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# The assessment of placental sharing using X-ray angiogram versus digital photograph: A prospective study

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ABSTRACT

*Introduction:* We aim to compare two different methods for the assessment of placental sharing in monochorionic diamniotic twins: X-ray-angiogram and digital photograph of the placenta.
*Method:* We included the placentas of a prospective series of twins that were followed from the first trimester onward and resulted in a double live birth or double stillbirth between April 2016 and February 2019. Injection was performed after delivery and an X-ray angiogram was made, as well as a digital photograph. On both of these, the territory of each twin was measured two investigators (IC and LL). Placental sharing discordance was determined using the following formula: (larger territory – smaller territory)/larger territory. We calculated the intra-class correlation coefficients for intra-observer and inter-observer reliability and used Bland-Altman analysis to compare both methods.
*Results:* 77 placentas were included in the analysis. For both methods, there was an excellent intra- and inter-observer reliability. The mean difference in sharing (bias) on the X-ray and digital photograph using Bland-Altman analysis was 3,7% (95% CI 1,1% - 6,3%), where the digital photograph tends to overestimate the discordance. Limits of agreement were between −19% and 26%.
*Conclusion:* Delineation of the placental sharing on a digital photograph slightly overestimates the discordance. Since the venous territory on X-ray angiogram physically determines where each twin gets its oxygenated blood, X-ray angiogram may be a better method to determine placental sharing, although the digital photograph constitutes a valid alternative.

## 1. Introduction

Unequal sharing of the placenta in monochorionic twins plays a role in discordant fetal growth and adverse pregnancy outcome. Several previous studies suggested that placental territory discordance is associated with birthweight discordance [1–6]. However, different researchers have used different methods to determine placental sharing. In previous studies by our group, all arteries and veins were injected. Then, the artery-to-vein (AV) anastomoses were identified and used to delineate the equator and to measure the placental territory of each twin on a digital photograph [1,2]. Several other groups have used photographs of the placental surface after injection to assess placental sharing [3–8]. It is unclear which method is superior.

We hypothesized that placental sharing may be more accurately determined on an X-ray angiogram than on a photograph. When the umbilical vein of one twin is injected, the angiogram will show the exact area of the placenta that drains towards its umbilical vein. As the vein transports the oxygenated blood, the X-ray angiogram can define the exact placental part that is responsible for the growth and development of this twin. Also, the measurements on a photograph may be more inaccurate as there are often large areas without anastomoses where it is difficult to judge to which twin the territory belongs. The purpose of this study is to compare the measurement of placental sharing on an X-ray angiogram with that on a photograph of the placental surface in a prospective series of monochorionic diamniotic twin pregnancies recruited in the first trimester.

## 2. Methods

Patients who were diagnosed with an ongoing monochorionic diamniotic twin pregnancy between 11.0 and 14.0 weeks in the University Hospitals Leuven were invited to take part in the TWINSHARE study. As

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part of this prospective cohort study, referring physicians are requested to send the placenta after birth in a dry container for injection studies (ClinicalTrials.gov Identifier: NCT03024918). This study received ethical committee approval from the University Hospitals Leuven (S58744).

Only placentas of pregnancies resulting in the livebirth or stillbirth of both twins were included in the analysis [3–6]. Placentas of pregnancies complicated with single demise remote from delivery (> 48 h) were excluded because of placental maceration of the part of the demised twin, as were placentas that were too damaged for injection or stored in formalin. We did not exclude placentas with double stillbirth close to delivery because it is still possible to accurately assess the individual venous territory that supplied each twin with oxygenated blood during their intrauterine life. Also, the vascular anastomoses and placental sharing remain unchanged regardless of both twins being liveborn or stillborn.

Placentas were kept in a dry container at +4 °C after delivery. The arteries and veins were catheterized as described previously [9]. First, the umbilical vein of one twin was injected with barium. Vein to vein anastomoses (if present) were tied off at the level of the vascular equator before injection to prevent injection of both territories at once.

After injection of the venous territory of one twin, a digital X-ray angiogram was taken (Luminos dRF, Siemens, Erlangen, Germany). The X-ray tube was positioned at 115 cm, with machine settings at 45 kV and 2,5 mA. Subsequently, the vein of the co-twin and the arteries of both were injected with color dye and a regular digital photograph was taken. All images were taken perpendicular to the placental surface .

