

## **Diagnostic Amniocentesis in Singleton Pregnancy**

### **Guidelines Suggested by EFSUMB'S Educational And Professional Standards Committee**

#### **Indications**

- a) Increased risk of chromosomal abnormality
- b) DNA analysis, biochemical analyses for hereditary diseases
- c) Suspicion of intra-amniotic infection
- d) Suspicion of iso-immunisation

#### **Comments**

**Re a)** After individual genetic counseling with regard to the risk of miscarriage caused by the invasive procedure and the risk of fetal chromosomal abnormality, every pregnant woman/couple decides whether an amniocentesis should be performed. From a strictly medical point of view, it is desirable that the risk of chromosomal abnormality be at least as high as that of miscarriage caused by the invasive procedure. An individual risk of fetal chromosomal abnormality can be calculated on the basis of maternal age, personal and family history, serum-biochemistry, nuchal thickness, and fetal anatomy.

**Re b)** Many hereditary diseases can be diagnosed in the fetus by DNA analysis or biochemical analyses in fetal cells from amniotic fluid.

**Re c)** Culture or polymerase chain reaction (PCR) technique can be used to show the presence of viruses and bacteria in the amniotic fluid.

**Re d)** Cordocentesis with analysis of fetal hemoglobin has replaced amniocentesis with analysis of extinction quotient in most centres.

#### **Contraindications**

- a) gestational age < 15 completed gestational weeks
- b) insufficiently informed couple
- c) vaginal bleeding (?)
- d) current infection in the mother or operator (?)

#### **Comments**

**Re a)** There is plenty of evidence that amniocentesis should not be performed at 10-13 gestational weeks. Randomized controlled trials have shown chorionic villus sampling (CVS) to be safer at that gestational age, early amniocentesis being associated with higher risk of amniotic fluid leakage<sup>1-3</sup> congenital talipes<sup>2-5</sup> miscarriage<sup>1, 3, 5</sup> and total fetal loss<sup>1, 3, 5</sup>. The risk of performing amniocentesis at 14 gestational weeks has not been studied in any randomized trial, and the risks at this gestational age are insufficiently known. Therefore, until we know more, amniocentesis should not be performed before 15 completed gestational weeks.

**Re b)** It goes without saying that the parents-to-be must be well informed about the risks of the procedure and the information that it can provide.

**Re c)** To the best of our knowledge, there is no scientific evidence that ongoing vaginal bleeding increases the risk of complications of amniocentesis, but it seems reasonable to postpone the procedure until one week after the bleeding has ceased, in order not to "irritate" the uterus in a situation already associated with an increased risk of miscarriage, i.e., bleeding.

**Re d)** To the best of our knowledge, there is also no scientific evidence that amniocentesis should not be carried out if the pregnant woman or the operator are currently infected. However, chorioamnionitis has been reported to be a complication of amniocentesis<sup>6</sup>. Theoretically, the risk of causing chorioamnionitis might be increased if the amniocentesis is carried out when the mother or the operator have clinical signs of infection.

#### **Complications/risks**

- a) miscarriage
- b) amniotic fluid leakage
- c) respiratory problems in the newborn
- d) talipes (after amniocentesis at 10 — 13 gestational weeks)
- e) feto-maternal haemorrhage (possibly causing Rhesus iso-immunization)
- f) fetal damage by the needle
- g) contamination of the sample with maternal cells

#### **Comments**

**Re a, b, c, d)** The well-known randomized controlled trial by Tabor and colleagues<sup>7</sup> where women <35 years old were randomized to amniocentesis or not amniocentesis showed that amniocentesis at 16 completed gestational weeks (range 14 — 20; only 3.6% had amniocentesis at 14 gestational weeks or earlier) was associated with an increased risk of miscarriage of 1%, the miscarriage rate in the amniocentesis group being 1.7% and that in the control group 0.7% (difference 1%, 95% confidence interval of difference 0.3-1.5%). The same study also showed an increased risk of amniotic fluid leakage within 6 weeks of the procedure (1.7% vs. 0.4%) and of respiratory problems in the newborn (respiratory distress syndrome and pneumonia; 1.9% vs. 0.8%). No increased risk of talipes or congenital hip dislocation was found after amniocentesis at 16 gestational weeks.

