Ultrasound Soft Markers

Lauren A. Miller, MD, MPH

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Disclosures

None ☺

Learning Objectives

Correctly identify soft markers on routine / detailed anatomic surveys at the mid-2nd trimester

ID and Understand Soft Markers that require follow-up

Understand the utilization of soft markers in the world of non-invasive prenatal testing for the common aneuploidies

What is a “soft marker”?

Ultrasound finding that:

Is not a structural abnormality/may be normal	Has been *associated* with increased chance of aneuploidy

Originally meant to improve detection of T21 when only age-based risk assessment, serum screening, and amniocentesis were available.

Role of soft markers has been called into question

- Cell free DNA has high sensitivity and specificity for aneuploidy at all maternal ages.
  - At lower maternal age, same sensitivity → lower PPV.
  - ACOG and SMFM (2020): Baseline risk should not limit screening options, endorsed use of cfDNA or serum screening regardless of age.

- Serum screening = any of the following:
  - first trimester screen, quad screen, integrated screen, sequential screen

- Preimplantation Genetic Testing – aneuploidy (PGT-A)
  - Cell-free DNA screening still recommended

Soft markers to discuss

- Thickened Nuchal Fold
- Absent or hypoplastic nasal bone
- Shortened humerus, femur, or both
- Echogenic Bowel
- Urinary Tract Dilation
- Echogenic intracardiac focus
- Single umbilical artery
- Choroid Plexus Cysts

What is an “isolated” soft marker?

- **No** other structural abnormality
- **No** growth restriction
- **No** other soft markers

Canadian Calculator

Ultrasound Markers

Please indicate if the ultrasound markers listed below are absent or present.

If a particular ultrasound marker was not assessed, select unknown.

Ultrasound Marker	Present	Absent	Unknown
Echogenic intracardiac focus (EICF)	○	○	○
Mild pyelectasis	○	○	○
Short femur	○	○	○
Echogenic bowel	○	○	○
Increased nuchal fold	○	○	○
Aberrant right subclavian artery (ARSA)	○	○	○
Absent nasal bone	○	○	○
Ventriculomegaly	○	○	○

Please note: This tool should NOT be used in the following situations:

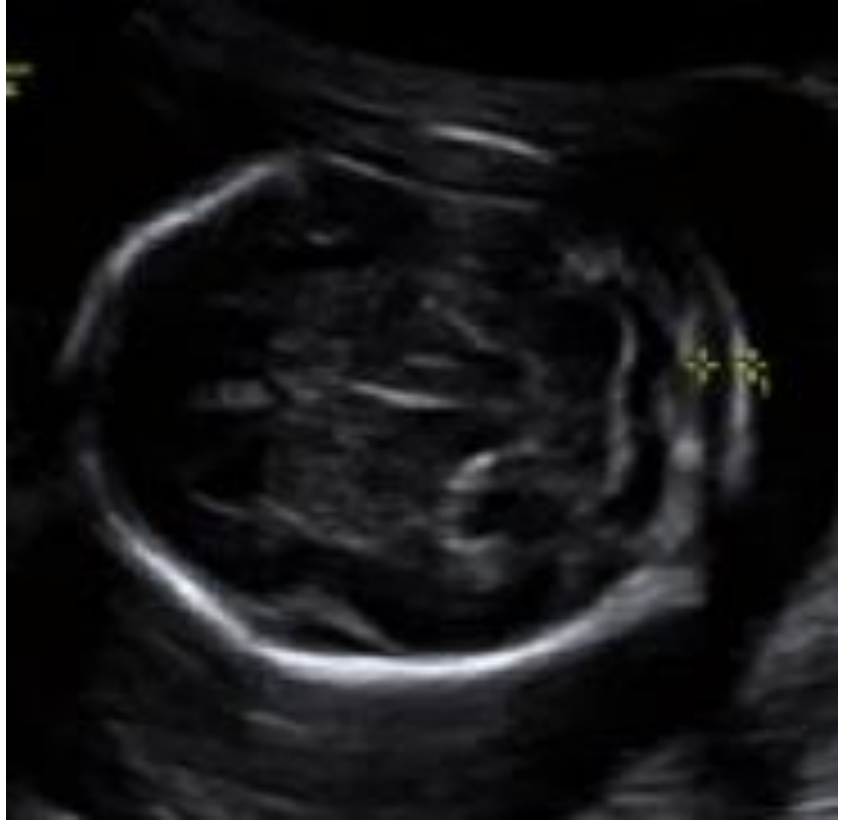
- Multiple gestation (eg. twin pregnancy).
- Major malformation(s) in the fetus.
- Woman with a negative NIPS screen result.

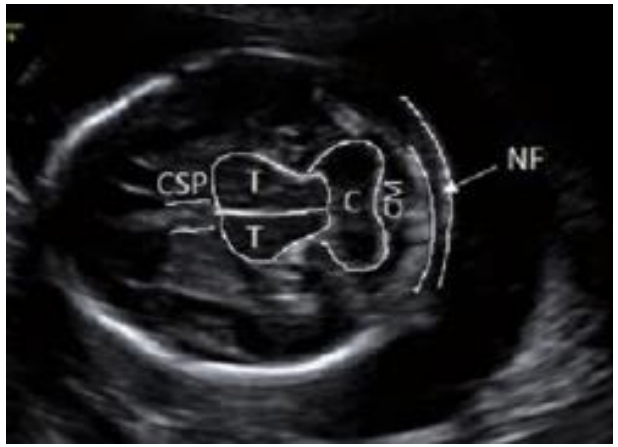
Perinatology.com Calculator

Calculation of Age Adjusted Ultrasound Risk Assessment	
Mid trimester apriori risk of Down Syndrome is	1 in [ ]
Use midtrimester risk for Down syndrome by maternal age of [20] [Use Maternal Age for Apriori Risk [5]]	
Ultrasound Marker [6]	* Likelihood Ratios [7]
☐ Nuchal fold Thickened soft tissue at the fetal occiput > = 6 mm between 15 to 20 weeks'	17
☐ Hyperechoic bowel Bowel echogenicity comparable to bone	6.1
☐ Short humerus Measured to Expected Humeral Length is < 0.9 Expected Humeral Length = -7.9404 + 0.8492 * BPD Measured Humeral Length [ ] BPD [ ] [Calculate >] MHL/EHL = [ ]	7.5
☐ Short femur Measured to Expected Femur Length is <= 0.91 Expected Femur Length = -9.3105 + 0.9028 * BPD Measured femur Length [ ] BPD [ ] [Calculate >] MFL/EFL= [ ]	2.7
☐ Echogenic intracardiac focus Discrete echogenic spot as bright as bone	2.8
☐ Pyelectasis Anterior posterior dimension of the renal pelvis >= 4 mm between 16 to 20 weeks'	1.9
Total post-ultrasound likelihood ratio [Calculate Likelihood Ratio]	[ ]
Patient-specific risk for Down syndrome posterior probability [Calculate Posterior Probability]	1 in [ ] [Clear] *

Likelihood Ratios (when isolated)

