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# Twin-to-twin transfusion syndrome: diagnosis, treatment, and long term outcomes

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# TWIN-TO-TWIN TRANSFUSION SYNDROME:

# DIAGNOSIS, TREATMENT, AND LONG TERM OUTCOMES

by

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**TWIN-TO-TWIN TRANSFUSION SYNDROME:**

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**ARISHA ANSARI**

ABSTRACT

Twin to twin transfusion syndrome is a rare complication that can develop in monochorionic twin pregnancies where abnormal placental connections lead to hemodynamic imbalance between the two fetuses. The twin receiving the surplus of blood experiences polyhydramnios whereas the twin donating their blood experiences oligohydramnios. Diagnosis of this syndrome is done based off of the Quintero Staging scale, which consists of five categories of criteria ranging from non-critical diagnoses to diagnoses involving demise of one or two fetuses. The gold standard for treatment involves ablating abnormal vessel connections via a laser therapy. This therapy has shown to reduced short term and long term complications within the twins, and be most efficient at ceasing the disproportionate blood supply between the fetuses. Long term outcomes of twin to twin transfusion syndrome mainly involve neurodevelopmental impairment, but cardiovascular and renal complications can also be present. Adverse neurodevelopmental outcomes should be the ones to most closely monitor postnatally in all TTTS survivors. For recipient twin survivors, cardiovascular outcomes should be most closely watched via blood pressure monitoring and routine echocardiograms. For donor twin survivors, creatinine levels should be routinely checked in order to detect signs of chronic kidney disease in early childhood. Long term outcomes of twin to twin transfusion syndrome still need further investigating due to the difficulty of gathering

information postnatally. Limitations that further increase the complexity of this research include lack of education and decreased opportunities for underserved communities to access the advanced medical care required to treat and monitor this disease. Shedding light on this disparity can lead mothers to be more aware of the signs and symptoms of this disease, leading to early detection and more positive outcomes.

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## LIST OF ABBREVIATIONS

AA.....arterioarterial

AKI .....acute kidney injury

ASQ.....Ages and Stages Questionnaire

AV.....arteriovenous

CHD ..... congenital heart disease

CKD ..... chronic kidney disease

CRL..... crown rump length

DCDA ..... dichorionic diamniotic

DV..... ductus venosus

DVP.....deepest vertical pocket

FLP.....fetoscopic laser photocoagulation

MC ..... monochorionic

MCDA..... monochorionic diamniotic

MCMA..... monochorionic monoamniotic

MFM .....maternal fetal medicine

MRI.....magnetic resonance imaging

NDI ..... neurodevelopmental impairment

NT .....nuchal translucency

OB/GYN ..... obstetric/gynecological

RAS.....renin angiotensin system

RRT.....renal replacement therapy

TTTS ..... twin-to-twin transfusion syndrome

VV ..... venovenous

# INTRODUCTION

Twin-to-twin transfusion syndrome (TTTS) is a complication that can arise in monochorionic (MC) twin pregnancies and is highly fatal if untreated. TTTS occurs when abnormal blood vessel connections form within the shared placenta of the twin fetuses, causing an uneven distribution of blood. The twin that is giving away too much blood is called the donor twin, and the twin that is receiving an excess of blood is the recipient. This imbalance sparks a multitude of complications for both fetuses, and if left without intervention, can be up to 90% fatal to one or both twins. TTTS affects 10-15% of MC twin pregnancies that are between 12-26 gestational weeks (Sun et al., 2021). While predictive markers are still unclear, TTTS can be detected as early as 10 to 14 weeks based on amniotic fluid levels and nuchal translucency (NT).

Current studies have shown focus on the most efficient diagnostic techniques and treatments for TTTS. Diagnosis is done using ultrasound to measure bladder filling, amniotic fluid volume, and blood flow between the donor and recipient twins. The Quintero Staging System then quantifies the severity of the syndrome in order to establish proper prognosis and management. The gold standard for TTTS treatment is Fetoscopic laser ablation, a minimally invasive surgical procedure that uses a fetoscope to seal off abnormal anastomoses.

TTTS is a rare disorder that, if properly detected and treated, is likely to result in a positive short term outcome for the mother and fetuses. The long term outcomes, however, is a topic of increasing interest and research. This paper will break down the

various long term outcomes in order to determine which area of development faces the most severe risks, and therefore should be most closely monitored postnatally. Attention to outside factors such as inadequate access to medical care will also be discussed due to the fact that TTTS requires advanced medical treatment and monitoring.

**Anatomy**

Pregnancies involving multiples require extra attention to the developmental anatomy of the fetus. Two parts of gestation that are variable for multiple pregnancies are the amniotic sac and the placenta. The amniotic sac is a thin membrane that surrounds the fetus during pregnancy. It is filled with amniotic fluid which cushions the fetus during growth. Amniotic fluid also facilitates exchange of nutrients, biochemical products, and water between the mother and fetus. The amniotic sac and amniotic fluid are surrounded by the chorion, which is the outermost fetal membrane. The chorion connects the amniotic sac and fetus to the placenta. The placenta is an organ that develops only during pregnancy, and is expelled from the uterus after birth. It provides the fetus with oxygen and nutrients and also removes waste products from the fetus' blood (Marceau et al., 2016). Since the chorion connects to the placenta, if twins are monochorionic, they will share a placenta. If they are dichorionic, they will each have their own placenta. Within the chorion, twins can share an amniotic sac or each have their own amniotic sac. This is referred to as monoamniotic and diamniotic, respectively. **Figure 1** shows the breakdown of the three different types of twins: monochorionic-monoamniotic (MCMA), monochorionic-diamniotic (MCDA), and dichorionic-diamniotic (DCDA).

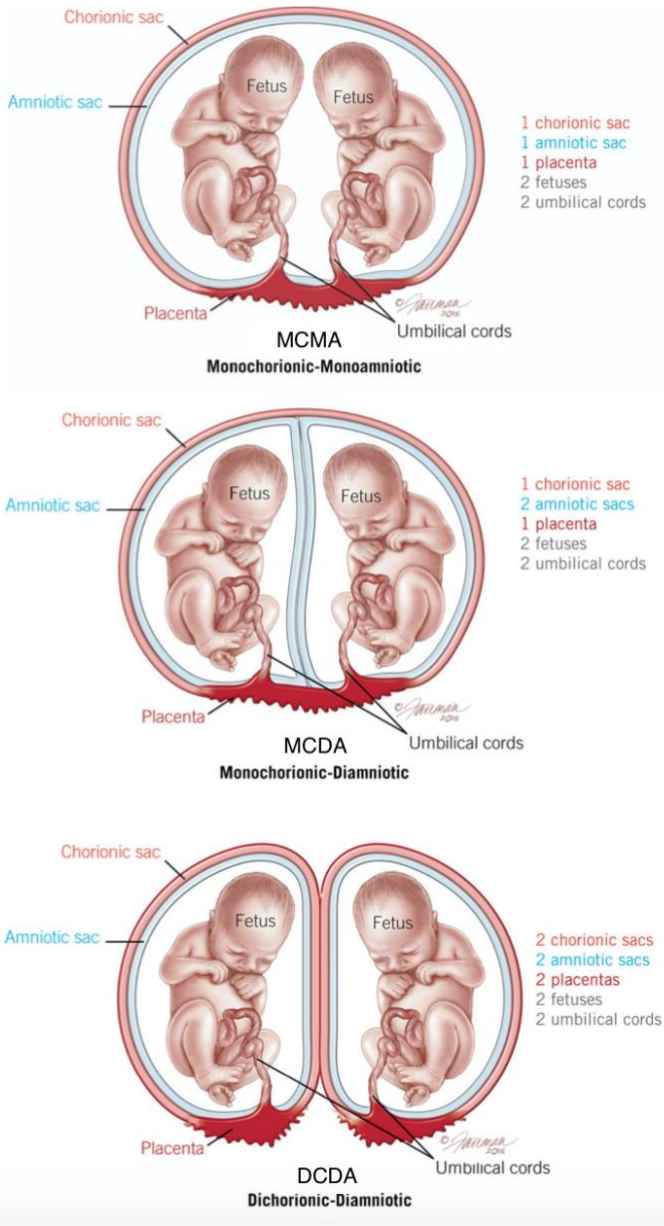


**Figure 1. Anatomical Differences Between MCMA, MCDA, DCDA Twins.** Monochorionic-monoamniotic twins (MCMA, shown in the top image) have 1 chorion and 1 amnion. Monochorionic-diamniotic twins (MCDA, shown in the middle image) have 1 chorion and 2 amnions. MC twins (whether MCMA or MCDA) share the same placenta. Dichorionic-diamniotic (DCDA, shown in the bottom image) twins have two chorions and two amnions. Diamniotic twins can have the same or different placentas. Figure adapted from (Marceau et al., 2016).

**Pathophysiology**

The key etiologic problem underlying TTTS lies within the placental structure shared between the monochorionic twins. In MC twin placentation, chorionic spread radially and are actively developing by the fifth embryonic week. These connections initially overlap, and occasionally form intertwin anastomoses. If both twins start this chorionic spreading process around the same time, the inter-twin anastomoses that develop will balance and thus cancel out any imbalance in blood flow. However, certain types of connections move unidirectionally, causing imbalance. If these connections are numerous, this imbalance in fluids will ultimately lead to TTTS.

The three primary types of anastomoses in MC placentas are as follows: venovenous (VV), arterioarterial (AA), and arteriovenous (AV). AA and VV anastomoses are superficial connections that lie directly on the surface of the placental. Both of these connections have the ability to conduct bidirectional flow, moving both back and forth between the donor and recipient twin. AV connections, however, the vessels are planted deep in the placenta in an area known as the cotyledon. AV connections consist of a chorionic artery from one twin draining into the chorionic vein of the other twin. This occurs due to the deep implantation of the connection dipping into the placental substance of the contralateral twin (Galea et al., 2005). The presence of the AV connections in the cotyledon not only makes them harder to detect, but also is what causes unidirectional flow of blood to occur, potentially leading to TTTS. Visualization of all three types of connections can be seen in **Figure 2**.

