Prediction of risk of trisomy 18 in fetuses with isolated choroid plexus cysts: a novel patient-specific algorithm

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Prediction of Risk of Trisomy 18 in Fetuses with Isolated Choroid Plexus Cysts: A Novel Patient-Specific Algorithm

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Myriam Almeida Fernandes

2004
Abstract

PREDICTION OF RISK OF TRISOMY 18 IN FETUSES WITH ISOLATED CHOROID PLEXUS CYSTS: A NOVEL PATIENT–SPECIFIC ALGORITHM. Myriam Almeida Fernandes, Inane Mendilcioglu, Utku Oz, and Ray Bahado–Singh. Section of Maternal Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT.

The purpose of this study was to develop a screening algorithm for Trisomy 18 risk estimation in mid-trimester fetuses with isolated choroid plexus cysts, i.e. cysts with no gross anomalies on ultrasound. Maternal age, serum triple screen marker levels, femur length, as well as soft sonographic markers were documented in all cases of isolated choroid plexus cysts. The data series included nine Trisomy 18 and 1,045 normal fetuses with isolated choroid plexus cysts. Stepwise logistic regression analysis of the data was performed to identify which marker variables were significant predictors of Trisomy 18 risk in this population of fetuses. The resultant screening algorithm allowed for the calculation of patient–specific risk estimates based on the significant maternal serum markers, unconjugated estradiol and human chorionic gonadotropin, as well as maternal age. The diagnostic accuracy of the model was determined from the receiver operating characteristics (ROC) curve. The sensitivity and false positive rates were 66% and 0.7%, respectively, with the area under the ROC curve of 0.92 (P < .00001). In conclusion, we report a novel, highly sensitive screening algorithm, which combines maternal age and serum levels of unconjugated estradiol and human chorionic gonadotropin, for Trisomy 18 risk estimation in mid–trimester fetuses with isolated choroid plexus cysts.
Acknowledgments

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I. Introduction

A central question in the field of Maternal–Fetal Medicine is when and how to screen for, diagnose, and prevent illnesses which lead to perinatal mortality. With the continued improvement of imaging resolution, prenatal sonography now routinely identifies subtle structural variations of controversial significance. One such example is the presence of isolated choroid plexus cysts in the mid–trimester sonography, i.e. in cases of choroid plexus cysts with no gross anomalies on ultrasound. Research conducted over the past two decades has not only extensively characterized these fetal structures, but have unveiled a significantly increased risk of Trisomy 18, or Edward’s Syndrome, in fetuses with such cysts. Much debate remains, however, on whether “isolated” choroid plexus cysts alone are associated with a significant risk of Trisomy 18 or are an indication for such invasive diagnostic procedure as genetic amniocentesis. Further a precise method of estimating the patient–specific Trisomy 18 risk level in fetuses with isolated choroid plexus cysts is currently lacking.

In this study, we set to establish a new algorithm based on variables such as maternal age, serum screen markers, as well as ultrasonographic biometry to derive patient–specific risk estimates of Trisomy 18 in a population of fetuses with choroid plexus cysts. It is proposed that such multivariate algorithm will allow for a more sensitive detection of Trisomy 18 risk in fetuses with isolated choroid plexus cysts. Lower false–positive rates and higher sensitivity values are related with several potential benefits, including: reduced medical costs related to a lower number of genetic amniocentesis that will be performed in the future; reduced iatrogenic loss of normal
fetuses due to amniocentesis, improved detection rate for mid-trimester Trisomy 18 fetuses presenting with choroid plexus cysts on prenatal ultrasound; as well as higher level of reassurance of normal karyotype in the vast majority of women with choroid plexus cysts in the mid-trimester of pregnancy. The most significant benefit of precise risk estimation of Trisomy 18 in cases of isolated choroid plexus cysts is that it allows for patients to make informed decisions regarding the acceptance or refusal of invasive prenatal diagnostic procedures.

A. Characterization of choroid plexus cysts

The first published reports of choroid plexus cysts suggested that these are common findings in brain autopsy studies. In 1899, Findlay published a series of postnatal brain autopsies from 64 previously asymptomatic patients and established the prevalence of choroid plexus cysts as 57 percent in that cohort (1). The formation of choroid plexus cysts, however, was not elucidated until 1966, when Shuangshoti and Netky studied the histogenesis of the lateral ventricle at different stages in development (2, 3).

Fetal choroid plexi begin forming between the seventh and ninth weeks of gestation as a thick pseudostratified ependymal layer, which invaginates into the medial walls of ventricles and appear as lobulated protrusions. This portion of the ventricle neuroepithelium covers a stroma of mesenchymal tissue derived from the pia mater, and is rich in rapidly proliferating capillary nets with fenestrated vascular walls. During this period, as its underlying capillary nets change into wavy longitudinal non-branching loops, the lobulated protrusions of choroid plexus transform into undulated structures.
At the ninth week of gestation, the pseudostratified cells of the epithelium are replaced with simple columnar and cuboidal cells. As the neuroepithelium grows and thins, the choroidal stroma becomes increasingly loose and its lining forms villi which project into the ventricles. By the eleventh and sixteenth weeks of gestation, the plexi usually reach maximal size and occupy approximately three-fourths of the ventricles. The large amount of stroma mesenchyme, produced up to the sixteenth week of gestation, regresses and is interspersed with connective tissue fibers between the seventeenth to the 28th weeks of gestation. From the 29th week of gestation to term, the stroma is progressively replaced by connective tissue fibers (Figures 1–4) (2, 3, 4).

It is hypothesized that cyst formation is intrinsically related to the histologic development of the choroid plexus. The unifying theory is that invagination of neurepithelium villi, coupled with extensive growth of loose mesenchymal stroma, occasionally lead portions of the choroidal projections to become buried in the stroma or to “pinch off” the ventricle walls resulting in choroid plexus cysts. It is believed that these cysts are filled with cellular debris from the choroid plexus mesenchyme, as well as with a cerebral spinal fluid that is subsequently produced by its secretory ependymal cells (3, 5, 6). If these structures are too large and grow adjacent to the plexus epithelium they might not be readily visualized. As the amount of stroma regresses, during the seventeenth to the 28th weeks gestation period, the cysts temporarily become more noticeable, but most eventually regress as well. This accounts for the transient nature of the majority of cystic structures in the choroid plexus and correlates with the time course of cyst formation and disappearance in clinical cases (5, 6). Moreover, studies done in brains of children and adults show that choroid plexus cysts are lined by a fibrous outer
layer and an inner membrane of cuboidal epithelium which is consistent with the known histological elements of choroid plexi at full term (6).

More recently, it has been reported that the wall of choroid plexus cysts are lined with angiomatous capillaries, and that this irregular vessels might reflect a failure in the vascular transformation from the lobulated to the wavy pattern of choroid plexus structures. Furthermore, it has been suggested by the same group that in some instances ultrasonographically diagnosed choroid plexus cysts are in actuality “pseudocysts” with no true epithelial lining that resulted from fluid accumulation and stromal expansion in areas of angiomatous capillary malformation (Figure 5-6) (4, 5).

