Twin-Twin Transfusion Syndrome
The Influence of Intrauterine Laser Photocoagulation on Arterial Distensibility in Childhood

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Background—In twin-twin transfusion syndrome (TTTS), the donor and recipient fetus are exposed to differing volume loads and show discordant intertwin vascular compliance in childhood despite identical genotype. We hypothesized that discordance is prevented by intrauterine endoscopic laser ablation of placental anastomoses, which abolishes intertwin transfusion. We tested this by examining pulse wave velocity (PWV) in brachial arteries of twin survivors of TTTS treated with and without laser therapy.

Methods and Results—One hundred children (50 twin pairs, 27 with TTTS) were studied. Group 1 comprised 14 monochorionic (MC) twin pairs with TTTS treated symptomatically; group 2 comprised 13 MC twin pairs with TTTS treated by laser. The control groups comprised 12 MC twin pairs without TTTS (group 3) and 11 dichorionic twin pairs (group 4). Fetal cardiovascular data, predictive factors for, and duration of TTTS and cord blood were collected prospectively. We measured blood pressure and PWV photoplethysmographically at a median corrected postnatal age of 11 months (range, 1 week to 66 months). Both TTTS groups showed marked intertwin PWV discordance, unlike MCDA control subjects. The PWV discordance seen in laser treated twin pairs resembled that of dichorionic control subjects (heavier individual with higher PWV), whereas group 1 showed the opposite (negative) intertwin discordance (ANOVA $F(1,45) = 4.5, P = 0.04$). No significant differences in blood pressure or intrauterine growth were observed between TTTS groups.

Conclusions—Vascular programming is evident in monozygotic twins with intertwin transfusion and is altered but not abolished by intrauterine therapy to resemble that seen in dichorionic twins. (Circulation. 2003;107:1906-1911.)

Key Words: vasculature ● elasticity ● arteries ● lasers ● pregnancy

Twin-twin transfusion syndrome (TTTS) complicates 10% to 15% of monochorionic (MC) multiple pregnancies. Its perinatal mortality rate of 80% to 100% has been halved over the last decade by intrauterine treatment including serial amnioreduction, laser coagulation, and cord occlusion for selective feticide (improving survival of the remaining fetus). Placental vascular anastomoses provide the basis for intertwin transfusion ("donor" to "recipient") and failure to compensate for the circulatory imbalance set up by deep unidirectional arteriovenous anastomoses, as the result of a paucity of superficial bidirectional arterioarterial anastomoses, has been proposed as the pathogenic mechanism. Placental vascular anastomoses provide the basis for intertwin transfusion ("donor" to "recipient") and failure to compensate for the circulatory imbalance set up by deep unidirectional arteriovenous anastomoses, as the result of a paucity of superficial bidirectional arterioarterial anastomoses, has been proposed as the pathogenic mechanism.

Both donor and recipient twins have dynamic changes in volume loading during cardiovascular development constituting a hostile intrauterine environment. The hypervolemic recipient fetus has polyuria and polyhydramnios and hypertrophic cardiomyopathy and hydrops may develop. Echocardiography demonstrates ventricular hypertrophy, tricuspid regurgitation, and right ventricular outflow obstruction that may persist, requiring treatment, and an increased incidence of cardiac malformations. Conversely, the donor has chronic hypovolemia and poor renal perfusion with oliguria and oligohydramnios.

The two main treatments are amnioreduction and fetoscopic laser photocoagulation of placental vascular anastomoses. The primary aim of amnioreduction is to reduce amniotic fluid volume and pressure, thereby reducing the risk of preterm labor or ruptured membranes. However, this technique fails to address the underlying cause of TTTS. Laser therapy reduces or abolishes intertwin transfusion by ablating chorionic plate anastomoses, producing functionally dichorionic pregnancies.
In a preliminary study, we reported that the donor has a 2-fold increase in pulse wave velocity (PWV) (reflecting increased arterial stiffness) in infancy compared with its cotwin.10 We hypothesized that increased donor PWV values are a consequence of chronic hypovolemia resulting from unbalanced intertwin transfusion, which might be reversed or prevented by definitive treatment. We investigated this hypothesis by examining PWV in twin survivors of TTTS treated by laser, which, by abolishing or restricting intertwin transfusion at an early stage, might be expected to reverse or prevent vascular remodeling in utero.