In healthy MC pregnancies, there can be multiple AV connections, however they are balanced on both sides. Furthermore, even if there is a small excess of AV connections, the presence of many superficial AA and VV anastomoses serves a protective effect by redirecting flow back to the initiating side of the AV connections (Galea et al., 2005). AA connections in particular are the most compensatory because they have the lowest pressure gradient and therefore have a greater ability to create balanced flow (Umur et al., 2002). TTTS pathology shows more AV connections on one side of the placenta than the other. On top of that, AV connections can be larger in diameter as well, increasing their odds at creating imbalance flow (Simpson, 2013). Studies have shown that the worst cases of TTTS were associated with many AV anastomoses paired with the absence of AA or AV connections. Overall research on the exact mechanisms of these connections has proved difficult to investigate due to the lack of animal models with hemochorial placentation similar to humans (Galea et al., 2005).

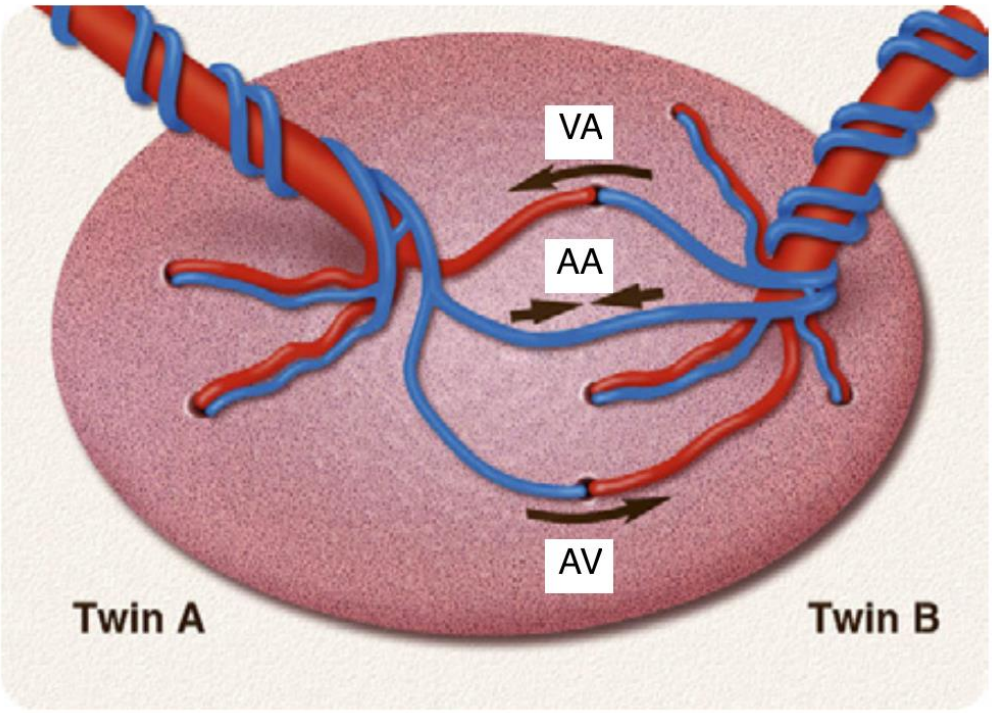


**Figure 2. Selected Anastomoses in Monochorionic Placentas.**
This figure shows different types of anastomoses in MC placentas: AA, arterioarterial anastomosis; AV, arteriovenous anastomosis; VA, venous-arterial anastomosis. Figure adapted from (Simpson, 2013).

**Diagnosis and Staging**

TTTS is commonly diagnosed based on the length of the deepest vertical pocket (DVP) of amniotic fluid for each amniotic sac in MCDA twins. This measurement shows the discordance of amniotic fluid volume on either side of the membrane. If the DVP is <2cm in one amniotic sac and >8cm in the other in an MCDA pregnancy, the criteria for TTTS has been met (Washburn et al., 2016). Having a small DVP measurement means there is too little amniotic fluid to support the growing fetus. This is referred to as oligohydramnios. The opposite, having too much amniotic fluid, is polyhydramnios (**Figure 3**).

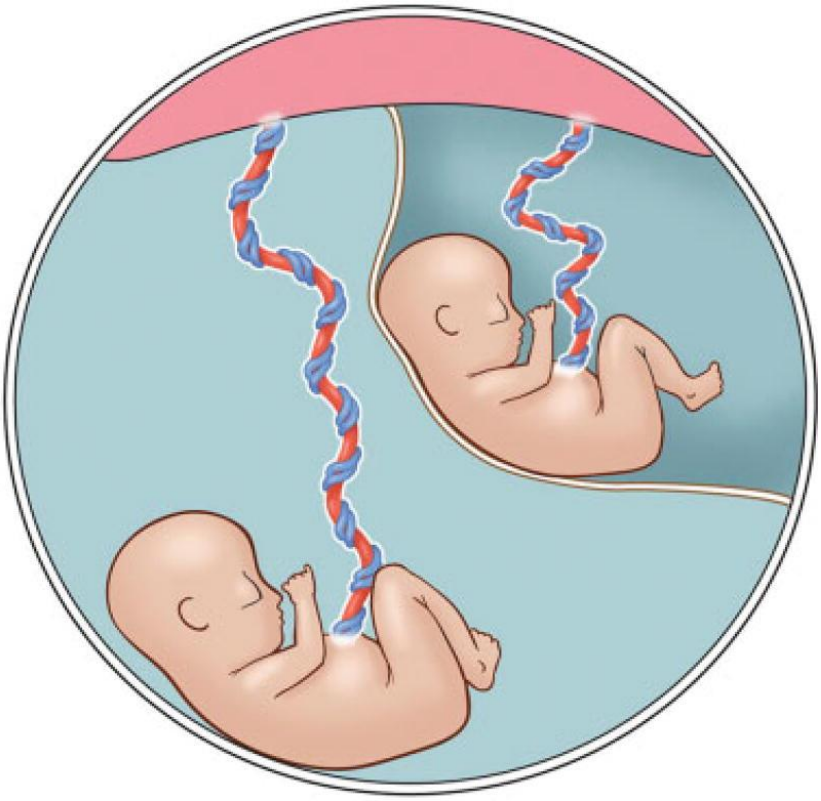

**Figure 3. Simplified illustration of polyhydramnios vs oligohydramnios.**
The lighter blue area on the left shows a greater amniotic fluid volume, known as polyhydramnios. The darker blue area on the right shows a smaller amniotic fluid volume, which is oligohydramnios. Figure taken from (Washburn et al., 2018).

This diagnosis occurs typically between 16 and 26 weeks' gestation and signs of the condition are detected through routine prenatal ultrasounds. TTTS can also however be detected early on based on observation of the mother's acute symptoms related to polyhydramnios such as uterine distension, uterine contractions, and dyspnea (Chalouhi et al., 2011). Current guidelines suggest screening via ultrasound every two weeks in all MCDA twin pregnancies in order to detect TTTS. This is because an interval of greater than 14 days between ultrasounds has been associated with detection of higher stage TTTS based on the Quintero Staging System. Therefore, staying consistent with biweekly

ultrasounds would increase the chance of early detection and intervention (Thorson et al., 2011).

While this recommendation is ideal, it's not always practical due to lack of resources or awareness of this rare condition. Therefore, efforts to find alternate ways to detect TTTS early are sought after. Methods such as measuring NT thickness, abnormal flow in the ductus venosus (DV), and crown-rump length (CRL) have been shown to potentially detect TTTS. These three factors together can be detected as early as 11-14 weeks and signifies high-risk of developing TTTS in MCDA twins (Matias et al., 2010). Examples of ultrasound findings showing these parameters can be seen in **Figure 4a** and **Figure 4b**.

When abnormalities have been detected via ultrasound, it is then time to determine how progressed the syndrome is. This is done by the Quintero Staging System. This system was developed by Ruben A. Quintero and his associates, describing definite sonographic criteria necessary for prognosis and management of monochorionic pregnancies with risk of TTTS. It consists of five stages (**Table 1**) based on amniotic fluid volumes, bladder filling, critical Doppler values, and cardiac function/hydrops (Durbin, 2011). Stage 1 represents the most benign symptoms of TTTS, a simple imbalance of amniotic fluids. In this stage, the Doppler findings will still show the bladder of the donor twin, who is experiencing oligohydramnios. Stage 2 is when the bladder of the donor twin is no longer visible via ultrasound. This indicates that the bladder is no longer filling, since the donor twin is lacking the blood needed to produce enough urine. Stage 3 is when Doppler studies show critical arterial abnormalities. This

can consist of absent end-diastolic velocity in the umbilical artery of the donor twin, or venous abnormalities such as reverse flow in the ductus venosus or pulsatile umbilical venous flow in the recipient twin (Quintero et al., 1999). Stage 4 is when hydrops develop. Hydrops is a condition that consists of pleural effusion, pericardial effusion, skin edema, and overall fluid overload in different parts of the fetal body (Chang et al. 2019). The progression of hydrops eventually leads to Stage 5: fetal demise of one or both twins.

Diagnosis of TTTS can also involve other varieties of perioperative care than just ultrasound findings. A sonographer may also perform a fetal echocardiogram to determine if cardiovascular complications are present in one or both of the twins. Specifically, hypertrophic cardiomyopathy in the recipient twin is a marker of severe TTTS (Stirnemann et al., 2010). This is when the heart muscle thickens in both ventricles, causing systolic and diastolic dysfunction (Pedra et al., 2002). Furthermore, a maternal fetal medicine (MFM) specialist can schedule a magnetic resonance imaging scan of the patient's uterus to assess fetal brain status. This will show any abnormalities present before treatment. This is helpful as studies have shown presence of brain lesions on one or both twins after laser ablation treatment (Hochberg et al., 2021). The MFM specialist may also suggest a genetic counseling appointment if fetal anomalies or birth defects are identified in either fetus before treatment. These perioperative steps along with consistent ultrasound appointments will best prepare the patient for treatment.

