thus detected T cell immunodominant epitope sites on HAFP [5, 7, 19, 33, 37]. These various groups have reported that both major MHC class I epitopic immunodominant and minor sub-dominant sites can be identified throughout the three domain molecule of HAFP (Fig. 1). Computer-generated HAFP AA sequences (9–10 AA in length) comprising multiple potential peptides were screened; these produced immunoreactive peptides representing major immunodominant histocompatability complex (MHC) sites of the HLA-A type such as HAFP #542–550 (GVALQTMKQ) [6, 7, 25]. Several peptidic segments of the latter positive group corresponded to known major T cell epitope binding sites that had been described in other reports; the remaining antigenic determinants were classified as minor epitopic (subdominant) sites [23] (Fig. 1). Each peptide proved capable of inducing specific T cell lymphocyte responses in vitro from normal human HLA-A donor lymphocytes; they also recognized HLA-A/AFP-primed tumor cells in both cytotoxicity and interleukin/interferon induction assays. AFP peptide-specific T cells were further identified by these researchers in the spleens of mice immunized with dendritic cells transduced with an AFP-expressing adenovirus. Such studies and others concluded that the human T cell repertoire is capable of recognizing AFP in the context of MHC Class-I immune responses, even in an environment of low to moderate circulating HAFP levels, in both hepatoma and cirrhotic patients. Several of the peptides of HAFP-origin are currently in Phase I and II clinical trials for the treatment of hepatocellular carcinoma [8, 9] (Table 2). Although the AFP epitopes provide the rationale for T cell based immunotherapy against hepatomas (AFP-producing cells), the eventual side effects of circulating antibodies of HAFP epitopes aside from the T cell responses described above, have yet to be determined. One study suggested that the induction of liver autoimmune disease may be a consequence of sensitizing T cells against HAFP, a self protein [16]; however, another study showed no effect [37]. Nonetheless, the recent reports discussed above have continued to pursue the identification of major and minor epitopes from AFP that can activate high-avidity T cells; such cells can be detected and clonally expanded in hepatoma patients. Such findings served as the initial impotus to employ AFP-peptides as targets for T cell-based immunotherapy in hepatoma patients [6].

