بنام خداوند بخشنده و مهربان

Screenings for Fetal Aneuploidy

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دی 1400

Why screening?

- Trisomy 21 is the most common autosomal trisomy among live births prevalence 1 in 340

- Biochemical marker screening for Down syndrome provide risk assessment for 18 and 13 T

Advantages and disadvantages

- Tests are available
- Gives parents options: an opportunity to plan for the birth of an affected child or pregnancy termination
- Adverse psychological effects
  - Affected fetus
  - Procedure-related loss of a normal fetus

Whome to screen?

- (ACOG): All women should be offered aneuploidy screening in early pregnancy
- All women should have the option of invasive diagnostic testing instead of screening

گروه هدف

- سن بالای 35
- سابقه جنین با تریزومی
- اختلالات ژنتیکی یکی از زوجین

در سایر موارد تقاضای غربالگری کاملا اختیاری است

اصول انجام غربالگری بر اساس پروتکل بازنگری 1400

لازمه انجام غربالگری به بیمار دادن مشاوره می باشد

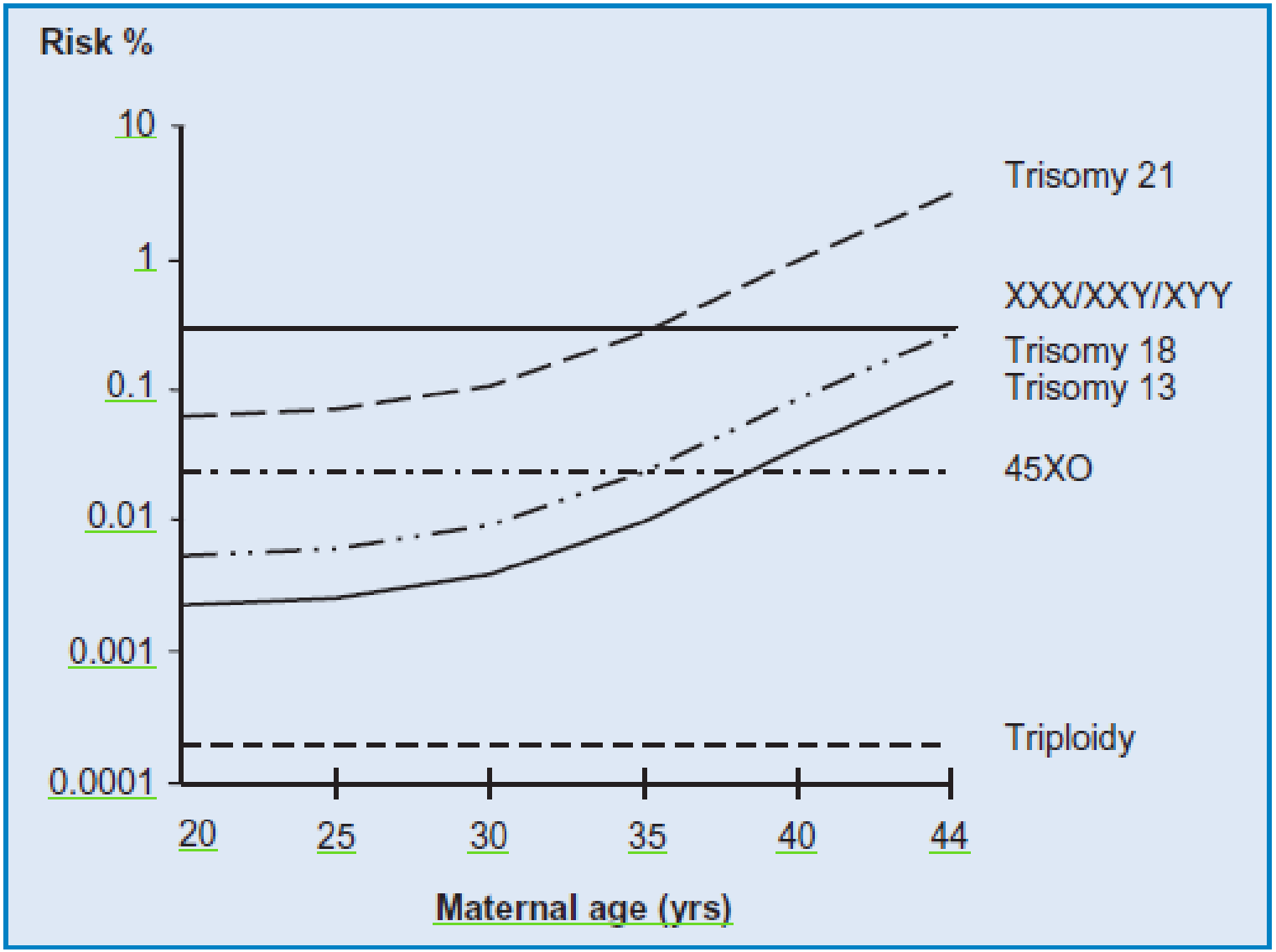
- توضیح علت و روند انجام غربالگری
- عدم امکان درمان اختلالات کروموزومی
- مشکلات مربوط به سقط جنین از نظر شرعی و قانونی
- اختیاری بودن غربالگری
- و امکان انصراف ازان درهر مرحله

