OBSTETRICS

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

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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.

Key words: management, maternal serum alpha-protein, pregnancy complications

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Alpha-fetoprotein (AFP) is a glycoprotein produced during pregnancy by the fetal yolk sac and the fetal liver, which crosses the placental barrier, the fetal membrane and the decidua to reach the maternal circulation.[1] It is also produced by the choroid plexus and is released into the cerebrospinal fluid.[2] First used in screening for neural tube defects,[3,4] maternal serum AFP assay was combined with human chorionic gonadotropin hormone (hCG) in second-trimester biochemical screening for chromosomal abnormalities.[5-7] An increased level of maternal serum AFP (MSAFP) was subsequently recognized as being associated with an increased risk of pregnancy complications as intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), preterm birth, and preeclampsia.[8-14] An elevation of MSAFP is also seen in several fetal anomalies such as ventral wall defects, and rare congenital disorders, including among others nephrotic syndrome and epidermolysis bullosa.[15-19] In France, the national consensus threshold used is 2.5 multiples of the median (MoM) and concerns 1% of pregnancies.[20] Besides morphologic anomalies visible on ultrasound examination, there are many cases for which high MSAFP is not directly explicable. This can be worrying for the patient and physician. To our knowledge there are no explicit guidelines for pregnancies with abnormal MSAFP elevation. Some clinicians assay MSAFP again later in pregnancy, but this approach has never been evaluated. Our aim was to study the relevance of a second MSAFP assay and to propose a management strategy based on it.

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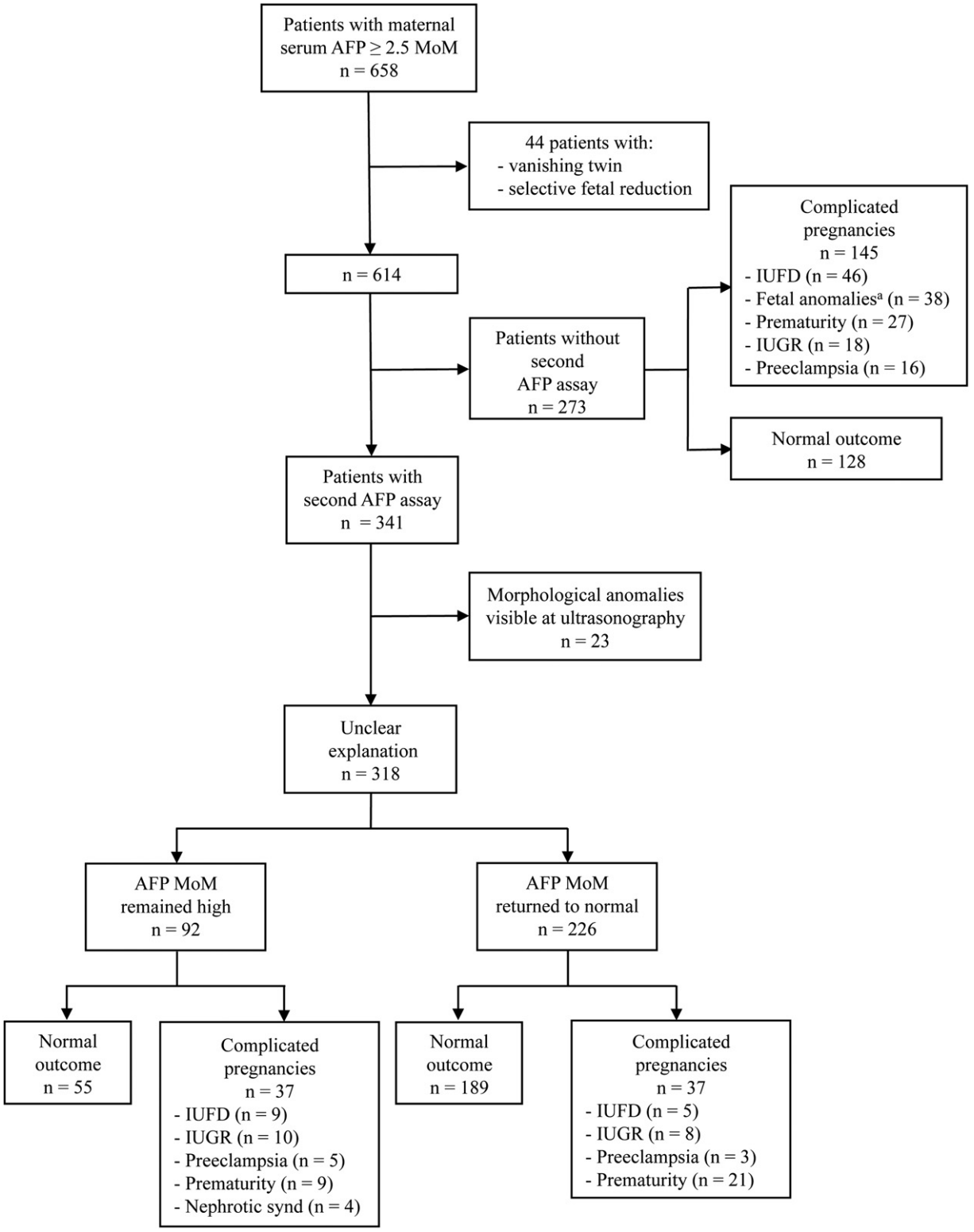
Materials and Methods