Amniocentesis at 10-13 gestational weeks is associated with higher risk of amniotic fluid leakage, talipes, miscarriage, and total fetal loss than CVS at the same gestational age<sup>1-5</sup>.

**Re e)** Maternal serum levels of alpha-fetoprotein (AFP) increase after ultrasound guided amniocentesis, especially after transplacental passage and after amniocentesis performed by a less experienced operator<sup>8</sup>. This AFP increase reflects fetomaternal hemorrhage or leakage of amniotic fluid into the maternal circulation. There are data suggesting a possible association between fetomaternal hemorrhage and procedure related miscarriage<sup>9</sup>. Because of the increased risk of fetomaternal hemorrhage the risk of maternal Rhesus iso-immunisation may also be increased. In the randomized trial by Tabor and colleagues, two of 283 women who underwent amniocentesis developed Rhesus immunization vs. none of 268 controls<sup>8</sup>. In another study by Tabor and coworkers, only five out of 361 Rhesus negative women who gave birth to Rhesus positive babies and who underwent amniocentesis at around 16 weeks developed positive test results with regard to anti-D antibody<sup>10</sup>. In three out of six patients with anti-D antibody in their serum before amniocentesis the titre of antibody increased after amniocentesis. Tabor and colleagues concluded that amniocentesis may have worsened the outcome of these three pregnancies, but that the risk of immunization in Rhesus negative women is small after midtrimester amniocentesis. In an early report from the British Medical Research Council, sensitization of Rhesus negative patients seemed to occur more frequently after amniocentesis than in a control group<sup>11</sup>.

**Re f)** Fetal damage (e.g., cardiac tamponade<sup>12</sup>, ocular trauma<sup>13</sup>, gangrene of fetal limb<sup>14</sup>, porencephaly<sup>15</sup>) suspected to have been caused by the amniocentesis needle has been described after amniocentesis performed without continuous ultrasound monitoring of the needle tip, i.e., after amniocentesis performed blindly or under ultrasound guidance (ultrasound guidance = marking a suitable puncture site on the maternal abdomen on the basis of ultrasound findings, then removing the transducer and immediately performing amniocentesis). However, when continuous ultrasound monitoring of the needle tip is used, the risk of damaging the fetus with the needle is likely to be small<sup>7</sup>.

**Re g)** Theoretically, contamination of the amniotic fluid sample with maternal blood cells or other maternal cells could occur and result in an incorrect diagnosis.

### **Precautions to be taken**

- a) only after 15 completed gestational weeks
- b) always under real-time ultrasound guidance with continuous observation of the needle tip (ultrasound monitored)
- c) use the puncture site that gives easiest access to a pocket of free fluid; if placental puncture can be easily avoided, then avoid it
- d) use a needle guide (?)
- e) experienced operator ( 150 amniocenteses per year?)
- f) aseptic technique, sterile conditions
- g) no more than 2 needle insertions (preferably only one)
- h) discard the first 1 ml of amniotic fluid
- i) removal of no more than 15-20 ml of amniotic fluid
- k) Rhesus prophylaxis, if mother is Rhesus negative (?)

### **Comments**

**Re a)** Amniocentesis should only be performed after 15 completed gestational weeks because at 10 - 13 weeks it is safer to perform CVS<sup>1-5</sup>, and the risks of performing amniocentesis at 14 gestational weeks are insufficiently known (see above).

