EDITORIAL COMMENT

Pulmonary Arteriovenous Malformations and the Hepatic "Black Box"

Are We Emerging From the Darkness?*

Satish K. Rajagopal, MD,[a] Jeffrey R. Fineman, MD[a,b]

Surgical palliation of congenital heart diseases (CHDs) that are not amenable to a biventricular repair (complete septation) involves routing systemic venous blood directly to the pulmonary arteries (PAs), bypassing the heart. A prototypic example of this type of CHD is type 1c tricuspid atresia (ventricular septal defect, pulmonary valve stenosis with normal ventriculoarterial connections). The goal of staged surgical palliation is to restore normal or near-normal oxygen saturation and minimize ventricular workload. Systemic venous bypass of the heart typically requires 2 surgical steps. The first involves creation of a superior cavopulmonary anastomosis (Glenn shunt), usually accompanied by elimination of the previous source of pulmonary blood flow. Consequent to this operation, there is nonpulsatile flow in the pulmonary circulation and the lungs no longer receive blood flow directly from the inferior vena cava (IVC) nor its tributaries (e.g., hepatic veins). The second step, known as the Fontan procedure, is undertaken a few years later and involves routing IVC blood directly to the PAs. Flow in the PAs remains nonpulsatile, but then contains IVC and hepatic venous effluent.

The concept of routing blood flow from the superior vena cava (SVC) directly to the PAs was developed and refined by independent groups in the early 1950s. Meticulous investigators tracked the clinical course of early survivors for years, several noticing that many patients became progressively cyanotic after an initial period of improvement in systemic oxygen levels. In a remarkable report from 1973, Mathur and Glenn (1) catalogued the complications (and generated hypotheses for their development) in 63 patients who underwent a superior cavopulmonary connection. Using pulmonary angiography, they were the first to describe abnormal arteriovenous connections (later referred to as pulmonary arteriovenous malformations [PAVMs]) in the lungs of this patient population (n = 5) (1).

Over the next 2 decades, the natural history of PAVMs in those who have undergone interventions after a Glenn shunt has provided interesting insights into their development and maintenance. Specifically, PAVMs regress after: 1) incorporation of the IVC into the PAs (Fontan procedure); 2) placement of a systemic to PA shunt; 3) placement of a peripheral arteriovenous fistula; 4) incorporation of the hepatic veins into the PAs of those who had previously undergone an SVC-to-PA anastomosis in the setting of an interrupted IVC with azygous continuation (essentially a Fontan circulation without hepatic venous effluent); 5) redirection of the hepatic veins to the right atrium in a child born with isolated hepatic venous drainage to the left atrium; and 6) restoration of hepatic venous inflow to a lung that developed PAVMs despite Fontan completion (e.g., severe obstruction of the PA between the SVC and IVC anastomosis). In this last situation, the left lung is free of PAVMs, while the right lung is not, potentially because the obstruction between the caval anastomoses prevents hepatic venous inflow to

---

the right lung. Taken together, these observations suggest that hepatic venous blood suppresses the development of arteriovenous malformations in the lung and can make them regress, that the substance is present in arterial blood only until it reaches a capillary bed, and that pulsatile flow in the pulmonary arteries is not necessary to suppress development of PAVMs.

In 1995, Srivastava et al. (2) proposed that the exclusion of a hepatic factor was responsible for the development of PAVMs in patients with a Glenn shunt. The observation that PAVMs develop in some patients with cirrhosis (hepatopulmonary syndrome) lent more credence to the hepatic factor hypothesis. Since that time, several groups have attempted to isolate the hepatic factor without success. Advances in the understanding of angiogenesis and blood vessel homeostasis together with the availability of "omics" platforms and approaches may have rendered the problem tractable.

In this issue of *JACC: Basic to Translational Science*, Bartoli et al. (3) report on their attempt to identify the hepatic factor. They measured a panel of 60 "angiogenic and inflammatory" proteins, von Willebrand's factor antigen multimers and degradation products, and the von Willebrand's factor protease (ADAMTS-13) in plasma obtained from the proximal PAs and IVCs of children who previously underwent a Glenn shunt (n = 22). They compared the plasma concentrations of these proteins to each other (PA vs IVC) and to those in the peripheral venous blood of 20 healthy age-matched control cases. Additionally, they assessed the differential effects of PA, IVC, and control plasma on angiogenesis by measuring endothelial cell proliferation and tubule and sprout generation using in vitro human umbilical vein endothelial cell (HUVEC) assays.

To summarize their findings, the concentrations of several angiogenically active proteins were significantly different between plasma from Glenn patients and control cases with a much smaller number being different between PA and IVC plasma in Glenn patients (angiopoeitin-1 [Ang-1], C-X-C motif chemokine ligand 16 [CXCL16], and leukemia inhibitory factor [LIF]). Additionally, they found that the plasma of patients with a Glenn shunt stimulated endothelial cell proliferation more than control plasma, induced less HUVEC tubule and sprout generation, and resulted in shorter tubules. Interestingly and somewhat paradoxically, the plasma derived from the PA of those with a Glenn shunt had angiogenic effects on HUVECs that were more similar to those of control plasma than IVC plasma (i.e., more sprouts and more tubules that were also longer).