Choice of screening test

- Screening is optional
- Difference between screening tests and diagnostic tests
- Test performance DR, FPR,
- Test failure rate
- limitations of the test
- Timing for test
- Cost and whether their insurance covers

Prior risk

- Maternal age:
- Recurrence risk of aneuploidy is at least 1% if
- Recurrence risk of inherited (ie, translocation) is usually much higher

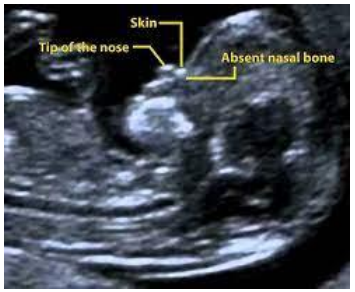

Risk %
10
1
0.1
0.01
0.001
0.0001
20
25
30
35
40
44
Maternal age (yrs)
Trisomy 21
XXX/XXY/XYY
Trisomy 18
Trisomy 13
45XO
Triploidy

FIRST-TRIMESTER SCREENING COMBINED TEST

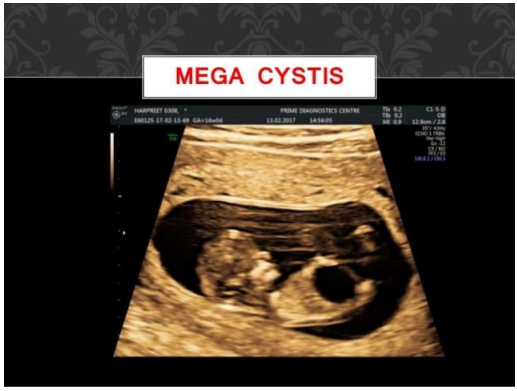
At 11+0 to 13+6 w

- ultrasound measurement of (NT) ,
  - NT provides 51% DR 5% FPR at 10 weeks, 59 percent at 11 weeks, and 62 percent at 12 to 13 weeks
- Serum screening test , (PAPP-A) and free-beta hCG
- combined screening DR for trisomy13 is 78 %

Additional sonographic markers



- **Nasal bone**
- **Ductus venosus flow**
- **Tricuspid flow**
- **Bladder size** (megacystis, bladder length ≥7 mm)



- **FHR** , In trisomy 18 the FHR is decreased and in trisomy 13 increased
- DR increase to about 95% and the FPR can be reduced to 3% by also these examinations

Genetic disorder	First-trimester markers		
	NT	PAPP-A	hCG/free beta
Down syndrome	↑↑	↓↓	↑
Trisomy 18	↑↑	↓↓	↓↓
Trisomy 13	↑	↓↓	↓

quadruple marker test

Perform at 15+0 to 18+6 weeks (but can be done as late as 22+6 w

Genetic disorder	Second-trimester markers			
	AFP	uE3	hCG/free beta	Inhibin A
Down syndrome	↓	↓	↑	↑
Trisomy 18	↓	↓↓	↓↓	↔
Trisomy 13	↔	↔	↔	↔