A retrospective cohort study was conducted over the period 2004-2008 in our biochemical laboratory, which is accredited for trisomy 21 screening. Second-trimester (14-18 weeks) maternal serum markers used in screening for Down syndrome in singleton pregnancies, including hCGb and AFP, were assayed (Autodelfia, dual kit; PerkinElmer, Turku, Finland). Maternal sera from an unselected population were routinely sent from 8 hospitals. Gestational age was determined by crown-rump length at first-trimester ultrasound examination (expressed in weeks and days). Patients with

FIGURE 1
Flowchart describing the cohort from prenatal diagnosis onwards



AFP, alpha-fetoprotein; *IUFD*, intrauterine fetal death; *IUGR*, intrauterine growth restriction; *MoM*, multiples of the median; *Nephrotic Sd*, nephrotic syndrome.

[a]One nephrotic syndrome.

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a high MSAFP (defined as AFP ≥2.5 multiple of median [MoM]) were offered a second AFP assay as part of the diagnostic work-up. This second assay was performed at least 18 days later (which represents approximately 3 times the half-life of AFP in maternal serum[21]). Reasons for not perform a second assay were as follows: (1) diagnosis of a severe fetal malformation at ultrasound examination or an IUFD before 18 days, and cases of vanishing twin or selective fetal reduction because of the artificial increase in AFP.[22] (2) Physician or maternal failure to adhere to the procedure. Patient management consisted of standard ultrasound surveillance and, if necessary, amniocentesis for fetal karyotyping, amniotic fluid AFP assay (Thermo Fisher Scientific, Hennigsdorf, Germany), and cholinesterase electrophoresis (AChE). No particular antenatal testing protocol for vascular follow-up was undertaken and patients were managed in their center of origin by the physician responsible. Consent for amniocentesis was obtained from all patients. A second MSAFP assay was part of the routine diagnostic work-up. For these reasons this study was exempt from ethical review board approval. Patients who underwent a second MSAFP assay were classified into 2 groups: (1) group with MSAFP remaining ≥2.5 MoM and (2) group with MSAFP returning to a normal level. Clinical data, ultrasonographic reports, laboratory findings, outcome, and final diagnosis were recorded. Pregnancy complications were classified in 5 groups: severe fetal anomalies, IUFD, IUGR <3rd percentile, preeclampsia, and spontaneous premature birth. Severe fetal anomaly was defined as a severe morphologic anomaly or syndrome, requiring possible extensive hospital care or to which French law about medical termination of pregnancy may apply. Only spontaneous preterm deliveries before 34 weeks of gestation were taken into account.

Pregnancy outcome was documented in all cases. In accordance with French law, termination of pregnancy was possible at the parents' request in cases of severe fetal anomaly and poor prognosis.

Results are presented as median, interquartile range (IQR), range, or proportions accordingly. The χ^2 test, or the Fisher exact test (numbers <5) were used for comparison of groups. A probability value of .05 or less was considered statistically significant. Statistical analyses were performed using *R* software, version 2.0.0 (www.r-project.org, Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Between 2004 and 2008, 658 second-trimester maternal serum marker samples with an MSAFP ≥2.5 MoM were retrieved. Cases of vanishing twin and selective fetal reduction were discarded (n = 44). In 341 of the remaining 614 cases, a second MSAFP assay was performed (Figure 1).

