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A Better Way for Ductal Stenting in Patients with Duct-Dependent Pulmonary Flow and Vertical and Tortuous Patent Ductus Arteriosus

ORIGINAL INVESTIGATION

ABSTRACT

Background: In this study, we aimed to compare the femoral route and the carotid artery route in terms of procedural success of ductal stent implantation in patients with duct-dependent pulmonary blood flow.

Methods: The study included 51 patients with duct-dependent pulmonary circulation who underwent ductal stent implantation upon their admission to our clinic between July 2017 and March 2021. In total, 23 patients (group I) underwent ductal stent implantation via the femoral route, while the remaining 28 (group II) underwent the procedure via the carotid artery. The groups were compared in terms of procedural success, time, post-procedural blood pH, lactate levels, and complications.

Results: Duct morphology was observed in group 1 as follows: type 1 in 12 patients, type 3 in 8, type 2 in 2, and type 6 in 1 patient. In group 2, 26 patients had type 3, 1 had type 2, and 1 had type 6. The tortuosity index of the patients in group 1 was 1 in 8 patients, 2 in 8 patients, and 3 in 7 patients, while in group 2, it was 1 in 5 patients, 2 in 15 patients, and 3 in 8 patients. The success rate was 69.6% (16/26) in group I and 93.5% (29/31) in group II ($P = .030$). The cumulative success rate was 88.2% (45/51). The procedural durations were 78.2 ± 34.1 and 52.1 ± 22.0 minutes in group I and group II, respectively ($P = .002$). The mean blood pH values upon the completion of the procedure were 7.26 ± 0.1 and 7.33 ± 0.0 in group I and group II, respectively ($P = .038$). The mean post-procedural lactate levels were 2.8 mmol/L and 2.3 mmol/L in group I and group II, respectively ($P = .038$). The 2 groups did not show any differences in terms of procedural complications.

Conclusion: The carotid artery route can be preferred, especially in vertical and tortuous ductus arteriosus, as it is associated with a high success rate and a short procedural time, as well as a better metabolic condition after the procedure.

Keywords: Duct-depended pulmonary flow, ductal stent implantation, carotid artery access

INTRODUCTION

Cyanotic newborns whose pulmonary circulation is dependent on ductus arteriosus usually require intervention to secure pulmonary blood flow. The first-line treatment is prostaglandin E1 infusion in such cases.[1-3] However, in cases of pathologies where complete repair is not feasible in the neonatal period, 2 options exist: either create a systemic-to-pulmonary shunt to ensure safe pulmonary blood flow or perform ductal stent implantation (DSI) to keep the duct open.[4-6] As an exception, palliative balloon valvuloplasty can sometimes be useful in the presence of antegrade flow or perforation and subsequent dilatation of the atretic valve in a small number of cases with appropriate pulmonary valve structure could be performed. Very rarely, right ventricular outflow tract stents may also be implanted for palliation. Despite extensive and long-term experience with systemic pulmonary shunts, it still has significant mortality and morbidity.[7] The implantation of a stent into the arterial duct (AD) to provide patency of it which was introduced by Gibbs in 1992 as an alternative method was a brilliant idea, but the initial results were disappointing.[5] As low-profile materials emerged owing to technology advancements and the interventional experience with this

Bedri Aldudak[1]
Osman Akdeniz[2]
Onur Doyurgan[3]
Nilüfer Matur Okur[4]
Özlem Gül Sivaslı[1]
Fikret Salık[5]

[1]Department of Pediatric Cardiology, Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey
[2]Department of Pediatric Cardiology, Faculty of Medicine, Fırat University, Elazığ, Turkey
[3]Department of Pediatric Cardiovascular Surgery, Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey
[4]Department of Neonatology, Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey
[5]Department of Anesthesiology and Reanimation, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

Corresponding author:
Bedri Aldudak
✉ draldudak@hotmail.com

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method grew further, the practitioners achieved superior results over time.[8-10]