B. Significance of choroid plexus cysts and the link to Trisomy 18

Incidental cysts of the choroid plexus have consistently been reported to occur in 57 to 77 percent of cases from serial postnatal autopsy studies (1, 2). Shuangshoti and Netky further demonstrated that the prevalence of choroid plexus cysts varied little throughout the human development as they examined choroid plexi of 124 brain autopsy cases. Thirty two of these cases were fetuses between thirteen weeks of gestation and full-term, and the remainder 92 cases consisted of patients one month of age to 90 years old. They reported a prevalence of cysts in 34 percent in fetuses and infants, 77 percent of cases in 1 to 30 year old group, 72 percent in the 31 to 60 year old group, and 76 percent in the 61 to 90 year old cohort. Rarely, choroid plexus cysts are clinically diagnosed in patients with intermittent obstruction of the cerebral spinal fluid circulation. In the vast majority of cases however, the cysts are usually less than one centimeter (cm) in diameter and do not cause obstruction of the cerebral spinal fluid within the ventricles.
Because of their transient nature in fetuses and high prevalence in asymptomatic patients, it was initially concluded that choroid plexus cysts were a normal variant in brain development (2, 4).

With advances in antenatal sonography, transient cysts of the choroid plexus were observed during screening surveys of mid–trimester gestations. In 1984, Chudleigh et al. were the first to report cases of choroid plexus cysts diagnosed by ultrasound in fetuses between the seventeenth and nineteenth weeks of gestation. The cysts were bilateral in the posterior horn of the lateral cerebral ventricles. All three female and two male fetuses underwent regression of the cysts between the 20\textsuperscript{th} and 23\textsuperscript{rd} weeks of gestation. At term, they had appropriate weight for gestation age, as well as normal neonatal ventricular sonographic screenings and neurologic examinations (7). Given the normal outcome of the fetuses and the previous autopsy studies, they also speculated that choroid plexus cysts were an incidental normal finding on mid–trimester ultrasound.

This belief was further supported by small studies that looked for possible postnatal sequelae of choroid plexus cysts diagnosed in utero. Digiovanni et al. followed 76 phenotypically normal infants diagnosed prenatally with choroid plexus cysts. After a period mean of $35.5 \pm 16.2$ months, all patients were reported to have normal Denver II Developmental Screening Tests (8). Similarly, Riebel et al. followed a group of 70 neonates and infants with neonatal ventricular sonographic screenings until they reached ten month of age. This cohort, which had a three percent prevalence of choroid plexus cysts also revealed that all patients were neurologically normal (9).

In accordance with the autopsy and neonatal surveys, it is not surprising that cysts of the choroid plexus have become relatively common findings on mid–trimester
ultrasound screenings. The reported incidence of fetal choroid plexus cysts, diagnosed by ultrasound between the fourteenth and 24th weeks of gestation, ranges from 0.18 to 3.6 percent, depending on the population surveyed (10–23). More recent and homogeneous meta-analyses, however, have established the incidence of choroid cyst between one percent (n=2,188/215,545) and 1.2 percent (n=1235/106,725) in the general population (24, 25). Similarly, the incidence of isolated choroid plexus cysts in women less than 35 years old is also reported as one percent (n=1016/106,725). However, no incidence data has been published in groups of women greater than 35 year old. The majority of cases in the current literature cite the conservative value of one percent as the overall incidence of isolated choroid plexus cysts (24, 25).

Following the publication of Chudleigh et al., several studies have explored the link between choroid plexus cysts and adverse neonatal outcomes. In 1986, Nicolaides and colleagues made the first report of choroid plexus cysts as a marker for aneuploidy. They described four cases of antenatally diagnosed bilateral choroid plexus cysts of the posterior horn of the lateral ventricles. One of the fetuses was diagnosed at eighteen weeks of gestation, underwent regression of cyst at 23 weeks of gestation, and was normal at term. The remaining three fetuses, diagnosed between the eighteen and 24th weeks of gestation, were found to have Trisomy 18. Of note, one of these fetuses with Trisomy 18 had isolated choroid plexus cysts, i.e. cysts with no other associated anomaly on ultrasound (26).

Despite a number of case reports and population surveys over the past two decades, the incidence of aneuploidy in mid–trimester fetuses with choroid plexus cysts ranges widely in the literature. Selecting for all series of fetuses between the fourteenth
and 24th weeks of gestation, the reported incidence of aneuploidy is 2.5 percent on average but ranges from zero and eleven percent (n=60/2411; range 0% to 11%). In the same cohort of fetuses, the overall incidence values for Trisomy 18 and Trisomy 21 (Down’s Syndrome) are 1.7 percent (n=41/2441; range 0% to 5.3%) and 0.5 percent (n=13/2441; range 0% to 5.2%), respectively (10–23, 27, 28). Such discrepancy is likely explained by the small number of cases that comprised some of the studies, as well as by inherent differences in the sensitivity of sonographic detection of choroid plexus cysts and in the heterogeneity of populations studied.

To further elucidate the characteristics of choroid plexus cysts in the setting of aneuploidy, some groups have looked at the cysts size and number, bilaterality, its persistence or resolution, and fetal sex, as possible prognostic indicators. Although a few small series (n <100) have described significantly larger cysts in fetuses with aneuploidy, series with more than 100 cases surveyed have shown no significant differences in the size of choroid plexus cysts between fetuses with and without aneuploidy (13, 27, 29, 30). In fact, 60 to 80 percent of cysts reported in all cases were less than one cm in diameter (17, 19, 31, 32). Similarly, no correlation with the risk of aneuploidy was unveiled when looking at whether cysts were unilateral or bilateral, persisted or regressed with gestation, and whether the fetuses were male or female (17, 19, 31–33).

Yet other groups have compared subpopulations of fetuses with cysts that occur with and without additional structural anomalies and their relation to aneuploidy. The well established fact that aneuploidy risk increases with advancing maternal age lead some to use a more clinically appropriate method such as likelihood ratio (LR) to calculate the increased risk of aneuploidy in fetuses with cysts. In 1997, Gupta et al
showed that the fetuses with isolated choroid plexus cysts have a Trisomy 18 risk increase by a factor of 9.04 (95% confidence interval (CI), 4.16–19.11) with regards to the a priori risk associated with maternal age. For fetuses with other structural anomalies in addition to the choroid cysts, the LR is increased up to 1754 (95% CI, 1261–2465) (34). The combined incidence of aneuploidy for mid-trimester fetuses with structural anomalies in addition to choroid plexus cysts averages 29 percent (n=32/109; range 11% to 75%). For Trisomy 18 and Trisomy 21, the incidence values average twenty percent (n=22/109; range 3.6% to 50%) and 6.4 percent (n=7/109; range 3.6% to 22%) respectively. Not surprisingly, the incidence of aneuploidy among mid-trimester fetuses with choroid plexus cysts alone is much lower and averages 0.8 percent (n=14/1673; range 0% to 4.1%). For Trisomy 18 and Trisomy 21, specifically, the mean incidence values are reported as 0.5% (n=8/1673; range 0% to 2.1%) and 0.3% (n=5/1673; range 0% to 1.4%) (18–20, 23, 27, 28, 35). Furthermore, Chitty et al. showed, in 1998, that when maternal age is considered, the incidence of aneuploidy in fetuses with isolated cysts where the mother is under 36 years of age was 0.36% (95% CI, 0.04–1.3%) compared to 2.4% (95% CI, 0.06–12.6%) in older women (23).

