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**Issue Date:** 2014-10-28
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American Journal of Obstetrics & Gynecology 2014;211
Chapter 8

Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial
Abstract

Objective:
Residual anastomoses after fetoscopic laser surgery for twin-to-twin transfusion syndrome (TTTS) may lead to severe postoperative complications, including recurrent TTTS and twin anemia-polycytemia sequence (TAPS). A novel technique (Solomon technique) using laser coagulation of the entire vascular equator was recently investigated in a randomized controlled trial (Solomon trial) and compared to the Standard selective laser technique. The aim of this secondary analysis was to evaluate the occurrence and characteristics of residual anastomoses in placentas included in the Solomon trial.

Study design:
International multicenter randomized controlled trial in TTTS, randomized 1:1 ratio to either the Solomon laser technique or Standard laser technique. At time of laser, surgeons recorded whether they considered the procedure to be complete. Placental dye injection was performed after birth in the participating centers to evaluate the presence of residual anastomoses.

Results:
A total of 151 placentas were included in the study. The percentage of placentas with residual anastomoses in the Solomon group and Standard group was 19% (14/74) and 34% (26/77), respectively (P = .04). The percentage of placentas with residual anastomoses in the subgroup of cases where the procedure was recorded as complete was 8/65 (12%) and 22/69 (32%) in the Solomon group and Standard group, respectively (P < .01).

Conclusion:
The Solomon laser technique reduces the risk of residual anastomoses. However, careful follow-up remains essential also after the Solomon technique, as complete dichorionization is not always achieved.
Introduction

Twin-twin transfusion syndrome (TTTS) occurs in 10% of monochorionic twins and is caused by imbalanced blood flow through placental vascular anastomoses.[1] The best treatment for TTTS is fetoscopic laser coagulation of the anastomoses and is associated with survival rates of both fetuses of 67%. [2] The goal of fetoscopic laser surgery is to coagulate all vascular anastomoses. However, inter-twin vascular connections may remain patent in up to 33% of TTTS cases. [3;4] These residual anastomoses can cause severe post-operative complications such as twin anemia-polycythemia sequence (TAPS) in 13-16% or recurrent TTTS in 7-14% of cases where both babies survive.[5;6]

To minimize the occurrence of residual anastomoses and their complications, we investigated in a randomized controlled trial a modified fetoscopic laser surgery technique called the “Solomon technique”. The aim of this technique is to draw a coagulation line along the entire vascular equator to reduce the risk of missing inter-twin vascular anastomoses, in particular the poorly visualized small anastomoses. In the first analysis of the Solomon study, we focused on the perinatal outcome and showed a significant improvement in clinical outcome after the Solomon technique.[6] The aim of this secondary analysis was to perform a detailed analysis of the placentas included in the Solomon study, and to determine the occurrence and characteristics of residual anastomoses. In addition we performed a sub-analysis of all cases in which the surgeon reported that the procedure was technically complete.

Materials and Methods

Study design:
The Solomon trial [6] was an open-label, randomized, controlled trial, performed in five European tertiary referral centres (University Hospital Leuven (Belgium), University Hospital of Strasbourg (France), Birmingham Women’s Hospital, University of Birmingham, Birmingham (UK), Buzzi Hospital Milan (Italy), and Leiden University Medical Centre (the Netherlands). The study was approved by the Ethics committee of the Leiden University Medical Centre (MEC P07.261) and each centre’s respective Institutional Review Board. The trial was registered with the Dutch trial registry, number NTR 1245. The background of the trial, methods and baseline characteristics have been reported previously.[6] In brief, the trial included 274 patients and were randomly assigned to the Solomon technique or Standard technique. All TTTS cases included in the Solomon trial were eligible for this study, except
cases treated in Birmingham. These cases (n=25) were excluded from the current study since placentas were not routinely injected. Exclusion criteria were placenta maceration following intrauterine death, placentas with disrupted architecture as a result of the delivery process or when placentas were placed in formalin. In case fetal demise occurred and the time period between fetal demise and birth was within a week the placentas were injected. Placentas were sent and injected in the participating centers of this trial. All other placentas were included in this study and injected with colored dye according to a specific technique reported below.