Despite the increasing experience in ductal stenting and the improved quality of the material used, the success rate of the procedure in patients with ductus-dependent pulmonary circulation varies between 82% and 95%.[6-8,11-13] Factors such as the exit point of the duct from the aorta, its curved or straight structure, the point where it enters the pulmonary artery, and whether it causes branch pulmonary artery stenosis affect the success of DSI. It is also necessary to devise various implantation strategies in which every single one of the existing characteristics is taken into account. Especially, in the vertical ductus, where the duct exits from the lower wall of the proximal aortic arch, the use of axillary or carotid arteries facilitates access to the AD and the passage of the guide wire and the balloon-stent complex through the AD. Apart from the increased success rate, accessing the AD through the axillary or carotid arteries shortens the procedural time, thereby reducing the radiation exposure of both patients and healthcare teams.[6] The aim of this study, which was carried out by retrospective evaluation of 51 patients who underwent DSI in our clinic, is to compare the effects of the use of carotid artery and femoral artery/vein access on the success of the procedure.

METHODS

Totally 51 patients with duct-dependent pulmonary circulation who needed palliation underwent DSI between July 2017 and March 2021. The pre-procedural, procedural, and post-procedural characteristics of these patients were evaluated retrospectively. Approval was obtained for the study from the Local Ethics Committee. For patients diagnosed with duct-dependent pulmonary circulation, the primary approach applied in our healthcare center is DSI. The exceptions for DSI were patients in whom there was an absolute contraindication for angiography (such as sepsis and severe bleeding diathesis), also where they willingly preferred surgery. Patients in whom additional surgical intervention was required due to the need for unifocalization, anomalies of pulmonary venous return, or septectomy were also exceptions. Patients having AD as a single source of pulmonary blood flow and/or those with branch pulmonary artery stenosis were not considered as a contraindication. Initially, in the first 8 patients, the femoral artery or vein route was used as the first choice in all ductal morphologies. When the femoral route approach failed, the team opted for a change of strategy upon successfully completing the procedure using the carotid route. For the patients in whom 2 different pediatric cardiologists evaluated the AD by detailed echocardiographic examination, the route to be used was decided based on their individual evaluations of the AD. When pulmonary valve perforation or pulmonary valvuloplasty was planned in addition to the DSI or where access to the AD was easily achieved via the antegrade pulmonary route, the femoral route was used. The femoral route was also used in straight and non-tortuous ductus arteriosus originating from the descending aorta. The femoral vein or arterial route was also preferred in patients with atypical AD originating from the subclavian artery. In cases with vertically located and/or tortuous AD, the carotid artery on the side, thought to be more suitable, was preferred. The patients were classified into 2 groups based on the DSI route preferred: Group I included 23 patients in whom the femoral vein or femoral artery route was used, while group II included 28 patients in whom the carotid artery route was used.

HIGHLIGHTS

- For a successful ductal stent implantation (DSI) procedure, the duct morphology should be well defined before the procedure.
- For DSI, the most suitable vascular approach for the ductal morphology should be preferred.
- In vertical ducts, carotid artery way is a method that increases the success rate.

Procedures

All procedures were performed under general anesthesia with endotracheal intubation directed by an anesthesiologist. All patients were completely monitored during catheterization (pulse oxymetric oxygen saturation, continuous electrocardiogram, and invasive arterial pressure) and also precautions were taken to prevent hypothermia by using a suitable heater for babies during the procedures. During all the procedures, the surgical team remained backup in case of emergency.

Femoral Route

The Seldinger technique was used to cannulate the femoral artery or femoral vein percutaneously. A 4 Fr radial sheath was placed in patients who did not require additional procedures. The femoral vein was used as the primary route of access in 2 patients with a primary diagnosis of pulmonary atresia with ventricular septal defect and 2 patients with a primary diagnosis of pulmonary atresia with intact ventricular septum who were scheduled for pulmonary valve perforation based on their echocardiographic evaluation. In 19 of the patients, the femoral arterial route was preferred. In the femoral artery approach, the first aortography was performed with retrograde catheterization of the descending aorta using pigtail catheter laterally at 90° and in left anterior oblique or cranial left anterior oblique views to show anatomic details of AD. Subsequently, the procedure was performed as explained in previous publications.[14,15]