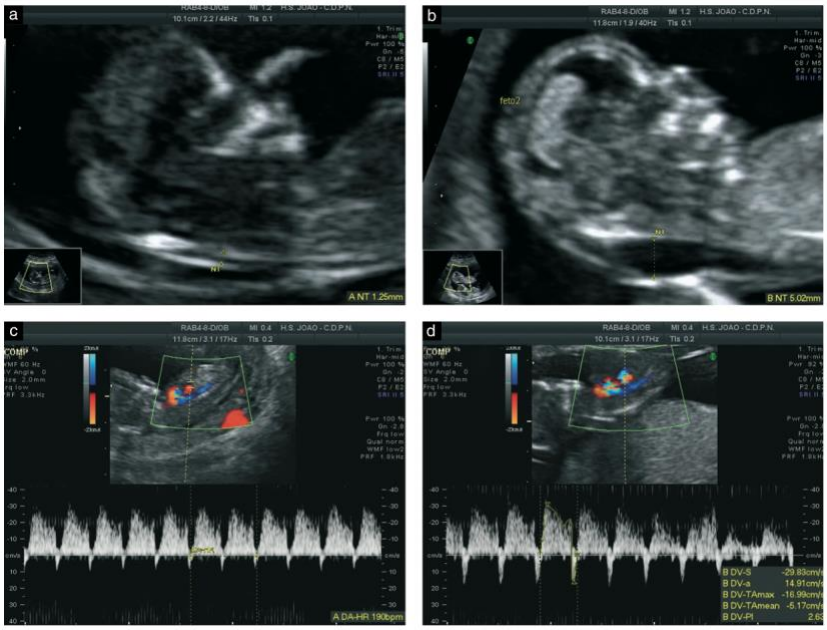

**Figure 4a. Ultrasound Imaging from a MCDA Pregnancy without TTTS Diagnosis.** These images were taken at 12 weeks' gestation. NT was measured at 1.06 mm (a,b) and Doppler waveforms were obtained in the DV and determined to be normal for both twins (c,d). Outcome for both fetuses was normal. Figure taken from (Matias et al., 2010).

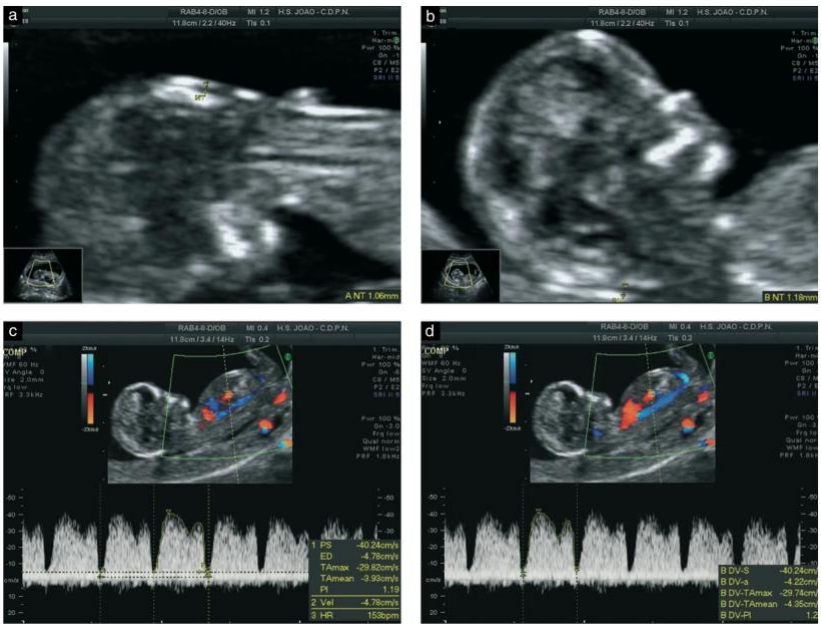

**Figure 4b. Ultrasound Imaging from a MCDA Pregnancy with TTTS Diagnosis.** These images were taken at 12 weeks' gestation. NT was measured at 1.25 mm (a,b) and DV examinations showed abnormal flow for both twins (c,d). TTTS developed at 17 weeks of gestation. Figure taken from (Matias et al., 2010).

**Table 1. Quintero Staging System of TTTS Based on Sonographic and Doppler Findings.** Adapted from (Quintero et al., 1999).

| Stage | Poly/ oligohydramnios | Absent bladder in donor | CADs | Hydrops | Demise |
|---|---|---|---|---|---|
| I | + | − | − | − | − |
| II | + | + | − | − | − |
| III | + | + | + | − | − |
| IV | + | + | + | + | − |
| V | + | + | + | + | + |

**Treatment**

Treatment for TTTS highly depends on the severity of the syndrome, gestational age, and chance of comorbidity. Patients should consult with their MFM specialist to determine which treatment option, if any, would be best for their case. There is a chance there might be no intervention needed but rather just consistent monitoring and tracking of symptoms associated with polyhydramnios and oligohydramnios. If intervention is recommended, the main and only treatment that successfully reverses TTTS is laser ablation therapy. Other treatments such as amnioreduction or amniotic septostomy help relieve symptoms of TTTS and will also be discussed.

As stated earlier, the gold standard for TTTS treatment is Fetoscopic laser photocoagulation (FLP, **Figure 5**). This can be performed safely between weeks 16 and 26 of gestation and specifically treats TTTS that has progressed to Stages 2 through 4 on

the Quintero Staging Scale. It is not common practice for Stage 1 severity because these cases often revert without intervention. FLP ablates the connections between abnormal blood vessels, restoring independent blood flow for each fetus. This laser ablation procedure is minimally invasive, requiring a small incision in the abdomen to provide percutaneous access to the uterine cavity (Cunningham et al., 2021). The equipment required for this treatment is an intrauterine fetoscope with a laser fiber, ultrasound, and a trocar or cannula to help with guidance within the placenta. The microscopic camera attached to the fetoscope first identifies the abnormal anastomosis and then the laser fiber is used to coagulate the vessels shut. The laser does this by converting electrical or chemical energy into light energy, leading to the production of a large amount of energy to a small target area. The heat produced from this conversion diffuses into the surrounding placental tissue, causing injury and retraction of the vessel walls. This helps further coagulate the red blood cells (Chalouhi et al., 2011). Coagulation of the vessels results in scar tissue formation which helps seal off the connections from reforming (Bamberg and Hecher, 2019, **Figure 6**). The laser should be distanced around 10 mm away from the placental surface. This distance helps yield an optimal focus for the laser beam, and avoids increased risk of puncturing the placenta (Gruijthuijsen et al., 2018). It is highly important that the laser is correctly angled and the vessels are appropriately visualized as the goal to ablate all intertwin anastomoses is crucial to meet.

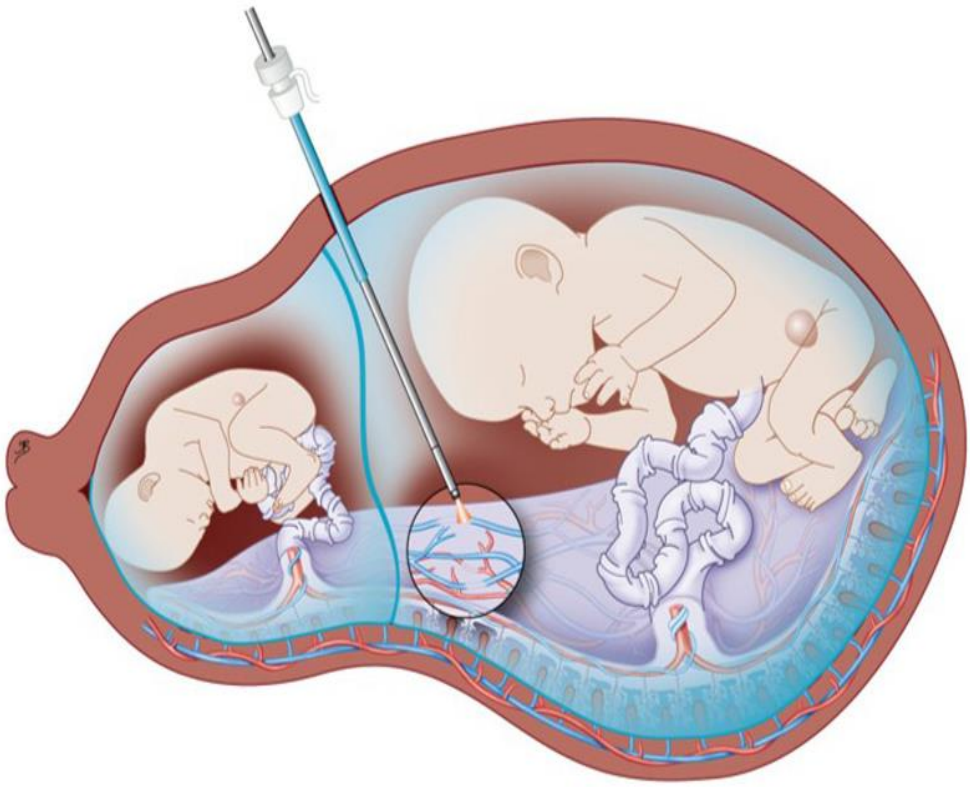

**Figure 5. Laser Therapy for Treatment of TTTS.**
This figure illustrates how FLP is performed on abnormal blood vessel connections. The recipient twin can be identified on the right due to the excess amount of amniotic fluid, and the donor twin can be seen on the left with a deficit of amniotic fluid. Figure taken from (Gruijthuijsen et al., 2018).

Amniocentesis, or amnio reduction, is a treatment for TTTS that helps relieve symptoms such as abdominal discomfort. It also helps prolong the gestational period by decreasing the risk of spontaneous rupture of the membranes or premature labor due to an enlarged uterus. The reduction in pressure on the placental surface can also help improve fetal hemodynamics, potentially reducing the number of abnormal blood vessel connections (Marie-Victoire et al., 2004). Results of amniotic reduction can have a 60-80% survival rate, however, the chance of major neurological abnormalities resulting from this treatment can be as high as 20% (Cincotta et al., 2000). Long term outcomes

from various TTTS treatments will be further discussed later in this paper. The method of amniocentesis involves an 18-gauge needle, ultrasonographic guidance, and some type of suction (Marie-Victoire et al., 2004). The needle is inserted in the polyhydramnios sac and up to 2-3 liters of fluid is gently removed. As the syndrome progresses, amniotic fluid can build back up in the recipient twin. Therefore, this procedure may need to be performed multiple times over the course of the pregnancy; this is referred to as serial amnioreduction.

Another symptom-relieving treatment is septostomy. This procedure should be compounded with amniocentesis to maximize relief for the patient. Septostomy involves the drainage of amniotic fluid, followed by a needle creating a small hole in the membrane between the donor and recipient twins' amniotic cavities. This allows amniotic fluid to flow into the donor twin' amniotic sac. While septostomy is a good technique for redistributing fluids between the twins, it has been shown to produce no perinatal survival advantage over serial amnioreduction (Akkermans et al., 2015). Overall, it's important to remember that neither of these treatments truly fixes TTTS due to the fact that they are not directly involving the abnormal anastomoses.