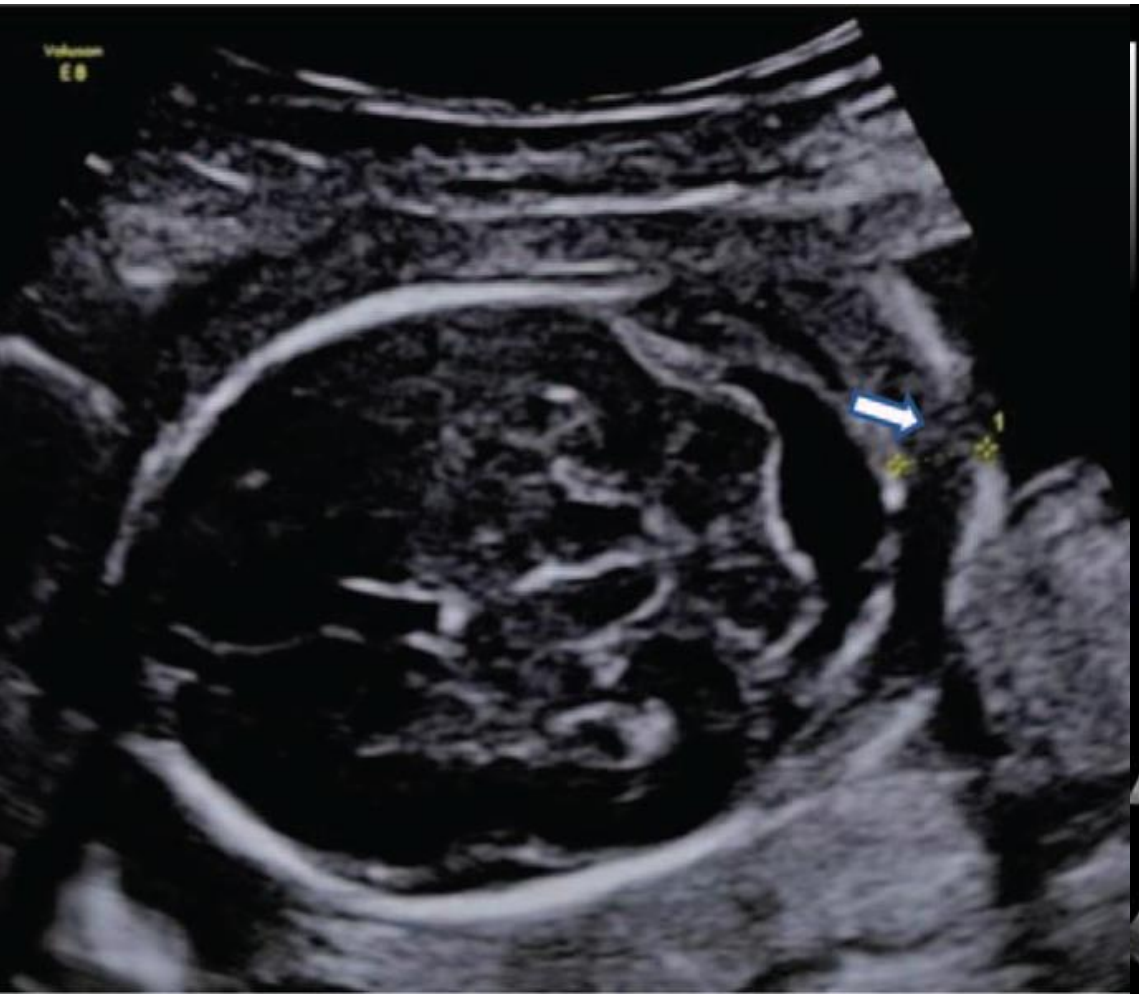
Single umbilical artery	1 (no increased risk)
Echogenic intracardiac focus	1.4 - 1.8 (crosses 1)
Urinary tract dilation	1.5 - 1.6
Choroid plexus cysts	< 2 (for T18)
Shortened femur	1.5 - 2.7
Echogenic bowel	1.7 - 8
Shortened humerus	5.1 - 7.5
Absent or hypoplastic nasal bone	6.6
Thickened nuchal fold	3.8 - 17

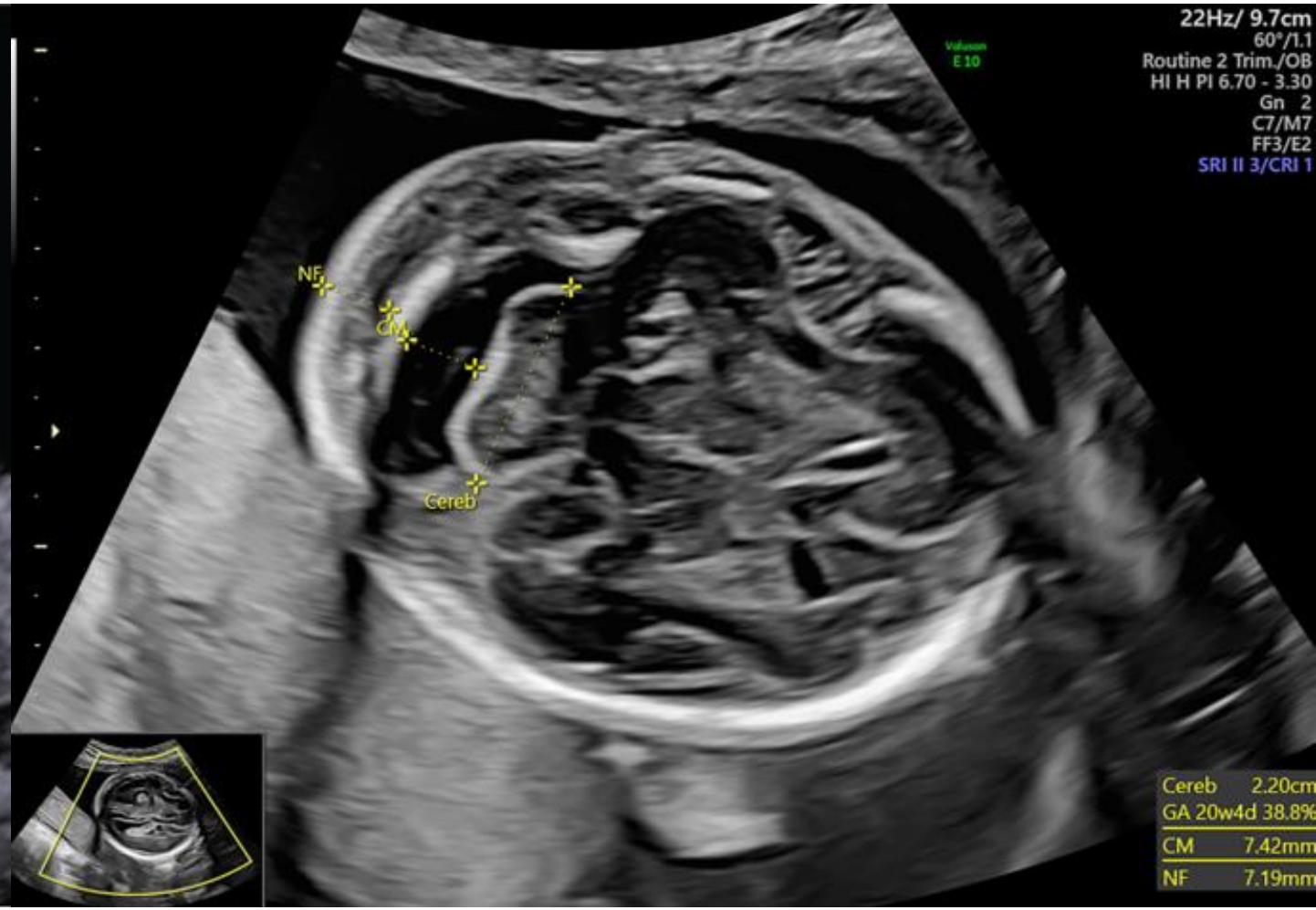




Thickened Nuchal Fold

- Imaged in the transverse plane, angled caudally to capture the cerebellum and occipital bone. CSP should be in the image.
- Place calipers between outer edge of skin and outer edge of occipital bone.
- A thickened nuchal fold is defined as ≥6 mm between **15** and **20** weeks of gestation.





Li L, Fu F, Li R, Liu Z, Liao C. Prenatal diagnosis and pregnancy outcome analysis of thickened nuchal fold in the second trimester. Medicine. 2018 Nov;97(46):e13334

Papamichail, M., Eleftheriades, A., Manolakos, E. *et al.* Prenatal diagnosis of 18p deletion and 8p trisomy syndrome: literature review and report of a novel case. *BMC Women's Health* **24**, 241 (2024).