**Placenta injection protocol:**
Placental storage and injection was performed according to a previously published report. [4;7] In brief, after birth, placentas were stored in a plastic bowl at a storage temperature of 4 °C (without being frozen or fixed in formalin) and injected within a week. Before injection the placenta was washed with warm water and amnions were removed for better visualization of the vascular anastomoses. The umbilical vein and at least one artery of each cord were cannulated. Syringes with four different colored dyes were connected to the cannulas and dye was gently injected into the placental vessels. A measuring tape was placed on the placenta and digital high resolution pictures were taken perpendicular to the placental surface. The following placental characteristics were recorded: number, localization, size and type of residual anastomoses. Measurements of the size and localization of the anastomoses were performed using Image J 1.45s equipment and software (Image J, National Institute of Health, USA). The placental injections and subsequent evaluation of residual anastomoses could not be blinded, as it is obvious from observing the placental surface which technique was used. In case of arterio-venous (AV) anastomoses, the caliber of the artery was measured. After measuring the total length of the vascular equator, we calculated its radius, by dividing the

<table>
<thead>
<tr>
<th></th>
<th>Solomon (n=74)</th>
<th>Standard (n=77)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at laser (weeks)</td>
<td>20±2.6</td>
<td>20±2.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Location of placenta:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>27 (37)</td>
<td>36 (47)</td>
<td>0.20</td>
</tr>
<tr>
<td>Posterior</td>
<td>47 (64)</td>
<td>41 (53)</td>
<td></td>
</tr>
<tr>
<td>Quintero stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I – n (%)</td>
<td>15 (20)</td>
<td>17 (22)</td>
<td>0.26</td>
</tr>
<tr>
<td>Stage II – n (%)</td>
<td>27 (37)</td>
<td>20 (26)</td>
<td></td>
</tr>
<tr>
<td>Stage III – n (%)</td>
<td>31 (42)</td>
<td>35 (46)</td>
<td></td>
</tr>
<tr>
<td>Stage IV – n (%)</td>
<td>1 (1)</td>
<td>5 (7)</td>
<td></td>
</tr>
<tr>
<td>Laser complete (opinion surgeon) n (%)</td>
<td>65 (88)</td>
<td>69 (90)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or n (%) unless otherwise specified

**Table 1:** Baseline characteristics
length of the vascular equator in two. The localization of residual anastomoses was recorded as the ratio of their distance and radius (distance/radius) as previously reported.[3] In case residual anastomoses were detected during fetoscopic re-intervention (due to TAPS or recurrent TTTS), these were analyzed in the group with residual anastomoses.

**Primary and secondary outcome:**
The primary outcome of the Solomon trial was based on the short-term clinical outcome,
and included the presence of at least one of the four following items: TAPS, recurrent TTTS, perinatal mortality or severe neonatal morbidity. One of the secondary outcomes of the Solomon trial was the incidence of residual anastomoses. In this placental study we analyzed the type, size and localization of residual anastomoses after colored dye injection. Comparison between the Solomon group and Standard group was performed in the total group of injected placentas, and in the sub-group of placentas from pregnancies in which the fetoscopic laser surgery was recorded as ‘complete’ according to the surgeon’s opinion directly after the laser intervention. This sub-group analysis was performed to determine the impact of the surgeon’s opinion on the final result after fetoscopic laser surgery, and whether this could be useful to direct postoperative management.