**Re b)** Although there are no randomized controlled trials showing that ultrasound monitored amniocentesis (i.e., amniocentesis under ultrasound guidance with continuous observation of the needle tip) is safer than ultrasound guided amniocentesis (see above) or amniocentesis done blindly, the risk of damaging the fetus with the needle tip is probably minimized if the procedure is carried out under continuous ultrasound monitoring. There is indirect evidence from non-randomized studies that ultrasound guided amniocentesis is safer than blind amniocentesis, because fetomaternal hemorrhage occurs less often if the procedure is performed under ultrasound guidance than if it is done blindly<sup>9</sup> and fetomaternal hemorrhage may be associated with an increased risk of miscarriage<sup>9</sup>. The technique of continuously monitoring the needle tip seems to be associated with fewer bloody and dry taps of the first needle insertion, fewer needle insertions and a lower incidence of blood-stained amniotic fluid than the ultrasound guided technique<sup>16, 17</sup>. Of course, this does not necessarily mean that the fetal loss rate is lower with the monitored technique. One non-randomized study showed a lower rate of spontaneous abortions after ultrasound monitored amniocentesis than after ultrasound guided amniocentesis<sup>16</sup>, whereas another similar study showed no difference in pregnancy loss between

ultrasound guided and ultrasound monitored amniocentesis<sup>18</sup>.

**Re c)** Possibly, transplacental passage of the needle should be avoided, because transplacental passage increases the risk of fetomaternal hemorrhage<sup>8</sup> and there may be an association between transplacental passage and procedure-related miscarriage, especially if there is a 'bloody tap'<sup>19</sup>. On the other hand, Giorlandino and colleagues<sup>20</sup> found that transplacental needle passage was associated with a lower risk of transient amniotic fluid leakage than non-transplacental passage and neither Giorlandino and coworkers<sup>20</sup> nor Marthin and colleagues<sup>18</sup> found a higher rate of miscarriage / pregnancy loss after transplacental passage of the amniocentesis needle than after non-transplacental passage. Possibly, the most important thing is to perform the procedure as atraumatically as possible. Therefore, the puncture site that gives easiest access to a pocket of free fluid should be chosen. If the placenta can be easily avoided, it is probably wise to avoid it.

**Re d)** See below under "Technique of ultrasound guidance"

**Re e)** It seems reasonable to assume the complication rate to be lower if the operator has performed a large number of invasive procedures than if he/she is inexperienced. There is indirect evidence of this, because the randomized trial of Tabor and colleagues showed fetomaternal hemorrhage to be less common after amniocentesis performed by an experienced operator than by a less experienced one<sup>8</sup>. Moreover, in several studies, fewer needle insertions and fewer bloody taps were reported when amniocentesis was performed by more experienced operators<sup>18, 21, 22</sup>. Despite this Wiener and colleagues<sup>21</sup> found the outcome of pregnancy not to differ between experienced and inexperienced operators. On the other hand Marthin and coworkers<sup>18</sup> reported fewer pregnancy losses for experienced operators (non-significant difference). The number of annual procedures needed for amniocentesis to be safe is not known, and the recommendation of at least 150 amniocenteses per year is not based on scientific evidence.

**Re f)** We know of no study that has compared the complication rate after amniocentesis performed under sterile conditions (sterile washing of the skin, sterile cover of the transducer or sterilization of the transducer, sterile cloths on the abdomen, sterile gloves) with that of amniocentesis performed under clean but not sterile conditions. Despite the lack of evidence, we think it is probably safest to do the procedure under sterile conditions because of the potential risk of

introducing pathogenic micro-organisms into the amniotic fluid thereby increasing the risk of chorioamnionitis.

**Re g)** In the NICHD study<sup>6</sup> and in the study by Marthin and colleagues<sup>18</sup>, there was some evidence that the rate of fetal loss after amniocentesis increased with the number of needle insertions. However, the larger number of needle insertions may have reflected a difficult procedure, and so the relationship between the number of needle insertions and fetal loss rate are not necessarily causal. Kappel and coworkers<sup>19</sup> found no association between the number of needle insertions and subsequent miscarriage. Nonetheless, to minimize trauma, it is probably wise not to allow more than two needle insertions in the same session.

