

Club foot, an adverse outcome of early amniocentesis: disruption or deformation?

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Abstract

An association between the occurrence of club foot and early amniocentesis has been reported. The largest of these randomised studies was the Canadian Early and Mid-Trimester Amniocentesis Trial. Data describing the neonatal outcome, focusing on this association, are presented. Possible mechanisms for the association and the implications for the development of club foot are discussed.

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There are many theories about the aetiology of isolated club foot and there is probably more than one cause of this congenital anomaly. Included among the possibilities are multifactorial inheritance,¹ deformation resulting from uterine factors,² skeletal and neurogenic problems, muscular anomalies, connective tissue differences, and vascular disruptions.³

Several randomised control studies⁴⁻⁶ have now shown an increased risk of club foot in association with early amniocentesis (EA). This association suggests there is a window of vulnerability to club foot, at the gestational age where EA was undertaken in these trials. In this report, the neonatal outcome data from the largest of these randomised trials, the Canadian Early and Mid-Trimester Amniocentesis Trial (CEMAT), will be reviewed and the possible causes of club foot are assessed in light of the association.

Materials and methods

Details of the trial procedures have been published previously.⁵ This large multicentered randomised trial was designed to compare the safety and accuracy of EA compared to MA. Women referred for prenatal chromosome testing for advanced maternal age (35 or older at delivery) were entered into the trial. They were eligible when there was agreement to randomisation and documentation of a viable fetus, with a crown-rump length of 20-50 mm before randomisation. Exclusion criteria included multiple gestation, three or more spontaneous pregnancy losses, a parental chromosome rearrangement with the risk of an offspring having a chromosomal anomaly of >5%, failed chorionic villus sampling (CVS), a non-viable or abnormal fetus, oligohydramnios, alloimmunisation, or the presence of an intrauterine contraceptive device. Upon agreeing to enter the trial, the woman was

randomised by computer to have either early amniocentesis (EA = 11+0-12+6 gestational weeks) or mid-trimester amniocentesis (MA = 15+0-16+6).

The primary details of the cytogenetic analysis and obstetric outcomes have been previously published.⁵ Neonatal outcomes were obtained by trained study coordinators using telephone contact during the pregnancy and after the birth. The mothers were asked questions regarding the health of their infants. All results were as reported by the mother.

Continuous variables were compared using a between groups *t* test. Binary variables were compared using a chi-square test of association, except when expected cell frequencies were less than 5, in which case the Fisher's exact test was used. In the case of categorical variables, where categories were equally spaced, a linear trend chi-square test was used to compare the groups. Other methods of statistical analysis have been described previously.⁵

Results

There were no differences between the demographic, social, and maternal characteristics of each group.⁵ Of 4374 women eligible, 2187 were randomised to each group. CEMAT described the losses which occurred between randomisation and final outcome analysis.⁵

In this trial, all diagnoses were self-reported by the mothers and not verified by the investigators. Therefore, the terminology is that used by the mothers. However, the accuracy of this reporting is supported by the fact that the incidence in the MA group matched the expected population rates. Further supporting the accuracy of self-reporting, in the EA club foot group the sex distribution and the increase in bilateral cases matched the expected ratios.^{3 7 8} Finally, all of the children with reported club foot underwent casting, consistent with a significant anomaly.

There were 29 (1.3%) cases of club foot among the 2172 EA followed pregnancies but only two (0.1%) in the 2162 pregnancies in the MA group ($p=0.0001$). One of the two cases in the MA group was diagnosed with spinal muscular atrophy type 1. The rate of club foot in the EA group is 10 times the liveborn population risk of 0.1%.⁷ Bilateral club foot occurred in 14 cases (52%) and 13 were unilateral in the EA group. (The total number of cases for bilateral versus unilateral is smaller than the total case number because there were incomplete data for two cases.) The proportion of bilateral versus unilateral is similar to that previously

Table 1 Factors analysed in association with club foot in EA cases

Factor	With club foot	Without club foot	p value
<i>Maternal</i>			
Height (cm)	161.1	162.1	0.37*
Weight (kg)	67.5	64.9	0.39*
First pregnancy	0%	16.9%	0.07†
Previous premature delivery	10.3%	3.9%	0.08‡
Post secondary education	65.5%	75.1%	0.24§
Smoking	17.2%	10.2%	0.22§
Alcohol use	13.4%	10.3%	0.64§
<i>Procedural</i>			
Difficult procedure	6.9%	10.0%	0.58§
Uterine position retroverted	0.8%	6.7%	0.75‡
Gestation at amnio (days)	84.7	85	0.67*
Fluid volume sampled (ml)	11.3	11.4	0.59*
Membrane tenting	10.3%	10.6%	0.85‡
Transplacental approach	10.3%	22.2%	0.13§
Two needle insertions	6.9%	5.3%	0.67‡
Cramping	17.2%	14.0%	0.62§
Bleeding	13.8%	6.0%	0.10‡
<i>Perinatal</i>			
Median birth weight (g)	3152	3484	0.51*
Gestation at delivery (wk)	38.9	39.3	0.29*
Sex distribution (% male)	58.6	50.3	0.37§

*t test.