The placental territory measurements were performed independently by two investigators (IC and LL) using Image J software (Image J, National Institute of Health, USA). The placental surface of each twin was measured on the X-ray angiogram and on the digital photograph(Fig. 1 and 2. All measurements were blinded therefore the X-ray angiograms and photographs could not be linked to one another or to the pregnancy outcome.

We determined placental sharing discordance using the following formula: (larger territory – smaller territory)/larger territory. The closer the ratio to 100%, the more unequally the placenta is shared, while equally shared placenta will have a ratio close to 0%. We calculated the 90$^{th}$ centile for both methods and used this as a cut-off to define unequal placental sharing. We calculated the diagnostic accuracy of the photograph to diagnose unequal sharing in comparison with the X-ray angiogram. We then calculated absolute intra- and inter-observer variation of the X-ray and photograph method and compared these using a paired *t*-test. The intra-class correlation (ICC) for intra-observer variability of both methods was calculated based on a single-measurement, absolute-agreement, 2-way mixed-effects model. The ICC for inter-observer variability of both methods was calculated based on a single-measurement, absolute-agreement, 2-way random-effects model. Finally, we used Bland-Altman analysis to compare both methods by plotting the difference in sharing measured on the digital photograph and the X-ray angiogram against the average of the 2 measurements. The Shapiro-Wilk test was used to test for normality of the differences between both methods. All analyses were performed using STATA 13.1 (StataCorp. 2013. Stata Statistical Software: Release 13. College station, TX: StataCorp LP). A 2-sided p-value of $P < 0.05$ was considered significant.

## 3. Results

Between April 2016 and February 2019, 96 patients who were recruited as part of the Twinshare study in the first trimester had delivered. Of these, 3 placentas were not sent back for examination and 16 were excluded: 7 because of single demise, 5 were too damaged by delivery and 4 because of storage in formalin. This left 77 placentas available for the analysis. The clinical characteristics of the included pregnancies are presented in Table 1. Of the seven pregnancies that were complicated by twin-to-twin transfusion syndrome (TTTS), five underwent laser coagulation of placental anastomoses (5/77, 6%). Three pregnancies were complicated with intrauterine demise of both twins within 24 h of one another and all 3 were delivered within 24 h after the diagnosis.

The mean discordance in birth weight (calculated as [birth weight larger twin – birth weight smaller twin]/birth weight larger twin) was 13% ± 10%, while the mean discordance in placental sharing was 26% ± 18% % on X-ray angiogram and 29% ± 20% on photograph. The 90$^{th}$ centile of placental discordance for the X-ray method and for the photograph method in this series was 50% and 60%, respectively. If we use this 90$^{th}$ centile to define unequal placental sharing, 7 placentas were unequally shared for each method and 5 placentas were unequally shared according to both methods. Two unequally shared placentas according to X-ray angiogram, were not identified as such using the photograph method. Another two equally shared placentas according to X-ray angiogram were misclassified as unequally shared by the photograph method. If we consider the X-ray method to be the gold standard, then the photograph had a diagnostic accuracy of 95% (95%CI 87%–99%) with a sensitivity of 71% (95% CI 29%–96%) at a false positive rate of 3% (95%CI 0%–10%) to detect unequal sharing.

Table 2 shows the absolute intra- and inter-observer variation for both methods, as well as the absolute agreement intra-class correlation coefficients. Both methods have an excellent reliability. There was no significant difference in absolute intra- and inter-observer variation ($p = 0.333$ and $p = 0.061$ respectively). For the intra- and inter-observer ICC, there was a large overlap of the 95% confidence intervals for both methods, indicating the lack of a significant difference there as well.

While the discordance on X-ray angiogram and on photograph was

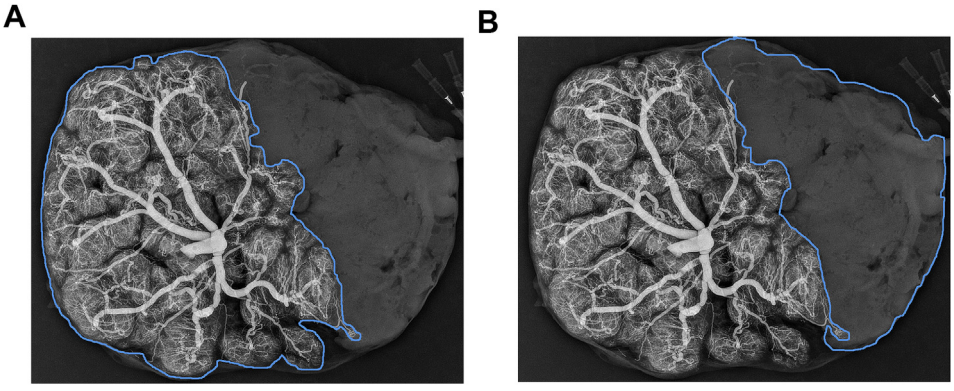


**Fig. 1.** X-ray image of the placenta. The vein of twin 1 is injected with barium sulphate (white on picture and on X-ray). Figures a and b show delineation of the venous territory of twin 1 and 2, respectively.

