Biochemical Composition of Amniotic Fluid in Pregnancies Complicated with Twin-Twin Transfusion Syndrome

P.N. Adama van Scheltema\textsuperscript{a} P.S In’t Anker\textsuperscript{a} A. Vereecken\textsuperscript{b}
F.P.H.A. Vandenbussche\textsuperscript{a} H.H.H. Kanhai\textsuperscript{a} R. Devlieger\textsuperscript{a, c}

\textsuperscript{a}Fetal Diagnosis and Therapy Unit, Department of Obstetrics, Leiden University Medical Centre, Leiden, The Netherlands; \textsuperscript{b}Algemeen Medisch Laboratorium, Antwerp, and \textsuperscript{c}Department of Obstetrics and Gynaecology, University Hospital Leuven, Leuven, Belgium

\textbf{Key Words}
Amniotic fluid \cdot Biochemical composition \cdot Fetoscopy \cdot Twin-twin transfusion syndrome

\textbf{Abstract}
\textbf{Objective:} To compare the electrolyte composition of pregnancies complicated with twin-twin transfusion syndrome (TTTS) with that of physiologic pregnancies.

\textbf{Materials and Methods:} Amniotic fluid samples from 16 pregnancies were studied. Specimens were obtained from recipient sacs in 10 pregnancies undergoing fetoscopy for severe midtrimester TTTS. Additionally, 6 amniotic fluid samples were obtained transcervically from legal second-trimester pregnancy terminations. The concentrations of sodium, potassium, chloride, bicarbonate, calcium, glucose, osmolality, pH, total protein content and albumin were determined in each sample.

\textbf{Results:} The mean gestational age at sampling was 20.2 weeks (range 17.2\textendash}27.1) in the TTTS group and 18.4 (range 16.0\textendash}22.0) in the control group (p = NS). We found significant lower levels of albumin (0.22 \pm 0.04 vs. 0.39 \pm 0.11, p = 0.01) and total protein (0.19 \pm 0.08 vs. 0.51 \pm 0.17, p < 0.001) and higher levels of bicarbonate (16.90 \pm 1.45 vs. 14.50 \pm 2.17, p = 0.02) in amniotic fluid samples taken from recipient sacs of TTTS pregnancies.

\textbf{Conclusion:} Amniotic fluid from the receptor in severe midtrimester TTTS differs significantly from control amniotic fluid samples in bicarbonate concentration, total protein content and albumin concentration. These findings may help to understand the pathophysiology of TTTS and to optimise therapeutic modalities.

\textbf{Introduction}

Twin-twin transfusion syndrome (TTTS) complicates 10\textendash}15\% of monochorionic twin pregnancies [1]. The syndrome is attributed to an imbalanced blood flow through vascular anastomoses in the monochorionic placenta between donor and recipient twin, but the exact pathophysiology remains incompletely understood [2]. Fetoscopic laser coagulation of the vascular chorionic anastomoses in cases of severe midtrimester TTTS is today the most frequently performed fetoscopic operation [3]. During this procedure, the recipient sac is entered by the fetoscope, and the vascular equator is identified for selective laser coagulation of the inter-twin anastomoses [4\textendash}7]. During coagulation, most operators use fluid infusion to increase visibility and reduce heat production.
The aim of this study was to evaluate the biochemical composition of amniotic fluid samples taken from the recipient sac of TTTS pregnancies and to compare this with the biochemical composition of samples taken from physiologic pregnancies.

**Materials and Methods**

**Sample Collection**

Ten millilitres of amniotic fluid were obtained from 10 pregnancies undergoing fetoscopy for severe midtrimester TTTS (TTTS group, n = 10). The fluid was obtained by direct puncture in the amniotic sac of the recipient twin before the start of the operation (at the time of the introduction of the trocar). We compared these with amniotic fluid samples obtained from physiologic pregnancies (control group, n = 6). These amniotic fluid samples were obtained transcervically from legal second-trimester pregnancy terminations. The institutional ethical committee approved the study protocol.

No TTTS patient had undergone previous amnioreduction, septostomy or other treatment, which could alter the biochemical properties of the amniotic sac of the recipient twin.

**Analyses**

Biochemical parameters in amniotic fluid were measured with a Vitros 950 analyser (Johnson & Johnson, Clinical Diagnostics, Rochester, N.Y., USA) according to the manufacturer’s recommendations and using assay slides obtained from the manufacturer. Parameters outside the normal assay range were measured spectrophotometrically according to Waddell [8]. After centrifugation, the amniotic fluid was diluted 1:100 with saline. All determinations were performed on a Spectronic Genesys 5 (Milton Roy, distributed by Analis, Gent, Belgium) continuous-recording ultraviolet-visible spectrophotometer. Three millilitres of diluted fluid were read against 3 ml of saline in the reference cuvette. The continuous ultraviolet spectrum from 190 to approximately 310 nm was scanned. This included the primary analytical peak between 200 and 270 nm as well as the secondary protein peak in the range of 280 nm. The net absorbency at 225 nm was subtracted from the net absorbency at 215 nm as using the difference in absorption rather than a single wavelength reading lessened the effect of absorbency of non-protein constituents. This difference was multiplied by a factor, experimentally determined and calculated individually on each day of determination and obtained by utilising commercial lyophilised serum controls treated in a manner identical to that of the samples. Multiplication of the absorbency of a given sample by the factor yielded the concentration of protein of the undiluted sample expressed as grams per 100 ml. Coefficients of variations within and between runs were lower than 5 and 10%, respectively.