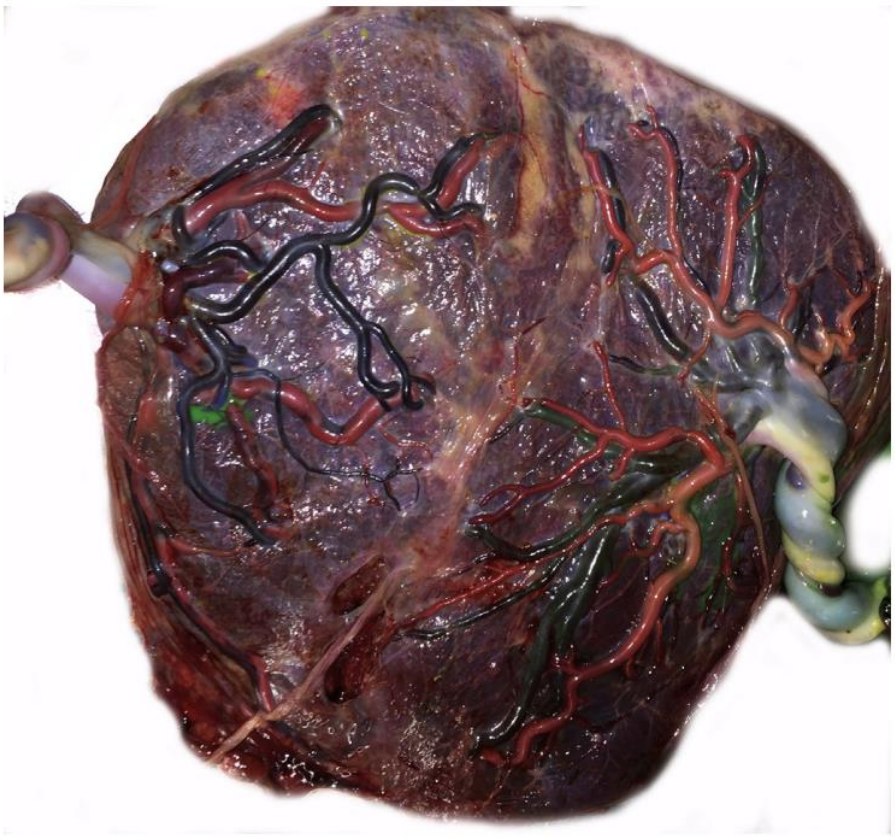

**Figure 6. MCDA Placenta with TTTS Successfully Treated with FLP.**
This is a visualization of FLP being conducted along the vascular equator, which can be seen running through the middle of the placenta. The red lines are arterial connections and the black lines are venous connections. Figure taken from (Bamberg and Hecher, 2019).

**Long Term Outcomes**

TTTS can have a significant impact on its survivors even after being successfully treated. Neonatal morbidities of TTTS are still being investigated, but seem to mainly involve neurodevelopment. Other areas that can be affected include cardiac and renal function. Studies have shown that long term outcomes can vary based on the type of treatment received, the gestational age the fetuses were treated and born, and whether the fetus was a donor or recipient.

When diagnosed with TTTS, it is imperative to continue its monitoring and regulation through the neonatal period and onward in order to best prepare for long term complications that can arise. Providing early intervention for the potential morbidities will help the neonate have the best prognosis. The way to achieve this goal of early intervention is to have a better understanding of the long term outcomes of TTTS. This will help clinicians accurately counsel parents of these children, allowing for more targeted interventions and optimization of child development. This section will explore the types of pathology that can be shown during the neurological, cardiovascular, and renal development of a TTTS survivor in a long term setting.

*Neurodevelopmental Outcomes*

Neurodevelopmental impairment (NDI) is the most common long term complication to arise from neonates born after TTTS. NDIs are caused by cerebral injury, which can manifest either antenatally or postnatally. Antenatal injury can be secondary to hemodynamic and hematological imbalance, which can lead to hypoxic-ischemic insults (Salomon et al., 2010). This means that the lack of blood flow to the brain can deprive it of the proper amount of oxygen it needs in order to function. Postnatal injury occurs after the neonate is born, and depends highly on how long their gestational period was, and whether they had a low birth weight. The World Health Organization defines low birth weight as less than or equal to 2500 grams, or 5 pounds 8 ounces (Cutland et al., 2017). In other words, if the TTTS survivor is born prematurely, the chance of postnatal cerebral injury greatly increases.

Being born prematurely is one of the biggest risk factors for cerebral injury. With regards to MCDA twin pregnancies specifically, they have a 50% chance of being delivered preterm (Gheorghe et al. 2020). One study demonstrated that 100% of TTTS patients born before 28 weeks experienced major neurologic sequelae after successful FLP treatment. When the patients were born after 32 gestational weeks, no major sequelae occurred (Wagner et al., 2013). This emphasizes the importance of following up on and closely monitoring maternal and fetal health even after TTTS has been successfully subsided. A way to provide early intervention for TTTS survivors that could potentially have neurological damage is by conducting routine cranial ultrasounds once they are born. While the effectiveness of this is not concretely determined due to lack of research, it provides a greater chance of screening for cerebral injury than if no type of monitoring was performed (Bamberg and Hecher, 2019).

The cause of cerebral injury can also differ depending on whether the neonate was a TTTS donor or recipient. Donor twins are the ones susceptible to the hypoxic-ischemic damage mentioned earlier, and recipient twins are more likely to experience hyperviscosity and polycythemia. These abnormalities can be detected antenatally via Doppler ultrasound. The ultrasound will show the speed of the peak systolic velocity in the middle cerebral arteries of both twins. The donor twin will have increased velocity, which means there is low density of red blood cells, also known as fetal anemia (Slaghekke et al., 2014). The recipient twin will show decreased peak systolic velocity, indicative of polycythemia, a surplus of red blood cells. Polycythemia is harmful to the fetus because it can cause vascular sludging, which is a buildup of clotted blood and

blood components, and leads to blockages in perfusion (Lopriore et al., 2008). Additionally, brain magnetic resonance imaging (MRI) is crucial for detecting cerebral injury both prenatally and postnatally in TTTS patients. **Figure 7** shows MRIs of TTTS survivors where one twin suffered from cerebral injury and the other did not. It is important to note that unlike cardiovascular outcomes, which will be discussed later, the donor and recipient twins are equally at risk for cerebral injury. The cause of cerebral injury is what is different between the twins, not the chance of one getting more damage than the other.

Cerebral injury can be manifested in several ways. It can take the form of cerebral white matter cysts, severe intraventricular hemorrhage, ventricular dilation, cerebral atrophy, or arterial ischemic stroke. All of these kinds of cerebral insults lead to NDI. TTTS sequelae is classified as an NDI if it involves one of the following: cerebral palsy, speech delay, global developmental delay, visual impairment, auditory impairment, and fine motor delay (Klink et al., 2016). Measuring NDI is done by a physical neurological examination, and assessments of motor and cognitive development through tests such as the Bayley Scales of Infant and Toddler Development. This assessment is specifically designed for infants who are at high risk for developmental delays and covers the domains of cognitive, language, adaptive, motor, and social-emotional development (Lennon et al., 2008).

The severity of NDI has also shown to differ based on the type of TTTS treatment performed on the MCDA fetuses. Before the discovery of the effectiveness of FLP, serial amnioreduction was the go-to treatment for TTTS. Studies have now shown

that serial amnioreduction poses great risks to the fetuses, even if executed successfully. When compared to FLP, amnioreduction leads to a threefold increase in severe cerebral injury (Wagner et al., 2013). Additionally, those treated with amnioreduction are at greater odds of intraventricular hemorrhage or periventricular leukomalacia during their neonatal period and also have a greater risk of development delay by the time they are two years old. Furthermore, fetuses with TTTS that are treated with amnioreduction are born on average at 29 weeks' gestation, whereas those treated with FLP are born at 32-33 weeks (Senat et al., 2004). Therefore, the former are more likely to face the grave complications that accompany premature birth.

The reported chance of cerebral injury is 6-38% and 8-18% for serial amnioreduction and FLP treatments, respectively (Klink et al., 2016). The reason for the large discrepancy is due to considerable differences in methodologies between conducted research, which leads to high levels of heterogeneity. Other factors affecting heterogeneity of TTTS studies will be elaborated on further in the Discussion section. Overall, it is a widely accepted claim that FLP is the more desired type of treatment based on its minimizations of adverse outcomes.