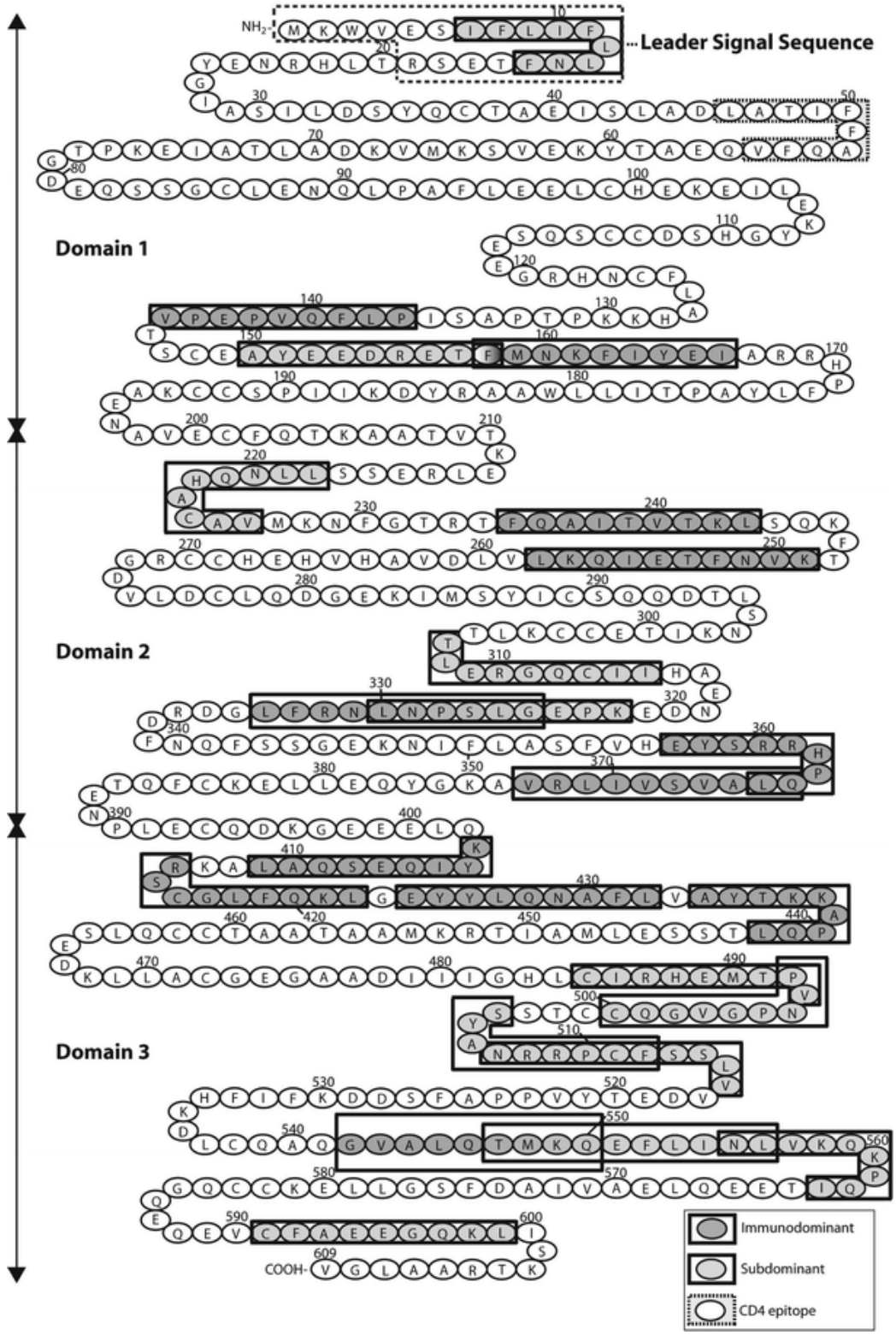

Fig. 1
The 609 amino acid sequence of the entire human alpha-fetoprotein (HAFP) molecule is displayed including the 19-amino acid leader signal sequence at the amino-terminus. The HAFP-derived immunodominant 9–10 amino acid peptide epitopes (see text) are represented by dark grey boxed circles, while the subdominant peptide epitopes are shown by light grey boxed circles. The amino acid are displayed in the single letter code. Some peptide segments, produced by proteolytic cleavage of the HAFP molecule, can serve as HLA-A class-1 antigens when processed and

presented to the T cell receptor by the MHC proteins positioned on antigen-presenting cells, that is, the macrophages, phagocytes, and dendritic cells. Although the leader signal sequence exhibits a subdominant epitiope, HAFP Domain-1 seemingly exhibits less peptide epitopes than domains −2 and −4. However, these are the HAFP published epitopes to date, and more have yet be identified and reported. It is of interest that domain −1 of HAFP contains more amino acid sequences specific to the human, than do domains −2 and −3 [32]; therefore, fewer peptide epitopes may be capable of inducing an immune response in human T cells. Note also that AFP amino acid sequences # 46–55 represent an epitope recognized by CD4+ cells as reported in References. [2, 3]. The CD4+-responsive epitope is represented by the boxed white circle on a grey background

As shown above, AFP-derived peptide epitopes can be recognized by heterologous human T cells in the context of MHC class I antigens. Researchers have now determined the identity of additional AFP-derived peptides, presented as HLA-A*0201 restricted antigens, that could be recognized by the human T cell repertoire [7]. After screening 74 peptides, the group identified three new AFP epitopes, HAFP #137–145, HAFP #158–166, and HAFP #325–334, in addition to the previously reported HAFP #542–550 epitope. (Table 3, Fig. 1) Each peptide possessed two anchor hydrophobic AA residues and each stabilized HLA-A*0201 antigens on T cells in a concentration-dependent MHC class I binding assay. The AFP peptides showed stability for 2–4 h in dissociation-kinetic assays. The peptides also induced epitope-specific primed T cells in vitro from several normal HLA-A*0201 donors. Importantly, the HAFP peptide-specific T cells also were capable of recognizing HLA-A*0201(+)/AFP(+) tumor cells in both cytotoxicity and IFN-gamma induction assays. The immunogenicity of each peptide was assayed in vivo with HLA-A*0201 in Kb-transgenic mice following immunization with each peptide emulsified in adjuvant; also, draining mouse lymph node cells were shown to produce IFN-gamma cytokines on recognition of cells stably transfected with HAFP. AFP peptide-specific T cells were also identified in the spleens of mice immunized with dendritic cells transduced with an AFP-expressing adenovirus (AdVhAFP). Three of the four AFP peptides were identified by mass spectrometric analysis of cell surface peptides derived from an HLA-A*0201 human HCC cell line. In summary, the authors presented compelling immunological and physiochemical evidence which showed that at least four HAFP-derived epitopes were immunogenic, naturally processed, and presented in the context of MHC class I antigens. Thus, these investigators confirmed that such AFP epitopic sites could serve as prime targets for hepatoma immunotherapy [7].

The immune response of lymphocytes was further studied after activation of dendritic cells (DCs) sensitized by a cytotoxic T lymphocyte (CTL)-based peptide from the second domain of human alpha-fetoprotein (HAFP #218–226 LLNQHACAV) (Table 3, Fig. 1). High purity DCs were obtained from plastic-adherent monocytes cultured from healthy human donors of HLA-A2(+) peripheral blood which had been co-incubated for seven days with granulocyte-monocyte colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). Self-lymphocytes were also stimulated with DCs treated with the HAFP #218–226 peptide in a culture medium containing interkeukin-2 (IL-2) for 7 days. IL-12 and TNF levels were analyzed in culture medium and specific lysis activity of lymphocytes was assayed against four strains of primary hepatocellular carcinoma cells. After stimulation by DCs that were activated with HAFP #218–226 peptide, lymphocytes appeared unchanged and the culture medium of the activated lymphocytes contained high levels of Th1-type cytokines namely, IL-12 and TNF. Activated lymphocytes not only specifically lysed HLA-A2(+) HepG2 tumor cell line, but also showed cytotoxicity against three other primary hepatocellular carcinoma cell lines and against T2 hepatoma target cells activated with the HAFP peptide. The results of these experiments provided the underpinnings for developing a DC-based vaccine with a HLA-A2 restricted peptide epitope derived from an HAFP epitope directed against AFP positive primary HCC cells [23].

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

(1) Source: Prenat Diagn. 2013 May;33(5):457-61.