**Results**

The characteristics of the studied pregnancies are listed in table 1. The mean gestational age at sampling was 20.2 weeks (range 17.2–27.1) in the TTTS group and 18.4 (range 16.0–22.0) in the control group (p = NS). The de-
gree of polyhydramnios in the recipient twin is reflected by a mean deepest vertical pocket of amniotic fluid of 11.3 cm (SD 3.23) in that group.

The biochemical composition of the samples for the studied parameters in both groups is listed in table 2. We found significant lower levels of albumin, total protein and higher levels of bicarbonate in amniotic fluid samples from TTTS pregnancies (fig. 1). The variation in electrolyte composition within each group was small, except for one outlier in each group (remarkably lower amniotic fluid concentrations for sodium, potassium, chloride and osmolality in one TTTS pregnancy and one physiologic pregnancy, respectively).

### Table 2. Composition of amniotic fluid in 16 investigated pregnancies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TTTS (n = 10)</th>
<th>Control (n = 6)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
</tr>
<tr>
<td>Na, mEQ/l</td>
<td>134.60</td>
<td>6.20</td>
<td>134.00</td>
</tr>
<tr>
<td>K, mEQ/l</td>
<td>3.72</td>
<td>0.17</td>
<td>3.82</td>
</tr>
<tr>
<td>Cl, mEQ/l</td>
<td>107.80</td>
<td>5.14</td>
<td>108.67</td>
</tr>
<tr>
<td>Ca, mEQ/l</td>
<td>3.35</td>
<td>0.35</td>
<td>3.25</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>26.91</td>
<td>11.12</td>
<td>28.65</td>
</tr>
<tr>
<td>HCO₃, mEQ/l</td>
<td>16.90</td>
<td>1.45</td>
<td>14.50</td>
</tr>
<tr>
<td>Osmolality, mosm/kg</td>
<td>269.50</td>
<td>10.53</td>
<td>269.83</td>
</tr>
<tr>
<td>pH</td>
<td>8.33</td>
<td>0.27</td>
<td>8.35</td>
</tr>
<tr>
<td>Total protein, g/dl</td>
<td>0.19</td>
<td>0.08</td>
<td>0.51</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>0.22</td>
<td>0.04</td>
<td>0.39</td>
</tr>
</tbody>
</table>

NS = Not significant.

### Discussion

To the best of our knowledge, the amniotic fluid composition of TTTS pregnancies has never been studied before. However, this information could give valuable insight into the incompletely understood mechanisms leading to disturbed fetoplacental haemodynamics in TTTS. We found significant differences in bicarbonate concentration, total protein content and albumin concentration in amniotic fluid obtained from recipient sacs of TTTS pregnancies. As amniotic fluid proteins are mainly a transplacental filtrate of maternal plasma proteins, the lower albumin and total protein levels could be explained...
by a dilution of the filtrate due to polyuria of the recipient twin. However, in a series of 109 patients undergoing placental laser surgery for TTTS at midpregnancy, De Lia et al. [9] found hypoproteinemia in all TTTS mothers. This suggests that our findings could be at least in part a reflection of the changed maternal protein content.

In 1 TTTS pregnancy we found remarkably lower amniotic fluid concentrations for sodium, potassium, chloride and osmolality. Interestingly, this was the severest case of TTTS (Quintero stage IIIR) [10] with signs of cardiovascular decompensation in the recipient twin. As the composition of amniotic fluid is closely related to the urinary production of the fetus, it can be hypothesised that the capacity of the fetal kidney to maintain normal electrolytes in increased urine production is affected by the volume overload and subsequent cardiovascular decompensation, leading to the observed low values. Similar findings in one of the control samples are probably not due to physiologic variation. We rather think this could also have been an abnormal pregnancy. Unfortunately, this remains unclear as this pregnancy termination was performed on maternal request in a specialised family planning clinic and no karyotyping or detailed structural ultrasonography was performed prior to termination.

Besides the differences described above, we found the biochemical composition of amniotic fluid in the recipient compartment of TTTS pregnancies to be comparable to that in control situations at a comparable gestational age. Interestingly, the pH of the samples did not differ significantly between TTTS pregnancies and controls. This is a clinically important finding because it has been demonstrated that an acidic environment could weaken the amniochorion surrounding the fetus [11, 12]. When fetoscopy with amnio-infusion is performed in pregnancies complicated by severe midtrimester TTTS, besides the direct trauma to the fetal membranes the amnio-infused solution could alter the pH of the amniotic fluid and thereby affect the fetal membrane integrity in an indirect way. The pH of the solution could thus influence the risk of iatrogenic preterm premature rupture of membranes.

Our findings confirm that fluids used for amnio-infusion for other reasons than TTTS (with a pH in the normal range for amniotic fluid) could also be suitable for amnio-infusion during fetoscopic placental surgery in TTTS. Despite the small differences found, we believe that further investigation into the amniotic fluid composition in TTTS pregnancies is needed to gain a better understanding of the pathophysiology of this disorder.

References