Carotid Artery Route

A cardiovascular surgeon performed carotid artery cannulation by using the direct cut down from the side with the closest and the straightest angle to the ductus arteriosus. The Seldinger technique was employed to cannulate the carotid artery with a 22G needle, which was followed by the placement of a 4 Fr 7 cm radial sheath (Figures 1 and 2). To assess the morphology and sizes of the AD and the pulmonary artery, angiography was performed in lateral and left anterior oblique positions. In cases where the images were not helpful to have a definite conclusion, angiographic images of the right anterior oblique position were also obtained. In

Figure 1. Preparing and marking of 4 Fr radial sheet for carotid route.

Figure 2. Inserted and fixed 4 Fr radial sheet for carotid route.

most of the cases, imaging was performed by hand injection directly from the side arm of the sheath. Then, an open-end catheter was placed in AD ampulla, and the AD was passed through by using a 0.014-inch floppy coronary guide wire. A 0.014-inch extra support wire was also passed through the AD to the pulmonary artery both to straighten the AD (buddy wire) and to carry the stent. In the cases where the AD presented multiple turns and a complex morphology, re-imaging was performed following the extra support wire, and the measurements were repeated accordingly. Later, coronary bare-metal stents (Ephesos II, Alvimedica, Turkey) selected over the extra support wire in all cases were introduced into the duct through the short sheath without using a long sheath. The floppy wire was retrieved after ensuring that the stent had been advanced to the appropriate position in the AD. After confirming the location of the stent with manual contrast injections from the side arm of the short sheath, the stent implantation was performed by inflating the balloon with an indeflator. The balloon was carefully removed after implantation. Control injection was made to evaluate both the status of the stent and the flow, and when it was ensured that the position and flow are acceptable, the guidewire was carefully removed and the procedure was terminated. In the last 6 patients whose procedures were performed in the carotid artery, the procedure was performed using the flip technique described by Bauser-Heaton et al.[16]

To compare the advantages and disadvantages of each access route, the duration between the first angiographic imaging and the routine post-implantation angiography at the end of the procedure was accepted as the procedural time.

The procedure-related complications and the inotropes used were recorded in the catheter report. Arterial blood gas analysis was performed immediately after entering the vessel assigned for the procedure and just before exiting the vessel at the end of the procedure.

The stent diameters were preferred according to the body weight of the patients. The procedure was performed using coronary stents with a diameter of 3.5 mm in patients weighing 2.5-3.0 kg and stents with a diameter of 4-4.5 mm in patients weighing 3.0-5 kg. The lengths of the stents to be used were decided to be 2-3 mm longer than the measured ductal length so that both the aortic and pulmonary sides of the AD were covered.

After entering the vein, 50 U/kg heparin was administered, and the heparin infusion was continued at the rate of 20 U/kg/h on the first day and 10 U/kg/h on the second day for patients in whom the procedure was completed successfully. Additionally, acetylsalicylic acid at a dose of 3-5 mg/kg was started on the first post-procedural day.

During the first follow-up examination after their discharge, patients in whom the stenting was performed via the carotid artery were examined for stenosis and obstruction of the carotid artery using Doppler ultrasonography. For those in whom the procedure was performed via the femoral route, manual pulse control was performed.

Study Variables

After dividing the patients into 2 groups according to the method used in DS, group I (femoral artery/femoral vein route) and group II (carotid artery route) were compared retrospectively. The data used for comparing the 2 groups included age, body weight, sex, cardiac pathology, and procedural time. Furthermore, pH and lactate levels measured in the arterial blood gas analyses following the procedure, inotropic use during and after the procedure, success rate of the procedure, and the complications encountered were included.

The angiographies of all patients were reviewed retrospectively, and the ADs were divided into 6 types, as defined by Roggen et al[12], based on the aorta origin and pulmonary artery insertion. According to Qureshi et al[17] the classifications of the ADs are type I (straight), type II (single turn), and type III (multiple turns) based on the tortuosity index.[17]

Statistical Analysis

Statistical Package for the Social Sciences (version 24, IBM Corp., Armonk, NY, USA) software was used for all the statistical analyses. The numerical variables were presented as mean ± standard deviation (SD), while the categorical variables were expressed as frequencies and percentages. The Kolmogorov–Smirnov test was performed to determine whether the numerical data were normally distributed. Depending on the type of variable, unpaired *t*-test, Mann–Whitney *U* test, chi-square test, or Fisher's exact test were used for the variables related to the vascular route employed in DS. A *P* value of <.05 was considered statistically significant.