To date Trisomy 18 has been the only aneuploidy shown to have a statistically significant link with choroid plexus cysts per se. Recent meta–analyses of the published literature have reported LR values of 7.09 (95% CI, 3.97–12.18), 9.04 (95% CI, 4.16–19.11), and 13.8 (95% CI, 7.72–25.14, P <0.001) related to the increased risk of Trisomy 18 in fetuses with isolated choroid plexus cysts. The differences in LR values are reflective of variations in the inclusion criteria for studies in the meta–analyses, such as the type population surveyed, the prevalence of choroid plexus cysts, as well as the
accuracy of sonographic screenings (34, 36–38). It also suggests the lack of a single study that is statistically powerful enough to be considered alone.

Given the demonstrated increased risk of Trisomy 18 when fetuses with choroid plexus cysts have additional structural anomalies on ultrasound, it has become standard of care to offer amniocentesis for prenatal karyotyping in such cases.

C. The role of amniocentesis in cases of isolated choroid plexus cysts: When is it acceptable to offer or withhold invasive antenatal diagnosis testing?

Despite a myriad of publications on the topic and the well established association described above, it remains unclear when the risk of Trisomy 18 from fetuses with isolated choroid plexus cysts is high enough to warrant invasive diagnostic testing such as amniocentesis.

Such debate has endured for a number of pertinent reasons. First is the incidence of choroid plexus cysts, which is reported to be as high as one in 100 pregnancies during mid–trimester ultrasound screenings. It is not infrequent, thus, that the issue of counseling on choroid plexus cysts and Trisomy 18 risk arises in routine sonographic screenings (15, 17, 39–41).

Second is the relatively low incidence of Trisomy 18. Despite being the most common mid–trimester autosomal aneuploidy after Trisomy 21, Trisomy 18 usually occurs in one in 3,000 to 5,000 cases of second trimester fetuses screened and one in 8,000 births (42–45). Therefore, there is a need for large enough studies (n ≥1,000) with the statistical power necessary to estimate patient–specific risks of Trisomy 18 in cases of isolated choroid cysts. Most prospective series published on choroid plexus cases have
less than 250 choroid plexus cysts cases per study (10–19, 21, 27–29, 31, 35, 46–50), a few have between 250 and 850 cases (20, 22, 23, 32, 41, 51), and only one sonographic study has more than 1,000 cases of choroid plexus cysts (45). Furthermore, it is difficult to interpret the majority of data published because some groups convey the overall, rather than patient-specific, risk of Trisomy 18 in the setting of isolated cysts. In fact, the few studies that have looked at factors such as maternal age, serum or sonographic markers, as modifiers of the a priori individual risks of Trisomy 18 in fetuses with isolated choroid plexus cysts, have demonstrated a more practical and sensitive screening approach for Trisomy 18 (34, 36, 38, 45, 51–55).

Third is the procedure-related risk of invasive testing used in prenatal diagnosis. Genetic amniocentesis is the most frequently performed invasive diagnostic procedure in mid-trimester fetuses and carries an associated risk of miscarriage. The most cited study regarding amniocentesis-related fetal loss is the 1976 NIH collaboration with reported a 0.3% procedure-related fetal loss rate (56). A better estimate of excess pregnancy loss, however, is a prospective study involving 1,040 cases and 992 controls published by Taber and colleagues in 1986. They reported an increased risk of miscarriage of 0.8% over controls (2.1% versus 1.3%, relative risk (RR) 1.6, 95% CI, 1.02 to 2.52) in a population of women between 25 and 34 years of age (57). More recent surveys have reported a procedure-related risk of fetal loss between 0.4 and 0.6 percent (58,59). Interestingly, when women with a history of first or second trimester abortions or vaginal bleeding during the current pregnancy are excluded, the procedure-related fetal loss rate was only 0.03%, and was statistically comparable with that of controls (59). Generally, however, the risk of miscarriage with such procedure is still quoted as one in 200 to 300
cases, and amniocentesis is recommended when the risk of aneuploidy is greater than or equal to one in 200 to 300 pregnancies.

Finally, the cost–benefit analysis of either offering or withholding amniocentesis has also been explored since, to a lesser extent, genetic counseling depends on the natural course of the illness in question and its related public health costs. Vintzileos et al. demonstrated in 1998 that it is not cost–effective to routinely offer amniocentesis in all cases which increased Trisomy 18 risk were derived solely from choroid plexus cysts or abnormal serum markers (60). Although this recommendation is adequate from a purely economic standpoint, it does not take into account the medical and psychosocial benefits that prenatal diagnosis can bring to subsequent decisions regarding care (51). In the case of Trisomy 18, only two thirds of fetuses diagnosed at mid–trimester are born alive, and these have a mean life expectancy of five to six days. Of the live born neonates, only 10 percent survive to six months, and less than five percent survive to their first year of life (42, 43, 61). Estimates of life expectancy of Trisomy 18 infants are crucial because practitioners and parents alike not only factor in the risks quoted, but also the expected life span and quality of life for the infants. Furthermore in practice, issues pertinent to the patient’s autonomy, such as their religious or personal beliefs, generally outweigh cost–benefit analyses when it comes to decisions regarding prenatal care (42, 62).

While there are numerous ethical, medical, and financial considerations underlying decisions in prenatal diagnosis, the overall goal is to target populations with the highest risk for diagnosis but minimize unnecessary normal fetal loss secondary to amniocentesis. Researchers have continued to refine the estimation of the patient specific–risk of Trisomy 18 in the case of isolated choroid plexus cysts. Some found that
women less than 35 years are at “low-risk” of aneuploidy compared to the risk of pregnancy loss associated with amniocentesis, and have defined a “high-risk” group as those with the risk of Trisomy greater than one in 132, which is the mid-trimester odds for all aneuploidy in a 35 year old woman (24). Other groups use the risk value from Snijders et al. (63) of one in 258 for maternal age–related risk estimates of Trisomy 18 to define a high–risk group, and some recommend amniocentesis starting at age 32 to 35 years old in cases of isolated fetal choroid plexus cysts (34, 36, 38, 53, 63).

D. Risk assessment variables in prenatal screening: maternal age, biochemical serum markers, and ultrasonographic biometry

Maternal age is the most common variable used for risk assessment in cases of suspected aneuploidy. This is largely due to meiotic non–disjunction, which is known to increase in frequency as patients age. As a screening tool, maternal age alone has a sensitivity of approximately 30 percent and a specificity of 95 percent for Trisomy 21 (64). The current standard of care is to offer amniocentesis to all women over the age of 35 because the procedure–related miscarriage risk approaches the odds of Trisomy 21 at maternal age 35. This risk value is approximately one in 258 at the mid–trimester and one in 385 at birth (53, 63, 65). Despite the above recommendation, one study has shown that up to two thirds of women 35 years old and older decline amniocentesis (62). Furthermore, the majority of aneuploidy cases occur in patients less than 35 years old since a much greater proportion of pregnant women belong to this younger age group. Additional sonographic markers have been incorporated to improve detection of pregnancies at higher risk of aneuploidy. As discussed earlier, in the case of isolated
choroid plexus cysts, advanced maternal age is an important parameter in the estimation of the overall risk of Trisomy 18 (22).