†Linear trend test.

‡Fisher's exact test.

§Chi square test.

described.⁷ The sex distribution was 17 males and 12 females, which is consistent with the known increased rate in males.

Maternal baseline, procedural, and perinatal factors were analysed in association with the occurrence of club foot. The maternal factors included age, weight, height, alcohol consumption, smoking habits, parity, education, history of premature delivery, or indication for amniocentesis. These factors were similar between the EA and MA groups. There was no association of club foot with procedural difficulty (as reported by the physician undertaking the procedure), number of needle insertions, transplacental approach, fluid volume sampled, post procedure cramping (from post procedure to time of reporting around 20-22 weeks), bleeding (from post procedure to time of reporting at 20-22 weeks), membrane tenting, or uterine position. There was a slightly greater frequency of club foot when EA was undertaken in the 12 week 0 day-12 week 6 day period (6/687 procedures, 0.9%) compared to the 11 week 0 day-11 week 6 day period (22/1040 procedures, 2.1%) ($p=0.046$, 95% CI 0.1-2.3).⁵ Gestations were based on crown-rump length assessment at the time of an ultrasound to date the pregnancy, done before entry into the trial. In addition, there was one case in the 13 week 0 day-13 week 6 day period (1/112 procedures, 0.9%). Birth weight, gestational age at delivery, chromosome result, and sex distribution did not differ between the EA and MA groups (table 1). The relationship of gestation and laterality of the club foot is described in table 2.

Amniotic fluid leakage increased the risk of club foot to 15% while it was 1.1% when leakage was not reported.⁵

Discussion

CEMAT⁵ showed a clear association between club foot and EA. The overall 1.3% frequency of club foot observed in the CEMAT EA group

Table 2 Age of fetus (as determined by ultrasound for determining trial eligibility) and type of club foot unilateral or bilateral

Gestation (wk)	Bilateral	Unilateral
11	3	7
12	11	6

$p=0.08$. Complete data were missing for two cases, not included in this table.

is well above the population risk of 1/1000 births, which was the frequency seen in the MA group. This prospective randomised trial had the largest number of women enrolled. In addition, it is the only trial comparing EA to mid-trimester amniocentesis, the gold standard of invasive prenatal diagnosis. The only other report showing clear evidence of an increased risk of club foot with EA was the trial of Sundberg *et al.*⁴ Talipes equinovarus (club foot) occurred in 1.7% of the EA group but there were no cases in the CVS group. This trial was discontinued before completion of enrolment because of the observed incidence of club foot in the EA group.

In other prospective randomised trials, the number of cases was too small to assess neonatal outcome adequately.⁶⁻⁹⁻¹³ Nicolaidis *et al.*⁹⁻¹³ compared CVS to EA in a study where not all women were randomised. In the EA group, the rate of club foot was 1.66% and in the CVS group it was 0.48%, which was not a statistically significant difference.

Nagel *et al.*⁶ observed a 3.1% incidence of club foot in their EA group but as some cases were not randomised and the numbers were small, the significance of these data is difficult to assess.

Non-randomised studies of EA have been published.¹⁴⁻²⁶ It is difficult to draw conclusions about the risk of lower limb anomalies from these.

During embryonic development, there is a period when the foot is in a position resembling club foot, starting at 9+0 weeks, with final reduction from the equinus, supinated, and calcaneovarus position to the neutral position occurring during 11+0 to 12+6 weeks. The foot moves out of the equinus position between 9+3 and 12+6 weeks. This is accomplished by flattening of the trochlea tali. Between 9+0 and 12+6 weeks, the lateral side of the distal tibia grows faster than the medial side. This results in a decrease in supination of the foot. Finally, these two mechanisms also result in a decrease in the calcaneovarus position.²⁷⁻²⁸ In CEMAT⁵ and in the study of Sundberg *et al.*,⁴ EA was done at a time when the fetal foot is still moving into its final neutral position.