RESULTS

In total, 51 patients who were scheduled for the DSI procedure were examined. The catheterization was performed in the carotid artery in 28 patients and 23 patients were catheterized via the femoral artery or femoral vein. In 3 patients where the femoral route failed, the route was changed to the carotid cutdown and the procedure was successfully completed (Figure 3). The success rate of the procedure was 69.6% (16/23) in group I, where the DSI was performed via the femoral vein route, and 93.5% (28+3=29/31) in group II, where it was performed via the carotid route (*P*=.030). The cumulative success rate was 88.2% (45/51).

The demographic and clinical characteristics of the patients are listed in Table 1. There was no statistically significant difference between the groups in terms of their demographic characteristics. The intervention-related characteristics are presented in Table 2.

Success rate in group II was statistically significantly higher than that of group I, whereas the mean procedural time in group II was lower than that in group I (the *P* values were .030 and .002, respectively). Withal, the pH values at the end of the procedure were found to be significantly higher, and the post-procedural lactate levels were found to be significantly lower in group II (*P* values.038 and .038, respectively). There was no procedure-related mortality. The acute

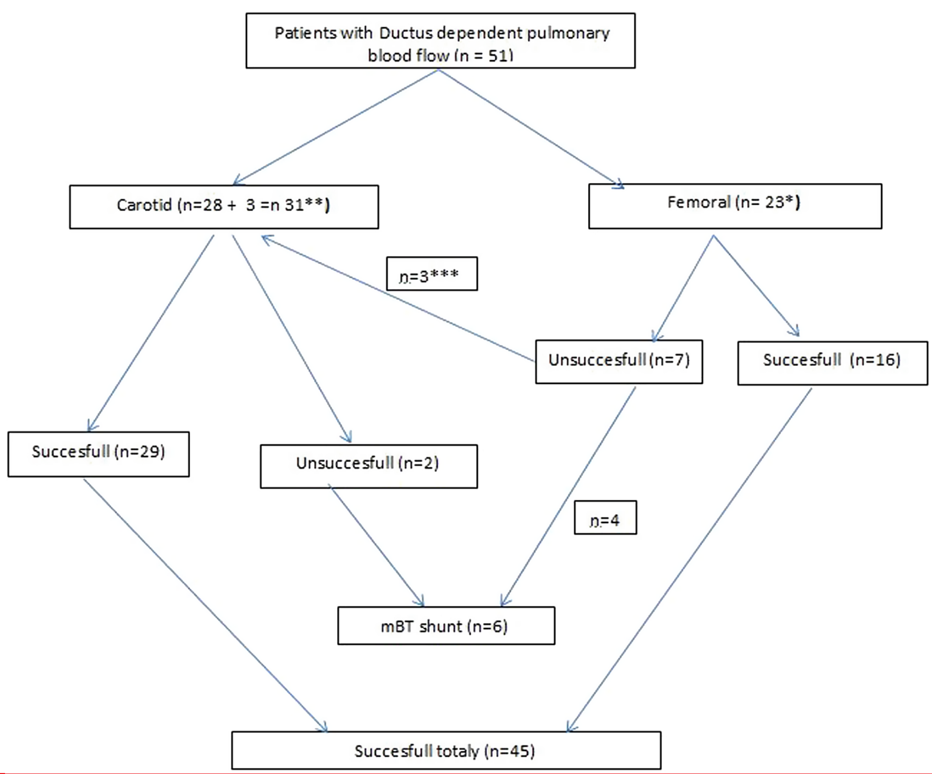

Figure 3. Patient flow diagram. *Ductus arteriosus types I-II=13 and type III=10. **Ductus arteriosus type I=2, type III=28, and type VI=1. ***Ductal stent implantation was performed via the carotid artery as the second approach in 3 patients failed with the femoral route.

