Pathology - Research and Practice 215 (2019) 152366

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

# Occurrence of a primary liver cancer with an unusual histologic appearance as a late Fontan complication

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ARTICLE INFO

*Keywords:*
Congenital cardiac anomaly
Congestion
Fontan procedure
Extracardiac complication
Liver cancer
Liver fibrosis

ABSTRACT

The Fontan procedure is an open heart procedure performed in pediatric patients with a particular congenital cardiac anomaly known as a univentricular heart. The procedure is used to reroute the systemic venous blood from the inferior vena cava directly to the pulmonary artery. It improves patients' prognoses, but various late-phase extracardiac complications that manifest when patients reach adolescence have been recognized. These complications, pulmonary arteriovenous fistula and protein losing gastroenteropathy, for example, present significant challenges in the management of adults with Fontan circulation. Liver fibrosis is another possible late-phase complication and one of the most serious. Development of a neoplasm, usually a hepatocellular carcinoma, is sometimes reported. We encountered a young patient in whom Fontan circulation led to the development of a histologically unusual liver cancer that resembled the poorly differentiated hepatocellular carcinoma or the combined hepatocellular-cholangiocarcinoma with stem-cell features described in the latest WHO classification.

## 1. Introduction

The Fontan procedure, a surgical procedure that redirects venous blood flow directly to the pulmonary circulation, improves the prognosis of patients with a single-ventricle type of congenital heart defect. However, various long-term multi-organ complications are associated with the Fontan circulation. These complications, which are due to the unique hemodynamics of the Fontan circulation, can manifest during adolescence and present significant challenges in the management of adult Fontan patients [1]. Liver fibrosis has been recognized as a reorganization that results in response to chronic venous congestion that arises from the unusual pulseless manner of venous return [2,3], and neoplastic lesions including liver cancer have sometimes been reported. Hepatocellular carcinoma is the most common histologic type of primary liver cancer, and this is the type most often found in patients in whom the liver cancer arises in the context of the Fontan circulation [4–6], but development of an intrahepatic cholangiocarcinoma has been reported recently [7]. This points to the possibility of multiple potentials for tissue differentiation of tumors arising in the liver after the Fontan operation. We encountered a unique liver cancer in a young woman with a Fontan circuit. The cancer was suspected to be a poorly differentiated hepatocellular carcinoma, but it resembled the rare histologic type described in the latest WHO classification as a combined hepatocellular-cholangiocarcinoma with stem-cell features [8].

## 2. Clinical summary

Our patient was a 21-year-old woman who, at age 1 year and 11 months, had undergone the Fontan procedure (intracardiac lateral tunnel Fontan with an intraatrial conduit) for a single ventricle (double outlet right ventricle with pulmonary atresia) and left isomerism. Genetic tests had not been performed in this case, but there was no family history of congenital heart disease. No remarkable extracardiac complication occurred during the patient's early childhood. When she was 10 years of age, however, hepatic congestion resulting in liver fibrosis was indicated by the results of laboratory blood tests. No specific treatment was undertaken for the suspected congestion; we simply monitored the patient's central venous pressure. The patient was followed up regularly, and a mass lesion was found after 7 years. The mass was diagnosed as a liver cancer on the basis of its contrast computed

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https://doi.org/10.1016/j.prp.2019.02.016
Received 13 November 2018; Received in revised form 28 January 2019; Accepted 26 February 2019

tomography (CT) appearance and an increased serum concentration of α-fetoprotein (154 ng/mL), a marker for hepatocellular carcinoma. There was no clinical evidence of hepatitis virus infection. Transarterial chemoembolization (TACE) was performed, and the patient was followed-up regularly, but 16 months after the TACE procedure, she died suddenly from an unrelated subarachnoid hemorrhage that resulted from rupture of an intracranial aneurysm.

## 3. Pathological examination

Autopsy was performed 2 h after the patient's death. The heart and lungs and the liver, pancreas, and spleen were removed en bloc. The liver was sectioned for comparison with the CT images, which were obtained in the horizontal plane. The removed organs were cut and fixed in 15% buffered formalin solution (pH 7.4) and embedded in paraffin. Paraffin-embedded sections (5 mm thick) were stained with hematoxylin and eosin and then with Sirius red for identification of collagenous fibers so that acquired fibrosis could be evaluated.