Biochemical markers have been used in prenatal screening for almost three decades. Elevated maternal serum alpha–fetal protein (AFP) in was first used to detect fetal neural tube defects. In the 1980’s, it became a tool for aneuploidy screening and shortly thereafter two additional markers, unconjugated estradiol (uE3) and human chorionic gonadotropin (hCG), were incorporated into aneuploidy screening (66–69). The three analytes combined constituted the maternal serum triple marker test. Furthermore, the integration of maternal serum marker levels with maternal age risk, has allowed for the estimation of a patient–specific risk of aneuploidy. Triple serum markers as adjunct to maternal age were initially used for the detection of Trisomy 21. At risk threshold of approximately one in 200, the sensitivity in Trisomy 21 detection is approximately 60 percent with a specificity of 95.3 percent. Subsequent studies, by Palomaki and colleagues, showed the triple serum test was more effective in the detecting of Trisomy 18, and that significantly lower levels of serum AFP, uE3, and hCG levels were noted in such cases. Using the same risk threshold of one in 200, the sensitivity of identifying Trisomy 18 is 68 percent and the specificity 99.6 percent. Although, it has been shown that serum markers are reliable parameters in Trisomy 18 risk adjustment in fetuses with isolated choroid plexus cysts, the precise detection rate in this population remains to be determined due to the absence of large enough published series (53, 54).

Mid–trimester sonographic screening has become routine in the assessment of aneuploidy as well because associated anomalies are frequently visualized during this gestation period. Nicolaides et al. described chromosomal anomalies in fourteen percent
of fetuses with structural defects or growth restriction. Of these, four percent were diagnosed with Trisomy 18, 3.3 percent with Trisomy 21, and 1.5% had Trisomy 13. Although, the positive predictive value for detecting aneuploidy was increased to 29 percent when more than one defect was present, two percent of chromosomally abnormal fetuses had a single anatomical defect on ultrasound (73).

The main sonographic anomalies of Trisomy 18 fall into two categories: one consists of subtle or nonstructural findings, which are often nonspecific, and the other of gross anatomical defects. The most reported subtle markers of Trisomy 18 on second trimester ultrasound include nuchal fold thickness, pyelectasis, bowel ecogenicity, long bone shortening, brachycephaly or strawberry-shaped head, two-vessel umbilical cord, and the most debatable of all, the choroid plexus cysts (Figures 7 – 10). Major structural anomalies in fetuses with Trisomy 18 include congenital heart defects, cystic hygroma, neural tube defects, facial cleft, micrognathia, macroglossia, omphalocele, as well as skeletal defects such as clenched hands and club fleet (Figures 11 – 12). Intrauterine growth restriction (IUGR) is considered a major anomaly as well but is usually noted in the third trimester (73–79).

As with the biochemical serum levels, the sensitivity of these gross ultrasound defects in detecting Trisomy 18 has been well recognized. Several small studies have reported detection rates of anomalies ranging between 64 to 85 percent in fetuses with Trisomy 18. The differences in the sensitivity of sonographic screenings for structural defects often reflect disparities in the quality of ultrasonography, the inclusion criteria for positive findings, and gestational age of the fetus (75, 77–80). More recently, subtle ultrasound markers have been incorporated in sonographic screenings in an attempt to
improve the accuracy of ultrasound findings in mid-trimester fetuses (77, 81). This concept of the ‘genetic sonogram’ emerged in 1995 from studies which proposed that ultrasound screenings could be used to refine the a priori risk of aneuploidy (81–83). One approach to derive patient-specific risk estimates is to create multivariate algorithms combining sonographic findings with maternal age or serum triple screen marker data.

Among the most comprehensive studies using the concept of genetic sonogram and incorporating choroid plexus cysts is a series from Bahado-Singh and colleagues, which recently developed two mid-trimester ultrasound algorithms for Trisomy 18 risk estimation. Both algorithms were created from a database of over 1,200 cases. The first algorithm incorporated choroid plexus cysts, femur length, two-vessel cord, as well as maternal age, and had a sensitivity of 61.7 percent and false-positive rate of 1.5 percent. The second algorithm, which included gross anomaly, femur length, choroid plexus cysts, and maternal age, was diagnostically more accurate than both the first algorithm (sensitivity and false positive rates of 72.3 and 0.9 percent) and gross anomaly considered alone (sensitivity of 59.6 percent, false positive rates 0.8 percent). Not surprisingly, the best markers for Trisomy 18 were gross structural anomalies (sensitivity 59.6 percent, false positive rate of 0.8). The risk of Trisomy 18 was also increased by the occurrence of soft markers such as choroid plexus cysts or two-vessel umbilical cord (sensitivity 36.2 and 12.8 percent, false positive rate 4.2 and 1.1 percent, respectively). Unlike two-vessel cord, however, isolated cysts did achieve statistical significance when gross anomalies were considered in the regression analysis. The improved sensitivity of the second algorithm substantiates the fact that subtle markers help to refine the sonographic screening of Trisomy 18 in mid-trimester fetuses. Furthermore, because they
incorporated maternal age as one of the variables, these algorithms can provide more practical estimates of patient-specific, rather than general categories such as “high” vs. “low” risk groups (45).

Integrating maternal serum marker data with ultrasound algorithms, based on maternal age and specific sonographic markers, may further improve our ability to assess the risk Trisomy 18 in a population of fetuses with isolated choroid plexus cyst, thus reducing unnecessary invasive prenatal testing and much of the parental anxiety involved in decision making during pregnancy.
Figure 1. Embryonal Choroid Plexus. Sagittal view of fetal specimen with choroid plexus in the lateral cerebral ventricle at 10th weeks gestation. Magnification is approximately 10x. Adapted from Kraus et al. 2002.

Figure 2. Vessels of the Embryonal Choroid Plexus (arrow). India ink injected specimen of dissected choroid plexus at 10th weeks gestation. Magnification is 20x. Adapted from Kraus et al. 2002.
Figure 3. Choroid Plexus in the Lateral Cerebral Ventricles. Scanning electron micrograph image of embryo specimen at 12 weeks of gestation. At this stage the bulky embryonal structure begins folding into the wavy fetal structure. Adapted from Kraus et al. 2002.
Figure 4. Normal Fetal Choroid Plexus. Fluorescent picture under dissecting microscope, showing choroid plexus as wavy folds containing capillaries (arrow). Fetus specimen at 21 weeks of gestation. Magnification is approximately $30\times$. Adapted from Kraus et al. 2002.
Figure 5. Choroid Plexus Cyst. Fluorescent picture under dissecting microscope of a choroid plexus cyst with angiomatous capillaries in its walls. The normal portion of the plexus exhibits typical folds. Specimen derived from a fetus at 23 weeks of gestation. Magnification is approximately 40×. Adapted from Kraus et al. 2002.