Title: Maternal serum placental growth factor and α-fetoprotein testing in first trimester screening for Down syndrome

Authors: Donalson K, Turner S, Morrison L, Liitti P, Nilsson C, Cuckle H

Abstract: OBJECTIVE: The aim of this research was to evaluate the addition of first trimester maternal serum placental growth factor (PlGF) and α-fetoprotein (AFP) to the combined test for Down syndrome and a serum only protocol of PlGF, AFP, free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. METHODS: Samples were from 92 Down syndrome cases with 552 matched controls. All women had a combined test at 11-14 weeks gestation. PlGF and AFP were measured and expressed in multiples of the gestation-specific median (MoM), adjusting for maternal weight and smoking status. Multivariate Gaussian modeling was used to predict detection and false-positive rates. RESULTS: The median PlGF level in the cases was 0.694 MoM and controls 1.000 MoM ($p = <0.0001$). The corresponding values for AFP were 0.764 MoM and 0.990 MoM ($p < 0.0001$). Statistical modeling predicted that for a given false-positive rate, the addition of PlGF to the combined test increases the detection rate by 4-7%. For a given detection rate, the

false-positive rate could be almost halved. When both PlGF and AFP are used, the detection rate increase is 5-8%. A serum only protocol had a predicted a detection rate of 71% for a false-positive rate of 5%. CONCLUSIONS: Results suggest a substantial benefit of adding PlGF to the combined test.

(2) Source: Eur Rev Med Pharmacol Sci. 2013 Jul;17(13):1820-3

Title: First and second trimester biochemical markers in familial mediterranean fever

Authors: Turkcapar F, Engin-Üstün Y, Simsek OY, Deveer R, Danisman N, Dilmen U, Mollamahmutoglu L

Abstract: OBJECTIVES: Our aim was to investigate whether the maternal serum concentrations of first and second trimester serum analytes are altered in familial Mediterranean fever (FMF) pregnancies. MATERIALS AND METHODS: The screening tests were compared in a series of 16 serum samples from FMF pregnancies and in a cohort of 48 pregnant women with normal pregnancy. Serum samples were obtained between 11 and 13 weeks; 16 and 18 weeks gestation. RESULTS: Serum pregnancy-associated plasma protein-A (PAPP-A) levels, expressed as multiples of the median (0.9 ± 0.45 MoM) in the control group, were significantly higher than FMF patients (0.6 ± 0.3 MoM) ($p = 0.027$). Analyses of alpha-fetoprotein, human chorionic gonadotropin and oestriol levels showed no significant differences between FMF and normal pregnancies. CONCLUSIONS: Our study revealed that low levels of PAPP-A are associated with FMF.

(3) Source: Prenat Diagn. 2013 Jun 20:1-6.

Title: Amniotic fluid glial fibrillary acidic protein (AF-GFAP), a biomarker of open neural tube defects

Authors: Lopez J, Mikaelian I, Gonzalo P

Abstract: OBJECTIVE: Neural tube defects (NTDs) are usually identified by ultrasonography and confirmed by alpha-fetoprotein (AFP) assay and acetylcholinesterase (AchE) electrophoresis in amniotic fluid. Yet, both of these biomarkers can be found positive in other etiologies. Here, amniotic fluid glial fibrillary acidic protein (AF-GFAP), which was identified by a proteomic study, is shown to be a useful biomarker for NTD diagnosis. METHOD: Amniotic fluid glial fibrillary acidic protein was measured by an ELISA assay in 138 cases of NTDs. Seventy samples from normal pregnancies used as controls and 27 samples giving false positive or false negative results either for AchE or AFP and corresponding to fetal death ($n = 8$), gastroschisis ($n = 8$), and unexplained etiologies ($n = 11$) were also tested. RESULTS: Whatever the gestational age, GFAP was below 0.2 ng/mL in control samples, whereas 99.1% of open NTDs (29/29 in the anterior NTD group and 80/81 in the spina bifida group) were above this threshold. Closed NTDs were all negative (28/28). None of the other samples tested were positive, except in case of fetal death (8/8). CONCLUSIONS: Amniotic fluid glial fibrillary acidic protein is a sensitive biomarker for open NTD diagnosis with a good negative predictive value for closed NTD. Compared with AFP and AchE, our results indicate that AF-GFAP alone is more efficient than this classical association.

(4) Source: J Matern Fetal Neonatal Med. 2013 Jul 12. [Epub ahead of print]

Title: The umbilical cord alpha-fetoprotein levels for predicting hyperbilirubinemia in term neonates

Authors: Kuzdan C, Coban AC, Ince Z, Can G, Türkoğlu U

Abstract: OBJECTIVE: The aim of this study was to investigate the possible relationship between cord blood alpha-fetoprotein (AFP) level and development of subsequent neonatal hyperbilirubinemia. Study design: The term newborns born between March 2005 and October 2005 were included in the study. Infants with Coombs-positive ABO and/or Rh incompatibility and/or hemolytic jaundice, asphyxia, congenital anomaly and signs of bleeding were excluded from the study. Cord blood AFP levels were measured in 504 full term newborns in this period. Infants were followed-up for possible neonatal hyperbilirubinemia. The capillary bilirubin level (CBL) was examined expeditiously in newborns developing jaundice and in other infants at the time discharge while the screening test was being performed. RESULTS: The mean umbilical cord AFP level was 49.1 ± 44.9 mg/L (range 1.1-396.2 mg/L), mean CBL was 5.8 ± 3.1 mg/dL (range 1-19.4 mg/dL), and the mean bilirubin detection time was 37 ± 23.2 hours (range 12-144 h) of age. Although a significant positive correlation was found between umbilical cord AFP and CBL levels, it was weak ($r = 0.187$, $p < 0.001$). Comparison of AFP levels in terms of bilirubin percentile values appropriate for postnatal age also showed a

significant weak positive correlation ($r = 0.113$, $p < 0.001$). CONCLUSION: The umbilical cord AFP levels may not be used as a strong predictor for the determination of newborns at risk for hyperbilirubinemia.