To clarify the basic histologic components of the tumor (hepatocellular carcinoma or intrahepatic cholangiocarcinoma), immunohistochemistry was performed with primary antibodies against α-fetoprotein, cytokeratin (CK) 7, CK8, arginase 1 (Arg1), and glypican-3, CEA, CA19-9, and CK19, CD10, CD34, CD56 (neural cell adhesion molecule [NCAM]), c-kit (CD117). In addition, CD326 (epithelial cell adhesion molecule [EpCAM]) was performed for detection of stem/progenitor cells, and delta-like 1 (DLK1) for hepatoblstoma to identify whether the tumor was an ordinary hepatocellular carcinoma or another type of neoplasm. Sections were dewaxed in xylene and dehydrated through a graded series of alcohols. Endogenous peroxidase activity was blocked by 15-minute treatment with periodic acid. For antigen retrieval, sections were treated with citric acid at pH 6.0 (or 9.0) and autoclaved at 121 °C for 2 min or subjected to microwave heating. The immunohistochemical staining was performed with an EnVision kit (DAKO, Carpinteria, CA, USA) according to the manufacturer's instructions. After the sections were rinsed in phosphate-buffered saline, secondary antibody was applied, and diaminobenzidine was added as chromogen.

## 4. Pathological findings

### 4.1. Macroscopic findings

Disarrangement of the abdominal organs (visceral heterotaxy) was observed (Fig. 1A). The liver was located at the midline of the visceral space, and its surface was rough. In sections of formalin-fixed tissue obtained from the middle of the right lobe and middle of the left lobe, a 10 × 9-mm necrotic mass, due presumably to the TACE therapy, was observed (Fig. 1A). Furthermore, in the section obtained 10 mm in the diaphragmatic direction, a round, whitish, solid mass that measured 16 x 14 mm was found (Fig. 1B), and it was contiguous with the necrotic mass noted above. The cut surface of the background liver was of a non-uniform mosaic pattern reflective of severe fibrosis (Fig. 1B). Examination of the heart revealed that the inferior vena cava was posterior to the aorta; it was interrupted and continued via a hemi-azygos vein. The left atrial morphology was that of isomerism; polysplenia was not found. The single spleen was enlarged and weighed 56.0 g (Fig. 1A).

### 4.2. Histologic findings

We found that the main solid mass in the liver was not clearly demarcated by a fibrous component, although reticular fibrosis and fatty change were observed within the mass (Fig. 2A). Also within the mass were small round cells with clear cytoplasm and that proliferated in a solid or trabecular pattern (Fig. 2B), and the center of the tumor was characterized by necrosis and irregular fibrous replacement. Ductal invasion was observed in the adjacent portal area, indicating at least some malignant potential (Fig. 2C). Upon immunohistochemical examination, we found that the main solid liver mass was composed of small clear round cells, positive for Arg1 and CK8 (Table 1, Fig. 3A,B). Around the main tumor, diffuse interstitial fibrosis was seen, and irregularly sized pseudolobules had formed. The fibrous tissue was more extensive around the portal area than around the central veins of the lobules, and randomly distributed fatty deposits were seen (Fig. 4).

### 4.3. Histopathologic diagnosis of the main tumor

The main tumor showed potential for hepatocellular or cholangiocellular differentiation. Results of tests for markers of other types of tumor were negative (Fig. 34C,D). The unusual proliferation of small atypical cells was indicative of poorly differentiated hepatocellular carcinoma, but the test results pointed to the possibility of a combined hepatocellular-cholangiocarcinoma with stem-cell features, even though immunohistochemical staining that would identify stem/progenitor cells was negative. Histologically, the tumor resembled a hepatoblastoma, but this entity was ruled out on the basis of the patient's age, absence of hematopoietic cells, and results of immunohistochemistry (Figs. 2 and 3D).

Small, round, isolated tumors, up to 7 x 6 mm (not shown), were also found, and the borders between these tumors and non-tumorous tissue were clear despite the absence of surrounding fibrous components. The, mass consisted of hepatocellular cells that were increased in density but without nuclear or structural atypia. Immunohistochemistry did not yield a specific diagnosis, but an unclassified hepatocellular adenoma or focal nodular-hyperplasia-like lesion was suspected because of the increased cell density. In addition, multiple smaller, round masses were seen around the main tumor in the same section. These lacked conspicuous atypia and were thought to represent hyperplastic change without neoplastic potential.