Thickened Nuchal Fold

In some studies, it is **the most powerful** second trimester sonographic marker for Trisomy 21.

LR for T21 when isolated is 3.8 to 17

SMFM recommendation different than for most other soft markers:

- If prior cfDNA: no further aneuploidy eval
- **If prior neg serum screening only**: offer no further aneuploidy eval, cfDNA, or amnio
- If no prior screening: offer cfDNA or amnio

Absent or Hypoplastic Nasal Bone

- Hypoplastic can be defined as:
  - < 2.5%ile
  - ≤ 2.5 mm
  - <1/10th or <1/11th the BPD
  - <0.75 or ≤0.7 MoM (best predictor of T21)

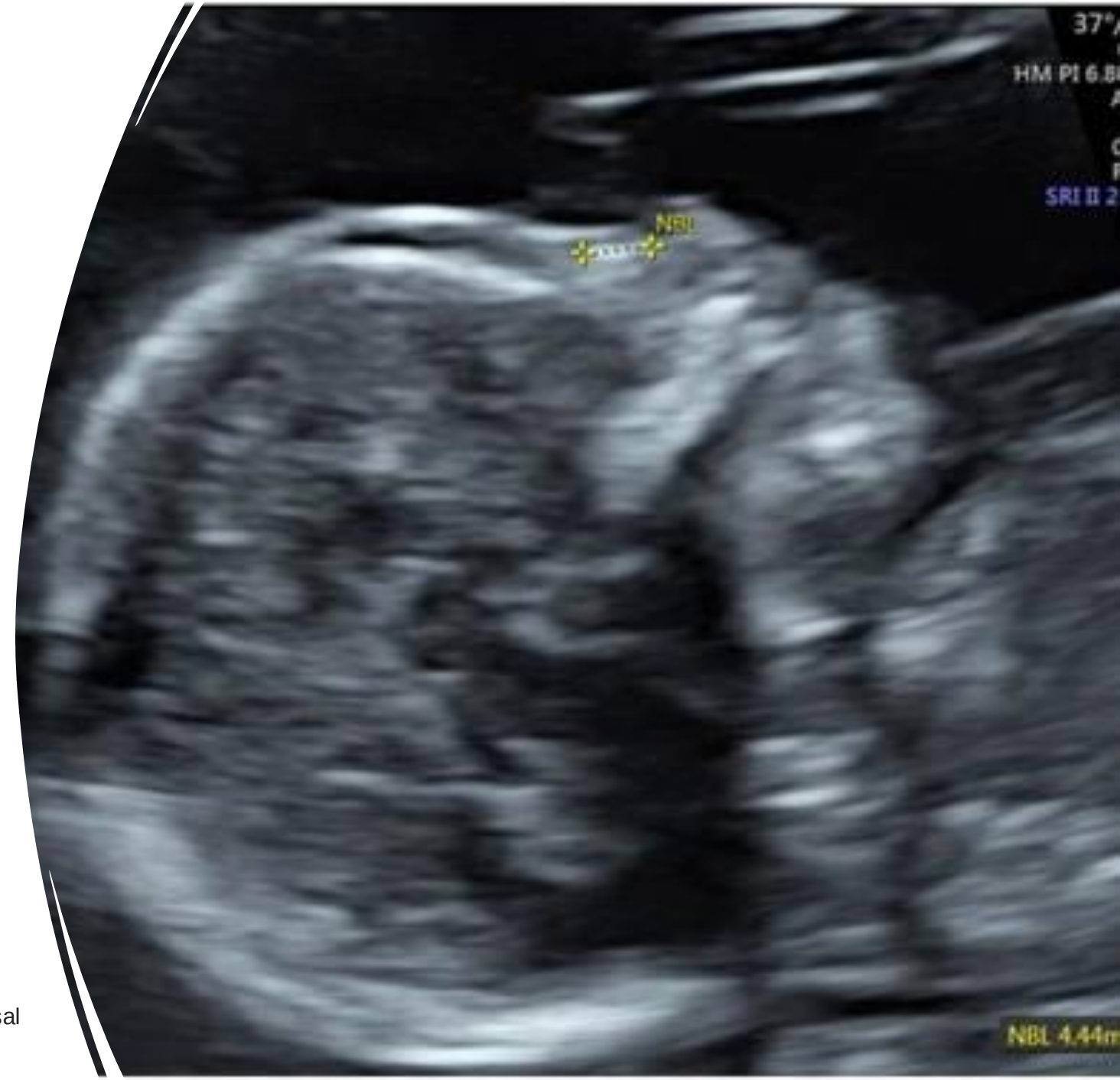
- Absent or hypoplastic nasal bone occurs in 0.1% to 1.2% of euploid pregnancies (9% of the Afro-Caribbean population)

- LR 6.6 for T21 in setting of isolated absent or hypoplastic NB in the 2nd trimester (for absent in the 1st trimester the LR is 27.8 during the NT window)

Absent or Hypoplastic Nasal Bone

- Because of the high likelihood ratio, SMFM recommendation different than for most other soft markers:
  - If prior cfDNA: no further aneuploidy eval
  - **If prior neg serum screening only**: offer no further aneuploidy eval, cfDNA, or amnio
  - If no prior screening: offer cfDNA or amnio

Same as for thickened nuchal fold



Moczulska, H.; Serafin, M.; Wojda, K.; Borowiec, M.; Sieroszewski, P. Fetal Nasal Bone Hypoplasia in the Second Trimester as a Marker of Multiple Genetic Syndromes. *J. Clin. Med.* **2022**, *11*, 1513.

Short Femur or **Humerus**

How is this defined?

- Ratio of the observed to expected bone length (based on BPD)
- Humerus: <0.90
- Femur: <0.92

OR

- Short Femur Z-score of -2 to -4

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Total post-ultrasound likelihood ratio Calculate Likelihood Ratio	
Patient-specific risk for Down syndrome posterior probability Calculate Posterior Probability	1 in Clear *

Short Femur or Humerus

- Short femur: LR from meta-analyses 1.5 to 2.7. CI of the lower estimate crossed 1, suggesting minimal risk.

- Short humerus: LR from meta-analyses 5.1 to 7.5, suggesting moderate risk.

- Parental race and ethnicity can lead to constitutionally short bones and should be considered in the differential diagnosis.
  - However, race and ethnicity specific definitions of shortened long bones have not improved prediction of T21.