**Re h)** To minimize the risk of contamination with maternal cells, it may be wise to discard the first ml of amniotic fluid. This recommendation is not based on scientific evidence.

**Re i)** Whether the amount of amniotic fluid removed has any effect on complication rates is not known, but it is probably wise to remove as little as possible (usually 15-20 ml is enough to obtain a diagnosis). In the NICHD study<sup>6</sup> there was no association between fetal loss and the volume of amniotic fluid removed, but the volumes removed were not specified.

**Re k)** Whether anti-D should be given prophylactically to Rhesus negative women to avoid Rhesus immunization at amniocentesis is controversial, because the risk of immunization in Rhesus negative women is small after midtrimester amniocentesis, and Rhesus prophylaxis is not without risks<sup>10</sup>.

### **Needles and other equipment**

0.7-0.9 mm external diameter with stylet.

#### **Comments**

To the best of our knowledge there are no randomized trials evaluating the effects on complication rates of needle size. The needle sizes used in the randomized trials cited<sup>1, 2, 3, 5,7</sup> vary between 0.7 and 1.2 mm external diameter (22 — 18 G). The needle size used today is usually 0.7 or 0.9 mm. Theoretically, a thin needle may have both advantages and disadvantages. One would expect a thin needle to cause a smaller hole in the membranes and to be less traumatic, thereby decreasing the risk of amniotic fluid leakage and fetomaternal hemorrhage. On the other hand, a thin needle increases the procedure time, and increased sampling time might be associated with an increased risk of chorioamnionitis<sup>23</sup>. In the

NICHD study the fetal loss rate was higher when a needle larger than 1.2 mm (18 G) was used<sup>6</sup>.

### Technique of ultrasound guidance

- a) Transabdominal approach
- (b) Use a needle guide (?)
- (c) Puncture under 45° angle (?)

### Comments

**Re a)** To the best of our knowledge there are no studies on any other route than the transabdominal for amniocentesis at 16 weeks. Any other approach would seem less practical.

**Re b)** We know of no randomized trials comparing freehand technique with needle guided amniocentesis. There is some evidence that the use of a needle guide might be preferable. When using the free hand technique the more freely movable needle might increase the risk of feto-maternal hemorrhage<sup>24</sup>. A needle guide was used in the randomized controlled trials of Tabor and coworkers<sup>7</sup>, Cederholm and Axelsson<sup>1</sup>, and Sundberg and colleagues<sup>2</sup>, whereas Nicolaidis and coworkers<sup>5</sup> used the freehand technique. In the CEMAT study<sup>3</sup> it was not stated whether a needle guide was used or not.

**Re c)** Possibly, one should try to puncture the membranes at an angle of 45° and not 90°. An *in vitro* experiment showed higher flow rate of fluid through the hole generated by needle puncture at 90° than by needle puncture at 45°<sup>25</sup>.

### Concluding remarks

The recommendation not to perform amniocentesis before 15 completed gestational weeks is based on firm scientific evidence. Most of the other recommendations are based on weak evidence (case-control studies<sup>6, 11</sup>, descriptive studies<sup>9, 10, 16-22</sup>, case reports<sup>12-15</sup>, experimental *in vitro* study<sup>25</sup>) or clinical experience, because there are no randomized trials investigating the effects of different procedures/techniques of amniocentesis. It goes without saying that because of the relatively low complication rate after amniocentesis a huge number of patients would have to be included in such trials in order to obtain reliable and generalizable results. Moreover, certain questions would not be suitable for answering in a randomized trial, e.g., whether the number of needle insertions affects the outcome.

### References

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10-13 weeks' gestation. *Prenat Diagn* 1997;17:311-317.

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