## 5. Discussion

The neoplasm we encountered was a histologically unusual liver

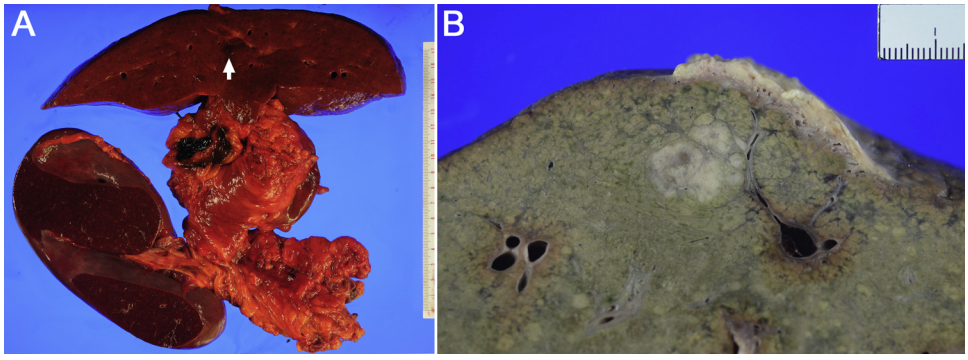


**Fig. 1.** Macroscopic appearance of the liver and spleen upon autopsy. A) Transverse cross sections of the liver and spleen. Heterotaxy of the abdominal organs and incomplete situs inversus (mid-line liver) were found. At the center of the right and the left lobes, a 10 × 9-mm necrotic mass, presumably the result of TACE, was observed (white arrow). The spleen was enlarged and weighed 560 g. B). Macroscopic view of the solid, whitish main liver mass after formalin fixation. The 16 × 14-mm round mass was located beneath the falciform ligament.