Short Femur or Humerus Follow Up

Prior low risk screening: no further aneuploidy evaluation

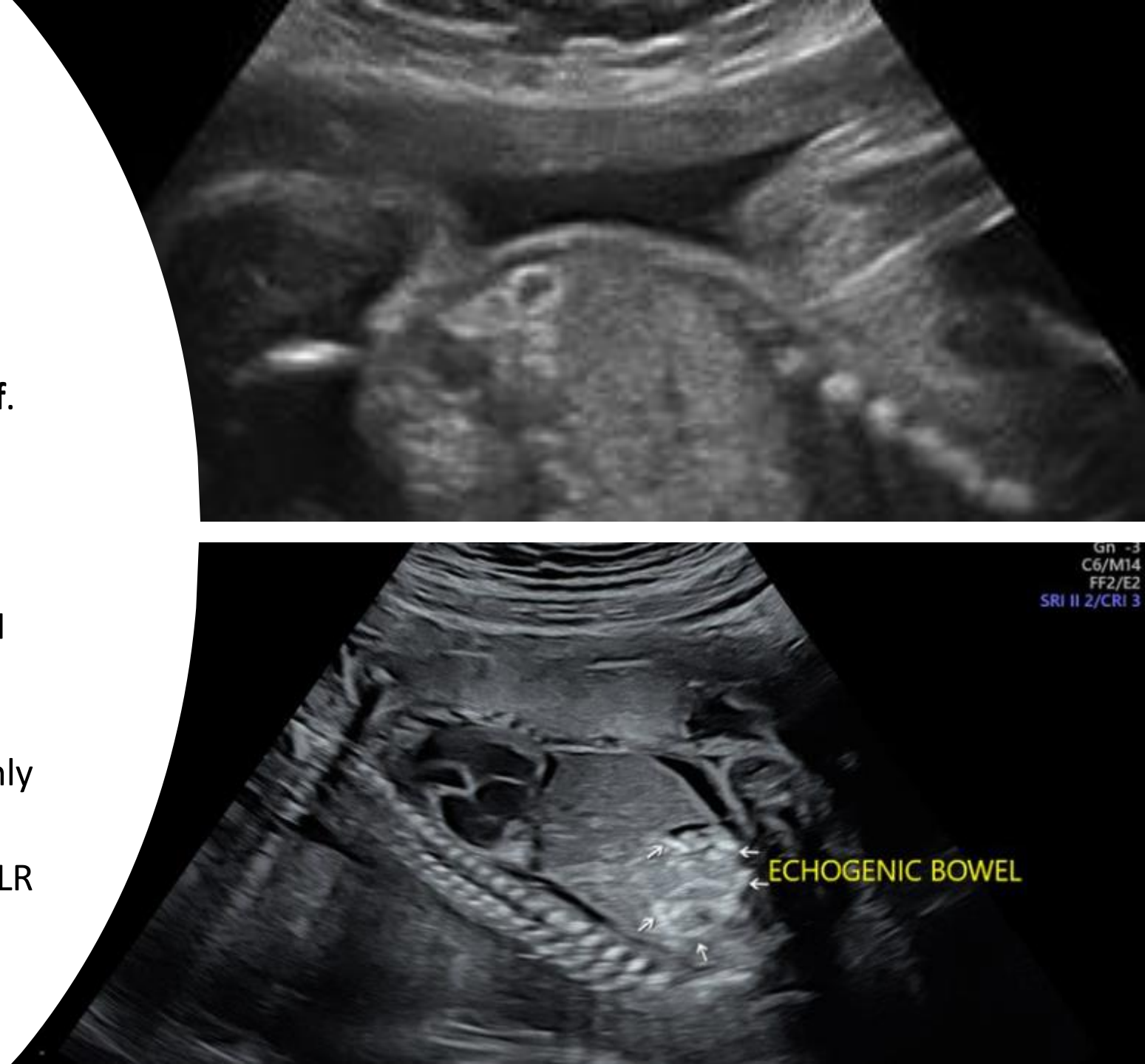
No prior screening: offer non-invasive screening

May be skeletal dysplasia → Measure all long bones, consider further genetic testing

May be impending FGR → Consider 3rd trimester growth

Echogenic Bowel

- Bright as bone with low frequency transducer (<5 MHz) and **harmonics off**.
- Seen in up to 1.8% of 2nd tri US.
- ~1/3rd transient and otherwise normal.
- ↑ risk of renal and cardiac anomalies, aneuploidy, CF, congenital infections, GI pathology, intraamniotic bleeding, and FGR.
- Risk of aneuploidy 3-5%. Most commonly T21, but others reported.
- LR for T21 is ~6-8 (1 meta-analysis had LR of 1.7).



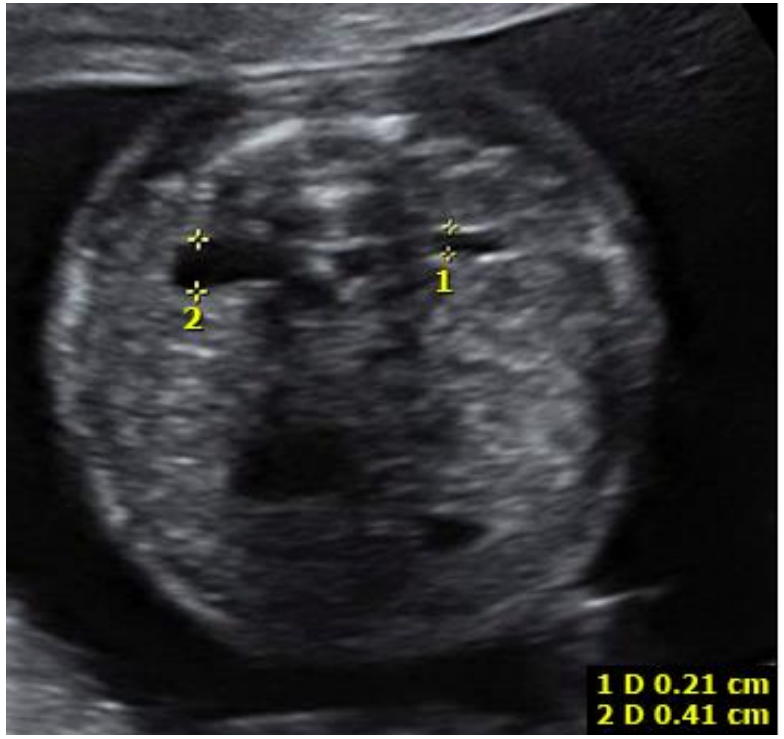
Echogenic Bowel

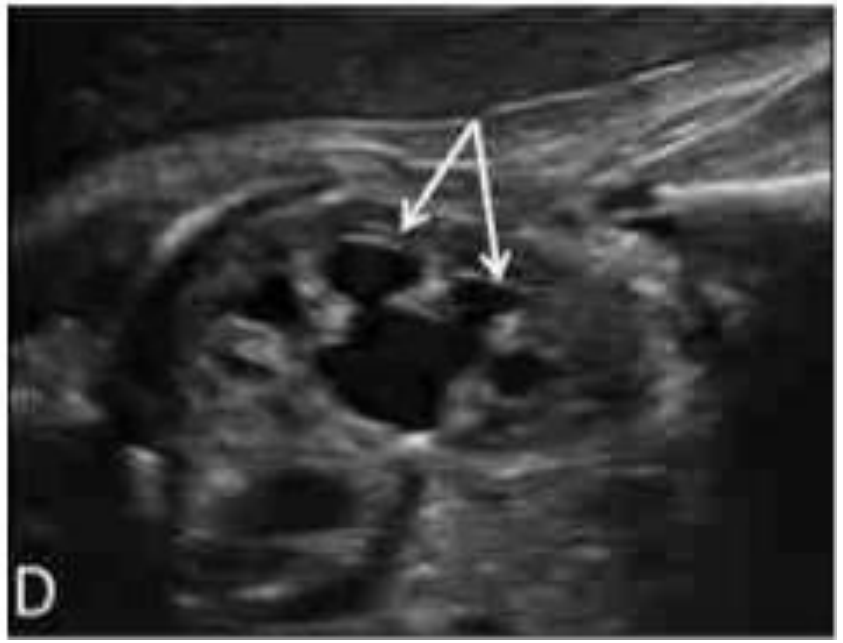
If no prior screening, offer non-invasive screening

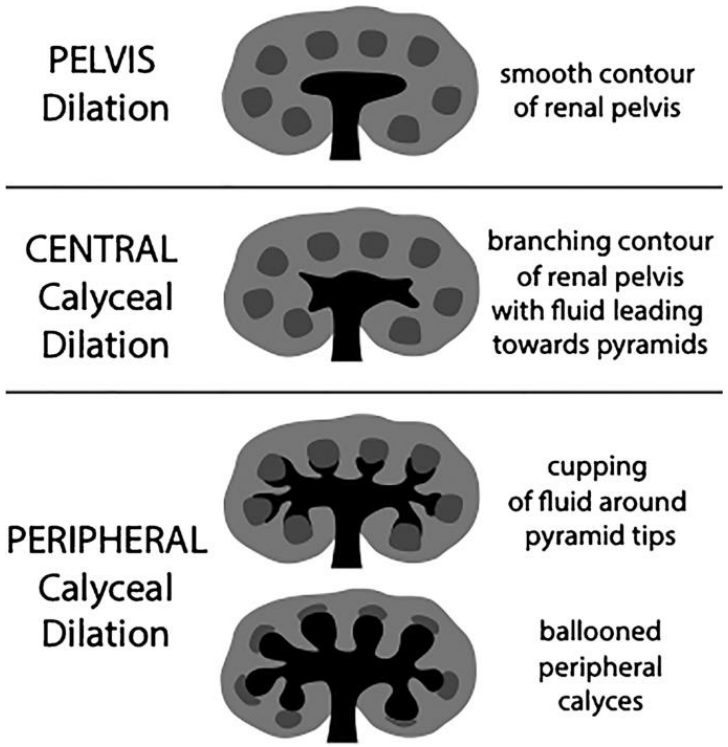
If prior low risk screening, no further aneuploidy evaluation.

