Brain Magnetic Resonance Immediately Before Surgery in Single Ventricles and Surgical Postponement

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Background. **Single-ventricle patients undergoing surgical reconstruction experience a high rate of brain injury. Incidental findings on preoperative brain scans may result in safety considerations involving hemorrhage extension during cardiopulmonary bypass that result in surgical postponement.**

Methods. **Single-ventricle patients were studied with brain scans immediately preoperatively, as part of a National Institutes of Health study, and were reviewed by neuroradiology immediately before cardiopulmonary bypass.**

Results. **Of 144 consecutive patients recruited into the project, 33 were studied before stage I (3.7 ± 1.8 days), 34 before bidirectional Glenn (5.8 ± 0.5 months), and 67 before Fontan (3.3 ± 1.1 years) operations. Six operations (4.5%), 2 before stage I, 3 before bidirectional Glenn, and 1 before Fontan, were postponed because of concerning findings on brain magnetic resonance imaging. Five were due to unexpected incidental findings of acute intracranial hemorrhage, and 1 was due to diffuse cerebellar cytotoxic edema; none who proceeded to operation had these lesions. Prematurity and genetic syndromes were not present in any patients with a postponed operation. Four of 4 before bidirectional Glenn/Fontan with surgical delays had hypoplastic left heart syndrome compared with 44 of 97 who did not ($p = 0.048$). After observation and follow-up, all eventually had successful operations with bypass.**

Conclusions. **Preoperative brain magnetic resonance imaging performed in children with single ventricles disclosed injuries in 4.5% leading to surgical delay; hemorrhagic lesions were most common and raised concerns for extension during the operation. The true risk of progression and need for delay of the operation due to heparinization associated with these lesions remains uncertain.**

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Patients with a single ventricle who undergo staged surgical reconstruction culminating in the Fontan procedure face a high risk of death and may sustain adverse outcomes such as cardiac transplantation [1], decreased ventricular performance [2], poor somatic growth [3], and development of pulmonary arterial venous malformation [4]. There exist well-known concerns for poor neurologic outcome in patients with a single ventricle due to brain injury and delayed maturation [5–9]. This has presumably led to the findings of poor neurodevelopmental outcome in this patient population [10–13].

In the course of discovering the causes of the neurodevelopmental challenges seen in this population, incidental findings on research brain magnetic resonance imaging (MRI), such as structural malformations or tumors, are discovered that require further workup, new medical therapies, or modifications of clinical care. Here we report a secondary analysis on the timing of operations in patients who participated in a research project sponsored by the National Institutes of Health that demonstrated brain lesions on immediate preoperative brain MRIs with the potential to be adversely affected by anticoagulation required for cardiopulmonary bypass. A neuroradiologist, neurologist, cardiologist, and a surgeon reviewed the MRI findings while the patient was still in

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the scanner, and a decision was made whether to proceed to the operation or postpone the operative date. Whether these previously occult findings contribute to poor outcomes in patients who do not receive preoperative brain MRIs or surgical postponement prevents harm is not known [14].

Material and Methods

Patients

This was a single-center, retrospective secondary analysis of a prospective study of cerebral blood flow in single-ventricle patients throughout staged surgical reconstruction. All patients were enrolled in one of two National Institutes of Health protocols, and all were enrolled from April 2009 to March 2012. The inclusion criteria included any patient aged younger than 10 years with single-ventricle physiology undergoing an operation at Children's Hospital of Philadelphia. The patient needed to be stable enough to undergo an MRI scan lasting approximately 1-hour under general anesthesia. Exclusion criteria included any contraindication to MRI. Demographics obtained included age, body surface area, gestational age, genetic disorders, diagnosis, and stage of surgical reconstruction. Informed consent for participation in the larger study was obtained from all participants' families. The hospital's Institutional Review Board has approved both prospective studies.