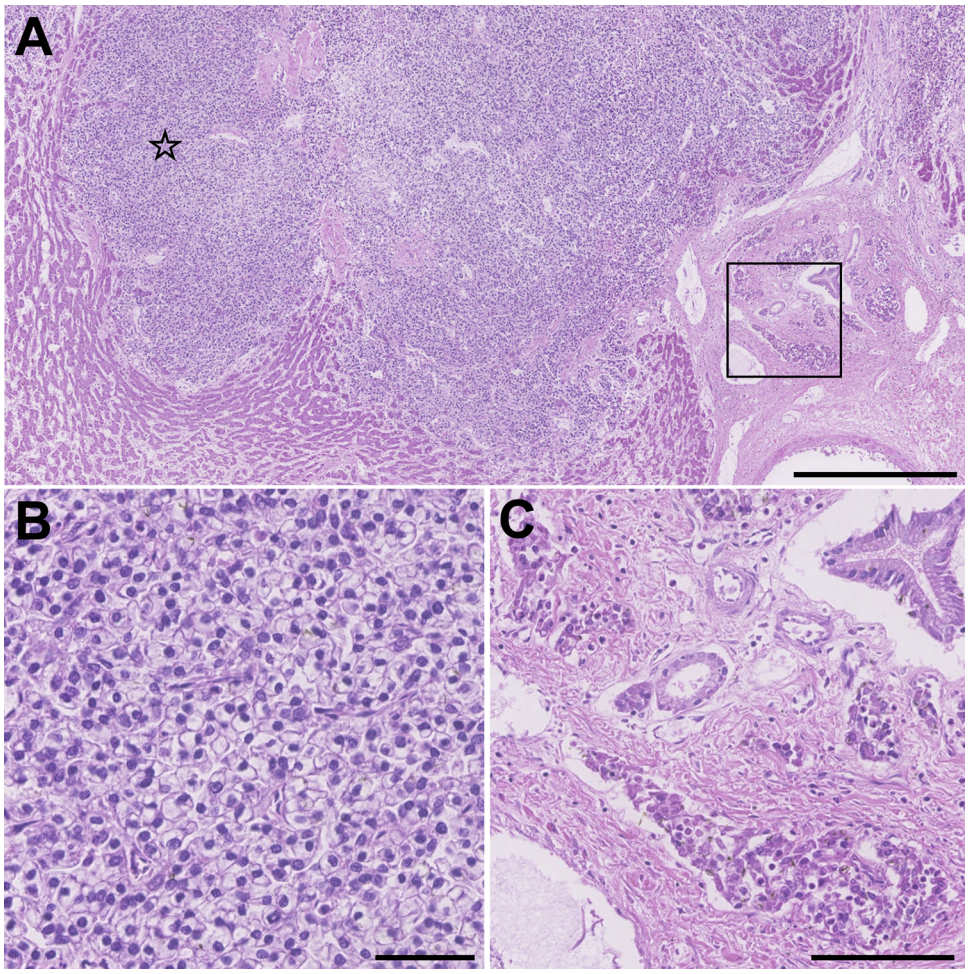


**Fig. 2.** Histologic features of the main liver mass. A) In this hematoxylin and eosin-stained section, the tumor does not appear to be surrounded by thin fibrous tissue. Fibrosis is seen in the center of the tumor. Bar = 0.5 mm. B) High-magnification view of the area marked with a star on the hematoxylin and eosin-stained section seen in Fig. 2A. The tumor consists mainly of a solid proliferation of small round cells with clear cytoplasm, and the border between the tumor cells and the normal hepatocytes is clear. Bar = 50 μm. C) In this high-magnification view of the square in Fig. 2A on the hematoxylin and eosin stained section, ductal invasion in the portal area is evident. Bar = 100 μm.

**Table 1**
Immunohistochemical profiles of the main tumor.

| AFP | GS | GPC3 | HSP70 | ARG-1 | CK7 | CK8 | CK19 | CA19-9 | CD10 | CD34 | CD56 | CD117 | CD133 | CD326 | DLK1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| – | – | – | – | +(diffuse) | – | + | – | – | – | – | – | – | – | – | – |

AFP. alpha fetoprotein, GS. glutamines synthetase, GPC3. glypican-3, HSP70. heat shock protein 70, ARG-1. arginase-1.
CD56. neural cell adhesion molecule, NCAM, CD117. c-kit, CD133. prominin-1, PROM1, CD326. epithelial cell adhesion molecule, EpCAM.
DLK1. delta-like 1.

cancer that developed as a long-term complication of the Fontan operation performed for a complex congenital heart anomaly, a single ventricle defect. Most liver cancers that have developed after the Fontan procedure have been ordinary hepatocellular carcinomas [4–6], but the neoplasm in our case was unusual in that we discovered a proliferation of small round cells with clear cytoplasm. Typical hepatocyte differentiation is rare in tumors, and poorly differentiated hepatocellular carcinoma was suspected in our case from the standpoint of the specific histologic identification of very small atypical cells with clear cytoplasm and the lack of immunohistochemical evidence for a hepatocellular carcinoma of greater differentiation. This histologic pattern may be indicative of non-differentiated or poorly differentiated hepatocellulcar carcinoma or cholangiocarcioma like the combined hepatocellular carcinomacholangiocarcinoma described in the WHO classification [**8**]. Results of our immunohistochemical analysis of Arg1 or CK8 expression were unfortunately insufficient for identification of this unique clinical entity. The tumor we encountered developed in the patient at a relatively young age, and tumors that have developed after the Fontan procedure in other, similarly young patients have shown signs of serious malignant potential, such as vascular invasion or both intra- and extra-hepatic metastases [6]. In our case, because vascular invasion was observed around the portal area, the tumor was thought to have malignant potential even though it appeared, by its size, to be an early-stage tumor. Although the interventional TACE had been performed more than 1 year previously, the remaining viable tumor component was enlarged. We believe that had more time passed, the clinical course would have been marked by further growth of the tumor and multiple metastases. Had the patient not died unexpectedly, more clearly differentiated hepatocellular or cholangiocellular carcinoma components might have been detected.