Other recommendations: Evaluate for CF, congenital viral infections (CMV), and history of intraamniotic bleeding.

Follow up ultrasound in the third trimester for growth and evaluation of the bowel.







Fetal Urinary Tract Dilation

- Occurs in 1-2% of second trimester ultrasounds
- Likelihood ratio of 1.5-1.6 for trisomy 21.
- Neg NIPS → SMSM say no additional testing

Fetal Urinary Tract Dilation

- 2014 Nguyen et al published a multidisciplinary consensus on classification of prenatal and postnatal UTD
  - UTD A1 (antenatal 1): low risk for postnatal uropathy
  - UTD A2-3 (antenatal 2-3): high risk for postnatal uropathy
  - UTD P1, 2, 3: postnatal UTD

Most UTD resolves in pregnancy or in first months of life, but some leads to conditions that can cause UTIs and renal dysfunction.

Predicting risk allows targeted evaluation of those at high risk of needing intervention while avoiding overevaluation of those who won't need intervention.

Fetal Urinary Tract Dilation

TABLE 2
Urinary tract dilation (UTD): Antenatal classification of findings

	UTD A1	UTD A2-3
Ultrasound findings		
AP RPD, 16–27 wk of gestation	4 to <7 mm	≥7 mm
AP RPD, ≥28 wk of gestation	7 to <10 mm	≥10 mm
Calyceal dilation	None or central	None, central, or peripheral
Parenchymal thickness	Normal	Normal or abnormal
Parenchymal appearance	Normal	Normal or abnormal
Ureters	Normal	Normal or abnormal
Bladder	Normal	Normal or abnormal
Unexplained oligohydramnios	Absent	Absent or present
Prenatal follow-up	Third-trimester ultrasound examination at ≥32 wks of gestation	Individualized follow-up ultrasound examination

Adapted from Nguyen et al.[89]

AP RPD, anterior-posterior renal pelvis diameter; *UTD*, urinary tract dilation.

Society for Maternal-Fetal Medicine. SMFM Consult Series #57: Evaluation and management of isolated soft ultrasound markers for aneuploidy in the second trimester. Am J Obstet Gynecol 2021.

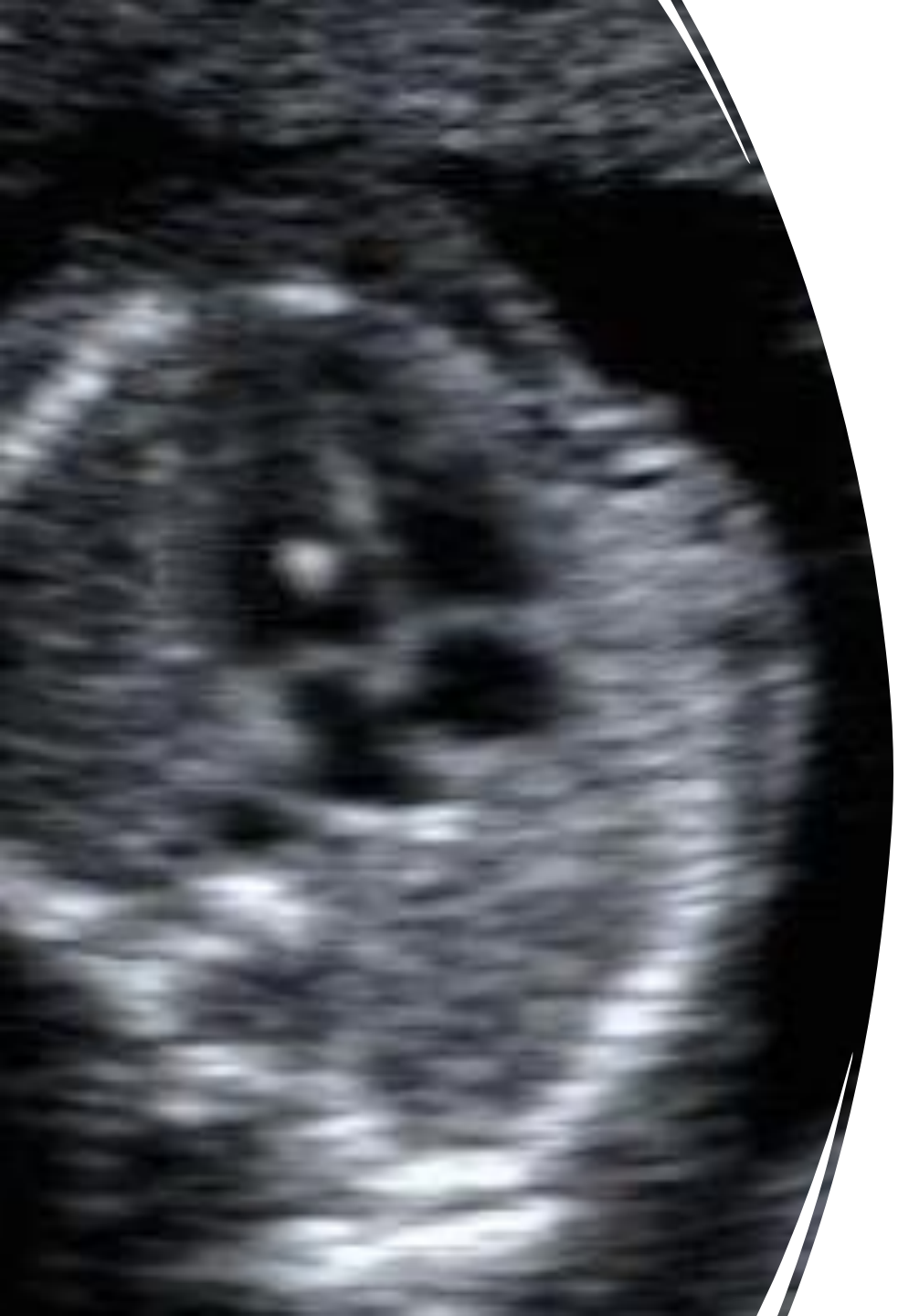
Fetal Urinary Tract Dilation

Zhang, Zhang, Guo (2020): Prospective cohort of antenatally diagnosed UTD (n=34; 24 with UTDA1, 10 with UTDA2-3)

- None of the A1s had postnatal abnormality
- All of the A2-3 had abnormal postnatal US:
  - 6 mild, 2 moderate, 2 severe

Singh et al (2021): Retrospective cohort of antenatally diagnosed UTD cases (n=70):

- None of the A1s had a postnatal abnormality
- 70% of the A2-3 had abnormal postnatal US
- 13% of the total cohort needed surgery, all in the A2-3 group