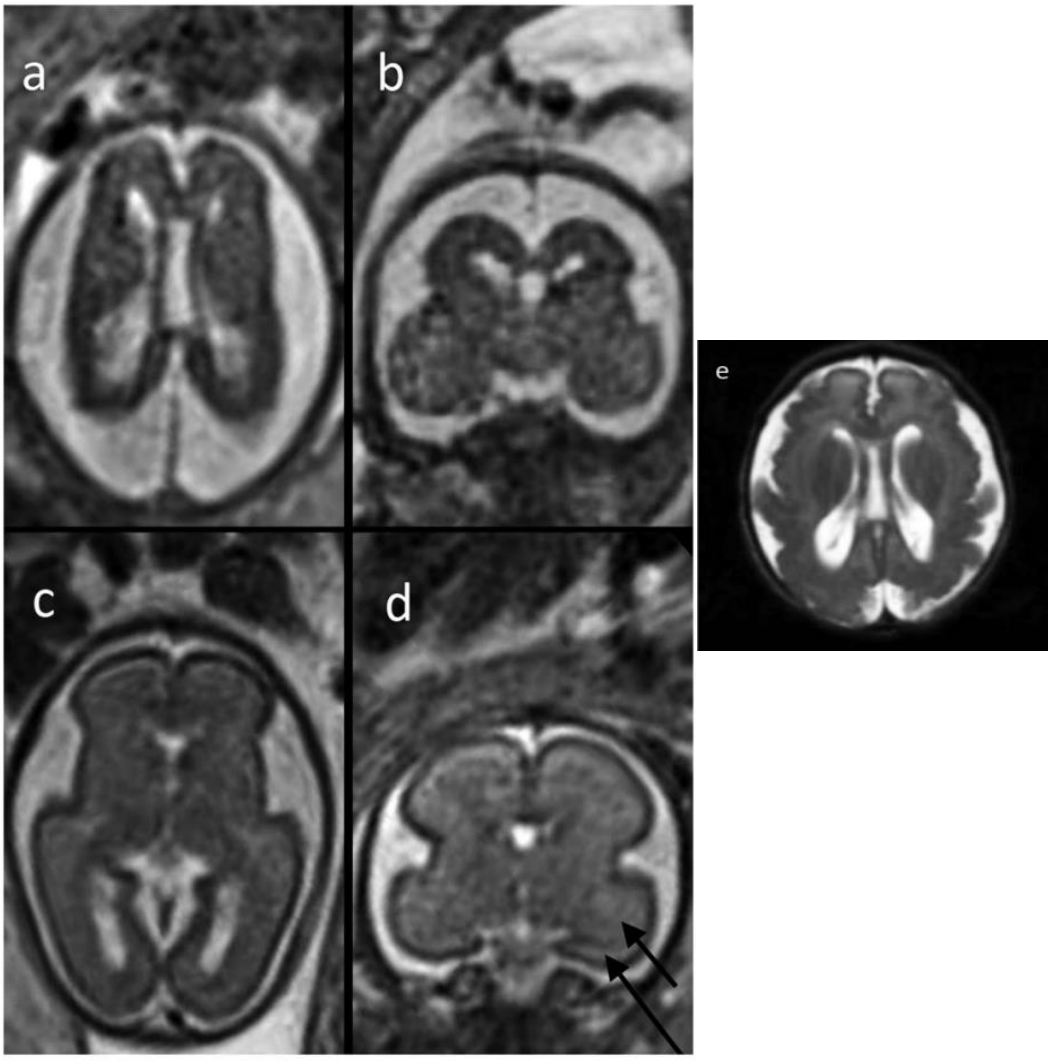


**Figure 6. Prenatal and Postnatal MRIs of TTTS Survivors.**
Images a and b show the abnormal co-twin who had microcephaly and dilated extra-axial cerebrospinal fluid according to prenatal ultrasound. MRI performed 2 weeks later at 24 weeks gestation (a and b) showed dilated extra-axial CSF areas, loss of normal zonal anatomy, and underdeveloped Sylvian fissures when compared to the co-twin (c and d). Normal subplate (long black arrow) and intermediate zone (short black arrow) can be delineated in the normal co-twin (d). Post-natal imaging of the abnormal twin was done at 6 weeks old and shows extensive polymicrogyria affecting the entire brain (e). Figure taken from (Robinson et al., 2017).

*Cardiovascular Outcomes*

Heart disease causes half of the deaths that occur in the postnatal period for TTTS survivors. When compared to unaffected populations, or even to uncomplicated MCDA twins, multiple studies have indicated that TTTS twins have a greater chance of developing congenital heart disease (CHD) throughout their life (Wagner et al., 2013). Unlike neurodevelopmental outcomes, severe cardiovascular long term effects are more relevant to the recipient twin. While still in utero, the recipient is suffering not only from hypervolemia, but also shows signs of systemic hypertension and pressure overload (Halvorsen et al. 2015). In order to compensate for the surplus in volume, the recipient has to produce large amounts of urine, which leads to polyhydramnios. Polyhydramnios, along with increased arterial pressures, initiates cardiac dysfunction which can have potential long term effects.

The antenatal consequences of TTTS lead to recipients having an increased risk for cardiomyopathy. Examples of the most common cardiomyopathy shown include cardiomegaly, biventricular hypertrophy, and atrioventricular valve regurgitation (Herberg et al., 2006). The key overall factors in the pathogenesis of this cardiomyopathy have shown to be increased arterial resistance and pressure, as opposed to the issue of volume overload. The mechanisms causing this resistance are suggested to be increased endothelin levels in the recipient and transplacental transfer of vasoconstrictor agents from the donor twin. This vasoconstriction is what ultimately leads to increased system hypertension. Early intervention in order to detect cardiomyopathy of the newborn recipient includes monitoring blood pressure and performing fetal echocardiograms.

Despite donor twins' volume depleted status, they exhibit minimal cardiac pathology post TTTS treatment. Antenatally, donor twins experience increased afterload due to raised placental resistance and alterations in arterial architecture (Rotar et al., 2020). Postnatally, they could be susceptible to hypotension, so it could be closely monitored. From a long term perspective however, systolic or diastolic dysfunction has shown to normalize over development.

Another key aspect to understand when considering the long term cardiovascular outcomes of TTTS relate to what kind of treatment the patients received. Similar to neurodevelopmental outcomes, amnioreduction shows greater long term risk for the fetuses than FLP does. Specifically, fetal heart failure is 2.7 times more likely in cases where amnioreduction is performed rather than FLP (Moon-Grady et al., 2011). Laser treatment is favored from a cardiac perspective most likely because it involves the direct progression of pathology by obliterating placental anastomoses. This is different from serial amnioreduction, which simply removes amniotic fluid in hopes of relieving hypervolemia in the recipient, as opposed to directly impacting the abnormal blood flow connections.

The overall incidence of CHD in the donor twin is 17% as compared to the recipient twin who has a 43% chance of CHD detection (Manning and Archer, 2016). Regardless of Quintero stage level, TTTS is one of the biggest risk factors for CHD in MCDA pregnancies. 6.9% of TTTS twins show some type of CHD, compared to 2.3% shown in uncomplicated MCDA pregnancies. To put this in perspective, uncomplicated singleton pregnancies have a 0.56% chance of CHD (Herberg et al., 2006).

Despite potential CHD complications that could arise in early life, there is a large chance that patients who survive the neonatal period are likely to have normalized cardiac examination results in the long term. In a study of 89 survivors of severe TTTS treated with FLP, cardiac function was normalized by 15 months of age (Wagner et al., 2013). This normalization can be accounted for by the fact that the pediatric heart has high plasticity. In other words, cardiac function is able to be restored once the stressors of systolic or diastolic dysfunction are gone. This plasticity however is not able to overcome the congenital developmental defects already in place from TTTS.

While the exact mechanism of CHD remains a mystery, clinicians have been able to rely on patterns to point to a cause. The two main CHD manifestations that have been seen at a significantly greater rate in TTTS survivors than the general population are pulmonary stenosis and atrial septal defects. Atrial septal defects are seen in both recipients and donors. Pulmonary stenosis is seen only in recipients and occurs due to the volume overload status present in the right ventricle before FLP treatment . All in all, despite the heart's remarkable ability to adapt during development, the prevalence of pulmonary stenosis justifies the need for high quality postnatal care involving echocardiographic surveillance.

*Renal Outcomes*

Kidney function plays a more minor yet still notable role in the postnatal period of TTTS. Antenatally, the kidneys can be affected by the imbalance regulation of the renin-angiotensin system (RAS). Namely, the RAS is upregulated in the donor twin and downregulated in the recipient twin's kidneys. Chronic renal hypoperfusion is what

causes the donor twin to have increased expression of the renin protein and its transcript (Galea et al. 2005). This increased expression could be beneficial to the donor twin at first, because it can allow the donor to adapt to hypovolemia. However, since increased renin leads to elevated angiotensin II, too much renin production can further cause fetoplacental vasoconstriction of the donor twin (Mahieu-Caputo et al., 2000). This can lead to worsening oliguria and oligohydramnios, ultimately exacerbating growth restriction.

On the other hand, the recipient twin has decreased expression of renin, which can be accounted for by the surplus of amniotic volume. Renin levels however are paradoxically raised in the recipient via the cord blood. This observation supports the idea that despite RAS downregulation in the recipient, the recipient is concurrently exposed to RAS upregulators present in the donor twin (**Figure 6**). These effectors are transferred from the donor twin to the recipient twin via placental shunts (Mahieu-Caputo et al., 2000). This paradox is hypothesized to contribute to the recipient's cardiomyopathy and hypotension, as mentioned earlier. **Figure 6** shows the manifestation of this increased higher staining intensity of renin in the recipient (Galea et al., 2008)

With regards to long term outcomes, the donor twin can develop acute kidney injury (AKI), possibly leading to the need for renal replacement therapy later in life. AKI can develop if the TTTS patient has chronic kidney disease (CKD) and presents itself as a sudden episode of kidney damage that can develop within a few hours or days. Unlike neurodevelopmental or cardiovascular sequelae, CKD development does not relate to TTTS survivors' birth weight, gestational age, or intrauterine growth restriction.

Research on long term survival and renal outcomes of CKD after TTTS are minimal, possibly due to the lack of variables measured during the neonatal period. The only criteria measured antenatally that relates to CKD research is serum creatinine levels and presence of AKI (Ho et al., 2017). This inhibits research to be efficiently conducted retrospectively on TTTS patients.

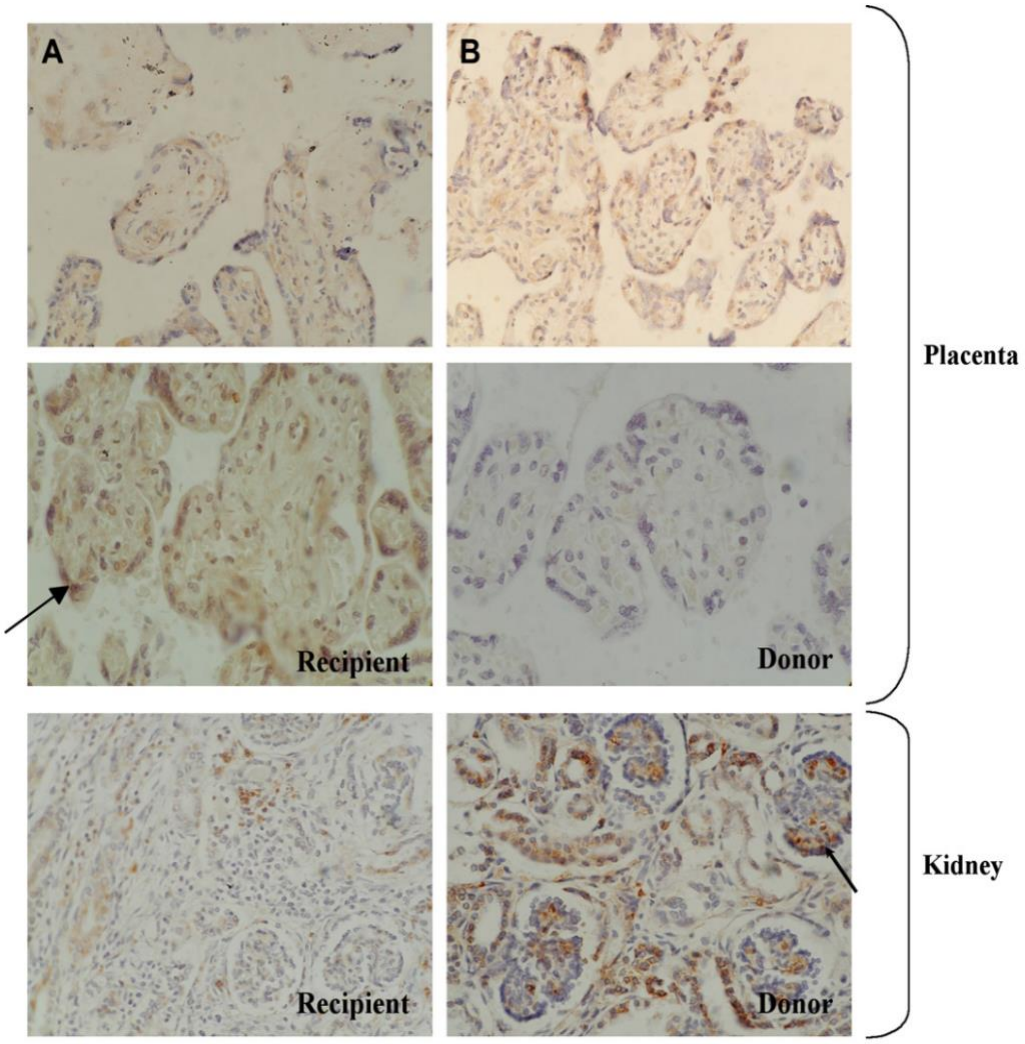


**Figure 8. Renin Expression via Immunolabeling in Controls and TTTS Placenta.** These stains are of renin antibodies in archived kidneys from TTTS pairs between 24 and 32 weeks of gestation. Renin-positive cells are shown by the black arrows. The slides labeled A and B represent controls. The black arrows indicate renin-positive cells. Magnification x 20. Figure taken from (Galea et al., 2008).