Figure 6. Angiomatous Capillaries in the Walls of a Choroid Plexus Cyst. Twenty-three week fetus specimen. Magnification is approximately 20×. Adapted from Kraus et al. 2002.
Figure 7. Normal Choroid Plexus. Axial sonography view of the fetal head at 17 weeks gestation. Courtesy of Wendy Shaffer. Yale New Haven Hospital Perinatology ultrasound database, Yale University.
Figure 8. Choroid Plexus Cysts. A. Unilateral cyst on an axial sonography image of a fetal head at 17 weeks gestation. B. Bilateral choroid plexus cysts on an axial view of a fetal head at 17 weeks gestation. Courtesy of Wendy Shaffer. Yale New Haven Hospital Perinatology ultrasound database. Yale University.
Figure 9. Normal Umbilical Cord. Doppler sonography image with color flow mapping of a mid-trimester fetus with a three vessel cord (the 2 umbilical arteries are in red). Courtesy of Wendy Shaffer. Yale New Haven Hospital Perinatology ultrasound database, Yale University.
Figure 10. Two–vessel Cord. A. Tranverse view of the umbilical cord of a fetus at 17 weeks gestation, showing a single artery (arrow) and one vein. B, Doppler sonography image with color flow mapping of a mid–trimester fetus with a two–vessel cord (artery in red, vein in blue). RT– right, LT– left. Courtesy of Wendy Shaffer. Yale New Haven Hospital Perinatology ultrasound database, Yale University.
Figure 11. Club Feet. Example of a Gross Structural Anomaly. Sonographic image of fetal lower extremity at 15 weeks gestation, showing abnormally foot. Courtesy of Wendy Shaffer. Yale New Haven Hospital Perinatology ultrasound database, Yale University.
Figure 12. Edward’s syndrome (Trisomy 18). A, Note features characteristic of Edward’s Syndrome including: low-set slanted auricles, underdeveloped ears, micrognatia, small eyes, nose, and mouth. B, Barrel chest with narrow pelvis. C and D, Underdeveloped first digits, hypertonicity as evidenced in contractures and clenched fists. E and F, Femur and radial aplasia, as well as rocker bottom feet. The patient also had a single umbilical artery (two vessel cord), a horseshoe kidney, and choroids plexus cysts (0.5x0.5x0.5cm). Courtesy of the Autopsy Service, Department of Pathology, Yale New Haven Hospital, Yale University.
II. Statement of purpose and hypothesis

We collected a large database of isolated choroid plexus cyst cases with the purpose of assessing of Trisomy 18 risk in this population of fetuses. A multivariate algorithm taking into account the risk of Trisomy 18 based on maternal age, maternal serum levels of AFP, hCG, and uE3, as well as significant sonographic markers can be generated from the database. We propose that such algorithm will allow for more precise patient-specific risk estimates of Trisomy 18 in fetuses with isolated choroid plexus cysts. Furthermore, it is expected that the increased sensitivity and decreased false positive rates of the new algorithms will carry several potential benefits, including:

1. Reduced medical costs related to the lower number of genetic amniocentesis that will be performed in the future;
2. Reduced iatrogenic loss of normal fetuses due to amniocentesis;
3. Improved detection rate for mid-trimester Trisomy 18 fetuses presenting with isolated choroid plexus cysts on prenatal ultrasound; and
4. Higher level of reassurance of normal karyotype in the vast majority of women with choroid plexus cysts in the mid-trimester of pregnancy.
III. Methods and Experimental Design

The study was conducted using data selected between September of 1989 and February of 2004 from the Genetics and Ultrasound databases at the Perinatal Unit, Yale University Department of Obstetrics and Gynecology in New Haven, Connecticut. In addition, cases between June of 1995 and August of 2002 from the Departments of Obstetrics and Gynecology at the University of Connecticut Health Center, Kaiser Permanente Center in Oakland, California, as well as the Reproductive Genetics Center in Denver, Colorado were also included in this series. Research funding was provided by the Medical Student Research Training Fellowship from the Yale University School of Medicine. Its protocol was approved by the Institutional Review Board at Yale University School of Medicine, in New Haven, Connecticut.

A. Description of the protocol for database creation and review

During this period, 1,149 cases of singleton pregnancies between 14 and 24 weeks of gestation were diagnosed with choroid plexus cysts on mid-trimester sonographic screenings, and also had maternal triple serum marker data available. Of these, 1,045 patients showed no gross anomalies on ultrasound. For the purpose of this study, only cases of isolated choroid plexus cysts, i.e. cases with no gross anomalies identifying on ultrasound, were considered. The study cases consisted of Trisomy 18 fetuses with isolated choroid plexus cysts.

The ultrasound information was recorded at the time of the sonogram on a computer database. The gestational age was based on last menstrual period (LMP),
rather than ultrasound measurements, since severe early-onset fetal growth restriction is a feature of Trisomy 18 fetuses. A systematic survey of all organ systems recognizable on ultrasound was performed, starting from the fetal cranium to the extremities and the umbilical cord. The survey was first performed by a sonographer and then repeated by a physician. There was no significant change in our survey technique over the course of the study. For each case in the database, the following variables were reviewed: maternal age, indications for the scan, LMP, gestational age estimate, presence or absence of gross morphological defects, and characteristic of soft markers findings such as choroid plexus cysts, two-vessel umbilical cord (2-VC), pyelectasis, intracardiac ecogenic focus, and abnormal fetal femur length (FL).

Outcome information was obtained by reviewing fetal karyotype data from patients which underwent genetic amniocentesis, as well as maternal and newborn medical records for those without karyotype information. Karyotype data was obtained by reviewing Giemsa-stained colonies of cells. This staining technique reveals specific banding patterns (G-band) in each homologous chromosome pair (Figure 13).

Maternal serum analyte levels were prospectively measured in all patients in the study; these included the AFP, hCG, and uE3 values.

B. Likelihood ratio calculations for specific markers

As with all continuous variables considered, the maternal serum analyte levels were expressed as gestation-specific multiple of the median (MoM) values and the distributions were normalized by log transformation of the MoM values. The measured analyte levels, expressed as MoM, were derived after population-based medians for
specific gestational ages at the respective laboratory testing center. All serum marker levels were appropriately adjusted at the laboratory testing centers for maternal body weight, type of gestation (single vs. multiple), diabetic status, and race (71).

Femur length is known to be significantly shortened in mid-trimester Trisomy 18 fetuses (84). This measurement is routinely obtained on all mid-trimester ultrasound screenings. In this study, the FL values were converted into multiples of the normal median by dividing the measured value by the expected normal median value based on gestational age. In addition, center-specific regression equations for LMP-based FL MoM values were calculated in order to obtain internal standardization of FL measurements, thus minimizing variability from differences in sonography equipments, measurement techniques, and population (race, body habitus, etc) that might arise between centers. Bahado-Singh and colleagues have previously generated femur length standardized values for Yale and the Reproductive Genetics Center in Colorado, from databases of 1167 and 226 normal mid-trimester fetuses, respectively. The reported inter-observer coefficient of variation for FL between these two centers was 4.1%. The MoM Trisomy 18 FL values from a particular site were similarly generated by dividing the measured value by the expected normal median from that center (45, 85).