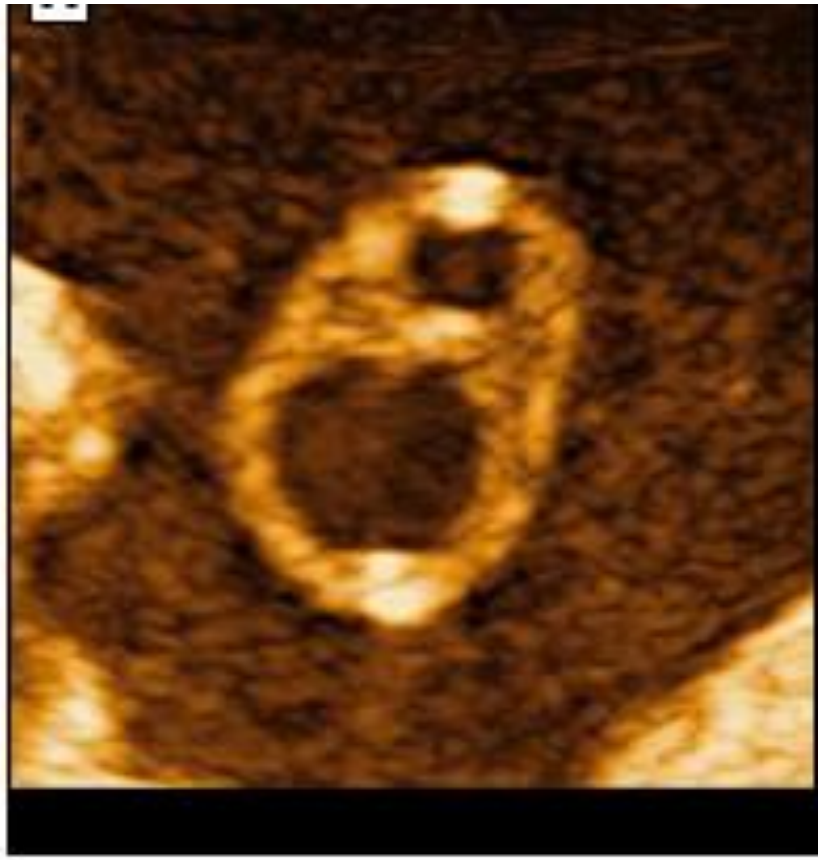


Echogenic Intracardiac Focus

- Calcium deposit in papillary muscle
- < 6mm, bright as bone, and seen in 2 planes
- Seen in 3-5% of euploid fetuses
  - Middle Eastern: 8%, Asian: 7-30%, Black: 7%, White: 3%
- LR for T21 1.4-1.8, but lower end of 95%CI crosses 1 → risk is “minimal”
- If no prior screening, offer non-invasive screening
- If prior low risk screening, no follow up needed as “**this finding is a normal variant of no clinical importance**.”

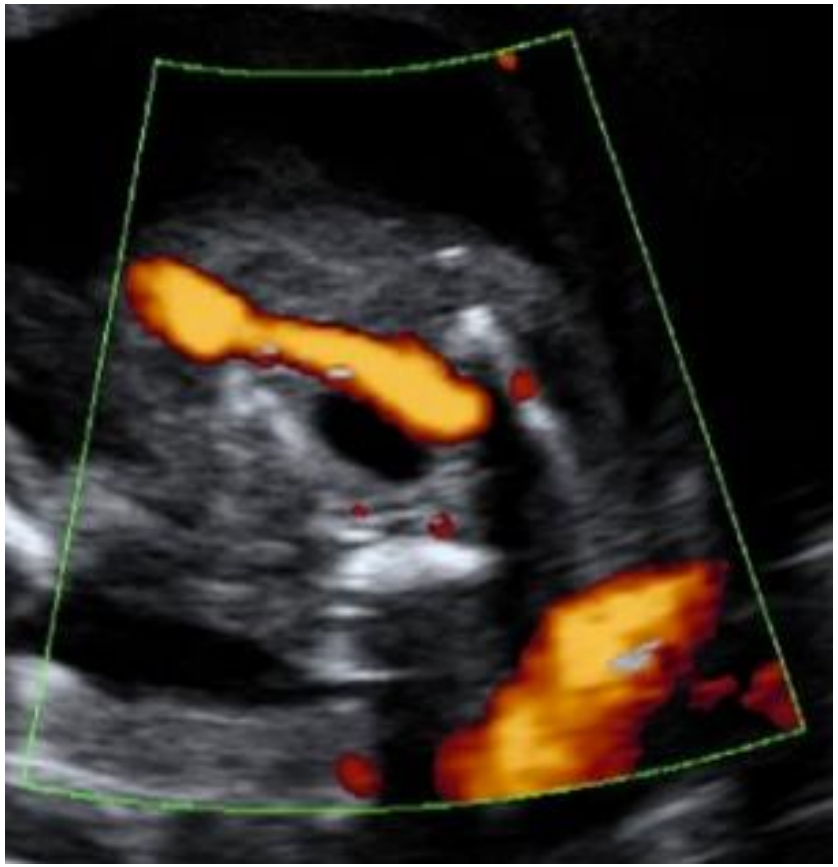
Single umbilical artery

- Seen in 1:100-1:400 singletons, up to 1:22 twins.
- Commonly associated with other abnormalities: most often cardiovascular and renal.
  - No echo if all views from 76811 are obtained.
- SUA and 1+ anomalies: aneuploidy rate 4-50%



Single umbilical artery

- If ISOLATED, NO increased risk of aneuploidy.
- SMFM recommends no aneuploidy evaluation even if patient has not had any prior screening!
- This is the **only** soft marker with this recommendation.



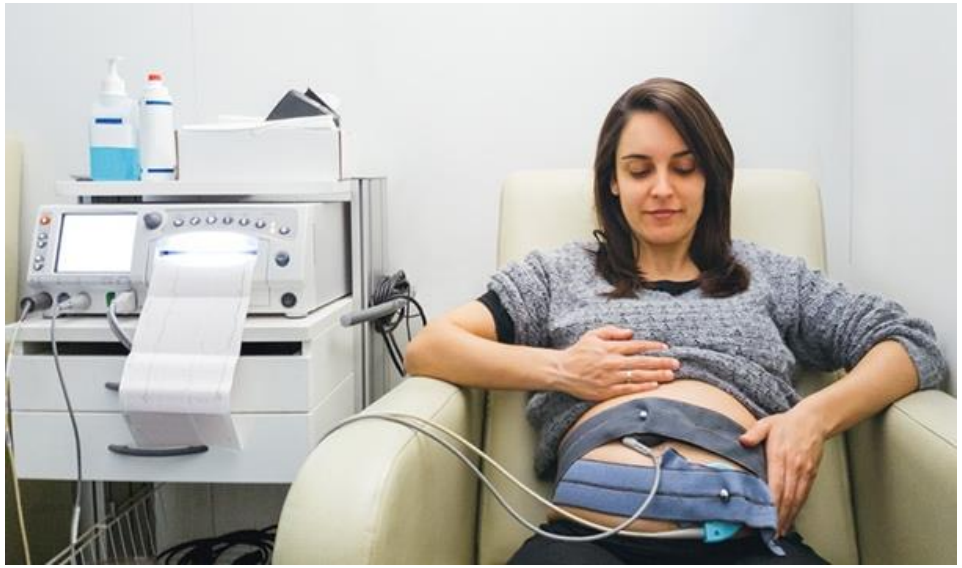
Single umbilical artery

SMFM recommends 3rd trimester growth US and “consideration of weekly antenatal surveillance at 36 0/7.” ACOG also “suggests” weekly surveillance at 36 0/7.

- Data regarding risk of FGR are conflicting

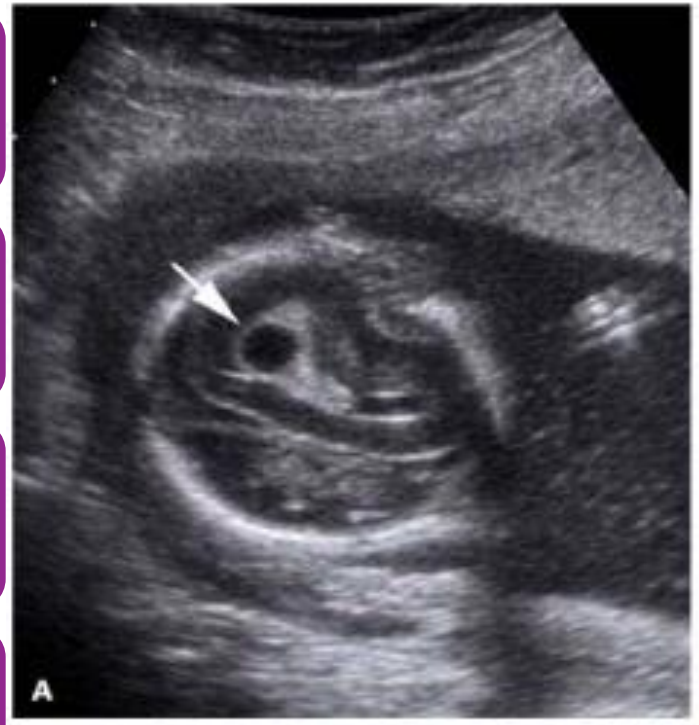
- What is the data for stillbirth risk?