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

(1) Source: Indian J Pathol Microbiol. 2013 Jan-Mar;56(1):54-6.

Title: Alfa-fetoprotein secreting ovarian sex cord-stromal tumor

Authors: Jashnani KD, Hegde CV, Munot SP

Abstract: Ovarian sex cord-stromal tumors are relatively infrequent neoplasms that account for approximately 8% of all primary ovarian tumors. They are a heterogeneous group of neoplasms composed of cells derived from gonadal sex cords (granulosa and Sertoli cells), specialized gonadal stroma (theca and Leydig cells), and fibroblasts. They may show androgenic or estrogenic manifestations. We report such a tumor associated with markedly raised serum alpha-fetoprotein (AFP) levels in a young female presenting with a mass and defeminising symptoms. Serum AFP levels returned to normal on removal of tumor.

(2) Source: Orphanet J Rare Dis. 2013 Aug 14;8:123.

Title: SETX mutations are a frequent genetic cause of juvenile and adult onset cerebellar ataxia with neuropathy and elevated serum alpha-fetoprotein.

Authors: Nanetti L, Cavalieri S, Pensato V, Erbetta A, Pareyson D, Panzeri M, Zorzi G, Antozzi C, Moroni I, Gellera C, Brusco A, Mariotti C

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. OBJECTIVES/BACKGROUND: Ataxia with oculomotor apraxia defines a group of genetically distinct recessive ataxias including ataxia-telangectasia (A-T, ATM gene), ataxia with oculomotor apraxia type 1 (AOA1, APTX gene) and type 2 (AOA2, SETX gene). Although, a few unique clinical features differentiate each of these forms, the patients also share common clinical signs, such as the presence of cerebellar atrophy, sensorimotor axonal neuropathy, and elevated alpha-fetoprotein (AFP) serum level. MATERIALS AND METHODS: We selected 22 Italian patients from 21 families, presenting progressive cerebellar ataxia, axonal neuropathy, and elevated serum AFP. We screened the coding regions of ATM, APTX and SETX genes for point mutations by direct sequencing or DHPLC, and searched genomic rearrangements in SETX by MLPA analysis. In selected cases, quantification of ATM and senataxin proteins was performed by Western blot. Clinical, neurophysiological, and neuroimaging data were collected. RESULTS: Thirteen patients (12 families) carried SETX mutations (AOA2, 57%), two were mutated in ATM (A-T), and three in APTX (AOA1). In three remaining patients, we could not find pathogenic mutations, and in one case we found, in homozygosis, the SETX p.K992R polymorphism (population frequency 1-2%). In AOA2 cases, we identified 14 novel and three reported SETX mutations. Signs at onset were gait ataxia and facial dyskinesia, and the age ranged between 11 and 18 years. None had obvious oculomotor apraxia at the latest examination (age 14-45 years). The patient carrying the p.K992R SETX polymorphism had a phenotype similar to that of the diagnosed AOA2 patients, while the other three undiagnosed subjects had a very late onset and a few distinguishing clinical features. DISCUSSION AND CONCLUSIONS: We describe a large series of 13 AOA2 Italian patients. The phenotype was consistent with previous descriptions of AOA2, except for a higher frequency of strabism, and for the absence of oculomotor apraxia. In our survey ~60% of juvenile-to-adult cases with cerebellar ataxia, sensorimotor neuropathy and increased AFP are due to mutations in the SETX gene, and a smaller percentage to APTX and ATM gene mutations.

(3) Source: Rare Tumors. 2013 Jun 3;5(2):83-4.

Title: Endodermal sinus tumor of vagina in infants

Authors: Chauhan S, Nigam JS, Singh P, Misra V, Thakur B

Abstract: Endodermal sinus tumor (or Yolk Sac tumor) of the vagina is a rare malignant germcell tumor which is seen exclusively in children younger than 3 years of age. We report two cases of endodermal sinus tumor of the vagina. In both cases no radiological investigation was done and serum alpha-fetoprotein was elevated. The histopathological examination of both the tumor masses revealed vaginal endodermal sinus tumor. Periodic-acid-Schiff stain with diastase showed diastase resistant hyaline globules. These findings confirmed the diagnosis of endodermal sinus tumor in both cases. Vaginal endodermal sinus tumor is both locally aggressive and capable of metastasis. The serum alpha-fetoprotein level is a useful marker for diagnosis and monitoring the recurrence of vaginal endodermal sinus tumor in infants. Early detection and therapy is important because of its aggressive nature and good response to chemotherapy.

(4) Source: Gan To Kagaku Ryoho. 2013 Jul;40(7):825-32

Title: Clinical features of solid malignant tumors in childhood

Authors: Koshinaga T, Ohashi K, Sugitou K, Ikeda T

Abstract: The pathogenesis of pediatric malignant tumors is associated with congenital abnormalities. Oncogenes and antioncogenes are identified in some of these cases. Neuroblastoma arises from the adrenal medulla and sympathetic ganglia. Most neuroblastomas produce catecholamine. Urinary vanillylmandelic acid(VMA)and homovanillic acid(HVA), metabolites of catecholamine, are sensitive tumor markers. Risk stratification according to tumor stage and a combination of prognostic factors helps determine the appropriate therapeutic strategy in clinical settings. Nephroblastoma(Wilms tumor)is the most common pediatric renal tumor and is often accompanied by congenital anomalies. Surgical resection of the tumor and the involved kidney is the initial treatment recommendation in the US and Japan. Consecutive chemotherapy and radiotherapy are administered after surgical staging and a definite histopathological diagnosis. Prognosis is relatively good for most nephroblastoma cases with a favorable histology. In addition to nephroblastoma, clear cell sarcoma of the kidney, characterized by a tendency to metastasize to the bone, is a renal tumor with poor prognosis. Rhabdoid tumor of the kidney is another tumor type; however, its pathogenesis is still unknown and it is associated with extremely poor prognosis because of the lack of effective therapeutic measures. Hepatoblastoma is the most common malignant liver tumor. The serum alpha-fetoprotein level is the most effective tumor marker. Complete surgical resection of the involved liver lobe is the definitive approach for cure. Preoperative chemotherapy increases the possibility of complete surgical resection. High-risk patients have a poor prognosis.