Overall population

In these 614 cases of elevated MSAFP, median gestational age at sampling was 16^{+1} weeks (IQR, 15^{+3}–17^{+5}). Median maternal age was 29 years (IQR, 25–34). Final diagnosis and pregnancy outcomes are presented in Table 1. No complication was observed in 372 of 614 cases (60.6%). Complications were observed in 242 of 614 pregnancies (39.4%). We observed 65 (10.6%) severe fetal anomalies, 60 (9.8%) IUFDs (15-30 weeks, median, 17 weeks), 36 (5.8%) IUGRs, 24 (3.9%) preeclampsias, and 57 (9.3%) spontaneous premature deliveries. The 65 severe fetal anomalies are detailed in Table 2. Termination of pregnancy was performed at the parents' request in 45 of 65 cases (69.2%). The main fetal anomalies involved were fetal cutaneous defect such as neural tube defect (n = 25), gastroschisis (n = 4) and cervical teratoma (n = 1), fetal glomerular defects such as nephrotic syndrome (n = 5), and placental anomalies such as triploidy (n = 3). There were 22 cases of undefined multiple malformation syndromes. Of the 65 fetal anomalies, 60 morphologic anomalies were visible at ultrasound examination, and only the 5 cases of nephrotic syndrome were undetectable at ultrasound examination. These 5 cases of nephrotic syndrome were confirmed by postnatal genetic testing. Amniocentesis was performed in 3 of these 5 cases and amniotic fluid AFP > 7 MoM associated with normal AChE electrophoresis findings was highly suggestive of nephrotic syndrome. The 2 other cases of nephrotic syndrome were diagnosed at birth.

TABLE 1
Outcomes of the 614 pregnancies with MSAFP ≥2.5 MoM (vanishing twin and selective reduction excluded)

Outcomes (n = 614)	n (%)
Pregnancies without complication	372 (60.6)
Complicated pregnancies	242 (39.4)
Severe fetal anomalies	65 (10.6)
IUFD	60 (9.8)
IUGR	36 (5.8)
Preeclampsia	24 (3.9)
Spontaneous preterm delivery <34 wks	57 (9.3)

IUFD, intrauterine fetal death; *IUGR*, intrauterine growth restriction; *MoM*, multiples of median; *MSAFP*, maternal serum alpha-fetoprotein.

Spaggiari. Pregnancy outcome according to AFP evolution. Am J Obstet Gynecol 2013.

Population without a second AFP assay

A second MSAFP assay was not performed in 273 pregnancies (Figure 1). A complication occurred in 145 of 273 of these pregnancies (53.1%). We observed 46 (16.8%) IUFDs, 38 (13.9%) severe fetal anomalies, 27 (9.9%) spontaneous preterm deliveries, 18 (6.6%) IUGRs, and 16 (5.9%) preeclampsia. In 21 cases termination of pregnancy because of severe fetal anomaly or severe IUGR was performed less than 18 days after MSAFP assay. In 31 of 39 cases, the IUFD occurred less than 18 days after MSAFP assay.

Population with a second AFP assay

A second MSAFP assay was performed in 341 pregnancies (Figure 1). Median maternal age was 29 years (IQR, 25–33), with no significant difference between the group with remained elevated MSAFP (median, 29 years; IQR, 25–33) and the group with decreased MSAFP (median, 29 years; IQR, 25–33). The median interval between the first and

TABLE 2
Description of the 65 severe fetal anomalies in the overall population

Anomalies	n
Neural tube defect	25
Multiple malformation syndrome	22
Nephrotic syndrome	5[a]
Gastroschisis	4
Cerebral anomalies	3
Triploidy	3
Tuberous sclerosis complex	1
Cervical teratoma	1
Bilateral renal dysplasia	1

[a] Not visible at ultrasound.