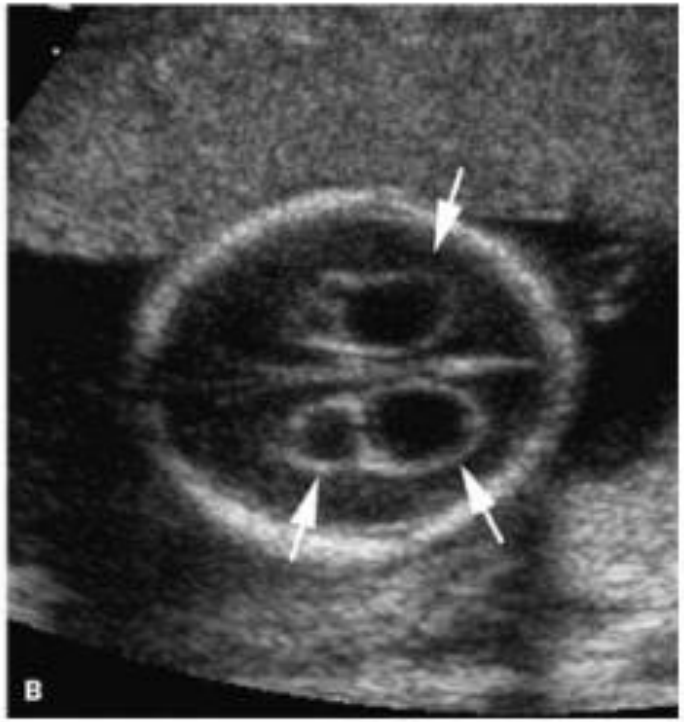
  “In a population-based case-control study, SUA was associated with an increased OR of stillbirth compared with live birth (OR, 4.80; 95% confidence interval [CI], 2.67–8.62).”



Choroid Plexus Cyst

- Can be single, multiple, unilateral, or bilateral
- Seen in 1-2% of 2nd trimester fetuses.
- LR for trisomy 18 is **<2 if isolated**, but 66 if associated abnormalities.
- **No** increased risk of T21.





Choroid Plexus Cyst

- If prior low risk serum screening, no further aneuploidy screening is recommended:

  "This finding is a normal variant of no clinical importance with no indication for follow-up ultrasound imaging or postnatal evaluation."

- If no prior screening, counsel on option of non-invasive screening

Key Takeaway Points

- All patients should be offered diagnostic testing regardless of aneuploidy risk.
- Diagnostic testing for aneuploidy is not recommended solely for the evaluation of an isolated soft marker **following low risk aneuploidy screening**.

EXCEPTION

If **serum** screening only and abnormal nasal bone or NSF, offer:

No further workup, cfDNA, or amnio

No prior aneuploidy screening

EIF, echogenic bowel, CPC, UTD, shortened HL, FL, or both	Counsel about non-invasive testing
Thickened NSF or abnormal nasal bone	Counsel about non-invasive testing vs amnio
Single umbilical artery	No further testing

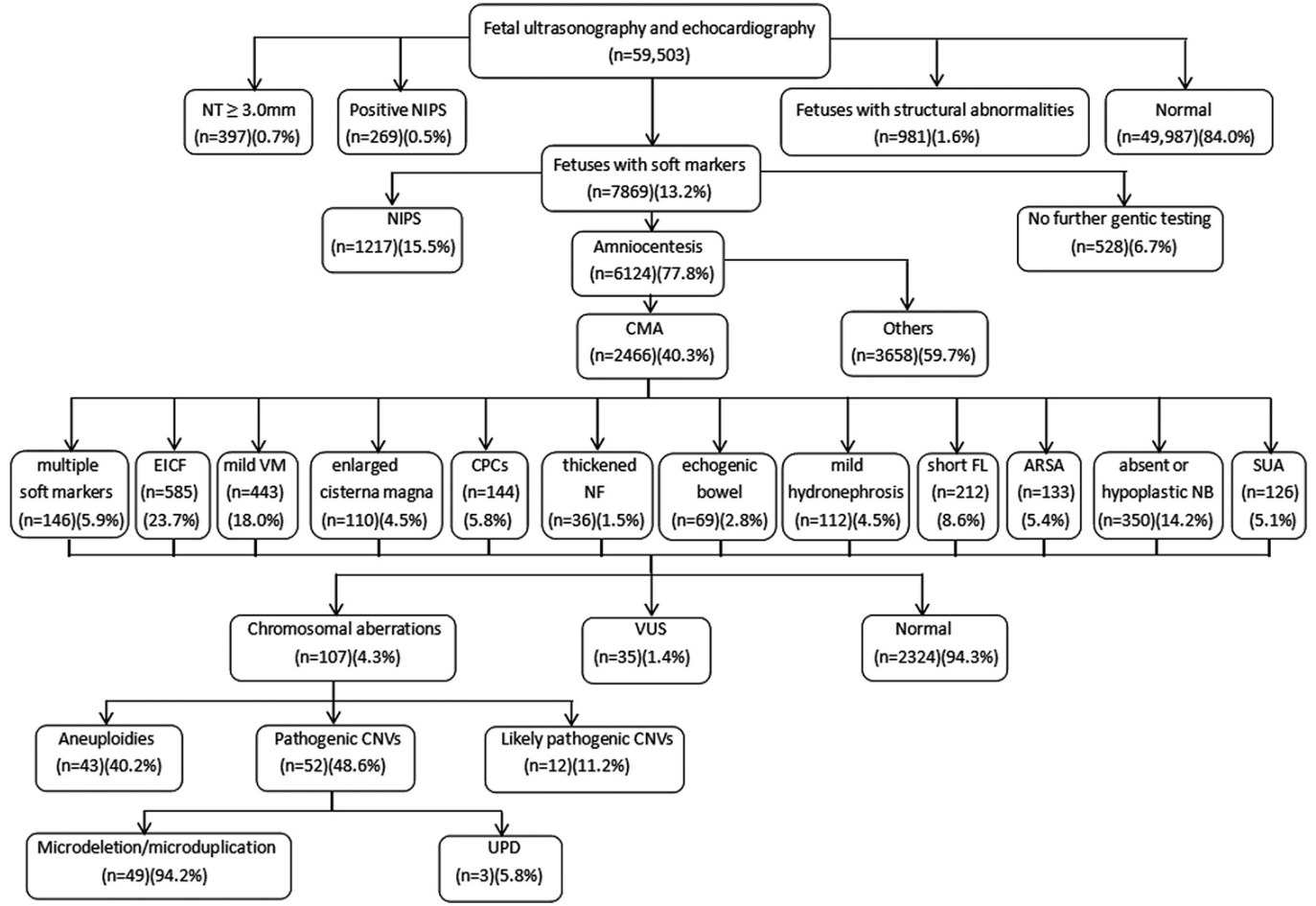
Emerging Data

Prenatal chromosomal microarray analysis in 2466 fetuses with ultrasonographic soft markers: a prospective cohort study

Ting Hu, Tian Tian, Zhu Zhang, Jiamin Wang, Rui Hu, Like Xiao, Hongmei Zhu, Yi Lai, He Wang, Shanling Liu,

AJOG, Volume 224, Issue 5, 2021, Pages 516.e1-516.e16,

FIGURE
The flow diagram of the study



ARSA, aberrant right subclavian artery; *CMA*, chromosomal microarray; *CNV*, copy number variant; *CPC*, choroid plexus cyst; *EICF*, echogenic intracardiac focus; *FL*, femur length; *NF*, nuchal fold; *NIPS*, noninvasive prenatal screening; *NT*, nuchal translucency; *SUA*, single umbilical artery; *UPD*, uniparental disomy; *VM*, ventriculomegaly; *VUS*, variant of uncertain significance.