Quadruple marker test

- It can also screen SLOS
  - DR 60% and FPR 0.3 %
  - Prenatal diagnosis of SLOS can be performed by
    - Amniotic fluid measurement of cholesterol and its precursors, 7- and 8 dehydrocholesterol
    - Molecular and mutation analyses
- Steroid sulfatase deficiency
- Trisomy 13 is not detected by the quadruple test

Factors can affect serum marker

- Maternal weight
- Multiple gestation
- Previous false-positive result
- IVF, the screen-positive rate for such pregnancies is approximately twice the expected rate
- Cigarette smokers AFP and InhA levels are higher and uE3, free beta hCG, and PAPP-A levels are lower
- **Diabetes mellitus** AFP decreased and uE3 are modestly reduced

Integrated screening tests

- Measure biochemical markers of Down syndrome in both the first and second trimesters and may or may not include NT measurements
- The serum-integrated test is highly efficient in identifying cases of trisomy 18 cut-off of 1 to 100

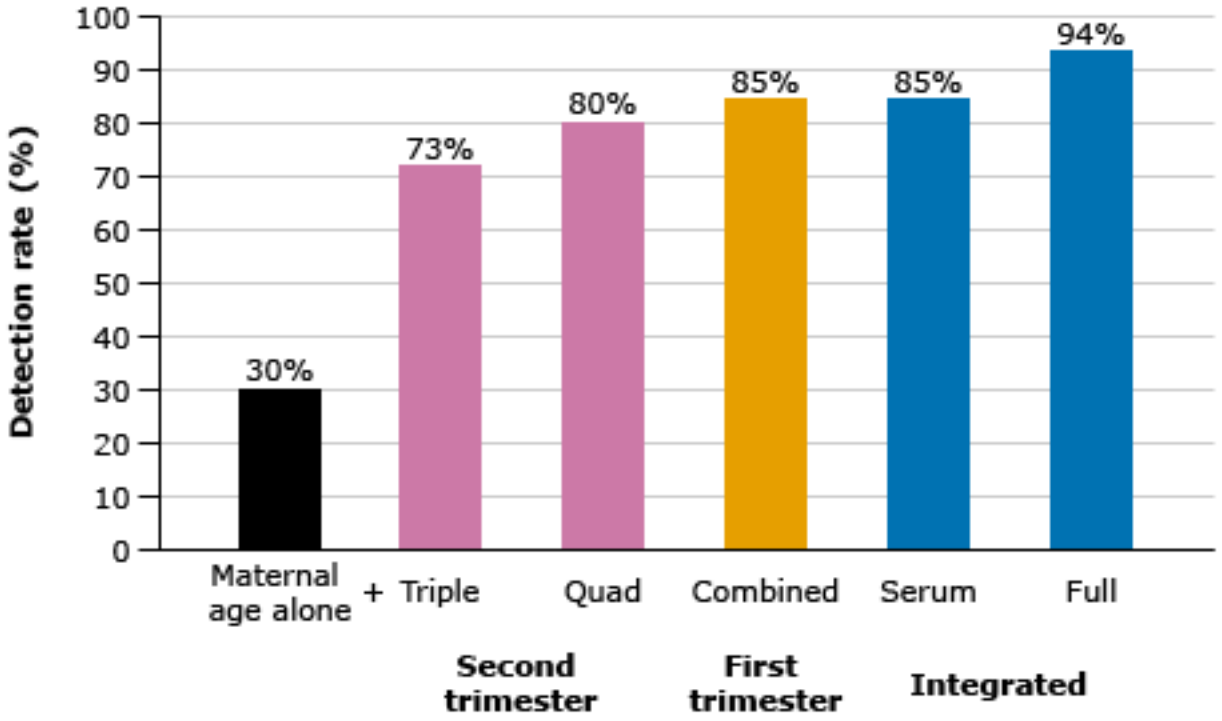
full integrated test

- Includes serum PAPP-A and sonographic NT at first step. These results are then integrated with results of the quadruple test performed on a second serum sample collected ideally at 15+0 to 18+6 weeks (but can be done as late as 22+6 weeks)

Stepwise sequential testing

- performing the first-trimester portion of the full integrated test, reporting risks of Down syndrome to the patient, and offering CVS to women whose results place them at very high risk of an affected fetus (eg, ≥1 in 50). Women whose screen does not place them at very high risk go on to complete the second-trimester portion of the test

Performance of maternal age and various second trimester maternal serum combinations in screening for Down syndrome



The bar graphs describe the detection rate attained at a fixed 5% false-positive rate for each of the screening tests.