Study Procedure

Patients underwent MRI immediately before the heart operation. The patient was prepared in the operating room with intravenous and arterial catheter placement. All participants were administered general anesthesia that varied according to age (eg, those who were at stage I or at the bidirectional Glenn/hemi-Fontan stage received nitrous oxide and sevoflurane of 1 minimal alveolar concentration or less), were paralyzed, endotracheally intubated, and mechanically ventilated using minute ventilation to achieve a partial pressure of carbon dioxide of 40 $\pm$ 2 mm Hg.

The patient was then transported by stretcher to the adjacent scanner, which was an Avanto 1.5-Tesla whole-body MRI system (Siemens Medical Solutions, Malvern, PA). The patient was placed supine, head first into the scanner using the 6-channel head coil and 8-channel body array coil. All imaging was performed at isocenter. Studies lasted approximately 1 hour; afterward, the patient was immediately removed from the scan room and transported to the operative suite where procedure was performed or to the hospital floor. On completion of the MRI, a staff neuroradiologist read the study, and a determination was made with the neurologist, cardiologist, and surgeon to proceed to the operation or to wake the child.

MRI Protocol

A stack of static steady state–free precession images were obtained from the diaphragm to the neck to assess cardiovascular anatomy. Gradient localizers were used to locate the brain and were used as a basis to perform the following anatomic brain imaging:

1. Three-dimensional (3D) volumetric T1-weighted magnetization prepared rapid acquisition gradient echo with repetition time (TR), 1,980 ms; echo time (TE), 2.65 ms; inversion time (TI), 1,100 ms; flip angle, 15 degrees; slice thickness, 1.5 mm; and matrix, 256 $\times$ 256.
2. 3D volumetric T2-weighted sampling perfection with application optimized contrasts using different flip angle evolution: TR, 3,200 ms; TE, 453 ms; slice thickness, 2 mm; and matrix, 256 $\times$ 254.
3. 3D susceptibility-weighted imaging: TR, 49 ms; TE, 40 ms; slice thickness, 2 mm; and matrix, 256 $\times$ 177.
4. Diffusion-weighted imaging: TR, 2,903 ms; TE, 86 ms, slice thickness, 4 mm; three b values of 0, 500, and 100 mm/s^2; and matrix, 128 $\times$ 128.
5. 2D T2-weighted coronal imaging: TR, 6,000 ms; TE, 112 ms; slice thickness, 4 mm; no gap; matrix, 448 $\times$ 336.

Imaging Review

A pediatric neuroradiologist immediately reviewed all images in their native form and in the multiplanar reformat while the patient was in the scanner. Any concerning abnormalities or injuries identified were brought to attention and the decision to proceed or delay the operation was made after discussions among the neuroradiologist (A.V.), the pediatric neurologist (D.J.L.), the cardiologist (M.A.F.), and cardiothoracic surgeon (T.L.S., J.W.G., S.F.).

No well-established protocols or previous studies are available to delineate the exact contraindications to an operation in this setting, but the abnormalities of concern were those with features that would increase the risk of intracranial hemorrhage as a result of anticoagulation in other relevant scenarios [15–20]. These included acute brain parenchymal hemorrhage, acute subarachnoid hemorrhage, acute or sizeable intraventricular hemorrhage beyond simple grade I intraventricular hemorrhage, acute subdural hemorrhage beyond the neonatal period, and large territories of acute infarct or cytotoxic edema (restricted diffusion on diffusion-weighted MRI).

The presence of small amounts of posterior convexity, tentorial, and posterior fossa subdural blood in patients undergoing operations in the first week of life was not considered a contraindication. These findings are very common in the perinatal period subsequent to normal vaginal delivery as a result of the birthing process and have been reported in up to 62% of patients before neonatal heart operations [21, 22].

Determination of recent hemorrhage was based on a combination of hyperintensity on T1-weighted images, hypointensity on T2-weighted images, and susceptibility blooming on susceptibility-weighted images. Tiny punctate foci of susceptibility without T1-weighted or T2-weighted signal abnormalities were not considered a contraindication.