### Methods
Fifty pairs of twins were studied serially before and during childhood in two tertiary referral fetal medicine units (London, UK, and Hamburg, Germany) by the same research team with portable PWV equipment. Chorionicity (the number of placentas) was determined at the first ultrasound scan and confirmed after birth. Monochorionic twins were scanned at least fortnightly from 18 weeks with an Acuson Sequoia, Aspen or XP10 or Siemens Elegra for fetal growth, liquor volume, and cardiovascular studies. Serial fetal parameters included (1) umbilical Doppler for absent/reversed end-diastolic arterial flow (AREDF), pulsatility index (UA PI) (an indicator of placental insufficiency) and venous pulsations, (2) fetal echocardiography for cardiothoracic ratio, atrioventricular valvar indicator of placental insufficiency) and venous pulsations, (2) fetal echocardiography for cardiothoracic ratio, atrioventricular valvar regurgitation (AVVR), or absence/reversal of flow during atrial contraction in the duc tus venosus (DV), (3) absence of functional arterioarterial anastomoses (AAA) on placental Doppler or at laser endoscopy as a predictor of TTTS and/or its severity, (4) fetal biometry, and (5) amniotic fluid indexes including volume removed at first amnioreduction. We compared PI at presentation with TTTS with that at similar gestation (mean, 22 weeks) in MC control subjects. Cord blood was taken at delivery for pH and hemoglobin measurement. Dichorionic (DC) twins were scanned every 4 weeks from 20 weeks until delivery.

TTTS was diagnosed by the presence of oligopolyhydramnios sequence in mid trimester in MC twins in the absence of other causes. Polyhydramnios and oligohydramnios were defined as deepest somatographic vertical pools of amniotic fluid of >8 cm and <1 cm, respectively.11 Each case was staged at diagnosis: stage I, oligohydramnios sequence only; stage II, “stuck” twin with absent visible bladder on ultrasound of the donor; stage III, AREDF of the umbilical artery or absence/reversal of flow during atrial contraction in DV; and stage IV, hydrops in either fetus.12

After delivery, PWV was measured with the use of a noninvasive photoplethysmographic technique in the brachioradial artery.13 PWV is inversely related to the square root of arterial distensibility and thus reflects arterial stiffness.14 Two probes, each containing an infrared emitting diode and a phototransistor, were placed over the right brachial and radial arteries to measure the pulse transit time. The PWV was derived by dividing the distance between probes, to the nearest millimeter, by the transit time (m/s). We validated this in 10 infants showing an intraobserver repeatability coefficient of 0.43 m/s and a coefficient of variation of 10.4%. Blood pressure was measured in the right arm by the Dinamap (Model XL, Critikon, Inc) with the use of an appropriately sized cuff, and transthoracic echocardiography was performed. All assessments were made in nonsedated infants.

Fifty twin pairs were studied at a median corrected postnatal age of 11.1 months (1 week to 64 months); 14 MC twin pairs with TTTS were treated by nonlaser methods (group 1, London), including amnioreduction (n=7), amniotic septostomy (n=1), and conservatively (n=6), and 13 pairs were treated by laser photocoagulation (group 2, Hamburg). Control subjects recruited in London were 12 MC twin pairs without TTTS (group 3) and 11 dichorionic (DC) twin pairs (group 4).

This study was approved by the institutional ethics committees in both centers, and parents gave written informed consent. Data are presented as mean±SD unless otherwise stated. The heavier or recipient twin was designated as twin 1. Birth weight was standardized for gestational age, based on growth data in singletons, and was expressed as z scores for comparison between groups. Postnatal age was corrected for prematurity and differences between the 4 cohorts for age at examination; umbilical Doppler, volume of first amnioreduction, cord pH and hemoglobin at delivery, birth weight z scores, and blood pressure were tested by 1-way ANOVA. For normally distributed variables, differences between cotwins within each group (PWV and birth weight) were assessed by paired Student’s t test. The data were log-transformed, and ANOVA of the log ratio and post hoc orthogonal contrasts used to detect intergroup differences in PWV. Nonnormally distributed and categoric variables (AREDF, abnormal DV, absence of AAA, and disease stage) were assessed by Fisher’s exact or Kruskall-Wallis tests. A probability value of <0.05 was considered significant. Data on a proportion of the nonlaser patients and control subjects have previously been reported in preliminary form.19 Statistical calculations were performed with Stata 6.0 software (Statistics Data Analysis).

### Results
Fetal data and postnatal variables are shown in Tables 1 and 2, respectively. The median time from diagnosis/laser treatment of TTTS to delivery was similar in groups 1 and 2 (9.0 [range 0 to 16] and 11.5 [range 6 to 18] weeks, respectively).