Data adapted from: Wald NJ, Kennard A, Hackshaw A, et al. J Med Screen 1997; 4:181.

UpToDate®

Choice of screening test

Statement	Screening test to consider
1. "I want the result of my screening test as early as possible in the pregnancy, while the pregnancy is still private and I have options of early prenatal diagnosis (CVS) because I would terminate an affected pregnancy."	First-trimester or cfDNA screening
2. "I want to have the test with the lowest chance of a screen-positive result."	cfDNA screening
3. "I would consider an amniocentesis if my test result shows a high chance of Down syndrome, but not a CVS because it has a higher risk for procedure-related complications."	Integrated or cfDNA screening

	procedure-related complications."	
4.	"I have been very anxious and want my results as soon as possible, whether or not I would terminate an affected pregnancy."	First-trimester or cfDNA screening
5.	"I am already in my second trimester of pregnancy."	Quad or cfDNA screening
6.	"Nuchal translucency ultrasound is not available in my area."	Serum-only integrated or cfDNA screening
7.	"CVS is not available in my area."	Integrated or cfDNA screening
8.	"My pregnancy is considered high risk for a chromosomal abnormality because of my age, my family history, a finding on my ultrasound examination, or a positive result on a screening test done on my blood."	cfDNA screening
9.	"I am carrying twins."	First-trimester or quad or integrated screening or cfDNA screening

consider an invasive diagnostic test

- For all genetic syndromes WES
- Unbalanced chromosomal rearrangement in parents
- Detect mosaicism
- Microdeletions/microduplications (microarray)
- Amniotic fluid AFP and acetylcholinesterase
- One or more structural anomalies(microarray)
- A previous pregnancy complicated by fetal trisomy
- At least one major or two minor fetal structural anomalies in the current pregnancy.

انتخاب تستهای غربالگری بر اساس دستورالعمل

- اقدام در سه ماهه اول combined test
  - لازمست شرایط تاثیر گذارمانند استفاده ازIVF تخمک اهدایی ،رحم اجاره ای ، سایقه قبلی بیماری ژنتیکی کتبا به اطلاع ازمایشگاه رسانده شود
  - در صورتیکه به سونوگرافی NTاستاندارد دسترسی نداشته باشد serum integrate انجام میشود
- اقدام در سه ماهه دوم در15 تا 16 هفته و 6 روز
  - کواد مارکر
- اقدام در هفته بیش از 17

کواد مارکر تا 17 هفته و 5 روز با تاکید به جواب سریع ازمایشگاه قابل انجام است و توضیح به بیمار در مورد زمان محدود سقط تا 18 هفته و6 روز

MANAGEMENT

MANAGEMENT OF SCREENING RESULTS

- Combind test
  - <1 in 250 is commonly used as a cutoff
  - After a low risk result, further testing for Down syndrome and trisomy 18 is not recommended
  - Biochemical marker screening tests should not be repeated it but it is prudent to double-check the laboratory

MANAGEMENT OF SCREENING RESULTS

- Definitive fetal chromosomal analysis, CVS
- Cell-free DNA
- Integrated test if patient desire amniocentesis instead CVS
  - A typical cut-off for the integrated test is ≥1 in 100 with an FPR of 1 to 2 percent

مدیریت تستهای غربالگری

- در صورت انتخاب Combind test برای غربالگری و نتیجه تست منفی (1/250) اقدام بعدی مراقبت روتین بارداری است (بررسی NTD با سونوگرافی یا AFP انجام گیرد)
- در صورت نتیجه غربالگری(پر خطر1/250 ≤)
- امنیوسنتز یا CVS
  - خطر بیشتر از 1/10
  - بارداری چند قلویی یا دوقلوی دی کوریون
  - زوجین با ترانسلوکاسیون
  - PAPPA, MOM زیر 0/26
- NIPT
  - خطر بین 1/11 تا 1/250