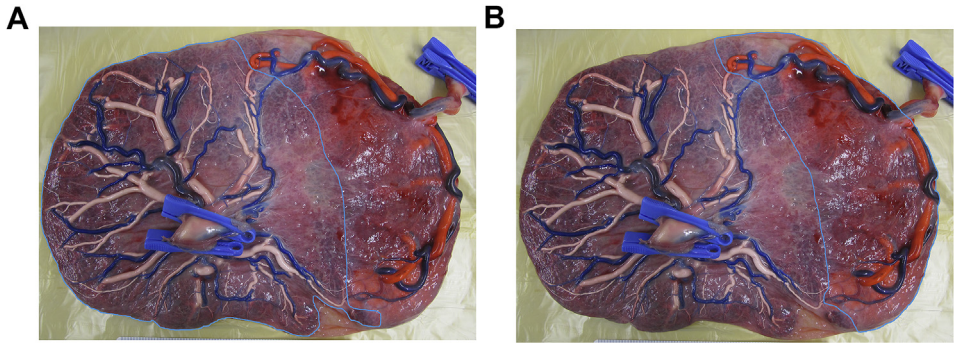


**Fig. 2.** Digital photograph of the placenta. Figures a and b show delineation of the venous territory of twin 1 and 2, respectively.

**Table 1**
Clinical characteristics of the study population.

| CHARACTERISTICS | N = 77 |
|---|---|
| **Maternal** | |
| Maternal age | 31 ± 1 years |
| Nulliparous | 30/77 (39%) |
| Spontaneous conception | 65/77 (84%) |
| **Pregnancy** | |
| Gestational age at delivery | 34 ± 1 weeks |
| Delivery before 24 weeks | 4/77 (5%) |
| TTTS | 7/77 (9%) |
| sIUGR | 11/77 (14%) |
| **Neonatal** | |
| Livebirth | 148/154 (96%) |
| Survival at 7 days | 145/154 (94%) |

Variables are expressed as means ± standard deviation and proportions (%).
TTTS = Twin-to-Twin Transfusion Syndrome.
sIUGR = selective Intra-Uterine Growth Restriction: defined as a discordance of 25% or more at birth in case of 2 livebirths or a discordance in estimated fetal weight of 20% or more on the last ultrasound prior to demise.

**Table 2**
Results of intra- and interobserver variation analysis of both the X-ray method and the photograph method.

| X-RAY METHOD | | | |
|---|---|---|---|
| Absolute variation in placental sharing discordance[a] ± SD | | Absolute agreement ICC (95% CI) | |
| *Intra-observer* | *Inter-observer* | *Intra-observer* | *Inter-observer* |
| 2,5% ± 0,3% | 3,3% ± 0,4% | 0.982 (0.972–0.988) | 0.968 (0.949–0.980) |
| **PHOTOGRAPH METHOD** | | | |
| **Absolute variation in placental sharing discordance[a] ± SD** | | **Absolute agreement ICC (95% CI)** | |
| *Intra-observer* | *Inter-observer* | *Intra-observer* | *Inter-observer* |
| 2,9% ± 0,3% | 4,5% ± 0,5% | 0.981 (0.970–0.988) | 0.952 (0.926–0.970) |

SD = standard deviation; ICC = intraclass correlation coefficient; CI = confidence interval.
[a] Expressed as the absolute value of the difference in placental sharing.

not normally distributed, the difference between the two methods was (p = 0.246). The mean difference in sharing (bias) on digital photograph versus X-ray angiogram using Bland-Altman analysis was 3,7% (95% CI 1,1% - 6,3%). As the line of equality (0) is not included in the confidence interval, the photograph appears to systematically overestimate the discordance. The limits of agreement were −19%–26%, meaning 95% of the differences between both methods were located within this interval [10] (see Fig. 3).