Table 1. Demographic Data and Anatomical Diagnosis

Variable	Femoral Artery/Vein (n = 23)	R/L Carotid Artery (n = 28)	*P**
Age (day)	21.4 ± 22.3	17.5 ± 19.5	.510
Weight (g)	3350 ± 584	3234 ± 448	.426
Sex (M/F)	13/10	18/10	.572
Physiology UV/BV	13/10	13/15	.473
Diagnosis			.214
PA + VSD	7	11	
PA+ IVS	6	2	
d-TGA + VSD + PS	4	3	
AVSD + PA	2	6	
Others	4	6	
Ductal anatomy			<.001
I, n, (success rate)	12 (62.5%)	0	
II, n, (success rate)	2 (50%)	1 (100%)	
III, n, (success rate)	8 (50%)	26 (92.3%)	
IV, n, (success rate)	0	0	
V, n, (success rate)	0	0	
VI, n, (success rate)	1 (100%)	1 (100%)	
Tortuosity index			.298
I, n	8	5	
II, n	8	15	
III, n	7	8	

*Statistically significant.
AVSD, atrioventricular septal defect; BV, biventricle; dTGA, d-transposition of the great arteries; IVS, intact ventricular septum; PA, pulmonary atresia; PS, pulmonary stenosis; UV, univentricle; VSD, ventricular septal defect.

Table 2. Comparison of the Findings of the 2 Groups

Variable	Femoral Artery/ Vein (n = 23)	R/L Carotid Artery (n = 28)	*P**
Stent diameter (mm)	3.8 ± 0.3	3.7 ± 0.3	.247
Stent length (mm)	15.2 ± 3.7	15.6 ± 2.5	.712
Intervention time (minutes)	78.2 ± 34.0	52.1 ± 22	**.002***
pH	7.26 ± 0.1	7.33 ± 0.0	**.038***
Lactate	2.8 ± 0.0	2.3 ± 0.6	**.038***
Inotrop use, n	10 (43.5)	3 (10.7)	**.025***
Success, n (%)	16/23 (69.6)	26/28 (92.9)	**.030***
Stay (day)	11.3 ± 9.3	12.7 ± 13	.666
Follow-up (months)	18.5 ± 14.6	12.0 ± 10.7	.074
Complications			.553
Stent migration	1	2	
Acute stent thrombosis	1	1	
Hemothorax	0	1	

*Statistically significant.
Bold values are show *P* < .05 and statistically significancy.

complications associated with the procedure were stent migration in 1 patient and acute stent thrombosis in 1 patient in group I. On the other hand, the acute complications related to the procedure in group II were stent migration in 2 patients, acute stent thrombosis in 1 patient, and ipsilateral hemothorax in 1 patient (Table 2). In group II, on the follow-up ultrasonography, 1 patient was found to develop mild stenosis of the procedure-related carotid artery, which was not clinically evident. This patient did not have any other vascular complications or symptomatology.

During the follow-up period, 13 patients underwent biventricular repair, 16 patients underwent univentricular palliation, and 18 patients were waiting for surgical intervention. Four patients died due to non-procedural causes within 2-48 days after the procedure.

DISCUSSION

Surgical palliation was performed on these patients until the 1990s, since complete surgical correction in the neonatal period is usually difficult and carries high risk in ductus-dependent pathologies. The first percutaneous DSI performed by Gibbs in 1992 introduced a less-invasive method in this area. Over the last 30 years, as the technology advanced, the quality of the material used has improved and low diameter but more expandable sheaths, low-profile stents of appropriate diameters and lengths, and more qualified guide wires have been developed.[5-10] The results of DSI have been improved as a result of these technological advancements and increased experience.[7-10] There has been a recent study reporting a success rate of 93% in DSI.[18] In addition to the experience of the operating center and the operator, ductal origin and morphology are the most important factors affecting the success of this procedure. The most appropriate access route to the origin of the duct and its anatomy is known to affect the success rate of DSI.[12-17] Our study revealed that high rate of DSI success was achieved through the carotid artery route, even if ducts were vertical and tortuous (31/29; 93.5%).