A recent study of the differential effects of SVC and hepatic vein serum obtained from children with a wide variety of CHD on angiogenesis and cell survival using human pulmonary microvascular endothelial cells (HPMECs) found that hepatic vein serum increased tube and sprout formation compared to SVC serum. Additionally, they found that hepatic vein serum promoted endothelial cell proliferation and reduced apoptosis of endothelial cells compared to SVC serum (4). It is unclear as to why these 2 groups had opposite findings regarding angiogenic properties of blood from the SVC versus the IVC (hepatic veins), but possibilities include different responses of HPMECs and HUVECs to serum and/or different protein composition of hepatic venous blood versus IVC blood. For example, differential responses between pulmonary arteries and umbilical veins are well demonstrated (i.e., the response to hypoxia).

It appears that the most compelling proteins for further study are those that were different between the PA and IVC samples given the observation that adding IVC/hepatic vein flow back to the PAs during Fontan completion results in near uniform regression of PAVMs. However, differential circulating half-lives of these proteins may compound this reasoning. The 3 proteins meeting this criterion are Ang-1, CXCL16, and LIF. Ang-1 is a secreted growth factor that is the major ligand for the endothelial specific tyrosine kinase receptor Tie-2. Ang-1/Tie2 signaling have complex roles in the vasculature. It is necessary for vasculogenesis and for maintaining the blood vessel homeostasis by restraining angiogenesis, maintaining blood vessel architecture, and preventing excessive vascular permeability, particularly under conditions of microvascular stress (5). This biologic profile combined with the current findings is intriguing and deserves further investigation.

The chemokine CXCL16 is a more recently identified autocrine angiogenic factor that has been studied most intensively in tumor angiogenesis. The protein is partially regulated by hypoxia and is upstream of the ERK, Akt, and p38 pathways. In studies using HUVECs, CXCL16 promotes tubule formation, lengthening, and sprouting (6). Finally, LIF is a member of the interleukin-6 superfamily with wide ranging biological effects, including on angiogenesis where its exact role remains unclear, with contradictory findings of both pro- and antiangiogenic effects (7).

In summary, Bartoli et al. (3) have identified several interesting candidate peptides that may play a role in the development of PAVMs in children who have undergone a Glenn shunt, the biological origin

of which has remained a mystery for decades. Several next steps seem to be warranted: 1) replicate the findings in an independent cohort; 2) use PA plasma from Glenn patients in which candidate protein concentrations have been normalized to perform in vitro assays using HUVECs and HPMECs and assess whether angiogenesis approximates that of control plasma; 3) the plasma concentrations of the candidate proteins should be measured in patients with the hepatopulmonary syndrome; and 4) the effects of candidate protein manipulations (supplementation and pharmacological inhibition) on PAVMs in large animal models of the Glenn shunt should be studied.

The search for the elusive hepatic factor, whose exclusion from the pulmonary vasculature remains a great source of morbidity for children with single ventricular physiology, continues. Congratulations to Bartoli et al. ([3]) for potentially paving a road toward discovery.

ADDRESS FOR CORRESPONDENCE: Dr. Jeffrey R. Fineman, Department of Pediatrics, University of California, San Francisco, 505 Parnassus Avenue, Box 0106, San Francisco, California 94143, USA. E-mail: jeff.fineman@ucsf.edu.

REFERENCES

1. Mathur M, Glenn WW. Long-term evaluation of cava-pulmonary artery anastomosis. Surgery 1973; 74:899–916.

2. Srivastava D, Preminger T, Lock JE, et al. Hepatic venous blood and the development of pulmonary arteriovenous malformations in congenital heart disease. Circulation 1995;92: 1217–22.

3. Bartoli CR, Hennessy-Strahs S, Dowling RD, Gaynor JW, Glatz AC. Abnormalities in the von Willebrand–angiopoietin axis contribute to dysregulated angiogenesis and angiodysplasia in children with a Glenn circulation. J Am Coll Cardiol Basic Transl Science 2021; 6:222–35.

4. Spearman AD, Gupta A, Pan AY, et al. Hepatic vein blood increases lung microvascular angiogenesis and endothelial cell survival-toward an understanding of univentricular circulation. Semin Thorac Cardiovasc Surg 2020; 32:980–7.

5. Jeansson M, Gawlik A, Anderson G, Li C, Kerjaschki D, Henkelman M, Quaggin SE. Angiopoietin-1 is essential in mouse vasculature during development and in response to injury. J Clin Invest 2011;121:2278–89.

6. Yu X, Zhao R, Lin S, et al. CXCL16 induces angiogenesis in autocrine signaling pathway involving hypoxia-inducible factor 1α in human umbilical vein endothelial cells. Oncol Rep 2016; 35:1557–65.

7. Santos GC, Silva DN, Fortuna V, et al. Leukemia inhibitory factor (LIF) overexpression increases the angiogenic potential of bone marrow mesenchymal stem/stromal cells. Front Cell Dev Biol 2020;8:778.

KEY WORDS angiopoeitin-1, Glenn operation, pulmonary arteriovenous malformations, von Willebrand's factor