Hu et al. Prenatal chromosomal microarray analysis in fetuses with ultrasonographic soft markers. Am J Obstet Gynecol 2021.

Emerging Data

Prenatal chromosomal microarray analysis in 2466 fetuses with ultrasonographic soft markers: a prospective cohort study

TABLE 5
Prevalence rates of chromosomal aberrations in different soft marker groups

Ultrasound category	n	Chromosomal aberrations	*P* value	Aneuploidies	*P* value	P/LP CNVs	*P* value
Multiple soft markers	146	16 (10.96)	<.0001[a]	8(5.48)	.001[a]	8(5.48)	.046[a]
EICF	585	15 (2.56)	.016[b]	7(1.20)	.247	8(1.37)	.032[b]
Mild ventriculomegaly	443	16 (3.61)	.407	7(1.58)	.772	9(2.03)	.410
Enlarged cisterna magna	110	2 (1.82)	.276	1(0.91)	.755	1(0.91)	.406
CPCs	144	2 (1.39)	.073	1(0.69)	.504	1(0.69)	.227
Thickened nuchal fold	36	4 (11.11)	.110	3(8.33)	.024[a]	1(2.78)	.615
Echogenic bowel	69	1 (1.45)	.371	0(0.00)	.512	1(1.45)	.823
Mild hydronephrosis	112	6 (5.36)	.761	1(0.89)	.738	5(4.46)	.332
Short femur length	212	20 (9.43)	<.0001[a]	6(2.83)	.322	14(6.60)	<.0001[a]
ARSA	133	8 (6.02)	.329	1(0.75)	.577	7(5.26)	.087
Absent or hypoplastic nasal bone	350	15 (4.29)	.958	7(2.00)	.693	8(2.29)	.694
SUA	126	2 (1.59)	.120	1(0.79)	.626	1(0.79)	.309
Total	2466	107 (4.34)		43(1.74)		64(2.60)	

Values are number (percentage) unless indicated otherwise.

ARSA, aberrant right subclavian artery; *CPC*, choroid plexus cyst; *EICF*, echogenic intracardiac focus; *P/LP CNVs*, pathogenic/likely pathogenic copy number variants; *SUA*, single umbilical artery.

[a] The prevalence rate was significantly higher than those in the other groups; [b] The prevalence rate was significantly lower than those in the other groups.

Hu et al. Prenatal chromosomal microarray analysis in fetuses with ultrasonographic soft markers. Am J Obstet Gynecol 2021.

Emerging Data

Prenatal chromosomal microarray analysis in 2466 fetuses with ultrasonographic soft markers: a prospective cohort study

TABLE 3
Characteristics of P CNVs among the 2466 fetuses with soft markers

Number	Ultrasound findings	P CNVs (GRCh37)	Size of CNVs, kb	Copy number	Known syndromes	OMIM gene	Inherited or de novo	Karyotyping/FISH results	Outcomes
1	Absent nasal bone, mild hydronephrosis	arr 1q21.1q21.2 (146586249_147844778) x3	1259	Gain	1q21.1 recurrent microduplication (possible susceptibility locus for neurodevelopmental disorders)	GJA5	De novo	/	TOP
2	CPCs, mild hydronephrosis	arr 1q21.1q21.2 (145895746_147933973) x1	2038	Loss	1q21.1 recurrent microdeletion (susceptibility locus for neurodevelopmental disorders)	GJA5 (HI score, 1), GJA8 (HI score, 1)	Inherited from normal mother	/	Born
3	Short femur length, SUA	arr 7q11.23 (72653992_74154209)x1	1500	Loss	Williams-Beuren syndrome	ELN (HI score, 3)	N/A	Confirmed by FISH	TOP
4	Absent nasal bone, mild hydronephrosis	arr 8q21.11q21.12 (76427726_78583918)x1	2156	Loss	8q21.11 Microdeletion Syndrome	/	N/A	/	TOP
5	Mild ventriculomegaly, short femur length	arr 16p11.2 (29581101_30190029)x3	609	Gain	16p11.2 microduplication syndrome	TBX6	De novo	/	TOP
6	Echogenic bowel, mild hydronephrosis	arr 16p11.2 (29351826_30176508)x1	825	Loss	16p11.2 microduplication syndrome	TBX6	De novo	/	TOP
7	Absent nasal bone, echogenic bowel	arr Xp21.1 (31795363_32083020)x0	288	Loss	/	DMD (HI score, 3)	Inherited from normal mother	/	TOP

TABLE 4
Characteristics of LP CNVs among the 2466 fetuses with soft markers

Number	Ultrasound findings	LP CNVs (GRCh37)	Size of CNVs, kb	Copy number	Known syndromes	OMIM gene	Inherited or de novo	Karyotyping/FISH results	Outcomes
1	Mild ventriculomegaly, absent nasal bone,	arr 10q22.3q23.2 (81630468_88973570)x3	7343	Gain	10q22.3q23.2 recurrent region (LCR_3/4_flanked) (includes BMPR1A)	BMPR1A	De novo	/	TOP

Emerging Data

Prenatal chromosomal microarray analysis in 2466 fetuses with ultrasonographic soft markers: a prospective cohort study

TABLE 2
Characteristics of numeric chromosomal abnormalities among the 2466 fetuses with soft markers

	Microarray results						
Ultrasound category	Trisomy 21	Trisomy 18	XXY	XYY	Monosomy X	Mosaicism	Total
Multiple soft markers	8	-	-	-	-	-	8
EICF	2	-	3	2	-	-	7
Mild ventriculomegaly	4	-	2	-	-	1[a]	7
Enlarged cisterna magna	-	-	-	-	-	1[b]	1
CPCs	-	1	-	-	-	-	1
Thickened nuchal fold	3	-	-	-	-	-	3
Echogenic bowel	-	-	-	-	-	-	-
Mild hydronephrosis	1	-	-	-	-	-	1
Short femur length	3	1	-	1	1	-	6
ARSA	-	-	-	-	-	1[c]	1
Absent or hypoplastic nasal bone	6	-	1	-	-	/	7
SUA	-	1	-	-	-	/	1
Total	27	3	6	3	1	3	43

ARSA, aberrant right subclavian artery; *CPC*, choroid plexus cyst; *EICF*, echogenic intracardiac focus; *FISH*, fluorescence in situ hybridization; *SUA*, single umbilical artery.

[a] Mosaic trisomy 12, arr[GRCh37] 12p13.33q24.33(173786_133777562)x2.22; [b] Mosaic trisomy 21, arr[GRCh37] 21q11.2q22.3(15016486_48093361)x2.51; [c] arr[GRCh37] Xp22.33q28(168551_155233098)x2.25, confirmed by FISH: XXX[86]/X[11]/XX[3].

Hu et al. Prenatal chromosomal microarray analysis in fetuses with ultrasonographic soft markers. Am J Obstet Gynecol 2021.

An ethical question:

- Should an isolated soft marker be documented and discussed with patients?

"Given the low likelihood of aneuploidy…, some providers or practices may decide that **for pregnant people who have previously declined aneuploidy screening after counseling**, identification of an isolated soft marker will be treated as a normal variant and neither acknowledged nor discussed, except for those associated with a need for further imaging follow-up (eg, UTD, echogenic bowel)."