Cases of enlarged NT

- Aneuploidy
- Congenital heart disease (septal defects)
- Noncardiac anomalies 4 to 10 percent
  - Hydrocephalus
  - Agenesis, hypoplasia, and dysplasia of the lung
  - Atresia and stenosis of the small intestine
  - Osteodystrophies
  - Diaphragm anomalies
- Genetic syndromes
  - Noonan s
  - Multiple pterygium s
  - Congenital adrenal hyperplasia
  - Spinal muscular atrophy
  - DiGeorge syndrome
  - Smith-Lemli-Opitz syndrome
  - Variety of skeletal dysplasias
- Twin-twin transfusion

Enlarged NT

- NT> 3.0 to 4.0 mm proceed directly to karyotype (some choose microarray) assessment
- Genetic testing(by targeted molecular genetic testing or WES)for some of the associated conditions, such as the RASopathies(Noonan syndrome) especially with NT ≥5 mm
- Anomaly scan
- Fetal echocardiography

Enlarged NT

- In one study, for example, first-trimester NT 3.5 to 4.4 mm was associated with a normal outcome in 70 percent of fetuses whereas NT 5.5 to 6.4 mm was associated with a normal outcome in only 30 percent of cases

اقدامات بر اساس نتیجه NT

- NT کمتر از 3/5میلیمتر و نسبت NT/CRL کمتر از 95 پرسانتایل باشد ادامه فرایند combind test
- اگر کمتر از 3/5میلیمتر و نسبت NT/CRL بین 95 تا 99 پرسانتایل باشد nipt میتواند پیشنهاد گردد
- اگرNT بالای 3/5میلیمتر و نسبت NT/CRL بیشتر از 99 پرسانتایل بشد تستهای تشخیصی انجام می شود

Cell-free DNA

- The primary source of cfDNA in the maternal circulation is thought to be apoptosis of placental cells (syncytiotrophoblast)
- Is done ≥10+0 weeks of gestation
- DR
  - **Trisomy 21** – DR 99.5 percent, FPR 0.05 percent
  - **Trisomy 18** – DR 97.7 percent, FPR 0.04 percent
  - **Trisomy 13** – DR 96.1 percent, FPR 0.06 percent
  - **Sex Chromosome**– DR are lower and FPR rates are higher

It is a screening test for trisomy's 21,18,13 and sex chromosoms **D**oes not screen for open neural tube defects and other chromosomal abnormality

Cell-free DNA

- cfDNA screening is often used as a secondary screening test in the following high-risk groups
  - Maternal age ≥35 y
  - Fetal ultrasound finding of a soft marker
  - History of prior pregnancy with a trisomy detectable by cfDNA screening
  - Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or 21
  - Screen-positive biochemical-based test for Down syndrome.

Cell-free DNA

- ACOG supports consideration of cfDNA for all pregnant patients as a screening test option in single and in twin pregnancies
- The use of cfDNA as a primary screening test in the United States is limited by some practical concerns

Low fetal fraction

- **Early gestational age**
- **Suboptimal sample collection** EDTA) tube and centrifuged within six hours; the resulting plasma is stable with -80°C freezer storage
- **Obesity** (81 kg or more)
- **Fetal karyotype** ,FF is lower in 18, 13 T and Turner and extremely low in triploid F (>4%) but higher with trisomy 21
- **Maternal use of low molecular weight heparin**
- **IVF**
- **Twin gestation, as the per fetus**

Contingent model

- In this model, all patients are offered first-trimester combined screening with two risk cutoffs
- The "high risk" (eg, >1:150) identifies a group that could choose between going directly to invasive testing or to secondary cfDNA
- The low-risk group (eg, <1:1000) would receive routine prenatal care
- Intermediate-risk group (eg, 1:151 to 1:1000) offered cfDNA screening after counseling

RESULTS

- Minimum level of fetal fractionis is 3 to 4 percent
- Borderline" call should be considered screen positive and not a test failure (parental consanguinity)
- Test failure(No call or no result)
  - **Repeat the cfDNA test after seven days**
  - **Standard serum marker or combined serum marker and ultrasound screening, if not already done.**
  - **Invasive procedure** some studies offering ultrasound evaluation and diagnostic tests