Spaggiari. Pregnancy outcome according to AFP evolution. Am J Obstet Gynecol 2013.

second MSAFP assays was 35 days (IQR, 26−55). Pregnancy complications were observed in 97 of 341 (28.4%) in the group with a second MSAFP assay. In 23 of these 341 pregnancies, a diagnosis was possible by ultrasound examination because of morphologic abnormalities. In the remaining 318 pregnancies with MSAFP ≥2.5 MoM there was no clear explanation (Figure 1). No statistical difference between the 2 groups was observed for the first MSAFP value (2.85 MoM when AFP returned to normal vs 3.11 MoM when AFP remained elevated).

When AFP returned to normal, pregnancies were complicated in 37 of 226 cases (16.4%), and when AFP remained high, pregnancies were complicated in 37 of 92 cases (40.2%) ($P < .001$). Among these 37 complications, there were 4 nephrotic syndromes, of which 2 had been prenatally suspected because of amniotic fluid AFP > 7 MoM.

The relationship between pregnancy outcomes and MSAFP evolution after exclusion of cases with a clear ultrasound explanation is presented in Table 3. Pregnancies with a MSAFP remaining ≥2.5 MoM had significantly higher rates of IUFD (21-31 weeks; median, 26 weeks) ($P = .005$), IUGR ($P = .01$), and preeclampsia ($P = .047$) and would have allowed identification of cases of fetal nephrotic syndrome ($P = .006$). The rate of spontaneous preterm delivery was high in the 2 groups (9%), but did not differ significantly between the groups ($P > .99$).

TABLE 3
Outcomes according to MSAFP evolution, in the population with a second MSAFP assay and without clear ultrasound explanation

Variable	AFP remained high n = 92	AFP returned to normal n = 226	*P* value
Initial MSAFP (MoM), median (range)	3.11 (2.5–48.4)	2.85 (2.5–38.7)	
Repeat MSAFP (MoM), median (range)	3 (2.50–43.7)	1.6 (0.12–2.47)	
Outcomes			
Pregnancies without complication	55 (59.8%)	189 (83.6%)	< .001
Complicated pregnancies	37 (40.2%)	37 (16.4%)	< .001
IUFD	9 (9.8%)	5 (2.1%)	.005
IUGR	10 (10.9%)	8 (3.5%)	.01
Preeclampsia	5 (5.4%)	3 (1.3%)	.047
Spontaneous preterm delivery <34 wks	9 (9.8%)	21 (9.3%)	> .99
Congenital nephrotic syndrome	4 (4.3%)	0 (0%)	.006

AFP, alpha-fetoprotein; *IUFD*, intrauterine fetal death; *IUGR*, intrauterine growth restriction; *MoM*, multiples of median; *MSAFP*, maternal serum alpha-fetoprotein.

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COMMENT

In agreement with the literature, our results reveal a higher rate of adverse maternal and fetal outcome in pregnancies complicated by an elevated MSAFP. Besides fetal morphologic anomalies and chromosomal defects, an elevated MSAFP is associated with an increased risk of preeclampsia, IUGR, preterm delivery, and IUFD.[8-14] This association may result from placental malfunction and fetal maternal placental barrier disruption.[23,24]

In our overall population, it was possible to diagnose fetal anomalies at a second ultrasound examination in 60 of 614 cases (9.7%). These fetal anomalies were mainly neural tube defects, multiple malformation syndromes and gastroschisis, with morphologic abnormalities visible on ultrasound examination. In the 554 remaining cases, the elevation of MSAFP was not clearly explained by ultrasound examination. Complications subsequently observed were mainly gestational vascular anomalies (such as preeclampsia or IUGR), IUFD, and spontaneous preterm birth. A closer prenatal surveillance could be proposed for this high-risk population. Gestational vascular complications potentially leading to IUFD should be screened for based on fetal biometrics, umbilical and uterine artery Doppler, and maternal blood pressure monitoring. The risk of preterm delivery could be determined based on monitoring of cervical length. We observed 5 cases of congenital nephrotic syndrome, a rare renal disease that can be screened for by assaying MSAFP, further evidence being provided by elevated total protein and AFP in amniotic fluid, whereas AChE electrophoresis findings are normal.[18,25] We strongly suspected antenatally the diagnosis in 3 of the 5 cases. Fetal blood sampling for albumin assay can also be offered to strengthen the diagnosis.