In order to derive the Gaussian distribution curves of normal and Trisomy 18 populations, the probability graphs of MoM $\log_{10}$ transformation were plotted for each variable. Straight lines, resulting from each probability graph, suggest that the log transformed parameters follow a Gaussian distribution over a wide range of values. The truncation limits were defined as $\pm$ 2.5 standard deviation (SD) to exclude the range of values in which the parameters do not have a Gaussian distribution. At a given MoM
parameter, the likelihood ratio (LR) or odds of Trisomy 18 can be determined by dividing the height measurement of the Trisomy 18 population curve by that of the normal population curve at the corresponding MoM value (45, 71, 84) (Figure 14).

Maternal age–related Trisomy 18 risk estimates were derived as in previously published data as one–tenth the age–related risk of Trisomy 21. Although such estimation is likely oversimplified, it does correlates with the incidences of Trisomy 18 and 21 at birth (one in 8,000 and one in 750, respectively), and it takes into account the miscarriage rate of Trisomy 18 fetuses during the third trimester (65, 71, 84).

For non–continuous variables, such as the presence or absence of a 2–VC, the odds risk of Trisomy 18 was as defined in both the positive (+LR) and negative (−LR) likelihood ratios. The +LR were utilized when the variable was present, while in the absence of such variable, the −LR were used. The 2VC variable was included in the study because is known as a sensitive predictor of Trisomy 18 cases (90–93). Bahado–Singh and colleagues have calculated both the positive (+LR) and negative (−LR) likelihood ratios for 2VC in a population of Trisomy 18 fetuses. The +LR, defined as the ratio of sensitivity over false positive rate, was reported as 11.46, while the −LR, or the ratio of false negative rate to specificity, was only 0.88. (45, 86).

C. Generation of individual Trisomy 18 risk estimates

Stepwise logistic regression was the statistical method of choice in determining whether maternal age, serum analyte levels, and ultrasound markers (independent variables) were significant predictors of Trisomy 18 (dependent variable) risk in mid–trimester fetuses with isolated choroid plexus cysts (87, 88). The mean, standard
deviation (SD), and correlation coefficient for each of the parameters in both normal and Trisomy 18 populations were determined. The median was used to estimate the mean. To minimize the effect of outliers, we calculated the SD by dividing the difference between the 10th and 90th percentiles by 2.53 (84).

For each independent variable, the Wald value was calculated. The Wald statistic determines whether a specific variable has no effect given that other variables are in the model. The null hypothesis is that the coefficient of the tested variable is zero, if rejected; the variable should not be removed from the model algorithm. For categorical variables, the Wald statistic has a degree of freedom equal to one less than the number of categories. If the variable has one degree of freedom, the Wald output is equal to the square of the ratio of the coefficient to its standard error (45).

The regression formula, based on the statistically significant variables, was generated as a computerized screening algorithm and was used to combine individual LR risk estimates of Trisomy 18. The overall probability values were expressed as an odds risk.

D. Statistical analysis and considerations

The sensitivity and FPR values of the algorithm at several threshold risk levels (i.e. greater than one in 50, or one in 100, etc) were generated using numerical integration and plotted as a receiver operating characteristics (ROC) curve. The optimal threshold risk level that maximizes the detection rates with low FPR was subsequently determined.

The overall performance of the algorithm as a screening method for Trisomy 18 was determined by the Hosmer–Lemeshow $\chi^2$ (Goodness–of–Fit) test, as well as by
calculating the area under the ROC curve and its corresponding $P$ value (88). The *null hypothesis* was that the area under the ROC curve was less or equal than 0.5.

Calculations were performed by the Statistical Program for Social Sciences (SPSS Inc., Chicago, IL) software under the guidance of Dr. Oz. All tests were two-tailed and a $P$ value of less than 0.05 was the threshold for statistical significance.
Figure 13. Karyotype of a Trisomy 18 Male (47 XY +18) Fetus. G–banding preparation of amniotic fluid cells shows an extra copy of chromosome 18 as the only consistent karyotypic change in this cell population (arrow).
Figure 14. Log Gaussian Distribution of FL MoM Values for Normal (solid squares) and Trisomy 18 (open squares) Populations. The Y-axis indicates frequency distributions in these populations. FLMoM – femur length multiples of the median.
IV. Results

The database consisted of a total of 1,149 cases of choroid plexus cysts that underwent mid-trimester sonograms and also had serum triple screen markers and fetal karyotype or postnatal exam. Of these, 104 cases were excluded because of gross anomalies on the ultrasound data or abnormal karyotype other than Trisomy 18. The remaining 1,045 cases with isolated choroids plexus cyst constituted the study group. Nine cases of isolated choroid plexus cysts had Trisomy 18, and the remainder cases either had a normal karyotype, or were presumed normal based on postnatal evaluation. No distinctions or subcategorization of the study group were made based the size, bilaterality or multiplicity of the choroid plexus cysts. Fifty one percent of the cases were obtained from the Yale ultrasound database. The remainder of the cases was from the collaborating institutions in California (18%), Colorado (14%), and Connecticut (17%) mentioned in the Material and Methods section.

The equivalence-of-means t-test revealed no significant difference in either the mean maternal age or gestational age at ultrasound between the normal and study cases (Table 1). Soft sonographic markers were noted among 62 karyotypically normal fetuses and one Trisomy 18 fetus. Please refer to Table 2 for the frequency of soft sonographic markers among normal and Trisomy 18 cases.

The main indication for referral to amniocentesis in this population was the detection of CPC on a prior scan. Table 3 gives the breakdown of indications for prenatal ultrasound in the study population.
Stepwise logistic regression analysis of the data revealed that the statistically significant predictors of Trisomy 18 in study group were maternal age, as well as maternal serum uE3 and hCG levels. The other serum and sonographic markers were not found to be statistically relevant in estimating Trisomy 18 risk in fetuses with isolated choroids cysts (Table 4).

The individual risk for Trisomy 18 based on maternal age, uE3, and hCG was calculated. Different risk threshold levels were used to screen for Trisomy 18 and the corresponding sensitivity, false positive rate, and estimated odds risk values associated with each threshold level were calculated (Table 5). The most appropriate Trisomy 18 risk threshold value was established as one in 147, rounded off to greater than one in 150, based on the receiver operating characteristic curve. At this threshold level, the algorithm sensitivity and corresponding false-positive rate are 66% and 0.7%, respectively. The model is a significant predictor of Trisomy 18 with a calculated area under the curve value of 0.92 (SE 0.08, \( P < 0.00001 \)). Please refer to Figure 15 for the receiver operating characteristic curve.
<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Trisomy 18</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of cases</strong></td>
<td>1,045</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><strong>Maternal age mean (SD)</strong></td>
<td>30.40 (6.58)</td>
<td>32.17 (4.83)</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Gestational age mean (SD)</strong></td>
<td>18.31 (2.16)</td>
<td>17.37 (1.33)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Table 1.** Mean Maternal Age and Gestational Age Data for Normal and Trisomy 18 Cases. SD – Standard deviation. n.s. – Non-significant.
Table 2. Frequency of Soft Markers among Normal and Trisomy 18 Cases. A total of 62 soft sonographic markers were reported in this study population.