We defined TTTS using the Eurofoetus criteria, with a cut-off at a deepest vertical pocket (DVP) of amniotic fluid in the donor ≤2 cm; in addition, we used a cut-off for the DVP in the recipient of ≥8 cm within the first 20 weeks of gestation or ≥10 cm after week 20.[8] The definition of recurrent TTTS was based on the same parameters used to define TTTS. Recurrent TTTS is including cases of reversal of TTTS. The presence or absence of TAPS was identified using previously published criteria.[5] In brief, antenatal TAPS was defined as present when Doppler ultrasound examination revealed an increase in Middle Cerebral Artery - Peak Systolic Velocity (MCA-PSV) of >1.5 Multiples of the Median (MoM) in one fetus that coincided with a decrease in MCA-PSV of <0.8 MoM in the co-twin. Postnatal TAPS was defined as both an inter-twin Hb difference of ≥8 g/dL at birth and at least one of the following: reticulocytosis in the donor with an inter-twin reticulocyte count ratio >1.7

<table>
<thead>
<tr>
<th></th>
<th>Total Solomon (n=74)</th>
<th>Standard (n=77)</th>
<th>p value</th>
<th>Laser complete (surgeon’s opinion) Solomon (n=65)</th>
<th>Standard (n=69)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placentas with residual anastomoses</td>
<td>14 (19)</td>
<td>26 (34)</td>
<td>0.04</td>
<td>8 (12)</td>
<td>22 (32)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Overall no. of residual anastomoses per placenta</td>
<td>2 (1-24)</td>
<td>2 (1-8)</td>
<td>0.94</td>
<td>2 (1-4)</td>
<td>2 (1-5)</td>
<td>0.90</td>
</tr>
<tr>
<td>Diameter of residual anastomoses (mm)</td>
<td>1.3±1.8</td>
<td>0.8±0.9</td>
<td>0.07</td>
<td>2.1±3.0</td>
<td>0.9±1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Placentas with arterio-venous residual anastomoses</td>
<td>10 (71)</td>
<td>21 (91)</td>
<td>0.11</td>
<td>6 (75)</td>
<td>17 (90)</td>
<td>0.33</td>
</tr>
<tr>
<td>Placentas with arterio-arterial residual anastomoses</td>
<td>6 (43)</td>
<td>4 (17)</td>
<td>0.09</td>
<td>3 (38)</td>
<td>2 (11)</td>
<td>0.10</td>
</tr>
<tr>
<td>Placentas with veno-venous residual anastomoses</td>
<td>7 (50)</td>
<td>4 (17)</td>
<td>0.04</td>
<td>4 (50)</td>
<td>3 (16)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data are median (range), mean ± standard deviation or n (%) unless otherwise specified.

*Table 2: Prevalence, number and size of residual anastomoses*
and/or the presence of only small (<1 mm in diameter) residual anastomoses seen at the
time of postnatal placental injection studies.[9] Severe neonatal morbidity was defined as the
presence of at least one of the following: chronic lung disease (defined as oxygen dependency
at 36 weeks gestational age), patent ductus arteriosus requiring medical therapy or surgical
closure, necrotizing enterocolitis grade 2 or higher, retinopathy of prematurity stage III or
higher, ischemic limb injury, amniotic band syndrome or severe cerebral injury. Severe
cerebral injury includes at least one of the following: intraventricular haemorrhage grade
III or higher, cystic periventricular leukomalacia grade II or higher, ventricular dilatation
greater than the 97th percentile, porencephalic or parenchymal cysts, or other severe cerebral
lesions associated with adverse neurological outcome.[10] Neonatal follow-up was done by
the treating neonatalogist. Length of follow-up was until term age or at discharge (which ever
came first); neonatal outcome in this study only included short term outcome.

Statistical analysis

Continuous variables are reported as the mean ± standard deviation; group differences were
compared using the Student’s t-test. Proportions were compared using the Chi-square test or
the Fisher’s exact test, where appropriate. Differences with a p-value <0.05 were considered
to be statistically significant. All analyses per fetus or neonate were performed using the
Generalized Estimated Equation module to account for the effect that observations between
co-twins are not independent. All statistical data were analyzed using SPSS version 20.0
(IBM, Armonk, NY, USA).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Laser complete (surgeon's opinion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solomon (n=74)</td>
<td>Standard (n=77)</td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>31±5</td>
<td>32±4</td>
</tr>
<tr>
<td>Twin anemia-polycythemia</td>
<td>3 (4)</td>
<td>17 (22)</td>
</tr>
<tr>
<td>sequence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent TTTS</td>
<td>1 (1)</td>
<td>4 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twin anemia-polycythemia</td>
<td>4 (5)</td>
<td>20 (26)</td>
</tr>
<tr>
<td>sequence or recurrent TTTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal survival</td>
<td>122/148 (82)</td>
<td>135/154 (88)</td>
</tr>
<tr>
<td>Severe neonatal morbidity</td>
<td>14/131 (11)</td>
<td>17/144 (11)</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or n (%) unless otherwise specified. TTTS = twin-twin transfusion syndrome