C) News of Note: Abstracts of New Markers:

(1) Source: Am J Obstet Gynecol. 2013 May 11. pii: S0002-9378(13)00503-6.

Title: Combined screening for open spina bifida at 11-13 weeks using fetal biparietal diameter and maternal serum markers

Authors: Bernard JP, Cuckle HS, Bernard MA, Brochet C, Salomon LJ, Ville Y

Abstract: OBJECTIVE: Screening at 11-13 weeks with ultrasound biparietal diameter (BPD) can detect half of open spina bifida cases. Maternal serum α-fetoprotein (AFP) levels at 15-19 weeks are increased 3- to 4-fold, in open spina bifida. We assessed whether combined screening using BPD, AFP, and other serum markers at 11-13 weeks would increase detection. STUDY DESIGN: Maternal AFP levels were measured on serum stored at 11-13 weeks in 44 open spina bifida and 182 unaffected pregnancies, and results were expressed in multiples of the median (MoM) for gestational age. All samples had been measured for free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP)-A. A multivariate Gaussian model was used to predict screening performance from the serum data and BPD measurements on 80 cases, including 36 previously published. RESULTS: The median AFP level in cases was 1.201 MoM, significantly higher than in unaffected pregnancies ($P < .01$, 1 tail). The median free β-hCG was significantly reduced to 0.820 MoM ($P < .02$), but the median PAPP-A was similar in cases and controls. Modeling predicted the following: BPD alone would detect 50% of cases for a 5% false-positive rate or 63% for 10%; adding AFP increases detection by 2%; and a combined test with BPD, AFP, and free β-hCG detects 58% for

5% or 70% for 10%. CONCLUSION: Combining AFP and BPD with free β-hCG as part of first-trimester aneuploidy screening would also allow early detection about two-thirds of cases with open spina bifida.

(2) Source: J Matern Fetal Neonatal Med. 2013 Aug 7. [Epub ahead of print]

Title: Effect of aspirin in prevention of adverse pregnancy outcome in women with elevated alpha-fetoprotein

Authors: Khazardoost S, Mousavi S, Borna S, Hantoushzadeh S, Alavi A, Khezerlou N

Abstract: BACKGROUND: To evaluate the effect of low-dose aspirin in prevention of adverse pregnancy outcomes (APO) in women with second trimester alpha-fetoprotein (AFP) >2.5 multiple of median (MOM) and to compare aspirin effect on women with normal and abnormal uterine artery (UtA) Doppler. The primary outcome was the adverse pregnancy outcome. METHODS: This randomized controlled trial was conducted in singleton pregnant women, who had unexplained AFP >2.5 MOM and gestational age between 15 and 18 weeks of gestation. They were assigned randomly to receive either aspirin (N = 65) or control (N = 68). UtA Doppler velocimetry studies were performed at the time of targeted ultrasonographic exam. RESULTS: Two groups were comparable regarding the maternal characteristics. The frequency of APO in aspirin and control groups were 26.1% versus 44.1% (p = 0.045), the frequency of preterm delivery before 34 weeks were 3.2% versus 22.0% in aspirin and control group, p = 0.001. Other outcomes were similar in both groups. The frequency of adverse outcomes in women with abnormal UtA Doppler was 39.1% in aspirin and 60.0% in control group, p = 0.556. CONCLUSION: Low-dose aspirin reduces APO and delivery before 34 weeks of gestation in pregnant women with unexplained elevated AFP.

(3) Source: Prenat Diagn. 2013 Jul 8:1-6. [Epub ahead of print]

Title: First-trimester Down syndrome screening using additional serum markers with and without nuchal translucency and cell-free DNA

Authors: Johnson J, Pastuck M, Metcalf A, Connors G, Krause R, Wilson D, Cuckle H

Abstract: OBJECTIVE: This study aimed to evaluate serum-only four-marker first trimester (1T-Quad) Down syndrome screening, alone or contingently to select 10-20% with highest risk for nuchal translucency (NT) or cell-free (cf)DNA. METHODS: Stored maternal serum samples (-80 °C) from 90 pregnancies with fetal Down syndrome and 1607 controls were retrieved and measured for placental growth factor, α-fetoprotein, pregnancy-associated plasma protein and free β-human chorionic gonadotropin. Samples were from singleton pregnancies (9-13 + 6 weeks), and NT was measured between 11 and 13 + 6 weeks. Markers were expressed in multiples of the normal median (MoM) for gestation. Gaussian models were fitted to the distribution of log MoMs by using observed parameters, standardized maternal age distribution (mean 27, SD 5.5) and published cfDNA results. RESULTS: The model-predicted detection rate (DR) for 1T-Quad was 74% [5% false-positive rate (FPR)]. When used contingently to select for NT, the DR was 89% at 5%. When used to select for cfDNA, the DR was 91% (FPR < 0.05%). CONCLUSION: The 1T-Quad test can achieve a similar DR to a second-trimester Quad test. When used contingently to select for NT, the DR is similar to the Combined test. Used contingently to select for cfDNA would achieve even higher detection.