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### Table 1. Fetal Demographics and Clinical Variables in TTTS Groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Nonlaser) n=28</th>
<th>Group 2 (Laser) n=26</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at referral/diagnosis, wk</td>
<td>23.5 (16.3–34.4)</td>
<td>22.3 (17.1–24.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>No AAA detected</td>
<td>4 (29%)</td>
<td>6 (46%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Stage of disease</td>
<td>II (I–III)</td>
<td>II (I–III)</td>
<td>0.1</td>
</tr>
<tr>
<td>First amnioreduction volume, mL</td>
<td>1850 (400–4300)</td>
<td>3250 (1600–5600)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Values are median (range) or mean±SD. AAA indicates arterioarterial anastomosis; AREDF UA, absent or reversed end diastolic flow in umbilical artery; AREDF DV, zero or reversed velocities of flow during atrial contraction in ductus venosus; UA PI, umbilical artery pulsatility index; Hb, hemoglobin.
Although the median disease staging did not differ between the TTTS cohorts (stage II), a greater volume of amniotic fluid was removed at laser therapy than at the amnioreduction procedure, suggesting more severe disease in group 2 (range, II to III versus range, I to III in group 1). Both cohorts showed no significant differences in cord hemoglobin or pH at birth (Table 1).

Groups 1 and 2 were born significantly more prematurely compared with those in groups 3 and 4 ($P=0.0005$), and there was no significant difference in the median corrected age at study between the groups ($P=0.07$). Twin pairs in groups 1 and 2 had greater mean birth weight discordance (23% and 16%) than groups 3 (10%) or 4 (14%). Birth weight $z$ scores did not differ significantly between the TTTS groups, but the donor twin in group 1 was significantly lighter than MCDA control subjects (ANOVA $F(1,45)=2.87$, $P=0.007$).

The absolute PWV values for each twin pair are shown by group in Figure 1 (a through d) and the mean intertwin PWV differences (Figure 2) and log ratios for each group in Table 3. The ANOVA of the log PWV differences just failed to achieve statistical significant difference ($P=0.084$), but post hoc tests comparing the two TTTS groups showed significant differences between those receiving conservative and laser therapy (ANOVA $F(1,45)=4.5$, $P=0.04$).

**Discussion**

This study confirms our previous work and demonstrates that in TTTS, abnormal fetal hemodynamic loading is associated with discordant intertwin arterial wall stiffness in childhood despite identical genotype. Midtrimester laser therapy did not prevent this discordance but altered the pattern of PWVdiscordance to resemble that seen in nonidentical dichorionic twin pairs. In twins treated conservatively (group 1), the trend was toward a negative discordance (donor twin with higher PWV), whereas those treated by laser (group 2) showed a positive discordance similar to DCDA control subjects (heavier fetus with higher PWV), suggesting that separation of the fetal circulation resulting from intrauterine therapy encourages a pattern of arterial development similar to that seen in nonidentical DCDA twins.

We measured PWV in the brachial artery by using this photoplethysmographic technique because we thought it may be an early indicator of altered cardiovascular performance. PWV is relatively easy to measure and is reproducible in small children, but examination of the muscular brachial artery may not be ideal because its compliance depends on vascular smooth muscle activity as well as its structure and material properties. This may be one explanation for the lack of differences observed in blood pressure between the groups because systolic blood pressure, although related to aortic compliance, is unlikely to be strongly related to brachial artery elasticity as measured in this study.

PWV increases in the fetus with gestational age and throughout postnatal life, reflecting maturation of the aortic wall and deposition of connective tissue leading to a progressive age-related reduction in arterial distensibility. There are few data on PWV values in young age, but post hoc tests comparing the two TTTS groups showed significant differences between those receiving conservative and laser therapy (ANOVA $F(1,45)=4.5$, $P=0.04$).
and early calcification and atherosclerosis in the other. Consistent with this is the finding of reduced distensibility in the brachial arteries of young adults after neonatal repair of coarctation of the aorta, where flow about the isthmus is markedly reduced, even in utero.22

Probable mechanisms include those mediating the effects of differential volume loading on arterial wall structure. A rapid increase in intravascular pressure in animals (equivalent to the recipient twin) results in vascular smooth muscle contraction and increased resistance and medial thickness. Over a month, as the smooth muscle tone returns to normal, this increased medial thickness is maintained by remodeling23 to preserve a constant wall stress. These changes imply that the recipient twin exposed to an acute transfusional load should have increased PWV (as seen in 1 recipient of the 3, with an acute intertransfusional process in group 1). However, in humans, the influence of immediate delivery on this adaptation process is unknown, as is the volume of intertwin transfusion in those with apparently more chronic disease. Several of the donor twins in group 1 showed reduced arterial compliance compared with their cotwin, and in those with a chronic intrauterine course, it is likely that active hormonal and cardiovascular reflex responses to fetal hypovolemia, as seen in animal models, provide an explanation.24,25 Human studies suggest additional plausible mechanisms for discordant PWV in childhood survivors of TTTS such as the finding of increased endothelin levels in recipient fetuses compared with their codonor and control subjects26 and recipient neonatal hypertension.27 The finding of renin gene and protein expression in donor kidneys28,29 but not in those of their cotwins suggests that the renin-angiotensin system is activated in the donor but not the recipient. This may be one explanation for the relatively lower PWV seen in some of the recipients in group 1 in addition to increased deposition of elastin in the fetal aorta associated with increased volume flow. However, verification of structural responses to chronic volume loading and the effects of increased endothelial shear stress could not be measured in this study.