Even though research supporting long term impact of CKD on TTTS patients is minimal, the mechanisms of pathology are well understood. Renal impairment is instigated by chronic ischemia and hypoxia, mainly in the donor twin's developing kidney. This severe lack of oxygen can lead to renal tubular dysgenesis. Autopsies of placental transfusion disorder victims show this abnormal kidney development, which specifically involves poor formation of the proximal tubules (Gubler, 2014). This can lead to abnormal activation of the RAS, such as absence of angiotensin II. Absence of production or lack of efficiency of angiotensin II can result in mutations, ultimately leading to reduced glomerular filtration rates, low blood pressure, and renal hypoperfusion.

Overall, the impact of CKD caused by AKI has much potential for further investigation. With regards to short term outcomes, renal dysfunction has shown to occur less frequently in patients treated with FLP than serial amnioreduction (Melhem et al., 2019). This follows the common theme of how FLP is the advantageous treatment option due to its ability to minimize risks and have protective effects on organ function. Long term implications of renal dysgenesis have a spectrum of potential effects. They could manifest as catastrophic end stage kidney disease requiring early renal replacement therapy, or they could progress more slowly as CKD.

## PUBLISHED STUDIES

This section will provide an in-depth breakdown of research that has been pivotal to the current understanding of TTTS. These studies vary in methods, demographics, and statistical analysis, and provide a broad range of evidence for the long term outcomes of TTTS. Most of the focus on long term outcomes relates to childhood neurodevelopment, however, there are also significant cardiac and renal potential outcomes. The following studies also shed light on the importance of having good quality healthcare, and how underserved backgrounds could be at a disadvantage for this. Analysis of this research will eventually lead to the conclusion of which outcomes clinicians and parents should most closely monitor, and how future research can address the obstacles families can face with a TTTS diagnosis.

### Neurodevelopmental Outcomes

Research on neurodevelopmental outcomes for TTTS survivors is the most widely studied topic regarding the long term effects of this disease. Studies show a variety of age ranges used as target populations. A study published in 2015 by Jawish et al. evaluated long term NDI on TTTS survivors at 18 and 24 months old. This prospective study's objective was to see how NDI presented based on which Quintero stage level the patients were at when they received treatment. A parent-completed child monitoring system called the Ages and Stage Questionnaire (ASQ) was sent out to study subjects. The ASQ was used as a screening tool for abnormal development, and has been validated against the standard Bayley Scales of Infant Development as an alternate way of measuring NDI.

The assessment measures five domains: communication, fine motor skills, gross motor skills, personal social skills, and problem solving skills. When analyzing ASQ results from both 18 month old and 24 month old TTTS survivors, it showed a 10% rate of cerebral palsy, 36% rate of moderate to severe speech delay, and 4% of patients had visual impairment. When the 18 month old group was compared to the control group, Jawish et al. found that there was a significant reduction in fine motor scores for patients in Quintero stage III or IV. There was no significant findings between the two age groups of 18 and 24 months old. The control, or observation, group that was used as a comparison consisted of children who had TTTS but only at a Quintero stage I level, which means they did not go through any treatment for it.

While the Jawish et al. study supports the idea that TTTS survivors who went through treatment are at greater risk for NDIs, it has its limitations. Most of these limitations stay consistent throughout all of the studies being discussed, since they emphasize the rarity of TTTS. This study distributed 400 ASQs to parents of TTTS patients, and received only 97 back, or 24% (Jawish et al. 2015). This narrows the pool of subjects to gather data from, and creates a large discrepancy. Parental demographics should also be taken into account, which this study did not disclose or address. Parents who live in low-income areas or far from medical care are less likely to be educated about this disease and therefore not be aware of how to evaluate their child once they've gone through treatment and are past the woes of it.

The next study was conducted by Matsushima et al. and involves 3 year old TTTS survivors who have successfully gone through FLP surgery. This study was published in

2020 but conducted retrospectively, so all of the data is simply being gathered from events that have already occurred. All treatments were done at the National Center for Child Health and Development in Japan. All of the subjects had FLP treatment at 16 to 26 weeks' gestation between 2003 and 2014. The Kyoto Scale was used as the tool for developmental delay examination, which tests the three domains of postural-motor, cognitive-adaptive, and language-social skills (Aoki et al., 2016). This test is routinely administered by pediatric physicians and used conjointly with standardized physical and neurological examinations. Other data analyzed in this study was gathered from medical records and includes perinatal information such as Quintero stage, gestational age at FLP, gestational age at delivery, and birth weight of survivors.

Of the 424 MCDA pregnancies that went through FLP treatment due to TTTS, 188 children from 110 MCDA pregnancies were deemed eligible for this study. Exclusion criteria was if the birth was delivered outside of the study's center, if there was neonatal death or presence of trisomy 21. Additionally, it's important to note that all data was taken from children who were 3 years of age, which includes correction for prematurity. Of these 188 children, cerebral palsy and NDI were detected in 3.2% and 8.5%, respectively (Matsushima et al. 2020). Unlike other studies, no children in this subject pool suffered from severe hearing or visual impairments.

Strengths of this study include its large number of test subjects. Matsushima et al. had 188 participants, and to put into perspective, the largest study ever to be conducted on TTTS survivors between 3 to 6 years of age was by Graeve et al. in 2012 and had 190 children. Another strong point of the study is that imaging such as MRIs were used

alongside neurodevelopmental assessments. This was helpful to confirm any pathology caused by NDI. Abnormal MRI findings were seen in 56% of children with NDI, and 100% of children with cerebral palsy (Matsushima et al., 2020). A potential bias of this study is that all participants had to be treated at the same center, and participants who were unable to physically be at the center due to emergency complications were excluded from the study. These people who underwent obstetrical complications would've been beneficial to follow-up with as it could have provided valuable information on how TTTS pregnancies can present at delivery and the complications they could arise. These complications could potentially relate to NDI. It could be argued however that all of the participants being treated at the center results in consistent variables with regards to the treatment provided. Overall, the Matsushima study sheds light on how TTTS can cause NDI and cerebral palsy complications in survivors by the age of 3 in Japan.

Along with prospective and retrospective studies, there are rare cases of randomized clinical trials that have been conducted studying TTTS. Saloman et al. published a paper following up on the long term developmental outcomes of infants who participated in a randomized clinical trial for TTTS known as the Eurofetus trial. This trial's objective was to compare amniocentesis treatment with FLP to see which method reduced adverse long term developmental outcomes. The study consisted of 128 cases and was followed up in France. Similar to the Jawish et al. study, the parents of the survivors were given ASQs, which were used as the standardized neurological examination. Parents were given these when their children were at 12, 24, 48, and 60 months of age. The TTTS survivors were then prospectively followed up on by Salmon et

al. at 6 years of age using the Wechsler Intelligence Scale to evaluate their neurodevelopment. There was also another test used called the Goodenough Draw-a-Man test which determined the child's mental age based on their graphic capabilities (Saloman et al. 2010). It's important to note that the clinicals and psychologists in charge of administering these exams were blinded to the mode of treatment, negating the chance of bias.

This study was pivotal in establishing the three main conclusions that can be drawn about neurodevelopmental delay in children who survive TTTS. Firstly, they found that FLP treatment was associated with an almost 40% reduction in the risk of fetal death or major long term NDI when compared to serial amnioreduction. This idea has been repeatedly emphasized in research, mainly because TTTS treatment is a relatively new discovery, and this a great example of how science can modernize and raise the efficacy of treatments through clinical research. Secondly, Saloman et al. confirmed that there is an independent association between higher Quintero stage and level of NDI. This makes sense because a higher Quintero level relates to a higher severity of the TTTS. The third significant conclusion is the lack of different findings between donor and recipient twins. This suggests that both twins are at equal risk for developing NDIs.

There has also been a metanalysis conducted on TTTS that has produced noteworthy information on TTTS, and provides important recommendations on how to monitor presence of postnatal cerebral injury in TTTS survivors. This was done by Klink et al. in 2016, and focuses on neurodevelopmental outcome in TTTS patients treated with amnioreduction versus laser therapy. Based on their widespread search of published

experiments, they determined that there is a 7-fold higher risk of severe cerebral injury in patients treated with amniocentesis over FLP. This aligns with previous studies that demonstrate this as well. Alongside that, they performed normalizations on their data to find that the chance of long term NDI after amnioreduction is on average 20%, whereas it is 10% for FLP treatment.

I think the importance of the Klink et al. study is their emphasis on recommendations for minimizing risk of cerebral injury and consequent NDI, no matter which treatment the patients received. An important point they make is how MRIs should be taken both antenatally and postnatally in order to investigate their correlation for cerebral injury. Routine imaging will also help accurately evaluate origin, timing, and type of cerebral injury present. Furthermore, they shed light on an issue that several studies have been guilty of, which is testing NDI in TTTS survivors at too early of an age. They support this by saying how the average age of NDI testing in these studies is 2 years old. In reality, neurodevelopmental problems often do not become apparent until a child is at school age. Therefore, following up with these patients until at least 6 years or older is recommended (Klink et al., 2016). This, along with continued collaboration between clinicians and parents should help provide more accurate data on long term NDI in the future. **Table 2** and **Table 3** provide a summary of the outcomes gathered by Klink et al. based on whether the TTTS survivors were treated with amnioreduction or FLP.