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

(1) Source: J Perinatol. 2013 May 23. doi: 10.1038/jp.2013.55. [Epub ahead of print]

Title: Abnormal analyte preeclampsia: do the second-trimester maternal serum analytes help differentiate preeclampsia subtypes?

Authors: Critchfield AS, Paulus JK, Farez R, Urato AC

Abstract: OBJECTIVE: To determine if serum screen analytes identify preeclamptic patients at risk for small-for-gestational age newborns, maternal laboratory abnormalities and preterm delivery (<37 weeks gestation). STUDY DESIGN: Using a retrospective cohort of 102 preeclamptic patients, associations between serum screen analytes and newborn birth-weight percentile, gestational age (GA) at delivery and maternal pre-delivery laboratory abnormalities were evaluated using correlation coefficients and local polynomial regression. RESULTS: Inhibin-A and maternal serum alpha fetoprotein were inversely correlated with newborn birth-weight percentile (-0.27, P=0.006; -0.35, P=0.00004) and delivery GA (r=-0.42, P<0.0001; r=-

0.26, P=0.008) and positively correlated with pre-delivery aspartate aminotransferase (r=0.22, P=0.03; r=0.21, P=0.04) and lactate dehydrogenase (r=0.33, P=0.0007; r=0.29, P=0.004). A positive correlation was noted between both second-trimester beta human chorionic gonadotropin and estriol and maternal pre-delivery creatinine (0.28, P=0.004; 0.4, P<0.0001, respectively). Hundred percent of patients with ≥2 abnormal analytes delivered before 37 weeks gestation. CONCLUSION: Preeclamptic patients with abnormal serum screen analytes are more likely to have small-for-gestational age newborns, deliver preterm and have pre-delivery laboratory abnormalities.

(2) Source: Early Hum Dev. 2013 Sep;89(9):777-80

Title: Evaluation of the impact of maternal smoking on ultrasound and endocrinological markers of first trimester placentation

Authors: Jauniaux E, Suri S, Muttukrishna S

Abstract: OBJECTIVES: To study the effect of maternal smoking on 2D ultrasound measurements and maternal serum (MS) levels of endocrinologic markers of placentation. STUDY DESIGN: Prospective population-based cohort study of 32 smokers and 96 non-smoking controls with a normal pregnancy outcome. MAIN OUTCOME MEASURES: Placental thickness and 2D-volume and MS levels of pregnancy-associated plasma protein A (PAPP-A) and free-beta human chorionic gonadotrophin (fβhCG) at 11-13(+6)weeks of gestation and mid-trimester MS α-fetoprotein (AFP), unconjugated estriol (uE3) and inhibin A levels. RESULTS: The MS levels of fβhCG and PAPP-A were significantly (P<0.01 and P<0.001, respectively) lower in the serum and the level of inhibin A significantly (P<0.001) higher in the smokers than in controls. There was no significant difference for the MSAFP, MSuE3 placental thickness, basal plate surface and volume between the groups. CONCLUSION: The placental morphological alterations secondary to maternal smoking are mainly at the level of the villous trophoblast and are not associated with changes in the placental size or utero-placental interface during the first trimester of pregnancy.

(3) Source: Spectrochim Acta A Mol Biomol Spectrosc. 2013 Nov;115:136-44

Title: Fullerol-fluorescein isothiocyanate-concanavalin agglutinin phosphorescent sensor for the detection of alpha-fetoprotein and forecast of human diseases

Authors: Liu JM, Lin LP, Jiang SL, Cui ML, Jiao L, Zhang XY, Zhang LH, Zheng ZY, Lin X, Lin SQ

Abstract: Based on the reaction of the active -OH group in fullerol (F) with the dissociated -COOH group in fluorescein isothiocyanate (FITC) to form an F-FITC and the enhanced effect of N, N-dimethylaniline (DMA) on phosphorescence signal of F-FITC, a new phosphorescent labeling reagent (DMA-F-FITC) was developed. What's more, a phosphorescent sensor for the determination of alpha-fetoprotein variant (AFP-V) has been designed via the coupling technique of the high sensitivity for affinity adsorption-solid substrate-room temperature phosphorimetry (AA-SS-RTP) with the strong specificity reaction between DMA-F-FITC-Con A and AFP-V. The DMA-F-FITC increased the number of luminescent molecules in the biological target which improved the sensitivity of phosphorescent sensor. The proposed sensor was responsive, simple, selective and sensitive, and it has been applied to the determination of trace AFP-V in human serum and the forecast of human diseases using phosphorescence emission wavelength of F or FITC, with the results agreed well with those obtained by enzyme-linked immunoassay (ELISA). Meanwhile, the mechanisms for the labeling reaction and the sensing detection of AFP-V were discussed.