* Results measured per fetus using the Generalized Estimated Equation module

* Severe neonatal morbidity measured per live born neonate

Table 3: Perinatal outcome
A total of 247 placentas were eligible for this study (Figure 1). We excluded 65 placentas due to maceration following intrauterine fetal death of one or both twins (n=41), damaged (n=23), or placement in formalin (n=1). In cases where the placentas were damaged, this was often due to manual removal with disintegration of morphology. Of the 41 excluded cases were fetal demise occurred, two cases were double demise and 39 cases were single demise. Of the double demise one occurred within a week after laser and the other after a week. Of the single demise 24/39 (62%) occurred within one week after laser and 15/39 (38%) after a week. Data on fetal condition prior to fetal demise was not recorded. Finally, 31 eligible placentas were lost. This mainly occurred when patients delivered in referring hospitals. Two main reasons for delivering at a referral hospital were spontaneous premature delivery in which the patients could not be transferred to the treatment center or in cases with good recovery (normalization of amniotic fluid in both sacs and MCA-PSV Doppler measurements in both twins) in combination with the request of the patient to go back to the referral center (for logistic reasons). In these hospitals placental injection studies were not a standard procedure, and placentas were sometimes discarded or accidentally fixed for routine pathology. Gestational age at birth and the incidence of TAPS or recurrent TTTS was similar in the excluded group due to placental loss compared to the group of placentas included in the study (Table 4).

Complete placental dye injection was performed in 151 placentas, 74 (49%) placentas in the Solomon group and 77 (51%) placentas in the Standard group. Of the injected placentas, 19 placentas were after fetal demise, in 9/19 (47%) double fetal demise occurred and placentas were injected, in the remaining 10/19 (53%) placentas could be injected after fetal
demise of the co-twin. The laser procedure was recorded by the surgeons as complete in 65/74 (88%) in the Solomon group, and 69/77 (90%) in the Standard treatment group. There were no differences in baseline characteristics between the two study groups with respect to gestational age at fetoscopy, placenta localization and Quintero stage (Table 1).

A significant reduction of residual anastomoses was seen after using the Solomon technique. Residual anastomoses were detected in 19% (14/74) of placentas in the Solomon group compared to 34% (26/77) in the Standard group (P = .04). In the subgroup of cases in which laser surgery was recorded as complete by the surgeon, an even larger reduction of residual anastomoses was seen, 12% (8/65) in the Solomon group compared to 32% (22/69) in the Standard group (P < .01) (Table 2). In three cases with recurrent TTTS or TAPS requiring re-intervention with laser surgery, residual anastomoses were detected at time of re-intervention during fetoscopy. All three cases were initially treated with the Standard technique and analyzed in the group with residual anastomoses. Placental injection after birth in these three cases showed no residual anastomoses. Two (3%) placentas in the Solomon group had proximate cord insertions (distance < 5cm) and laser surgery was incomplete (though they were stated to be complete by the surgeon) resulting in various large residual anastomoses between the two cords. No placentas with proximate cord insertions were present in the Standard group.