Kawashima and Uthoff²⁸ suggested that developmental arrest, mainly of the talus at the stage of the physiological club foot, might be one of the determinants of club foot. Some authors have postulated other mechanisms involving primarily soft tissue anomalies (Sano *et al.*, personal communication).²⁹ In most tarsal joints, the joint cavities with synovial linings appear around 13+0 weeks.²⁷⁻²⁸

The sex distribution and prevalence of bilateral cases in the EA group were similar to those in other published reports on club foot. This

observation could suggest that the influences on the development of club foot associated with EA might be similar to those resulting in club foot in children not exposed to this procedure. In the CEMAT study, none of the children with club foot had other structural anomalies. This indicates that primary skeletal, neurogenic, connective tissue, or muscular causes were not the origin of the club foot in these children. This implies that the source of club foot in association with EA is not a malformation but instead is from a secondary process, a disruption or a deformation. During this crucial period of development, an element of the EA procedure might result in persistence of the physiological club foot as a deformation or alternatively cause a disruption of the normal evolution of position.

Traditionally, uterine restriction is thought to cause deformations.² The classical example, the oligohydramnios sequence, results in club foot but usually other joints and systems are affected. In CEMAT,⁵ amniotic fluid leakage before 22 weeks' gestation was the only significant factor associated with club foot. The chance of club foot was 15% (9/60) with leakage, but only 1.1% (19/735) without fluid loss. However, none of the cases of club foot had persistent oligohydramnios at a detailed fetal ultrasound done between 18 and 20 weeks. It should be noted that the volume of amniotic fluid removed was smaller in CEMAT,⁵ but unlike the trial of Sundberg *et al*,⁴ it was not returned to the amniotic sac. Since both CEMAT⁵ and Sundberg *et al*⁴ showed an association between EA, club foot, and transient amniotic fluid leakage, oligohydramnios could have contributed to the development of club foot. This does not readily explain why club foot also happened more frequently than expected in cases without clinically evident leakage. Nevertheless, unrecognised fluid loss might have occurred.

The transient nature of the oligohydramnios as well as the specificity of the site involved would suggest that the underlying process is disruptive rather than deforming. The question this poses is how uterine restriction from oligohydramnios could alter tissue development. One possibility is that oligohydramnios could cause a vascular event, disrupting blood vessels forming at that point in fetal development, with club foot as the consequence. Hootnick *et al*³⁰ have shown abnormal vascularity with club foot. Kawashima and Uhthoff²⁸ suggested that vascular factors should not play a primary role since vascular invasion of the talus starts only between 12+0 and 14+6 gestational weeks. However, disruption of the vasculature to the distal tibia could be postulated to result in a developmental arrest of the fetal foot evolving to the neutral position.

A second mechanism of disruption could be direct pressure upon developing tissue. This could cause irreparable damage. For example, pressure on the lateral side of the distal tibia, which normally grows faster than the medial side, could result in decreased growth of this bone contributing to an abnormal relationship

of the bones of the fetal ankle. If such pressure occurred at a later gestation, when the limb is fully formed, the effect might not be as severe.

The timing of the hypothesised disruption would be crucial to the observed effect. It is intriguing that there were more cases of club foot observed when the EA procedure was in the 12th week than in the 11th. However, as there were only a limited number of cases, the difference is not statistically significant. Also, there were more bilateral cases in the later week, with more unilateral cases in the earlier week. Although, the p value was not significant (0.08), it approaches statistical significance. The evidence from CEMAT suggests there might be a critical point in embryological development in the 11th to 12th weeks where there is increased susceptibility to club foot.

Mechanisms similar to those hypothesised to account for club foot in humans have also been proposed for experimental animal models of amniocentesis. The association between experimental amniocentesis/induced oligohydramnios and limb abnormalities can be seen in a review by Finegan.³¹ These include retraction of the nails with shortening of the digits, macroscopic haemorrhages in paws, fusion or absence of digits, phalanges, or metatarsals, and reduction of the long bones.

In the CEMAT trial,⁵ a large number of pregnancies were followed to delivery and with the exception of club foot an increased risk of neonatally detectable anomalies attributable to EA was not detected. The increased risk of club foot should be discussed if a woman is being offered EA at the gestation covered in this study.

The association of club foot with EA supports the theory of a disruption as a cause of isolated club foot. However, as the number of affected cases is small and anatomical detail is unavailable, the CEMAT study cannot fully resolve the question of whether club foot found in association with EA is a disruption owing to a vascular event or physical restriction of a developing tissue.

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