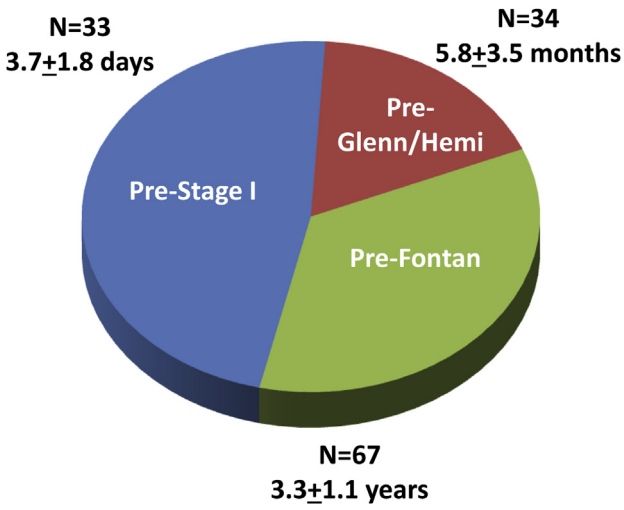

Fig 1. Pie chart shows distribution of patients at the various stages of surgical reconstruction (blue = pre-stage I; red = pre-Glenn/hemi-Fontan; green = pre-Fontan). Ages are at the time of magnetic resonance imaging.

Statistics

Descriptive statistics were used and recorded as mean ± standard deviation. A Fisher exact test was used to assess categoric variables. A p value of less than 0.05 was considered significant.

Results

Study Population

The study population comprised 134 single-ventricle patients. During the study period, the entire cardiac center performed 92 stage I, 76 bidirectional Glenn/hemi-Fontan, and 154 Fontan operations. The recruitment rate was 68% for the stage I patients and 48% for the bidirectional Glenn/hemi-Fontan and Fontan patients. Reasons for not being able to approach all patients included exclusion criteria, insufficient manpower, contraindication to MRI, and scanner availability. Figure 1 graphically demonstrates the distribution of the patients by surgical stage and by age.

Surgical Postponement

Tables 1 and 2 list data for the 6 patients (4.5%) whose operations were delayed due to brain injury demonstrated on MRI. Of these, 2 patients were delayed before stage I (1% of the total, 6% of patients before stage I), 3 before bidirectional Glenn or hemi-Fontan (2% of the total, 9% of stage I patients), and 1 before Fontan (1% of the total, 1% of bidirectional Glenn/hemi-Fontan patients). The age at the time of MRI was typical for the respective operative stage (Table 1). Operations for those patients before stage I was delayed 1 week compared with a delay of 7 to 105 days for those before bidirectional Glenn/hemi-Fontan and Fontan.

All patients whose surgical date was delayed eventually underwent successful operations with bypass and without neurologic injury. Demographic and surgical data for all patients are listed in Table 2. Because of the small numbers, a comparison of cardiopulmonary bypass, circulatory arrest, and cross-clamp times among groups precluded a definitive analysis.

MRI findings incurring a delay in surgical intervention were unexpected intracranial hemorrhage in 5 patients. These included acute temporal lobe parenchymal and