Persistent venous congestion is inevitable after the Fontan operation. The congestive state lasting several years is thought to be the main reason for the generation of liver neoplasms, and all patients who have undergone the Fontan operation are thought to be at risk for liver cancer. The liver is recognized as an organ that can tolerate and compensate for various pathologic conditions such as congestion and chronic inflammation, though beyond a certain point, liver regeneration is not possible, and fibrosis becomes the most common pathologic change. Such a pathological course is frequently thought to proceed asymptomatically even in hemodynamically well-compensated post-

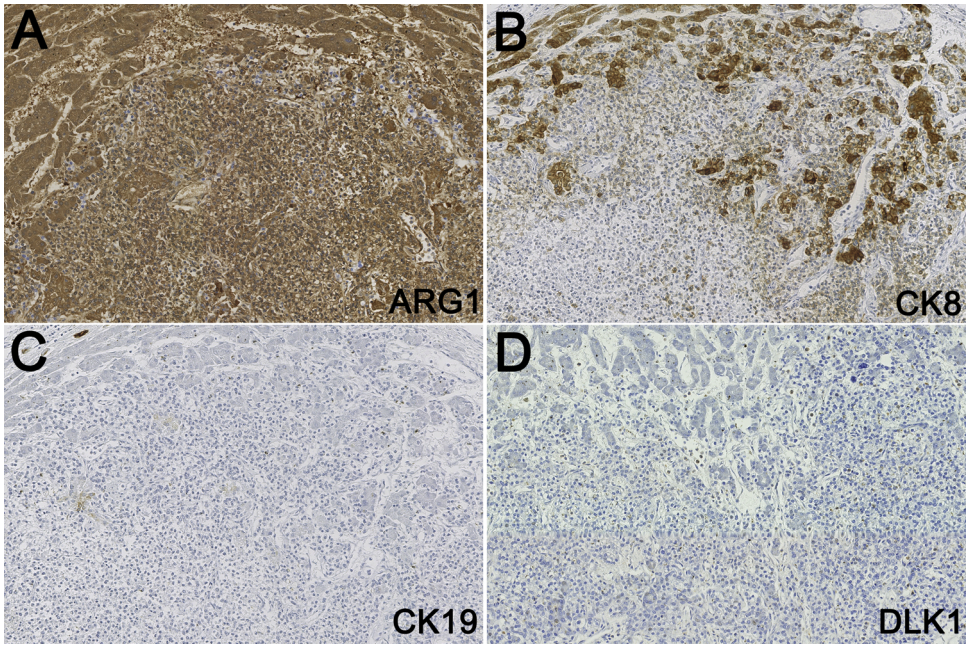


**Fig. 3.** Immunohistochemistry of the tumor component at the site shown in Fig. 2a.
A) arginase 1 (Arg1), B) cytokeratin 8 (CK18), C) CK19, D) delta-like 1 (DLK1)

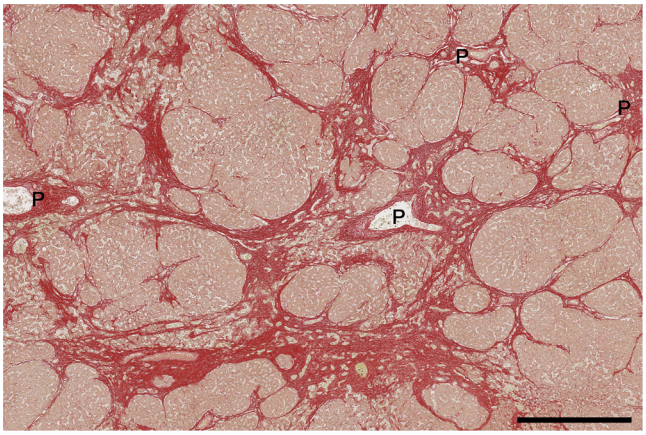


**Fig. 4.** Histologic features of the background liver tissue. In this Sirius red-stained section, irregular and reticular fibrosis is seen. The liver was cirrhotic nearly throughout, including the portal area. P. portal area, Bar = 1 mm.

Fontan patients. The fibrosis that occurs after the Fontan operation is usually severe not only around the central vein (zone 3), as is commonly seen with liver congestion, but also around the portal area (zone 1) [3]. This unusual pattern of fibrosis might be the result of the abnormality particular to the Fontan circulation, i.e., to the nonpulsatile, passive flow of caval blood in the absence of a functioning tricuspid valve and right ventricular contraction, which lead to longstanding congestion and a low perfusion-low cardiac output state resulting from the single-ventricle physiology [9].