<table>
<thead>
<tr>
<th>Twin Anemia-Polycythema Sequence (TAPS)</th>
<th>No TAPS</th>
<th>p value</th>
<th>Recurrent TTTS (n=3)</th>
<th>No rec TTTS (n=18)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of residual anastomoses (mm)</td>
<td>0.4±0.5</td>
<td>1.3±1.07</td>
<td>&lt;0.01</td>
<td>0.8±1.06</td>
<td>1.4±1.8</td>
</tr>
<tr>
<td>Placentas with residual arterio-venous anastomoses</td>
<td>17 (100)</td>
<td>13 (72)</td>
<td>0.05</td>
<td>2 (67)</td>
<td>13 (72)</td>
</tr>
<tr>
<td>Placentas with residual arterio-arterial anastomoses</td>
<td>1 (6)</td>
<td>7 (39)</td>
<td>0.04</td>
<td>2 (67)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Placentas with residual veno-venous anastomoses</td>
<td>1 (6)</td>
<td>9 (50)</td>
<td>&lt;0.01</td>
<td>1 (33)</td>
<td>9 (50)</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or n (%) unless otherwise specified. TAPS = twin anemia-polycythemia sequence, rec TTTS = recurrent twin-twin transfusion syndrome
* Sub-analyses performed in subgroup of the 37 placentas with residual anastomoses
* In the group "No TAPS" cases with recurrent TTTS were excluded
* In the group "No rec TTTS" cases with TAPS were excluded

Table 5: Sub-analysis of the residual anastomoses according to twin anemia-polycythemia or recurrent TTTS.

The median number of residual anastomoses was similar in the Solomon and Standard group (see Table 2). In the subgroup of cases in which laser surgery was recorded as complete by
the surgeon, the mean diameter of residual anastomoses was 2.1 (±3.0) mm in the Solomon group versus 0.9 (±1.0 ) mm in the Standard group (P = .01). The larger mean diameter of residual anastomoses in the Solomon group was mainly due to the two placentas with proximate cord insertion. After analyzing the data without the two placentas with proximate cord insertions, the mean diameter of residual anastomoses in the Solomon group and in the Standard group was similar, 1.4 (±1.3) mm versus 0.9 (±1.0) mm, respectively (P = .15). The localization of RAs was evenly distributed along the vascular equator. Placentas with residual anastomoses in the Solomon group where the surgeon stated to be complete were characterized by proximate cord insertion (n=2), discontinuous line (n=5) and Solomon line not along the vascular equator (n=1). Figure 2 shows a placenta treated using the Solomon technique with residual anastomoses near the margin of the placenta, causing TAPS. The risk of recurrent TTTS was 5% (4/77) in the Standard group compared to 1% (1/74)
in the Solomon group ($P = .19$). A significant reduction of TAPS of 22% (17/77) in the Standard group to 4% (3/74) in the Solomon group was seen ($P < .01$). A similar reduction in post-operative complications between Standard and Solomon group was detected in the subgroup of cases in which the laser procedure was recorded as complete at time of laser (see Table 3). Overall, the incidence of TAPS and recurrent TTTS in cases with residual anastomoses was 48% (19/40) and 13% (5/40), respectively. The risk of TAPS in the group with residual anastomoses was 21% (3/14) in the Solomon group and 62% (16/26) in the Standard group ($P = .02$). The risk of recurrent TTTS in the group with residual anastomoses was 7% (1/14) in the Solomon group compared to 15% (4/26) in the Standard group ($P = .64$). The residual anastomoses in TAPS cases were characterized by a smaller mean diameter 0.4 ($\pm 0.5$) mm compared to 1.3 ($\pm 1.1$) mm in cases without TAPS ($P < .01$). TAPS placentas had less arterio-arterial and veno-venous residual anastomoses compared to cases without TAPS, respectively 6% (1/17) versus 39% (7/18) ($P = .04$.) and 6% (1/17) versus 50% (9/18) ($P < .01$). There were no differences in size and type of residual anastomoses in the placentas with recurrent TTTS. Details are shown in Table 5.