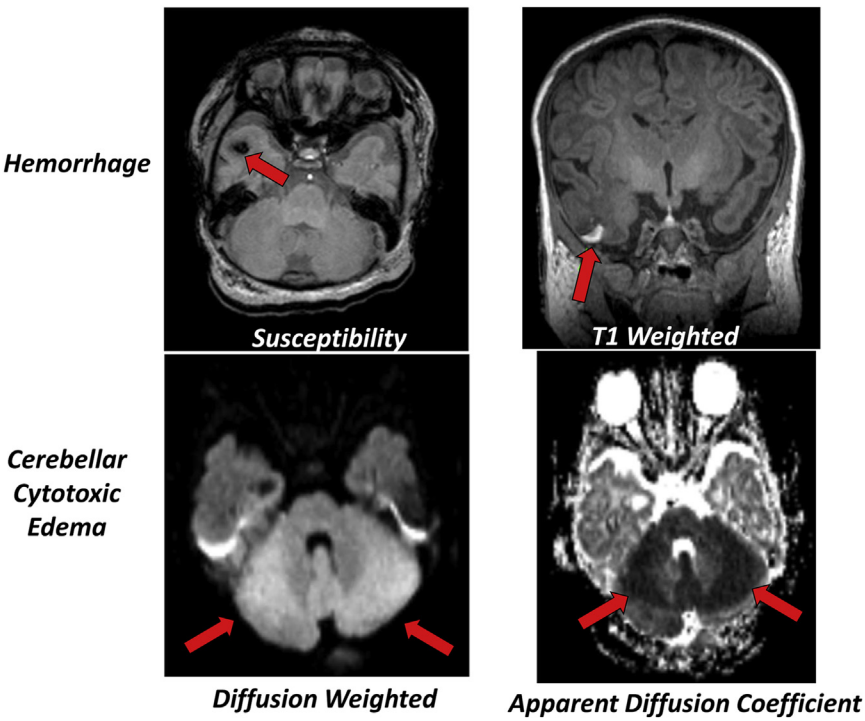

Fig 2. Examples are shown of two brain lesions that led to the decision to postpone the operation. The top two images are an example of hemorrhage from a patient with hypoplastic left heart syndrome: (Left) an axial view that used susceptibility imaging, and (Right) a coronal view that used T1-weighted imaging (red arrows point to the hemorrhage). The bottom 2 axial images are examples from a patient with cerebellar cytotoxic edema using (Left) diffusion-weighted and (Right) apparent diffusion coefficient imaging (red arrows point to the lesion).

Table 1. Patients Who Experienced Surgical Postponement Because of Brain Magnetic Resonance Imaging Findings

Patient	Age at Operation	Delay	Time From Last Operation	Gestational Age
Before stage I			(y)	(wk)
1	10 d	7 d	…	40
2	12 d	7 d	…	39
Pre-BDG/HF				
3	5.6 mo	1.6 mo	0.59	37
4	4.6 mo	0.73 mo	0.44	39
5	4.2 mo	0.23 mo	0.37	39
Pre-Fontan				
6	2.8 y	3.5 mo	2.66	39

BDG = bidirectional Glenn; HF = hemi-Fontan.

subpial hemorrhage in 1, acute subarachnoid hemorrhage in 1, sizeable acute intraventricular and choroid plexus hemorrhage in 1, and nonneonatal acute subdural hemorrhage in 2. Two patients also had small foci of cerebellar hemorrhage (some patients had more than one focus of bleeding). The other patient had an unusual diffuse cerebellar cytotoxic edema and restricted diffusion involving nearly the entire bilateral cerebellar hemispheres. No patients with surgical delay were receiving anticoagulation preoperatively. In addition, none of the patients without surgical delay had any of the lesions described above, and all patients with the lesions described above incurred a surgical delay. Figure 2 demonstrates representative MRI findings in 2 of these patients.

Follow-up imaging after the initial MRI in the 5 patients with intracranial hemorrhage showed a decrease or resolution of the initially detected lesions. The follow-up MRI of the patient with cytotoxic cerebellar edema demonstrated resolution.