(4) Source: Gene. 2013 Aug 8. pii: S0378-1119(13)00958-X. doi: 10.1016/j.gene.2013.07.050. [Epub ahead of print]

Title: Prenatal diagnosis and molecular cytogenetic characterization of mosaic ring chromosome 13

Authors: Chen CP, Tsai CH, Chern SR, Wu PS, Su JW, Lee CC, Chen YT, Chen WL, Chen LF, Wang W

Abstract: We present prenatal diagnosis and molecular cytogenetic characterization of de novo mosaic r(13). A 32-year-old woman underwent amniocentesis at 18weeks of gestation because of maternal anxiety. Amniocentesis revealed a karyotype of 46,XY,r(13)[33]/45,XY,-13[19]. aCGH on uncultured amniocytes at repeated amniocentesis detected a 4.22-Mb deletion at 13q34. Interphase FISH on 100 uncultured amniocytes showed the ratio of r(13):-13:idic r(13) as 85%:13%:2%. The cord blood had a karyotype of 46,XY,r(13)[91]/46,XY,idic r(13)[6]/45,XY,-13[3]. The placenta had a karyotype of

46,XY,mar(13)[31]/45,XY,-13[3]. Metaphase FISH confirmed that the marker chromosomes in placenta were derived from chromosome 13. aCGH on cultured placental cells detected a 77.81-Mb deletion at 13q13.3-q34. The fetus postnatally manifested facial dysmorphism. Prenatal diagnosis of r(13) should alert mosaicism for deletion/duplication of r(13) and distal 13q deletion. Fetoplacental chromosomal discrepancy of r(13) may exist in case of mosaic r(13) detected by amniocentesis.

E) Abstracts of New Assay Methodologies:

(1) Source: Analyst. 2013 Sep 21;138(18):5396-403..

Title: Dual signal amplification of zinc oxide nanoparticles and quantum dots-functionalized zinc oxide nanoparticles for highly sensitive electrochemiluminescence immunosensing

Authors: Zhang J, Liu S, Bao J, Tu W, Dai Z

Abstract: A novel electrochemiluminescence (ECL) immunosensor for highly sensitive detection of α-fetoprotein (AFP) based on a dual signal amplification strategy was developed. Zinc oxide (ZnO) nanoparticles were employed as the carriers for immobilizing the capture AFP antibody (Ab1) and CdSe quantum dots (QDs). CdSe QDs-functionalized ZnO nanoparticles were used as the tracer to label the signal AFP antibody (Ab2). CdSe QDs-functionalized ZnO nanoparticles were prepared through an amide dehydration reaction and they were characterized by transmission electron microscopy and Fourier transform infrared spectroscopy. The Ab2 was bound to the CdSe QDs-functionalized ZnO nanoparticles to obtain the detection probe. ZnO nanoparticles could accelerate electron transfer between the detection probe and the electrode, and their large surface area was beneficial for loading more CdSe QDs, leading to an enhanced ECL signal (0.9-fold increase) by a sandwich immunoreaction. This also indicated efficient association of the detection probe on the immunosensor surface. The designed immunoassay showed a wide linear range from 0.5 to 600 ng mL(-1) with a detection limit of 0.48 ng mL(-1) at a S/N ratio of 3 for AFP detection. The ECL immunosensor exhibited good analytical performance and was successfully applied to clinical sample detection, showing a promising application in ECL biosensing.

(2) Source: Biosens Bioelectron. 2013 Jul 4;50C:356-361.

Title: Electrochemical immunosensor for simultaneous detection of multiplex cancer biomarkers based on graphene nanocomposites.

Authors: Chen X, Jia X, Han J, Ma J, Ma Z

Abstract: In this work, a sandwich-format electrochemical immunosensor for simultaneous determination of carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) was fabricated using biofunctional carboxyl graphene nanosheets (CGS) as immunosensing probes, which were fabricated by means of immobilization of toluidine blue (TB) and labeled anti-CEA ($Ab_{2,1}$), Prussian blue (PB) and anti-AFP ($Ab_{2,2}$) successively on CGS. The capture anti-CEA ($Ab_{1,1}$) and anti-AFP ($Ab_{1,2}$) were immobilized onto the chitosan-Au nanoparticles (CHIT-AuNPs) modified electrode through 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and N-hydroxy succinimide (EDC/NHS). Experimental results revealed that this sandwich-type immunoassay enabled simultaneous detection of CEA and AFP with linear range of 0.5-60ngmL^{-1} for both analytes. The detection limit was 0.1ngmL^{-1} for CEA and 0.05ngmL^{-1} for AFP (S/N=3). The assay results of serum samples with the proposed method were in a good agreement with the reference values from the standard ELISA method. And the negligible cross-reactivity between the two analytes allows it to possess potential promise in clinical diagnosis.

(3) Source: Analyst. 2013 Jul 7;138(13):3697-704.

Title: Magnetic particle-based time-resolved fluoroimmunoassay for the simultaneous determination of α-fetoprotein and the free β-subunit of human chorionic gonadotropin

Authors: Hou JY, Liu TC, Ren ZQ, Chen MJ, Lin GF, Wu YS

Abstract: In this paper, a novel time-resolved fluoroimmunoassay (TRFIA) protocol using magnetic particles for the simultaneous determination of α-fetoprotein (AFP) and the free β-subunit of human chorionic gonadotropin (free β-hCG) in human serum is described. The new approach uses magnetic particles as an immobilization

matrix and means of separation, while the luminescent europium and samarium chelates are used as probes. The proposed method was evaluated via a single-step, sandwich-type TRFIA immunoassay of AFP and free β-hCG as model analytes in serum. With the advantages of magnetic particles, the TRFIA immunoassay exhibited a wide dynamic range for AFP of 0.1-750 ng mL(-1), with a lower detection limit of 0.05 ng mL(-1). The dynamic range for free β-hCG was 0.16-450 ng mL(-1), with a lower detection limit of 0.08 ng mL(-1). Satisfactory specificity, reproducibility, and recovery of the immunoassay were demonstrated. Good correlations were obtained in the analysis of 446 human serum samples between the proposed method and a commercial TRFIA kit. These results demonstrate the feasibility and potential of the new method as a rapid and highly sensitive immunoassay that could be developed into a platform for multi-analyte determinations in clinical practice.