**Comment**

In this secondary analysis of the Solomon trial, we showed that fetoscopic laser coagulation using the Solomon technique significantly reduces the incidence of residual anastomoses. This is the first randomized trial showing a reduction of residual anastomoses using a new laser technique in TTTS. In three recent retrospective studies, the Solomon technique was compared with the Standard technique, the investigators found a reduction of TAPS or recurrent TTTS in the Solomon group. Unfortunately these investigators did not report placental injection studies.[11-13] Importantly however, even after the Solomon procedure, we still found a clinically important number of residual anastomoses (19% in the overall Solomon group and 12% in the subgroup of placentas which were recorded as complete after the procedure). This highlights the fact that the Solomon procedure does not guarantee a complete dichorionization of the placenta. Therefore, careful follow-up with serial Doppler ultrasound measurements of the middle cerebral artery peak systolic velocity and of amniotic fluid volumes of both twins therefore remains of crucial importance, even after a laser intervention using the Solomon technique.

As shown in this study, the presence of residual anastomoses is associated with a 58% risk of developing TAPS or recurrent TTTS. Interestingly, the risk of TAPS or recurrent TTTS was lower in the Solomon group with residual anastomoses compared to the Standard group.
with residual anastomoses (29% versus 73% respectively). This could be explained by a trend towards higher rates of residual arterio-arterial anastomoses in the Solomon group. As previously shown, arterio-arterial anastomoses are known to protect against the development of TTTS or TAPS.[14;15] The relatively high rate of arterio-arterial and veno-venous anastomoses in the Solomon group was partly related to the presence of two placentas with proximate cord insertions in the Solomon group. As shown in a recent study, arterio-arterial and veno-venous anastomoses occur more frequently in monochorionic placentas with proximate cord insertions.[16]

The main reason for residual anastomoses in the Solomon group was the fact that the laser-line along the vascular equator was not continuous. We expected to find the residual anastomoses along the margin of the placenta. However, in the 8 placentas of pregnancies in which the procedure was complete according to the surgeon, residual anastomoses were spread along the vascular equator. A possible explanation could be that the energy used to coagulate the surface of the placenta was not always sufficient. More studies are needed to evaluate the effectiveness of coagulation using different laser energy settings. For the optimal laser effect, a careful balance should be sought between avoiding excessive tissue damage due to too much laser energy, and insufficient tissue damage (and possible residual anastomoses) due to reduced laser energy.

We found that the diameter of residual anastomoses in the Solomon group was increased compared to residual anastomoses in the Standard group. However, after exclusion of two of the placentas with residual anastomoses in the Solomon group with proximate cord insertions and large residual anastomoses no significant difference in diameter was found. As shown in a recent study[16] monochorionic placentas with proximate cord insertions treated with laser surgery have often an increased rate of residual anastomoses due to technical and surgical problems related to the identification of the vascular equator.[16;17]

The risk of TAPS or recurrent TTTS was significantly higher in the Standard group and was directly related to the presence of residual anastomoses. The incidence of residual anastomoses of 34% in the Standard group was similar to previous published reports by our own group and by Lewi et al (range 27-33%). [3;4;18] Other authors [19;20] reported a lower incidence of residual anastomoses in their series after using the Standard laser technique (4-6%). This difference might be related to operator-experience, although we believe that differences in placental injection technique are more likely. In the studies with lower incidence of residual anastomoses, placental injection was performed with air instead of colored dye, which hampers visualization of small missed anastomoses. As shown in this
and other studies, residual anastomoses in TAPS cases are very small (diameter <1.0mm) and may only be recorded reliably after accurate dye injection.[21-23]

One of the limitations of this study is the fact that we were unable to inject all placentas. Lost or damaged placentas mainly came from patients delivered at referring hospitals where placental injection studies were not a standard procedure. A selection bias based on the lost placentas is not likely since the outcome of these pregnancies did not differ from the included placenta group.

In conclusion, the Solomon technique reduces the incidence of residual anastomoses. Nevertheless, since the risk of residual anastomoses after using the Solomon technique is still existent, careful antenatal follow-up with Doppler ultrasound remains necessary.

Acknowledgement

The study was supported by research grant number ZonMw 92003545 from the Netherlands Organization for the Health Research and Development. The sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report.
References