Although we had no case of epidermolysis bullosa, it should be borne in mind that this rare condition difficult to diagnose prenatally is also characterized by high MSAFP and high amniotic fluid AFP.[26] Moreover, this disease is often associated with pyloric atresia and gastric dilatation at ultrasound examination and abnormally elevated digestive

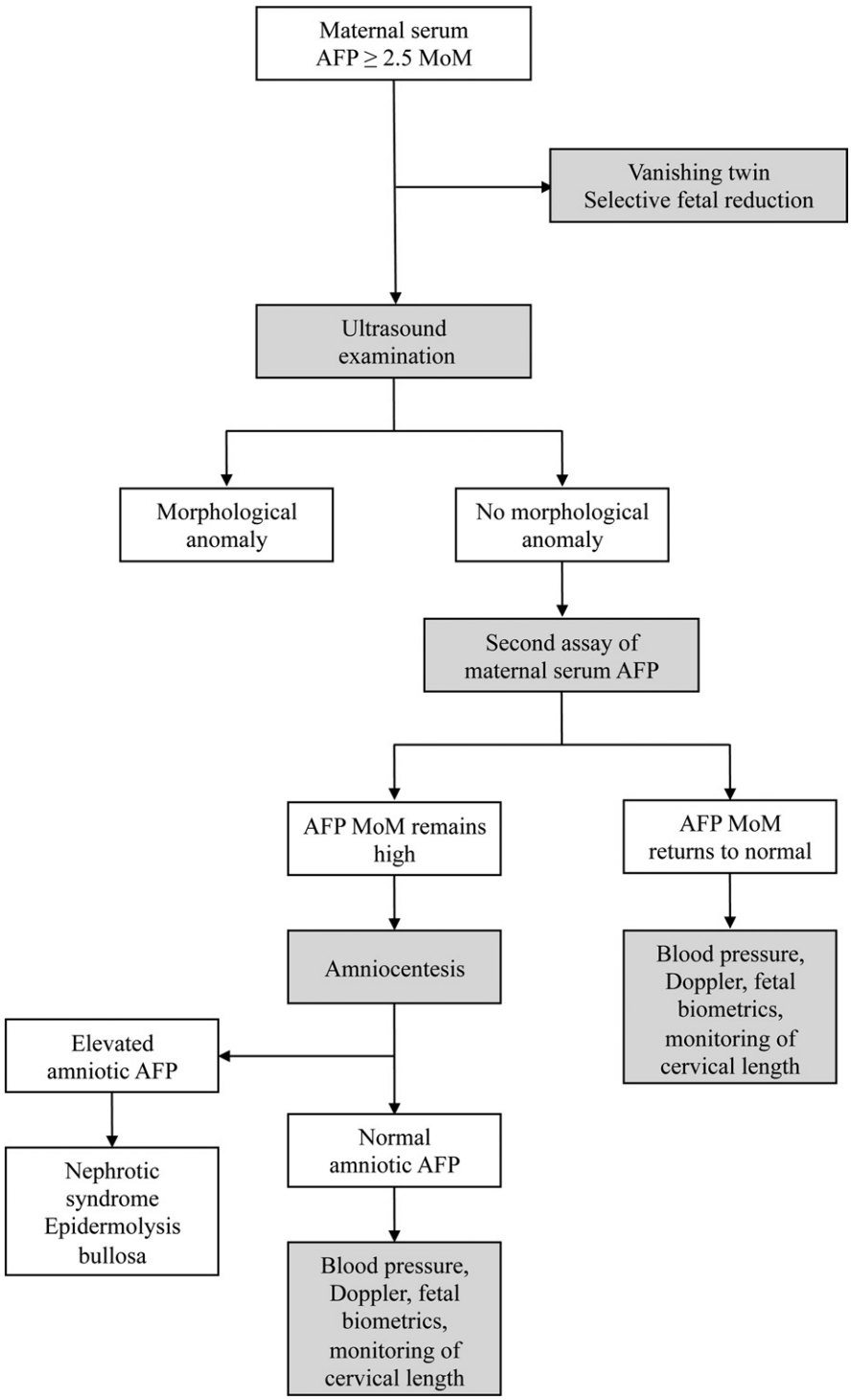
enzymes may be indicative of the diagnosis.[26]