No patients whose surgical date was postponed had a genetic syndrome or a premature birth. A contingency table was used to determine whether hypoplastic left heart syndrome was a risk factor for surgical postponement in those who were in the stage I or bidirectional Glenn/hemi-Fontan stage (Table 3). The 4 patients

Table 2. Demographics and Surgical Variables of Patients Who Experienced Surgical Postponement and Those Who Did Not

	Pre-Stage I		Pre-BDG or HF		Pre-Fontan	
Variables[a]	Delay	No Delay[b]	Delay	No Delay[b]	Delay	No Delay[b]
Sex						
Male	0	13	2	20	0	40
Female	2	18	1	11	1	26
Birth weight, kg	4.1 ± 0.1	3.1 ± 0.52	3.4 ± 0.4	3.1 ± 0.7	3.4	3.2 ± 0.7
Estimated gestational age, wk	39.6 ± 0.6	38.86 ± 0.85	38.5 ± 1.4	38.56 ± 1.3	39.1	38.3 ± 2.7
Weight at MRI, kg	4.1 ± 0.1	3.1 ± 0.52	5.8 ± 0.8	6.2 ± 1.2	10.9	14.0 ± 2.3
Age at MRI	4 ± 1.41 d	3.58 ± 1.74 d	0.40 ± 0.06 y	0.50 ± 0.30 y	2.84 y	3.33 ± 1.07 y
Delay of operation	7 d	N/A	0.86 ± 0.69 mo	N/A	3.50 mo	N/A
Stage I						
CPB time, min	…	…	121.7 ± 35.57	87.41 ± 28.50	76.00	84.89 ± 34.65
Circulatory arrest time, min	…	…	56.33 ± 17.21	41.95 ± 19.99	22.00	37.65 ± 21.18
Cross-clamp time, min	…	…	56.33 ± 17.21	39.91 ± 21.49	33.00	41.29 ± 20.22
BDG/Hemi						
CPB time, min	…	…	…	…	74.00	59.60 ± 20.37
Circulatory arrest time, min	…	…	…	…	27.00	15.43 ± 14.31
Cross-clamp time, min	…	…	…	…	29.00	20.40 ± 15.01
HLHS	1	30	3	19	1	25
Other single RV	1	1	…	9	…	32
Single LV	0	0	…	3	…	9

[a] Continuous data are shown as mean ± standard deviation and categoric data as the number. [b] None had the hemorrhagic lesions or cerebellar cytotoxic edema described in the patients who incurred a surgical postponement.

BDG = bidirectional Glenn; CPB = cardiopulmonary bypass; HF = hemi-Fontan; HLHS = hypoplastic left heart syndrome; LV = left ventricle; MRI = magnetic resonance imaging; N/A = not applicable; RV = right ventricle.

Table 3. Hypoplastic Left Heart Syndrome and Surgical Delay

Pre-BDG/HF or Pre-Fontan	Surgical Delay (No.)	No Surgical Delay (No.)	Total (No.)
HLHS	4	44	48
Non-HLHS	0	53	53
Total	4	97	101

BDG = bidirectional Glenn; HF = hemi-Fontan; HLHS = hypoplastic left heart syndrome.

with surgical delays in this subgroup had hypoplastic left heart syndrome (100%) compared with 44 of 97 (45%) with hypoplastic left heart syndrome without surgical postponement ($p = 0.048$).

Comment

This study was a secondary analysis reviewing our experience from two prospective studies of cerebral blood flow that involved preoperative brain MRI immediately before the operation in patients with single ventricle throughout staged reconstruction. This is one of the first studies to address incidental brain findings and the timing of the operation. Overall, 4.5% of single-ventricle procedures were rescheduled to a later date because of unexpected intracranial hemorrhage or diffuse cerebellar cytotoxic edema with a significant percentage before the bidirectional Glenn/hemi-Fontan stage. Prematurity and genetic syndromes were not present in patients whose operations were delayed; however, hypoplastic left heart syndrome at stage I and the bidirectional Glenn/hemi-Fontan stages were risk factors for surgical postponement.