**Table 2. Long Term Neurodevelopmental Outcome in TTTS Treated with Amnioreduction.** Adapted from (Klink et al., 2016).

| Author, year | Outcome measure | CP % (n/N) | NDI % (n/N) | Comments |
|---|---|---|---|---|
| Reisner et al., 1993 | Neurologic exam | 19 (5/27) | NA | No developmental tests, no controls, 19/27 < 18 months at follow-up |
| Mari et al., 2000 | Clinical record, discussion parent/pediatrician, speech, or physical therapy | 5 (2/42) | NA | No developmental tests, inclusion mild TTTS cases, follow-up based on clinical records, no controls, high neonatal death rate (16%) |
| Cincotta et al., 2000 | Neurologic exam, physiotherapy assessment, Griffiths scale | 13 (3/23) | 22 (5/23) | 18% neonatal death rate, inclusion gestation matched twin controls |
| Haverkamp et al., 2001 | Neurologic exam, Denver screening test, Griffiths scale | 23 (9/40) | 23 (9/40) | 18% lost to follow-up, incomplete follow-up, no controls |
| Frusca et al., 2003 | Neurologic exam, Griffiths scale | 16 (5/31) | 26 (8/31) | 35% (11/31) < 2 years at follow-up, no controls |
| Lopriore et al., 2003 | Neurologic exam, school functioning | 21 (6/29) | NA | No developmental tests |
| Dickinson et al., 2005 | Neurologic exam, general health questionnaire, vineland scales, child behavior checklist, Bayley scales, Stanford-Binet intelligence scale | 6 (3/52) | 14 (7/52) | Neurologic exam in very pre-terms only, behavioral outcome but only in pre-scholars, inclusion contemporaneous regional cohort |
| Lenclen et al., 2009 | Neurologic exam, ages stages questionnaire | 19 (4/21) | NA | No developmental tests, gestation matched dichorionic controls |
| Saloman et al., 2010 | Neurologic exam, ages stages questionnaire, | 13 (6/47) | NA | NDI not reported, 36% Neonatal death rate, no |

| Author, year | Outcome measure | CP % (n/N) | NDI % (n/N) | Comments |
|---|---|---|---|---|
| | Wechsler scales, goodenough draw-a-man-test | | | controls, inclusion twins treated with laser |
| Li et al., 2011 | Neurologic exam, Enjoji development scale, Wechsler scales | 15 (3/20) | 20 (4/20) | Small study size, preponderance mild TTTS cases |
| Total range | | 13.9 (46/332) 5-23 | 19.9 (33/166) 14-26 | |

**Table 3. Long-Term Neurodevelopmental Outcome in TTTS Treated with Laser Surgery.** Adapted from (Klink et al., 2016).

| Author, year | Outcome measure | CP % (n/N) | NDI % (n/N) | Comments |
|---|---|---|---|---|
| De Lia et al., 1999 | Neurologic exam | 3 (3/93) | NA | No developmental tests, mean age follow-up 14 months, no controls |
| Sutcliffe et al., 2001 | Neurologic exam, Griffiths scale | 9 (6/66) | 9 (6/66) | 19% lost to follow-up, 47% information general practitioner, 54% incomplete tests, no controls |
| Banek et al., 2003 | Neurologic exam, Griffiths scale, Snijder-Oomen intelligence test | 11 (10/89) | 11 (10/89) | Severe developmental delay not included as criterion for NDI, no controls |
| Graef et al., 2006 | Neurologic exam, Griffiths scale, Snijders-Oomen intelligence test | 6 (10/167) | 8 (13/167) | Suboptimal/incomplete use of developmental tests |
| Lenclen et al., 2009 | Neurologic exam, ages stages questionnaire | 10 (9/88) | NA | No developmental tests, preterm dichorionic controls matched for gestational age at birth |

| Author, year | Outcome measure | CP % (n/N) | NDI % (n/N) | Comments |
|---|---|---|---|---|
| Lopriore et al., 2009 | Neurologic exam, Bayley scales | 6 (17/278) | 18 (50/278) | 2 TTTS-pregnancies treated >26 weeks' gestation |
| Salomon et al., 2010 | Neurologic exam, ages stages questionnaire, Wechsler scale | 12 (9/73) | NA | NDI not reported, no controls |
| Gray et al., 2011 | Neurologic exam, Griffiths, and Bayley scales | 4 (5/113) | 12 (14/113) | Mixed developmental tests; e.g., second and third version Bayley scales, no controls |
| Chang et al., 2012 | Neurologic exam, Bayley scales | 5 (3/59) | 7 (4/59) | Small study size, corrected age follow-up 1 year |
| Graeve et al., 2012 | Neurologic exam, Griffits, and Bayley scales | 4 (5/113) | 12 (14/113) | Mixed developmental tests; e.g., second and third version Bayley scales, no controls |
| McIntosh et al., 2014 | Wechsler preschool primary scale of intelligence-III, general health questionnaire | 2 (1/50) | 4 (2/50) | 16% lost to follow-up, no neurologic exam, small sample size |
| Vanderbilt et al. 2014 | Amiel Tieson neurodevelopmental exam, Battelle developmental inventory | 3 (3/100) | 4 (4/100) | 50% lost to follow-up, majority lost to follow-up Quintero stage IV |
| van Klink, Slaghekke et al., 2015 | Neurologic exam, Bayley scales | 3 (6/216) | 10 (22/216) | Follow-up in two of the five participating centers, limited power to detect difference in long-term outcome |
| Total range | | 6.1 (82/1341) 3-12 | 9.8 (120/1228) 4-18 | |

**Cardiovascular Outcomes**

While studies on cardiovascular long term outcomes are not as abundant as ones on neurodevelopment, they've been imperative in providing insight on how hemodynamic imbalance can contribute to CKD in TTTS survivors. Herberg et al. studied the long term changes in cardiac morphology and function in survivors of severe TTTS that were treated with FLP. This was a prospective study conducted at Barmbek Hospital in Hamburg, Germany. It consisted of 89 survivors from 73 consecutive twin pregnancies after FLP treatment of severe TTTS. These survivors were born at a median gestational age of 33.7 weeks and their cardiac examinations were performed at a median age of 21.5 months. Investigational tools such as 12 lead electrocardiograms, Doppler echocardiography, and blood pressure measurements were taken in order to see if the survivors developed abnormal cardiopathy postnatally.

Results of the Herberg et al. study showed that 87% of survivors ended up having normal cardiac examination. This is a remarkable result since 55% of recipient twins had pathological findings during their prenatal assessment (Herberg et al. 2006). The power of ventricular remodeling is shown here as normalization of cardiac function occurred for most of the survivors. The study authors do however mention that this normalization doesn't avoid the chance of pulmonary stenosis occurring in recipients.

Limitations of this study include the fact that additional follow-up data on echocardiographic assessments were not available to the researchers due to the fact that patients followed up with their local referring units. This information would have provided insight on Doppler velocities and pulmonary valve diameter, which is critical

for detecting fetal pulmonary stenosis. Furthermore, new technology has developed since the start of this prospective study. This technology would have been able to describe additional parameters from tissue Doppler imaging, providing a more thorough assessment of systolic and diastolic function.

To add on to Herberg et al.'s findings, Halvorsen et al. performed a 10-year follow-up study of cardiac structure and function after TTTS. This study, taking place at the Clinic of Obstetrics and Gynecology at Sodersjukhuset, Stockholm, is unique in that it is one of the rare instances where follow-up was conducted well into childhood, as opposed to being while the child is still relatively young. The subjects of this study were 19 pregnant women who were diagnosed with TTTS in the second trimester. This study started before the introduction of laser therapy, therefore all women who were eligible were treated with amniotic reduction. From the 19 women, 12 cases ended up being eligible for this study due. Exclusive criteria involved fetal demise of one or both twins. All remaining cases were at Quintero stage I, II or IV. Cardiovascular parameters were measured with instruments such as 2-D echocardiography, Doppler tissue imaging, and Doppler flow velocimetry.

Results of Halvorsen et al. determined that diastolic and systolic function was within a normal range for all subjects when tested at around 10 years old. The unique finding of this study however is that they did find a significant difference between the donor and recipient's cardiac function. They found that recipient twins within the TTTS survivor pair had a decrease in early diastolic ventricular relaxation. Specifically, they observed that the peak velocities of early diastolic filling of both the left and right

ventricle walls were lower in the recipient twin when compared to their donor twin (Halvorsen et al. 2015). While the significance of this remains unclear and warrants follow-up, it shows that treatment of TTTS leads to unique recovery processes between the recipient and the donor twin. Halvorsen et al. suggests that this reduced function in the recipient twin warrants continuous monitoring and follow-up in the TTTS survivor. Overall, this study supports the idea that cardiovascular long term outcomes are not as detrimental or severe as potential neurological outcomes after successful TTTS treatment.

**Renal Outcomes**

Studies on how TTTS affects the renal system are being newly discovered through research on the RAS' impact on hemodynamic balance. Short term studies on TTTS outcomes have documented that non-laser treatment can lead to postnatal renal complications in the donor twin. These complications can include impaired renal perfusion, including histological renal changes such as microangiopathy and hypervascularization (Verbeek et al., 2017). Since long term studies on the impact of renal function on TTTS survivors is not as abundant as other fields of research, the first step to discovering potential long term adversities would be to understand current knowledge of short-term outcomes.

Verbeek et al. published a study in 2017 describing the impact on renal function in the short term on neonates treated with laser compared to non-laser treatment. This study was conducted in the Netherlands and collected data from all MC twin pairs with TTTS between 2009 and 2016. FLP was conducted for all TTTS cases that were Quintero

stage II or higher and for cases in stage I that had symptomatic polyhydramnios. All other cases were treated more conservatively through amniocentesis or expectant management. The study design tested various variables from each group and ultimately aimed to compare the results of the laser-group with the non-laser group.