It is known that in severe obstructive lesions of the right side, the AD morphology is complicated and varies in terms of origin, length, and tortuosity. In recent years, there have been various studies to develop a morphological classification for the AD in patients scheduled for DSI[12,13,16,17] Roggen et al[12] presented a morphological classification of the ductus arteriosus according to its origin from the aorta and its attachment to the pulmonary artery in patients with ductus-dependent pulmonary circulation, and 6 different types were defined.[12] Qureshi et al[17] introduced a tortuosity index based on the turns in ductus arteriosus, and the AD was classified into type I: straight, type II: single turn, and type III: multiple turns.

Many studies have reported low success rates for DSI via the classical femoral route in vertical ducts with a high tortuosity index.[12,15] It is recommended to perform the stenting procedure via the carotid artery or axillary artery route in such cases.[15] These alternative routes allow the practitioner to directly visualize the ductus arteriosus and to complete the procedure with fewer complications.[19] When the femoral artery route was used for DSI, long sheaths were found to result in femoral artery occlusion at a significant level.[19] The carotid and axillary arteries have larger diameters, and their short distance to AD does not require the use of a long sheath. Therefore, the vascular damage is reduced with the carotid and axillary route in this group of patients. Although certain studies have warned that the use of the carotid and axillary arteries for catheterization in newborns may lead to neurological damage, ischemia, and bleeding complications, there are other studies claiming that it is safer than the femoral artery.[16,20-22] While performing DSI via the axillary artery, it is recommended to be careful about the partial dissection that might potentially be caused by the existing anatomical angulation during the advancement of the sheath through the ipsilateral subclavian artery junction.[21] In the carotid artery route, the carotid cutdown technique has not been reported to be superior to the percutaneous direct puncture.[22] However, we preferred the former since we considered it to be a safer option because initially, we were not experienced in this regard. As we became more experienced with ultrasonography, in the last 6 of the cases, we performed the procedure via ultrasonography-guided percutaneous sheath placement. In these last 6 cases, no complications related to the access route were encountered.

The carotid artery way has some disadvantages, including the fact that its use is an unusual catheterization method in pediatric cardiology, as well as the differences in how the operator is positioned with regard to the patient and the operating table during the procedure. However, with the flip technique described by Bauser-Heaton et al,[16] it is possible to overcome this disadvantage. We preferred the carotid route for the balloon procedures due to critical aortic stenosis and palliation, requiring aortic coarctation in the neonatal cases treated at our clinic. We opted for this technique in the last 6 cases of DSI because the flip technique used during these procedures is more comfortable. We believe that this technique facilitates manipulation.

Our primary approach for patients diagnosed with duct-dependent pulmonary circulation was DSI during the study period, and it remains to be the same in our clinic. In the beginning, our preference was the femoral artery or vein route, regardless of the ductal morphology, because we were more acquainted with it and had not experienced it at the onset of the study (in the first 8 cases). Based on our own experiences and the literature data, it is evident that the success rate of implantation via the femoral route alone is low (69.6%) and the procedure time is longer. Since lactic acidosis was also frequently detected in the arterial blood gases measured at the end of the procedure in our first cases, we changed the strategy as stated in the method. When the femoral route was not successful in the eighth case, the attempt of the carotid route and its success was another reason that motivated us to change the strategy. In this new strategy, the patients underwent echocardiography in which a thorough examination of the ductal anatomy was performed to decide the route to be used. In patients with vertically located and/or tortuous AD, the carotid artery on the side, thought to be more suitable, was preferred. When we evaluated our choices retrospectively, it was found that we preferred the femoral artery or vein route in patients with types I, II, and VI ductal anatomy accompanied by the right aortic arch, subsequent to this strategy change. The carotid artery route was chosen in the AD of types III, IV, and V morphology. The right or left carotid artery was preferred according to the duct location. Types IV and V ducts were not encountered in our patient group. This strategy change was found to offer key advantages such as high rate of success, low procedural time, and more physiological metabolic status (low lactate, normal pH) at the end of the procedure, especially in type III ducts with a tortuosity index of 2-3. While the procedure was successful only in 4 (50%) of the 8 vertical ducts in the femoral group, it failed only in 2 (6.5%) of the patients in the carotid group, which included the 3 patients transferred from the first group (28 + 3 = 31). In 1 of the 2 patients with a failed procedure, it was terminated due to deterioration of the general condition because of recurrent ductal spasms after the AD was passed with the guide. In the remaining one patient, the AD could not be passed in due to severe tortuosity.