In the subpopulation who underwent a second MSAFP assay, the rate of pregnancies complications was significantly higher when MSAFP remained ≥2.5 MoM. After excluding morphologic anomalies diagnosed by ultrasonography, there were 318 cases without immediate clear explanation. The subsequent follow-up revealed complication rates of 16.3% vs 40.2% when AFP returned to normal or remained high, respectively ($P < .001$). Therefore, persistently elevated MSAFP at the second assay may be considered as a cause for concern. Moreover, if MSAFP returns to normal a diagnosis of nephrotic syndrome is unlikely. However, maternal and fetal surveillance should be continued because of the complication rate of 16.3%. Note that first-trimester vaginal bleeding can artificially increase MSAFP level,[27] but this should be a diagnosis of exclusion. Although the rate of spontaneous preterm delivery was not significantly different between the 2 groups, it remained high and should therefore be the subject of specific surveillance. There was no particular antenatal testing protocol for vascular follow-up, so it is not possible to exclude that patients with persistently elevated AFP had closer surveillance. However, the high level of complications we observed in the group with persistently elevated AFP is not in favor of a difference in management between the 2 groups. Moreover, the rate of complications as fetal anomalies, IUGR, and preeclampsia would not have changed according to the prenatal surveillance.

In the subpopulation who did not undergo a second MSAFP assay, the rate of complications was higher than in the subpopulation with a second MSAFP assay. This can be explained by the high rate of termination of pregnancy and IUFD before 18 days and, therefore, the lack of a need for a second MSAFP assay.

Given these results, we propose a management strategy in the case of second-trimester MSAFP ≥2.5 MoM (Figure 2). To our knowledge, no study

FIGURE 2
Flowchart summarizing the management strategy proposed in case of MSAFP ≥2.5 MoM



AFP, alpha-fetoprotein; *MoM*, multiples of the median; *MSAFP*, maternal serum AFP; *Nephrotic Sd*, nephrotic syndrome.

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has examined the value of a second MSAFP assay in the case of elevated second-trimester MSAFP and there is no well-supported management strategy.

First, it is necessary to exclude vanishing twins and selective fetal reduction. Second, ultrasound is used to exclude morphologic anomalies. Third, MSAFP is assayed a second time. When AFP returns to normal, this is relatively reassuring as normal outcome was noted in 83% of cases. However, complications are still possible and require continued maternal and fetal monitoring including blood pressure, Doppler flow, fetal biometrics, and cervical length. In this group, amniocentesis is not recommended if Down syndrome risk is <1 of 250. When AFP remains high, amniocentesis is performed for amniotic fluid AFP assay. Elevated amniotic fluid AFP is suggestive of congenital nephrotic syndrome or epidermolysis bullosa, and all direct and indirect signs of spina bifida should be screened for. Otherwise, close maternal and fetal monitoring is called for. Given that this is a retrospective study, this management is a suggestion, and a prospective study would be necessary to evaluate it. However, this management is based on our results and does suggest a possible course of action.

Although Down syndrome screening using second-trimester markers is being replaced by the first-trimester combined test (including ultrasound nuchal translucency and first-trimester biochemical markers) in most countries, second-trimester biochemistry is still of value in several situations, such as late pregnancy diagnosis, late patient admission, and inadequate nuchal translucency measurement preventing the combined test.

Moreover, because of the difficulty of ultrasound screening for spina bifida, MSAFP assay remains relevant in countries with a high prevalence of neural tube defects. Therefore, unexplained elevated MSAFP is not an exceptional situation.

Our aim is not to use MSAFP as a screening marker for pregnancy complications. MSAFP is part of the routine Down syndrome screening offered to all women and an unexplained elevated MSAFP can be worrying for the patient and physician. Our aim is to provide data and to suggest possible management to physicians when a high MSAFP level is observed fortuitously.

In conclusion, high MSAFP level is associated with a high rate of pregnancy complications. When MSAFP returns to a normal level on a second assay, the risk of adverse outcome decreases significantly, but these pregnancies are still at risk, and therefore need surveillance. Another major reason for repeating MSAFP assay is to identify patients who should be offered amniocentesis to evaluate the risk of nephrotic syndrome and epidermolysis bullosa. A strategy based on ultrasound examination, a second MSAFP assay and amniocentesis is proposed to improve prenatal counseling and management of these pregnancies. ■

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