Hemorrhagic brain lesions, usually local, are known to appear in a significant proportion of neonates and infants with congenital heart disease undergoing MRI before [23] and after operations [24]. One risk factor is preexisting lesions, and there may be good reason to be cautious in deciding to initiate cardiothoracic operations in patients with preexisting hemorrhagic lesions: cardiopulmonary bypass and hypothermic circulatory arrest could increase the cerebral damage by heparinization, causing expansion and progression of cerebral hemorrhage [25].

A number of studies have demonstrated the development of new lesions postoperatively associated with various risk factors, a few of which were preexisting hemorrhagic lesions [7, 24]. Tavani and colleagues [7] used MRI to study full-term newborns with congenital heart disease before and after operations; of patients with intracranial hemorrhage, 43% worsened after the operation. Dent and colleagues' investigation [24] concentrated on patients with hypoplastic left heart syndrome who underwent the Norwood procedure and found 53% with new or worsened focal ischemic or hemorrhagic brain lesions by comparing preoperative and postoperative brain MRIs.

There are a few dissenting investigations, one of which was published by Block and colleagues [14], who studied patients with transposition of the great arteries and single-ventricle physiology undergoing neonatal operations. This group did not find that preoperative injury worsened postoperatively. In addition, the relative risk of new postoperative lesions for patients with preoperative brain lesions compared with those without was not significant. That study, however, combined small and large brain lesions into one group, their statistical analysis combined patients with transposition of the great arteries and single-ventricle, and combined all brain lesions into one group (all white matter injury and stroke for example), making their study inapplicable to the neonatal population presented in the current study. Beca and colleagues [26] recently studied 153 infants before and after operations for varied congenital heart lesions and also did not find that cardiopulmonary bypass was a risk factor for extension of preexisting hemorrhage or stroke.

This study has some limitations. Our study cannot make a recommendation on the routine use of preoperative brain MRI throughout staged surgical reconstruction because of a lack of knowledge on whether these previously occult findings are associated with a risk of extension and further injury during the operation, contribute to poor outcomes in patients who do not receive preoperative brain MRIs, or whether surgical postponement prevents harm. Although this study suggests that the strategy of delaying the operation after imaging the brain by MRI preoperatively may be useful and have clinical implications, further investigation is needed to be able to make a definitive statement.

In conclusion, preoperative brain magnetic resonance imaging in children with single-ventricle congenital heart disease throughout surgical reconstruction disclosed incidental findings that generated clinical concern (abnormalities that would increase the risk of intracranial hemorrhage as a result of anticoagulation) leading to surgical postponement in 4.5%; hypoplastic left heart syndrome was a risk factor for this delay. The true risk of progression and need for delay of the operation associated with these lesions remains uncertain. Additional studies are needed to determine which patients are risk and therefore should undergo preoperative MRI.

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References

1. Ohye RG, Sleeper LA, Mahony L, et al. Comparison of shunt types in the Norwood procedure for single-ventricle lesions. N Engl J Med 2010;362:1980–92.
2. Fogel M, Weinberg PM, Chin AJ, Fellows KE, Hoffman EA. Late ventricular geometry and performance changes of functional single ventricle throughout staged Fontan reconstruction assessed by magnetic resonance imaging. J Am Coll Cardiol 1996;28:212–21.
3. Cohen MI, Bush DM, Ferry RJ Jr, et al. Somatic growth failure after the Fontan operation. Cardiol Young 2000;10:447–57.