AFP and NTD

- AFP, done at ≥15+0 weeks, up to 22+6
- Elevated AFP levels ≥2.0 or 2.5 MoM
  - NTD
  - Congenital nephrosis
  - Abdominal wall defects
  - Some tumors associated with elevated AFP
- When ultrasound screening is performed to detect NTDs, we believe MSAFP screening is not required
- If optimal images of the fetal spine or intracranial anatomy are not obtained MSAFP should be performed

AFP and NTD

- If ultrasound findings are uncertain or show an apparently normal fetus after a screen-positive MSAFP result, genetic counseling and further evaluation via amniocentesis are usually indicated.
- Amniotic fluid AFP and acetylcholinesterase (AChE) is performed with 96 percent accuracy; FPR 0.08 and 0.14 percent

AFP and NTD

- Although many NTDs are associated with genetic abnormalities that would be detected by a conventional G-banded karyotype, microarray has become the preferred genetic test
- Consider fetal magnetic resonance imaging in selected cases

PREDICTION OF ADVERSE PREGNANCY OUTCOMEA

- An unexplained elevated MSAFP (and some other marker) may be associated with pregnancies at increased risk of early fetal demise, fetal growth restriction and preeclampsia
- There is no evidence that more intensive fetal and maternal monitoring in otherwise normal pregnancies will improve outcome

Ultrasound structural anomalie markers for Down syndrome in the second trimester

- Cardiovascular defects (eg, septal and valvular defects)
- Central nervous system (ventriculomegaly Abnormal cisterna magna, absent corpus callosum, and/or cerebellar hypoplasia (NTDs)Strawberry shaped calvarium (pointed front and a flat occiput)
- Gastrointestinal system (eg, duodenal atresia [after 22 weeks]
- Facial defects (eg, clefts, micrognathia, low-set ears, microphthalmus)
- Urogenital defects (eg, horseshoe kidney, Polycystic kidneys
- Limb abnormalities
- Skeletal anomalies

Ultrasound soft markers

- Echogenic intracardiac focus (EIF)
- Choroid plexus cysts (CPCs)
- Single umbilical artery (SUA)
- Urinary tract dilation
- Slightly shortened long bones (humerus, femur)
- Hyperechoic bowel
- Thickened nuchal fold ≥6 mm between 15 and 20
- Absent or hypoplastic nasal bone

Ultrasound soft markers

- Echogenic intracardiac foci: in one or both ventricles If isolated finding, aneuploidy screen result is negative, no further evaluation is required
- Pyelectasis: Renal pelvis measuring ≥4 mm in anteroposterior diameter up to 30 weeks of gestation (>7 mm after 30 weeks)
  - If isolated finding, aneuploidy screen result is negative repeat ultrasonography in third trimester for potential urinary tract obstruction

Ultrasound soft markers

- Echogenic bowel:
  - Offer CMV, CF, and aneuploidy screening or diagnostic testing
- Thickened nuchal fold
  - Detailed anatomic survey
  - Further detailed genetic counseling and aneuploidy screening or diagnostic testing
- Mild ventriculomegaly Lateral ventricular atrial measurement between 10 to 15 mm
  - detailed anatomic ultrasound evaluation Consider diagnostic testing for aneuploidy and CMV Repeat ultrasound in third trimester

Ultrasound soft markers

- Choroid plexus cysts in one or both choroid plexus
  - Detailed anatomic survey including fetal cardiac ultrasound (echocardiogram not required) No further follow-up if isolated
- Short femur length Measurement $<2.5^{th}$ percentile for gestational age
  - Can be associated with aneuploidy, IUGR, short limb dysplasia
  - Second-trimester detailed fetal anatomic evaluation for short limb dysplasia
  - Further detailed counseling Consider repeat ultrasonography in third trimester for fetal growth

Ultrasound soft markers

- Two-vessel umbilical cord
  - Most experts do not recommend routine chromosomal analysis if there are no other malformations or other indications for genetic amniocentesis
  - R/O IUGR
  - Ecocardiography
- Fetal growth restriction
  - Associated with aneuploidy can occur as early as the first trimester if observed/expected CRL $\leq$0.86

Take home points

- Whome to screen?
- Type of screening test?
- Relevans of screening test?
- NIPT
- Follow up of screen positive patients?