(4) Source: Anal Chem. 2013 Aug 6;85(15):7102-8

Title: A near-infrared, surface-enhanced, fluorophore-linked immunosorbent assay

Authors: Furtaw MD, Steffens DL, Urlacher TM, Anderson JP

Abstract: The enzyme-linked immunosorbent assay is commonly used for research and clinical applications but typically suffers from a limited linear range and is difficult to multiplex. The fluorophore-linked immunosorbent assay is a closely related technique with good linear range and the ability to detect multiple antigens simultaneously but is typically less sensitive. Here, we demonstrate a near-infrared, surface-enhanced fluorophore-linked immunosorbent assay with sensitivity comparable to its enzyme-linked counterpart. A 59-fold enhancement to sensitivity (slope of linear fit) and an 8-fold improvement in LOD are demonstrated on a direct assay with rabbit immunoglobulin-G as a model system. The technique is also tested on a clinically relevant assay to detect alpha-fetoprotein, in which a 42-fold enhancement to sensitivity is demonstrated along with a 16-fold improvement in LOD. The technique enables these accomplishments while maintaining the entire traditional assay protocol and simply adding two steps at the end. This technique may prove superior to current protocols for biomarker research and clinical diagnoses, which require high sensitivity along with quantitation over an extended range.

F) Special Abstract Selection:

(1) Source: Am J Obstet Gynecol. 2013 Apr;208(4):303.e1-7

Title: Management strategy in pregnancies with elevated second-trimester maternal serum alpha-fetoprotein based on a second assay

Authors: Spaggiari E, Ruas M, Dreux S, Valat AS, Czerkiewicz I, Guimiot F, Schmitz T, Delezoide AL, Muller F

Abstract: OBJECTIVE: To assess maternal-fetal outcomes in pregnancies associated with persistently elevated second-trimester maternal serum alpha-fetoprotein. STUDY DESIGN: A retrospective cohort study in 658 patients with maternal serum alpha-fetoprotein ≥2.5 multiple of median, performed at routine Down syndrome screening. Maternal serum alpha-fetoprotein was assayed a second time in 341 of them. Outcomes were recorded in all cases. RESULTS: The group with unexplained maternal serum alpha-fetoprotein persistently ≥2.5 multiple of median was associated with more pregnancy complications 37 of 92 (40.2%) as fetal death, preeclampsia, intrauterine growth restriction, and congenital nephrotic syndrome, compared with the group with maternal serum alpha-fetoprotein that returned to a normal level 37 of 226 (16.4%) ($P < .001$). CONCLUSION: When maternal serum alpha-fetoprotein returns to a normal level on a second assay, the risk of adverse outcome significantly decreases, but these pregnancies are still at risk of complications and therefore need close surveillance. Repeat maternal serum alpha-fetoprotein assay allows identification of patients who should be offered amniocentesis to evaluate the risk of nephrotic syndrome and epidermolysis bullosa. Alpha-fetoprotein should be monitored in pregnancies associated with unexplained high maternal serum alpha-fetoprotein. A management strategy based on ultrasound examination, second maternal serum alpha-fetoprotein assay and amniocentesis is proposed to improve prenatal counseling and management of such pregnancies. However, a prospective study remains necessary to evaluate it.

(2) Source: J Genet Couns. 2011 Jun;20(3):287-93

Title: Reasons for adult referrals for genetic counseling at a genetics center in Izmir, Turkey: analysis of 8965 cases over an eleven-year period

Authors: Cogulu O, Ozkinay F, Akin H, Onay H, Karaca E, Durmaz AA, Durmaz B, Aykut A, Pariltay E, Kirbiyik O, Gunduz C, Ozkinay C

Abstract: A limited numbers of published studies evaluate the referral reasons for genetic counseling services in the literature. These studies are focused on prenatal genetic counseling services, in particular, prenatal diagnosis. In order to provide the most effective and helpful genetic counseling services, genetics professionals need adequate knowledge about the profile of individuals referred for these services. In addition, physicians need increased awareness of the nature of genetic issues in order to make appropriate referrals. This study was intended to provide a descriptive analysis of the referral reasons of patients that received genetic counseling at a genetics center in Izmir, Turkey during an 11-year period. A total of 8965 records generated between 1998 and 2008 from one genetic center (which consists of The Department of Medical Genetics and Division of Pediatric Genetics) were evaluated retrospectively. Of these, 6,258 involved referrals for prenatal reasons, and 2,707 involved referrals for postnatal reasons. Both prenatal and postnatal records were further classified into more specific categories of referral reasons. The most common reason for genetic counseling among the prenatal patients was advanced maternal age (42.0%), followed by high risk results on prenatal biochemical screening tests such as second trimester double test [(serum concentration of alphafetoprotein (AFP), beta-human chorionic gonadotropin (beta-HCG)], triple test (serum concentration of AFP, beta-HCG, oestriol) and integrated test (26.5%). The most common indications for postnatal patients were recurrent miscarriages (28.2%) and infertility (19.7%). A significant increase in number of specific categories of referrals for genetic counseling was observed for the last 3 years after the establishment of the Medical Genetics Department. These data provide useful information about the frequency of referrals to the genetics department, and the feasibility of genetic services. Organization of genetic services and systematic procedures for genetic counseling and genetic testing may improve the public's awareness of genetics and ensure a high standard of patient care.

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/AlphafetoproteinTesting.htm

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