4. Sundareswaran K, de Zélicourt D, Sharma S, et al. Correction of pulmonary arteriovenous malformation using image based surgical planning. JACC Imaging 2009;2:1024–30.
5. Mahle WT, Tavani F, Zimmerman RA, et al. An MRI study of neurological injury before and after congenital heart surgery. Circulation 2002;106(Suppl I):I109–14.
6. Licht DJ, Shera DM, Clancy RR, et al. Brain maturation is delayed in infants with complex congenital heart defects. J Thorac Cardiovasc Surg 2009;137:529–36.
7. Tavani F, Zimmerman RA, Clancy RR, et al. Incidental intracranial hemorrhage after uncomplicated birth: MRI before and after neonatal heart surgery. Neuroradiology 2003;45:253–8.
8. Licht DJ, Wang J, Silvestre DW, et al. Preoperative cerebral blood flow is diminished in neonates with severe congenital heart defects. J Thorac Cardiovasc Surg 2004;128:841–9.
9. Galli KK, Zimmerman RA, Jarvik GP, et al. Periventricular leukomalacia is common after neonatal cardiac surgery. J Thorac Cardiovasc Surg 2004;127:692–704.
10. Ravishankar C, Zak V, Williams IA, et al. Association of impaired linear growth and worse neurodevelopmental outcome in infants with single ventricle physiology: a report from the Pediatric Heart Network Infant Single Ventricle Trial. J Pediatr 2013;162:250–6.e2.
11. Wernovsky G, Stiles KM, Gauvreau K, et al. Cognitive development after the Fontan operation. Circulation 2000;102:883–9.
12. Mahle WT, Clancy RR, Moss EM, Gerdes M, Jobes DR, Wernovsky G. Neurodevelopmental outcome and lifestyle assessment in school-aged and adolescent children with hypoplastic left heart syndrome. Pediatrics 2000;105:1082–9.
13. Forbess JM, Visconti KJ, Hancock-Friesen C, Howe RC, Bellinger DC, Jonas RA. Neurodevelopmental outcome after congenital heart surgery: results from an institutional registry. Circulation 2002;106:I95–102.
14. Block AJ, McQuillen PS, Chau V, et al. Clinically silent preoperative brain injuries do not worsen with surgery in neonates with congenital heart disease. J Thorac Cardiovasc Surg 2010;140:550–7.
15. Monagle P, Chalmers E, Chan A, et al; for the American College of Chest Physicians. Antithrombotic therapy in neonates and children: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;133(6 Suppl):887S–968S.
16. Lazio BE, Simard JM. Anticoagulation in neurosurgical patients. Neurosurgery 1999;45:838–47.
17. Brouwers HB, Greenberg SM. Hematoma expansion following acute intracerebral hemorrhage. Cerebrovasc Dis 2013;35:195–201.
18. Hart RG, Boop BS, Anderson DC. Oral anticoagulants and intracranial hemorrhage. Facts and hypotheses. Stroke 1995;26:1471–7.
19. Chandra D, Gupta A, Grover V, Kumar Gupta V. When should you restart anticoagulation in patients who suffer an intracranial bleed who also have a prosthetic valve? Interact Cardiovasc Thorac Surg 2013;16:520–3.
20. Towfighi A, Greenberg SM, Rosand J. Treatment and prevention of primary intracerebral hemorrhage. Semin Neurol 2005;25:445–52.
21. Tavani F, Zimmerman RA, Clancy RR, Licht DJ, Mahle WT. Incidental intracranial hemorrhage after uncomplicated birth: MRI before and after neonatal heart surgery. Neuroradiology 2003;45:253–8.
22. Whitby EH, Griffiths PD, Rutter S, et al. Frequency and natural history of subdural haemorrhages in babies and relation to obstetric factors. Lancet 2004;363:846–51.
23. Miller SP, McQuillen PS, Hamrick S, et al. Abnormal brain development in newborns with congenital heart disease. N Engl J Med 2007;357:1928–38.
24. Dent CL, Spaeth JP, Jones BV, et al. Brain magnetic resonance imaging abnormalities after the Norwood procedure using regional cerebral perfusion. J Thorac Cardiovasc Surg 2006;131:190–7.
25. Woodman RC, Harker LA. Bleeding complications associated with cardiopulmonary bypass. Blood 1990;76:1680–97.
26. Beca K, Gunn JK, Coleman L, et al. New white matter brain injury after infant heart surgery is associated with diagnostic group and the use of circulatory arrest. Circulation 2013;127:971–9.