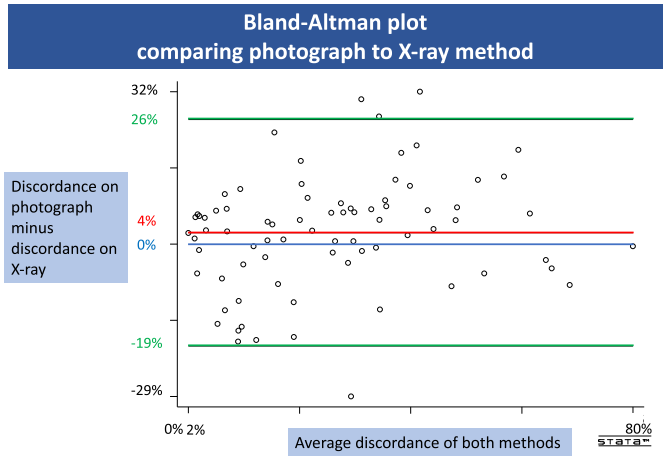


**Fig. 3.** Bland-Altman plot comparing both methods. The average placental sharing ratio of both methods is plotted on the X-axis, while the Y-axis shows the placental sharing ratio on the digital photograph minus the ratio on X-ray. The blue line indicates the line of equality for both methods. The red line (bias) is slightly above 0, demonstrating that the picture overestimates the discordance compared to X-ray. The green lines represent the limits of agreement, which contain 95% of the differences between both methods.

## 4. Discussion

Compared to X-ray angiogram as the gold standard, delineating the placental sharing on a digital photograph is an accurate method to diagnose unequal placental sharing using the 90$^{th}$ centile as a cut-off. Also, there is a high correlation and agreement within and between observers when measuring placental sharing on X-ray angiogram or on digital photograph. However, Bland-Altman analysis showed that there was a small but statistically significant overestimation of the discordance when comparing the photograph to the X-ray angiogram.

This is the first study to compare different methods of assessing placental sharing. While we expected to find a higher intra- and interobserver correlation when using the X-ray angiogram to determine placental sharing, the photograph analysis performed equally well in our series. This may be due to the fact that all measurements were performed by operators who were experienced in both placental injection and fetoscopic evaluation of intertwin anastomoses. Another weakness of this study is that there is a selection bias in the placentas that were used, as only these of double survival or double demise were suitable for the study. However, since our focus is on the technical evaluation of placental sharing measurement, we do not believe this to be a major problem. We did not exclude the placentas of the 5 patients that underwent laser ablation of the vascular anastomoses for TTTS, as they were part of our initial prospective cohort and assessment of

placental sharing remains relevant even after laser therapy. However, since the surgeon drew a coagulation line across the 2 placental shares, it may be easier to delineate the shares on the digital photograph and thus improve the inter- and intra-observer variability of the photographic measurement. Also, the coagulation line separates the 2 vascular territories, hereby increasing the agreement between the digital photograph and X-ray angiography.

The main advantage of the X-ray method is that it truly delineates the venous territory of 1 twin, which may be especially helpful in the monochorionic placentas with few (15%) [6] or no (6%) [1] anastomoses. Furthermore, only one vein needs to be catheterized and injected, limiting the time needed for injection. This method can also be used if one cord insertion site is severely damaged while the other is intact, which would make full injection impossible. Because the contrast delineates the entire venous territory of one twin, one can just measure the white and the black area on X-ray angiogram and no experience to visualize the equator is needed to measure the placental shares. Therefore, the X-ray method we propose here is easier than the method described previously [1,2] to assess placental sharing, but it does not give any information on the number, type and size of the anastomoses. Access to an X-ray machine and radiopaque dye is also mandatory, although both are readily available in most settings.

The photograph method has the advantage that no X-ray technology is required. However, injecting all arteries and veins is time consuming and assessing the location of the equator may be difficult, so a more experienced operator is needed for both injecting the placenta and for assessing the degree of placental sharing. However, injection of all the vessels is the only method to document the angio-architecture and detect potentially missed anastomoses after laser ablation for TTTS. Ideally, a combination of both methods is used, where the angiogram accurately delineates the sharing and the placental surface photograph gives detailed information on the vascular anastomoses, but adding an X-ray angiogram is more time-consuming and costly.

In conclusion, delineation of the placental sharing on a photograph of the placental surface is an accurate method to determine placental sharing. However, the photograph slightly overestimates the discordance. Since the venous territory measured on X-ray angiogram physically determines where each twin gets its oxygenated blood, the X-ray method may be used as the gold standard to determine placental sharing, although the digital photograph constitutes a valid alternative and remains the gold standard to document the angio-architecture.

## Disclosure statement

The authors report no conflict of interest.

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