<table>
<thead>
<tr>
<th>Sonographic Markers</th>
<th>Trisomy 18</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$</td>
<td>%</td>
</tr>
<tr>
<td>Intracardiac echogenic focus</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Two vessel cord</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pyelectasis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypoplastic fifth digits</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Indications</td>
<td>Trisomy 18</td>
<td>Normal</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>$N$</td>
<td>%</td>
</tr>
<tr>
<td>CPC on Prior Scan</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Advance maternal age</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td>Fetal Assessment or Screen</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Abnormal Triple Serum Screen</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td>Family History of Genetic Anomaly</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal AFP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Previous Pregnancy with Anomaly</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rule out Anomaly</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Potential Teratogen Exposure</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other Indications</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical or Placenta Assessment</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increase Trisomy Risk</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>Maternal Diabetes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None Reported</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3.** Indications for Amniocentesis. $N$ – Frequency, % – percentile. CPC – choroid plexus cysts. AFP – alpha-fetoprotein.
<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Wald $\chi^2$</th>
<th>Df</th>
<th>P</th>
<th>OR</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.12</td>
<td>6.59</td>
<td>1</td>
<td>0.01</td>
<td>1.12</td>
<td>1.03, 1.23</td>
</tr>
<tr>
<td>Log AFP</td>
<td>2.51</td>
<td>0.64</td>
<td>1</td>
<td>0.42</td>
<td>12.31</td>
<td>0.03, 5646.48</td>
</tr>
<tr>
<td>Log hCG</td>
<td>-5.23</td>
<td>11.54</td>
<td>1</td>
<td>0.001</td>
<td>0.005</td>
<td>0.002, 0.11</td>
</tr>
<tr>
<td>Log uE3</td>
<td>-17.75</td>
<td>14.95</td>
<td>1</td>
<td>.0001</td>
<td>0.00</td>
<td>0.00, .0002</td>
</tr>
<tr>
<td>Log FLMoM</td>
<td>-20.64</td>
<td>2.10</td>
<td>1</td>
<td>0.15</td>
<td>0.00</td>
<td>0.00, 1461.19</td>
</tr>
<tr>
<td>IEF</td>
<td>-0.80</td>
<td>0.06</td>
<td>1</td>
<td>0.80</td>
<td>0.45</td>
<td>.0008, 238.49</td>
</tr>
<tr>
<td>Pyelectasis</td>
<td>4.54</td>
<td>0.004</td>
<td>1</td>
<td>0.95</td>
<td>94.07</td>
<td>0.00, 4.38 E+61</td>
</tr>
<tr>
<td>Hypoplastic fifth digit</td>
<td>4.55</td>
<td>0.004</td>
<td>1</td>
<td>0.95</td>
<td>94.63</td>
<td>0.00, 3.18E+60</td>
</tr>
<tr>
<td>2-VC</td>
<td>8.59</td>
<td>0.03</td>
<td>1</td>
<td>0.86</td>
<td>5,395.97</td>
<td>0.00, 7.85E+43</td>
</tr>
<tr>
<td>Constant</td>
<td>-30.25</td>
<td>0.07</td>
<td>1</td>
<td>0.78</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Diagnostic Performance of Trisomy 18 Algorithm in Fetuses with Isolated Choroid Plexus Cysts. % – percentile; FPR – false positive rate.

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>FPR (%)</th>
<th>Risk (&gt;1:x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.11</td>
<td>0.00</td>
<td>3.38</td>
</tr>
<tr>
<td>22.22</td>
<td>0.00</td>
<td>4.23</td>
</tr>
<tr>
<td>33.33</td>
<td>0.00</td>
<td>8.82</td>
</tr>
<tr>
<td>44.44</td>
<td>0.00</td>
<td>36.05</td>
</tr>
<tr>
<td>55.56</td>
<td>0.38</td>
<td>76.55</td>
</tr>
<tr>
<td>66.67</td>
<td>0.68</td>
<td>146.91</td>
</tr>
<tr>
<td>77.78</td>
<td>2.90</td>
<td>508.36</td>
</tr>
<tr>
<td>88.89</td>
<td>3.86</td>
<td>730.60</td>
</tr>
<tr>
<td>100.00</td>
<td>38.23</td>
<td>29,223.05</td>
</tr>
</tbody>
</table>
Figure 15. Receiver Operating Characteristic Curve for Significant Markers of Trisomy 18: hCG and uE3. AUC – area under the receiver operating characteristic curve; SE – standard error; P – p-value.
V. Discussion

Despite the rarity of Trisomy 18, perinatologists are frequently called upon to assess patient-specific risks of having fetuses affected by Trisomy 18. Choroid plexus cysts are identified on approximately one percent of all pregnancies imaged in the mid-trimester. The reported overall risk of Trisomy 18 in mid-trimester fetuses with isolated choroid plexus cysts ranges widely from zero to one in 45 cases. Such discrepancy is due to the variations in the patient population, the quality of sonography screenings, as well as the number of cases per series (18, 20, 22, 23, 28, 31, 37, 40, 41, 44, 45, 54, 78). Most published studies report between 100 and 300 cases of mid-trimester fetuses with isolated choroid plexus cysts, and only three multi-center series report between 450 and 850 patients (20, 23, 41). In series published with more than 250 patients the overall risk varies form one in 548 to one in 45 cases. Not surprisingly, this risk is higher in series with patients “selected” for ultrasound and/or amniocentesis indications and those referred to tertiary centers (one in 274 to one in 45), and on average, it is lower in the studies with patients that underwent “routine” sonography screenings or in studies with a combination of the two patient population types (41).

The American College of Obstetrics and Gynecology recommends that patients with isolated choroids cysts on sonogram be screened for Trisomy 18 based on triple serum marker screen or maternal age. This recommendation, however, has not been adequately tested in clinical studies. Although many series in the literature have examined the risk of Trisomy 18 in the presence of isolated choroid plexus cysts, it
remains unclear how to estimate the individual patient risk of Trisomy 18 and when to offer amniocentesis. These studies almost universally suffer from inadequate patient numbers and from study designs that are unable to shed light on the central questions related to isolated choroids plexus cysts and Trisomy 18 risk.

The current series is the largest in the published literature with 1,045 cases of isolated choroids plexus cysts and provides an overall risk value of one in 116, which is consistent with the reported values from all series with more than 600 cases (Table 6). The current study is also unique in directly comparing serum and subtle sonographic markers that have been reported as effective in detecting the Trisomy 18 fetus.

In addition to providing the overall risk of Trisomy 18 in fetuses with isolated choroid plexus cysts fourteen to 24 weeks gestation, the present study also establishes an algorithm for more precise estimates of patient-specific Trisomy 18 risk. A numerical estimation of the individualized risk of Trisomy 18, rather than the use of nonspecific terms such as “high” vs. “low” risk, is therefore available in counseling patients. The end result is a more precise and informative counseling of Trisomy 18 odds which in turn facilitates the patient and physician decision-making process.