Another way of looking at the issue is that portal fibrosis has been seen in the majority of reported patients who died soon after the Fontan procedure, and such fibrosis may be the result of more than one factor at play in the specific physiology of the univentricular heart [[10]]. The persistent unnatural flow most certainly affects both the portal circulation and the pressure of the central hepatic veins. Accordingly, a widespread reticular fibrotic pattern that included portal areas but without significant inflammatory cell infiltration (as is commonly seen in chronic viral hepatitis) was seen in our patient. This suggests that persistent inflammation was not directly responsible for the liver tumor. However, unknown cytokines related to mild, undetected inflammation could have been related to the development of our patient's late Fontan complication.

With regard to the pathogenesis of this unusual type of carcinoma, its development as a **de novo** cancer is reasonable. However, multiple small hyperplastic nodules and a single adenomatous mass were present around the main tumor, so malignant transformation of a benign tumor (hepatocellular adenoma) or nodular hyperplasia cannot be ruled out.

## 6. Conclusion

We encountered an unusual histologic type of liver cancer in the young woman years after she had undergone the Fontan procedure. The pathogenesis of the tumor is uncertain, but it is clear that a poorly differentiated or unusual carcinoma can arise in the milieu created by chronic Fontan circulation.

## Funding

This work was supported by JSPS KAKENHI Grants 17K01366 and 16K08682.

## Disclosure statement

None.

## References

[1] L. Lastinger, A.N. Zaidi, The adult with a Fontan: a panacea without a cure? Review of long-term complications, Circ. J. 77 (2013) 2672–2681.
[2] C.H. Kiesewetter, N. Sheron, J.J. Vettukattill, N. Hacking, B. Stedman, H. Millward-Sadler, M. Haw, R. Cope, A.P. Salmon, M.C. Sivaprakasam, T. Kendall, B.R. Keeton, J.P. Iredale, G.R. Veldtman, Hepatic changes in the failing Fontan circulation, Heart 93 (2007) 579–584.
[3] F.M. Wu, M.M. Jonas, A.R. Opotowsky, A. Harmon, R. Raza, C. Ukomadu, M.J. Landzberg, M.N. Singh, A.M. Valente, G. Egidy Assenza, A.R. Perez-Atayde, Portal and centrilobular hepatic fibrosis in Fontan circulation and clinical outcomes, J. Heart Lung Transplant. 34 (2015) 883–891.
[4] A.A. Ghaferi, G.M. Hutschins, Progression of liver pathology in patients undergoing the Fontan procedure: chronic passive congestion, cardiac cirrhosis, hepatic adenoma, and hepatocellular carcinoma, J. Thorac. Cardiovasc. Surg. 129 (2005) 1348–1352.

[5] S. Kwon, L. Scovel, M. Yeh, D. Dorsey, G. Demgo, E.V. Krieger, R. Barkthavatsalam, J.O. Park, K.M. Riggle, K.J. Riehle, R.S. Yeung, Surgical management of hepatocellular carcinoma after Fontan procedure, J. Gastrointest. Oncol. 6 (2015) E55–60.
[6] C. Oh, J.K. Youn, J.W. Han, G.B. Kim, H.Y. Kim, S.E. Jung, Hepatocellular carcinoma after the Fontan procedure in a 16-year-old girl: a case report, Medicine (Baltimore) 95 (2016) e4823.
[7] D. Wang, D. Marshall, G. Veldtman, A. Gupta, A.T. Trout, J. Villafane, K. Bove, Intrahepatic cholangiocarcinoma after Fontan procedure in an adult with visceral heterotaxy, Pathol. Res. Pract. 214 (2018) 914–918.
[8] N.D. Theise, O. Nakashima, Y.N. Park, et al., Combined hepatocellular-cholangiocarcinoma, in: F.T. Bosman, F. Carneiro, R.H. Hruban, N.D. Theise (Eds.), WHO Classification of Tumours of the Digestive System, IARC, Lyon, 2010, pp. 225–227.
[9] H. Ohuchi, Adult patients with Fontan circulation: What we know and how to manage adults with Fontan circulation? J. Cardiol. 68 (2016) 181–189.
[10] M.C. Schwartz, L. Sullivan, M.S. Cohen, P. Russo, A.S. John, R. Guo, M. Guttenberg, E.B. Rand, Hepatic pathology may develop before the Fontan operation in children with functional single ventricle: an autopsy study, J. Thorac. Cardiovasc. Surg. 143 (2012) 904–909.