The limitation of this study is that TTTS is a complex model in which to study the differential effects of volume loading on the developing arterial tree. Three main factors contribute to the heterogeneity of response seen. First, the paired design of this study has resulted in recruitment of a greater proportion of pregnancies with placental AAA (71% of group 1 pregnancies compared with <25% in our total TTTS series) and thus these twin pairs represent the milder end of the spectrum of TTTS. The prevalence of AAAs provides one explanation for the heterogeneity of response to
volume loading in this study, as these anastomoses permit bidirectional flow; thus angiotensin produced by the donor may be active in corecipient fetuses and result in increased collagen synthesis, smooth muscle production, and vascular medial hypertrophy, resulting in higher PWV. In the pilot study, only half of the TTTS pregnancies recruited had an AAA, thus reducing the effects of this confounder, and we showed that those without an AAA tended to have greater PWV discordance (donor PWV greater than recipient).

Second, the chronicity and degree of volume loading is widely variable, and the potential programming effect of this process at differing gestations uncertain. In our pilot study, the degree of intertwin discordance was positively related to disease chronicity; however, individual responses may vary at different gestations. The exact onset of TTTS is sometimes uncertain and may stabilize, although balanced intertwin transfusion probably continues in most. Deterioration in late gestation is treated by immediate delivery and occurred in 3 pairs in group 1 who had been monitored biweekly. Intertwin PWV values illustrate the variability of vascular response to apparently similar stimuli. One pair showed no intertwin discordance in PWV after acute presentation at 30 weeks’ gestation, one a 43% negative discordance (higher PWV in the donor) and one a 41% positive intertwin discordance after acute deterioration and delivery at 34 and 32 weeks, respectively.

The third factor may be the influence of discordant fetal growth before the onset of TTTS, in which the resultant vascular development may reflect the balance of gestational age at onset or chronicity of each effect. Elastin deposition is greatest during late gestation and continues throughout early infancy, and a deficiency in this mechanism has been proposed to play a role in the reduced arterial compliance associated with growth restriction. However, our early studies have described lower PWV values in growth-restricted individuals. One twin pair (group 1) illustrates the complexity of events potentially affecting vascular development. They presented with growth discordance (32%) caused by fetoplacental insufficiency (increased UA PI, 2.58) and were delivered after acute intertwin transfusion. The sick recipient was ventilated for 14 days because of severe cardiovascular compromise secondary to acute volume overload. The children showed 41% PWV discordance at 27 months, with higher values in the recipient reflecting the effects of growth restriction, acute volume loading, and postnatal morbidity on vascular development. It is difficult to conceive that an animal model could be designed that would reflect the heterogeneity of the clinical situation in vivo.

Twin-twin transfusion syndrome provides a unique observational model to describe the effects of differing volume load on developing cardiovascular systems in genetically identical individuals. Because this study was cross-sectional.

### Table 3. Mean Absolute Pulse Wave Velocity, Intertwin Differences, and Log Ratios in Recipient/Heavier Twin 1 and Donor/Lighter Twin 2 by Group

<table>
<thead>
<tr>
<th>Group (Pairs)</th>
<th>PWV, m/s</th>
<th>PWV Difference, Twin 1–Twin 2</th>
<th>Log Ratio, Twin 1/Twin 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=14)</td>
<td>3.86±1.50</td>
<td>4.76±2.24</td>
<td>−0.90±2.56</td>
</tr>
<tr>
<td>2 (n=13)</td>
<td>5.50±1.80</td>
<td>4.59±1.75</td>
<td>0.91±2.23</td>
</tr>
<tr>
<td>3 (n=12)</td>
<td>3.62±1.12</td>
<td>3.77±1.06</td>
<td>−0.15±0.57</td>
</tr>
<tr>
<td>4 (n=11)</td>
<td>5.33±1.72</td>
<td>4.76±1.95</td>
<td>0.58±1.23</td>
</tr>
</tbody>
</table>

Values are mean±SD. PWV indicates pulse wave velocity.

Figure 2. Mean (95% CI) PWV differences in amnioreduction/conservative TTTS cohort (group 1), laser-treated TTTS cohort (group 2), monochorionic control subjects (group 3), and dichorionic control subjects (group 4).
in design, it cannot predict whether or not intertwin discordance persists into adult life. However, our results suggest that compared with MCDA control subjects, a period of unbalanced intertwin transfusion gives rise to altered arterial distensibility seen in childhood, consistent with the concept of fetal programming. In this study, we have shown that by abolishing intertwin transfusion at an early stage of development by laser photocoagulation, the pattern of PVV (and therefore arterial elasticity) resembles that seen in nonidentical twins who develop with separate circulations.

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References
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