A. Significant parameters in Trisomy 18 risk estimation in the presence of isolated choroid plexus cysts

Among the serum triple screen markers, the levels of uE3 and hCG were statistically relevant Trisomy 18 predictor variables, while AFP levels did not reach significance in predicting Trisomy 18 risk in a population with isolated choroid plexus cysts. Such observation is consistent with the Staples et al. study in 1991, which showed
that the levels of uE3 and the β-subunit of hCG were the stronger predictors of Trisomy 18 in the general population \((P = 0.0001)\). The AFP level, not statistically relevant in their algorithm, had significant pairwise correlation coefficients with uE3, but not with hCG \(95\). In a larger study by Palomaki *et al.* it was later established that AFP had stronger pairwise correlation with uE3 than with hCG for both the normal and Trisomy 18 populations. Furthermore, the markers that best predicted of Trisomy 18 risk were established in the following order of statistical significance: uE3, hCG, AFP, and maternal age \(71\). Brown *et al.* also examined 250 cases with choroid plexus cysts, 90% of which had isolated findings, and revealed that the triple serum screen values were significant risk modifiers in fetuses with isolated choroid plexus cysts. In view of the small sample size, they were unable to provide the sensitivity value for the combined marker test \(54\).

An important explanation for the lack of significance of AFP is that fetuses with Trisomy 18 frequently have associated neural tube defects, abdominal wall defects, renal and other anomalies that are associated with elevated AFP levels. In this scenario, AFP elevation is likely to have significant sensitivity for Trisomy 18 detection. In the current study, we were only interested in cases without such gross defects, which significantly weakens the contribution of AFP. With only nine cases of Trisomy 18 in the current study it is possible that the lack of AFP significance might reflect type II error in this sample population. However, the levels of uE3 and hCG were strong significant predictors despite the low number of Trisomy 18 cases, again confirming the limited utility of AFP for detection of Trisomy 18 cases in the presence of choroid plexus cysts but no gross anomalies on ultrasound.
The contribution of advanced maternal age was also statistically significant in calculating the risk of Trisomy 18 in this patient population. Although, Brown et al. did not find maternal age to be a relevant variable in aneuploidy risk estimation in fetuses with isolated choroid plexus cysts, other equally small studies have incorporated maternal age as significant variable in models for aneuploidy risk estimation in cases of isolated choroid plexus cysts (22, 53, 55, 78). In a practical sense, maternal age data is indispensable in the algorithm for the mathematical calculation of the individual risk of Trisomy 18 and so is generally included as a variable.

Soft sonographic markers, however, were not significant parameters in predicting Trisomy 18 risk in our sample population when maternal serum analyte data were simultaneously considered. Bahado-Singh et al. recently reported that mid-trimester soft ultrasound markers, such as choroid plexus cysts and femur length measurements, combined with gross structural anomalies and maternal age further increased the detection rate of Trisomy 18 when compared to the identification of gross sonographic structural anomalies alone (sensitivity of combined soft markers and gross defect algorithm is 72.3% and FPR 0.9%). Furthermore, the screening model, which only considered significant soft markers (femur length, choroid plexus cysts, 2–VC) and maternal age as variables, has a sensitivity of 61.7% and a FPR of 1.5% (45).

The current series is the first to evaluate the use of soft markers along with maternal serum markers in determining Trisomy 18 risks for of isolated choroid plexus cyst fetuses. It suggests that soft sonographic markers do not provide additional information above the serum analytes for the detection of Trisomy 18 among isolated choroids plexus cyst cases. From a practical perspective if amniocentesis is performed in
this context based on the mere presence of subtle markers the number of women exposed to unnecessary invasive procedure is likely to increase without corresponding improvement in the detection rate of Trisomy 18.

B. Algorithm for Trisomy 18 risk estimation in fetuses with isolated choroid plexus cysts

The current study provides a novel algorithm based on maternal age, uE3, and hCG levels, which is highly predictive of Trisomy 18 risk in fetuses with isolated choroid plexus cysts (AUC 0.92, SE 0.08, $P < 0.0001$). At a threshold risk level of greater one in 150, i.e. the cut–off level above which a screening test is considered positive, it is expected that 66% of the Trisomy 18 cases are detected from mid–trimester evaluations of fetuses with isolated choroid plexus cysts. A calculated FPR of 0.7% indicates that 7/1000 normal women would be exposed to amniocentesis in order to achieve the detection of 66% of the Trisomy 18 cases. Given the high pre– and postnatal lethality and low prevalence of Trisomy 18, it is desirable to establish a screening test such as the one we propose here with high detection and low false–positive rates.

Possible bias sources in the study are related to the fact that most of the isolated choroid plexus cases used resulted from a referral based population. Approximately nine percent of our patients were either lost of follow–up or did not have triple serum marker data available for review. The latter could mean that women at higher risk (e.g. based on age) underwent triple serum marker screening, and that the current study might have overrepresented the risk of Trisomy 18. In practical terms, women who undergo testing, biochemical or otherwise, might be at higher risk based on either their own perception or on objective standards, and are likely to be overrepresented in the perinatologist’s office.
The detail and accuracy of ultrasound for identifying structural defects are usually greater at the participating centers than at the average ultrasound facility, which might limit the generalizability of our results.

C. Future directions

In view of the frequency of isolated choroid plexus cysts in mid-trimester sonographic screenings, it is imperative to determine the reliability of the established model algorithm by comparing the predicted and actual detection rates and FPR values in larger sample population of Trisomy 18. Furthermore, the maternal serum marker inhibin, introduced in the quadruple serum marker test, should in the future be evaluated as a variable in predicting Trisomy 18 risk. Inhibin only recently became part of the new standard of quadruple serum marker test, and thus more patients need to be recruited for such a study.

Finally, it is possible that analysis of much larger populations of isolated choroid plexus cysts might reveal additional statistically relevant variables in Trisomy 18 risk estimation. The rarity of Trisomy 18 among isolated choroid plexus cyst cases, however, makes it unlikely that large enough series will be accumulated and reported on in the near future.

The current algorithm has high diagnostic accuracy which could translate to lower iatrogenic losses of normal fetuses, as well as reduced medical costs related to the lower number of genetic amniocentesis performed. Most importantly, a refined screening test of Trisomy 18 risk in cases of isolated choroid plexus cysts, as proposed above, allows
for obstetricians to provide individualized risk estimates and for patients to make more informed decisions regarding the acceptance of invasive prenatal diagnostic procedures.
### Table 6. Incidence of Trisomy 18 in Fetuses with isolated Choroid Plexus Cysts.

Series include all published studies with more than 100 cases of isolated CPC from which the above information could be extracted. The Demasio et al. (24) study was excluded from the table since it considered only cases of women > 35 years of age. Cases with karyotypes other than Trisomy 18 or normal were also excluded from the total number of cases. § - selected populations: include patient populations with indications for ultrasound and/or amniocentesis, as well as referral to tertiary centers. CPC – choroid plexus cysts.

<table>
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<tr>
<th>Study</th>
<th>Isolated CPC cases</th>
<th>Trisomy 18 cases</th>
<th>Trisomy 18 Risk (&gt;1:x)</th>
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<tr>
<td>Nadel et al. (31) §</td>
<td>220</td>
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<td>0</td>
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<tr>
<td>Snidjers et al. (37)</td>
<td>275</td>
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<td>275</td>
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<td>Walkinkshaw et al. (18)</td>
<td>140</td>
<td>3</td>
<td>47</td>
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<tr>
<td>Gupta et al. (20)</td>
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<td>1</td>
<td>548</td>
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<tr>
<td>Gray et al. (40) §</td>
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<td>Reinsch et al. (22)</td>
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<td>0</td>
<td>0</td>
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<td>Chitty et al. (23)</td>
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<td>Current Series §</td>
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VI. References


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