Our patient group is numerically similar to the series in the study where Alwi et al[6] used the antegrade or retrograde femoral route in all cases and reported an implantation rate of 91% in the study population that also included patients with a ductal morphology of type III. With a similar number of cases included in the present study, we achieved a success rate of 69.6% with the femoral route and 93.5% with the carotid artery route, as well as a mean success rate of 88.2%. The remarkably high rate of implantation success achieved by Alwi et al[6] when using the femoral route can be attributed to the operator being among the pioneers of this procedure and having a rich experience. However, our procedural time (group I: 78.2 minutes and group II: 52.1 minutes) was significantly shorter than the 95.7-minute procedural time of Alwi et al.[6] In suitable cases, despite having less experience, the carotid route facilitates higher success rates in implantation and shorter time for the completion of the procedure.

Roggen et al[12] achieved a mean success rate of 84% in their study population that included 98 patients of different ductal morphologies. In a study where the carotid and axillary artery route was used in 8 patients, they showed that success was significantly affected by the type of the AD.[12] In their study, while the success was 100% in type I, it was 64% in type III. The study by Alsawah et al[22] is quite similar to our study, except for the carotid cannulation that had been performed percutaneously. In their study, which divided 40 patients into 2 groups of 20 each, the carotid artery approach and the femoral artery approach were compared. The cumulative success rate was found to be 85%, and the procedural time and the fluoroscopy time were found to be significantly shorter in the carotid artery group than in the femoral artery group. Based on the comparison of the early and late vascular complications, the carotid artery was found to be superior.[22] The procedural time and success in our study were comparable to those in this study (Table 2).

In addition to vital signs monitoring during the procedure, hemodynamic effects of the procedure that were not presented as clinical abnormalities were evaluated in our patients by measuring pH and lactate level in arterial blood gas at the end of the procedure. It is known that lactate and metabolic acidosis are important parameters that might indicate circulatory failure and tissue hypoxia.[23,24] Our study is the first one to examine the pH and lactate levels while examining patients for their hemodynamics. As understood from the results in Table 2, the pH and lactate values in the carotid group were close to the normal physiological values and the difference between the 2 groups was statistically significant (lactate: 2.04 vs. 2.13 mmol/L, $P = .007$, pH: 7.33 vs. 7.26, $P = .019$).

Ductal stent implantation is not currently accepted as a class I indication in the widely used guidelines. However, it is likely to become more acceptable and be recommended in near future based on the results of our study and those from the above-mentioned studies, as well as comparative studies with the Blalock–Taussig shunt.[7,8,25] The successful results reported for the use of the carotid artery or the axillary route for vertical and long AD with multiple turns where DS is risky and not recommended will help reverse the claim that the procedure is contraindicated in these ducts. However, still further comparative and prospective studies should be conducted with a larger study population.

Study Limitations

The most important limitation of the study is that it was conducted retrospectively with a small number of patients in a single center that is still in the learning phase. Also, long-term data would be required to assess other long-term complications like carotid artery stenosis.

CONCLUSION

In patients with ductus-dependent pulmonary circulation, the success rate of DSI is strongly affected by the duct morphology. For a successful DSI procedure, the duct morphology should be well-defined before the procedure and the vascular access that provides the easiest access to the duct should be selected. Carotid artery way, especially in vertical (type III) ducts, will increase the success of the procedure, as well as ensure the completion of the procedure in a shorter time and better post-procedural metabolic condition. The carotid route can be used either by surgical cutdown or ultra sonographic-guided puncture depending on the experience of the clinic.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Gazi Yaşargil Research and Education Hospital (approval no: 07.09.2021-859).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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