Perinatally, variables such as mode of delivery, gestational age at birth, gender, birth weight, and birth weight difference between each pair of twins was recorded. Postnatally, routine measurements of heart rate, blood pressure, hemoglobin, creatinine and urea levels were taken. The infants were also tested for conditions such as hypotension, respiratory distress syndrome, patent ductus arteriosus, fetal distress, necrotizing enterocolitis, sepsis and neonatal mortality. The primary goal of the study was to test for short-term renal dysfunction, which was defined as having a creatinine level of >100 μmol/L in the first week of life (Verbeek et al. 2017). This study is unique in that it is the first to investigate short-term renal function in such a large scale of TTTS patients. 312 twins were included in this study, with 274 having been treated by FLP and 38 treated more conservatively and therefore were the non-laser group.

Results of the Verbeek et al. study showed that creatinine levels were much lower in patients who were in the laser-group as compared to the non-laser group. On the other hand, the incidence of renal failure or oliguria in twins treated without FLP is high. Specifically, the laser group and non-laser group had a 7.1% and 37.9% occurrence of short-term renal failure, respectively. Furthermore, neonatal mortality and morbidities occurred at a less frequent rate in the laser group when compared to the non-laser group. These findings suggest that laser therapy may have a protective factor on the renal

function of TTTS survivors. The impact of this short-term, large-scale study is that it shows the importance for long-term evaluation of renal function in order to see the true protective effects of laser therapy. This study also continues to support the ongoing theme of how FLP as a treatment for TTTS reigns supreme with regards to its decrease in long-term detriment in TTTS survivors.

Even though long term studies on renal function of TTTS are scarce, there is one study conducted by Melhem et al. that looked into the long term risk for CKD in TTTS survivors. All participants of this study were patients at Great Ormond Street Hospital in North London from 1998 to 2018. Data was extracted retrospectively in order to find which patients qualified for the study. Qualification included having TTTS as a primary diagnosis at birth, and having documented cases of either polyhydramnios or oligohydramnios. From this database the researchers were able to identify 34 eligible infants to be included for this study. This small sample size truly attests for the rarity of this complication. The average age of follow-up for these patients was 6.5 years old, which is an adequate age to tell if these survivors would develop signs of CKD. The first step to analyzing the renal conditions of the 26 infants was to split them into two groups: CKD group and non-CKD group. The CKD group consisted of participants who had stage 2-5 CKD according to Kidney Disease: Improving Global Outcomes guidelines . The remaining infants were ones who had normal serum creatinine levels. These participants were tested upon TTTS treatment follow up or had already been discharged from follow up.

After considering exclusion criteria for this analysis, 26 out of the 34 infants ended up being eligible for this study. Four participants were excluded because of in-utero demise of their co-twin, and the other four patients were excluded due to diagnostic uncertainty or structural anomalies of their urinary tract. Results of the Melhem et al. study showed that 8 out of the 26 children developed CKD and the 18 remaining children had no signs of CKD. 7 of the 8 patients with CKD also had AKI neonatally, suggesting that kidney injury early on in life can be a precursor for CKD. Additionally, this is the first study to provide evidence for the idea that CKD in TTTS survivors can lead to the need for long-term renal replacement therapy (RRT) throughout childhood. RRT can involve a range of methods for replacing normal blood-filtering function of the kidneys including hemodialysis, peritoneal dialysis, and continuous hemofiltration. Factors such as gestational age at birth or comorbidities such as NDI did not seem to affect long term renal outcome.

It was also determined that donor twins have a greater chance at developing CKD, however, this result did not have a significant p-value most likely due to small sample size (Melhem et al. 2019). Other limitations of this study included lack of data on postnatal TTTS care due to the study center not having an attached maternal unit, and difficulty with gathering follow-up data for the non-CKD group since they were not under active monitoring like the CKD group was. Despite these limitations, this study is still important in determining that CKD is a very possible outcome in TTTS survivors, potentially more so for donor twins. Because of this, signs of AKI in the early neonatal

period should be observed, along with active monitoring of creatinine levels throughout childhood.

## DISCUSSION AND CONCLUSION

Knowledge of TTTS has grown tremendously over the past decade, with emphasis being mainly on proper treatment protocols and short-term outcomes. Attention regarding the long term outcomes of TTTS revolve around the differences between the type of treatment the survivors received, and whether the symptoms are neurological or cardiovascular related. Focus should steer more towards how to monitor the neurodevelopmental outcomes, as they are the most prevalent and can affect the quality of life of the survivor to a severe degree. That being said, regular cardiac monitoring for CHD should be provided throughout the TTTS survivor's early childhood. Despite the current long-term studies showing a high chance of cardiovascular normalization once TTTS survivors reach childhood, monitoring should focus closely on the systolic and diastolic function of the recipient twin. Halvorsen et al.'s study showed that recipient twins had potential for decreased ventricular relaxation. Since there are no studies that have been conducted monitoring the progress of this past childhood, the meaning of this difference is unclear and therefore should stay on the radar of clinicians. An area of research that can definitely be expanded upon relates to the long term effects on the renal system after TTTS. Clinicians should pay special attention to the creatinine levels of the TTTS survivor that was the donor twin in order to monitor signs of CKD. If left untreated, CKD can lead to complications requiring RRT or possible kidney transplant. Meeting these long term goals for TTTS monitoring will greatly improve patient

outcomes and contribute to the current understanding of how short-term complications can impact the rest of an individual's life.

Conducting research on TTTS can be imperfect due to the lack of replicability caused by confounding variables. The biggest overall confounding variable present in most of the studies reviewed is the high lost-to-follow up rates. Failing to follow up with study participants can lead to skewed data and underestimation. Reasons parents of TTTS survivors may be hard to reach are multifaceted and relate to how variable each patient's condition is based on their background regarding geography, race, ethnicity, and overall socioeconomic status. Additionally, it is possible that parents with children suffering from severe NDI are less likely to keep up with study check-ins due to an already alarming amount of medical care to keep track of. Instances like these are an example of how obtaining research can be difficult when it requires patient participation and survey.

Another big limitation seen in the discussed studies is small sample size, which goes hand in hand with the high lost to follow-up rates. Sample size issues however are also due to the fact that TTTS is an overall rare disease. Studies can have access to millions of cases via patient databases, however, from all of that information there may only emerge a handful of TTTS cases to investigate. From those handful of cases researchers must go through further exclusion processes, narrowing the list down more. An example of this occurring is in the Melhem et al. where out of twelve million cases available from Great Ormond Street Hospital in North London, only 26 ended up being eligible for their retrospective study (Melhem et al., 2019). The rarity of TTTS means that in order to gather the most accurate information, cases must be taken from a broad

time period so that there can be enough to create an adequate sample size for statistical analyses.

An exclusion criteria for most long term studies is fetal demise of one of the twins. Having this being an exclusion makes sense because death of one of the twins can self-correct TTTS and lead to no adverse outcomes, however, that completely depends on when the death of the fetus occurs. If it happens close to when the twins are at an appropriate gestational age to be birthed, it's possible that the viable fetus will still have signs of adverse long term outcomes from TTTS. A possible solution to this criteria taking away potential samples would be to adjust the exclusion for which gestational age the neonatal death occurred. If one of the fetuses died close to full term, it should be okay for the other fetus to participate in these studies, granted they don't require comparing twin pairs against each other.

Current research has shown that there is still much to be discovered about the unique physiology of TTTS. Due to lack of complete knowledge about this syndrome, clinicians have to depend heavily on their own clinical judgment when diagnosing, treating, and monitoring their patients. This can lead to varying levels of education and awareness that parents of TTTS patients have about how to provide the highest quality care for their child. Other factors such as socioeconomic status, race, ethnicity and geography can also impact the level of care a patient of TTTS receives. While the etiology of these factors are still not well known, they are important to consider when thinking about why research on TTTS has shown limitations such as small sample size and low follow-up rates.

Healthcare disparities are ever present around the globe. The United States in particular has a prominent history of medical prejudice against minorities. The United States Census Bureau projects that by 2044 over half of the country's population will be non-white, therefore, it is of increasing importance for clinicians to be cognizant of the inconsistencies that minorities face (Mueller et al., 2020). A major contributing factor to these inconsistencies in medical care is implicit bias. Implicit bias is when clinical decisions are formed by behaviors in reaction to patient characteristics such as race, ethnicity, gender, sexual orientation, disability, or age (Blair et al., 2011). These biases are unconscious and therefore difficult to take away from a clinician's everyday judgement. Steps that can be taken to narrow disparities seen across obstetric/gynecological (OB/GYN) care includes enhancing communication between patients and providers, addressing implicit bias through unconscious bias training, performing regular maternal morbidity reviews, standardizing care on labor and delivery, and promoting an overall culture of equity across the care continuum (Howell and Ahmed, 2019). Addressing these gaps in OB/GYN care are key steps in providing equal healthcare to all. Achieving this goal would have a good chance at improving the motivation, accessibility, and likelihood of pregnant women seeking the medical care they need. Quality OB/GYN healthcare could also increase the odds of patients following up with their maternal care centers and participating in research studies.

Another factor that could improve the quality of TTTS research would be by addressing the inequalities for access to medical care in rural communities. Due to a grave shortage of health care workers, rural residents must travel long distances to

receive OB/GYN care, putting them at risk if they need to seek emergency intervention (Kozhimannil et al., 2019). Studies have shown that within a one-year frame, women living in rural communities had lower OB/GYN visit rates than women from urban areas (Lee et al., 2020). Therefore, it is concerning if a woman living in a rural area receives a TTTS diagnosis. Having a condition such as TTTS requires frequent appointments to maternal care centers in order to have imaging such as ultrasounds and fetal echocardiograms performed on the fetuses. These imaging appointments are essential in early detection and close monitoring of the progression of TTTS (Fischbein et al., 2018). Closing the gap in lack of healthcare resources for rural communities could potentially help women detect TTTS earlier, decreasing the rate of fetal demise and adverse long term outcomes.

TTTS complicates around 15% of all MCDA pregnancies and carries a great risk of fetal morbidity and mortality if left untreated. Diagnosis using ultrasound and monitoring of amniotic fluid volumes can swiftly detect TTTS, helping clinicians provide early intervention. FLP therapy is the key way to ablate abnormal vessel connections causing the imbalance of fluids between the two fetuses. Short term outcomes mainly relate to premature birth, cerebral injury, AKI, and CHD. Long term outcomes are still being understood through research, but mainly involve neurodevelopmental delays related to cerebral injury. Limitations on long term research for TTTS are vast and should be addressed through public health awareness of TTTS and greater access to medical care to underserved communities and minority populations. Continued close collaboration

between neonatologists, obstetricians, and childhood development specialists is critical in order to improve cases of children with TTTS.

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