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# Pulmonary hypertension in children and young adults with sickle cell disease

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**PULMONARY HYPERTENSION IN CHILDREN AND YOUNG ADULTS WITH  
SICKLE CELL DISEASE**

by

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Master of Science

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**PULMONARY HYPERTENSION IN CHILDREN AND YOUNG ADULTS WITH  
SICKLE CELL DISEASE**

**KRISTIN SHEPPARD**

**ABSTRACT**

**Introduction:** Primary pulmonary hypertension (PH) is a progressive disease that is characterized by restricted blood flow in the pulmonary vasculature, resulting in an increase in pulmonary vascular resistance (PVR) (McLaughlin et al., 2009). Patients with sickle cell disease (SCD), a monogenic blood disorder that causes red blood cells to form a sickle shape and decreases their ability to carry oxygen to the body's tissues, can develop secondary pulmonary hypertension. Although there is significant data showing that pulmonary hypertension secondary to SCD increases morbidity and mortality in adult patients, there is little information regarding how PH affects children with SCD (Klings & Machado et al., 2014). Although diagnosing PH requires right heart catheterization, the American College of Cardiology Foundation and the American Heart Association recommend using Doppler echocardiography as a screening test for PH (McLaughlin et al., 2009). A tricuspid valve regurgitant jet velocity (TRV) of 2.5 m/s or higher, measured by Doppler echocardiography, is suggestive of PH (McLaughlin et al., 2009). Because Doppler echocardiography is non-invasive, there is much more information available regarding TRV than right heart catheterization, an invasive procedure. This study examines the prevalence and incidence of elevated TRV ( $\geq 2.5$  m/s) in children with SCD as well as factors that may be associated with an elevated TRV.

**Materials and Methods:** We conducted a retrospective longitudinal cohort study chart review for 83 subjects followed by pediatric hematology at Boston Medical Center; examining demographic information, pediatric and adult echocardiogram reports, SCD complications previously identified as being related to PH, laboratory results that have previously been identified as being related to PH while each subject was between the ages of 8 and 30 between July 31, 2003 and June 30, 2015.

**Results:** We found that 11 (13%) of our study population had one or more elevated TRV value ( $\geq 2.5$  m/s). Elevated TRV values ranged from 2.5 – 2.83 m/s. The average age for elevated TRV was 15.9 years old. Of the subjects with elevated TRV 6 (55%) were taking HU, as compared to 53 (64%) of the general study population. No SCD complications previously reported to be related to PH were found to be significantly associated with elevated TRV in our study population. The only laboratory value that we found to be associated with elevated TRV was an elevated reticulocyte count, with an average value of 11% of red blood cells in subjects with elevated TRV and 8.41% of red blood cells in the general study population ( $p = 0.04$ ).

**Discussion/conclusion:** We found that 13% of children with SCD had at least one screening echocardiogram with  $TRV \geq 2.5$  m/s, which is in agreement with the prevalence estimates of 10-20% published by the American Thoracic Society (Klings & Machado et al., 2014). However, this is much lower than the 30% reported in the literature (Kato, Onyekwere, & Gladwin, 2007). This may be caused by more widespread use of hydroxyurea therapy at our academic institution. We also found no associated

factors other than elevated reticulocyte count, while other studies found associations with a history of sepsis/bacteremia, history of ACS, history of asthma, Hb SS genotype, low hemoglobin levels, and elevated reticulocyte count (Hagar et al., 2007; Minniti et al., 2009; Pashankar et al., 2008; Ambrusko et l., 2006; Kato et al., 2007)

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## LIST OF ABBREVIATIONS

cGMP .....	Cyclic Guanosine Monophosphate
GMP .....	Guanosine Monophosphate
GTP .....	Guanosine Triphosphate
Hb S .....	Sickle Hemoglobin
mPAP .....	Mean Pulmonary Arterial Pressure
NO .....	Nitric Oxide
NOS .....	Nitric Oxide Synthase
PAH .....	Pulmonary Arterial Hypertension
PDE-5 .....	Phosphodiesterase – 5
PH .....	Pulmonary Hypertension
PVR .....	Pulmonary Vascular Resistance
RAP .....	Right Atrial Pressure
SCD .....	Sickle Cell Disease
sPAP .....	Systolic Pulmonary Artery Pressure
TRV .....	Tricuspid Valve Regurgitant Velocity

## INTRODUCTION

Primary pulmonary hypertension (PH) is a progressive disease that is characterized by restricted blood flow in the pulmonary vasculature, resulting in an increase in pulmonary vascular resistance (PVR) (McLaughlin et al., 2009). This disease was first described in 1891 as “pulmonary vascular sclerosis” by German physician, Ernst von Romberg (Barst, 2008). However, very little research was in this area until the late 1960’s when the use of the appetite suppressant, aminorex fumarate, caused an epidemic of chronic pulmonary hypertension in Austria, Germany, and Switzerland (Gurtner, 1984). In 1981 the National Heart, Lung, and Blood Institute supported a national registry for idiopathic pulmonary arterial hypertension, a form of primary pulmonary hypertension, which fueled the characterization of the clinical features and the natural history of this disease (Barst, 2008).

Patients with sickle cell disease (SCD) can develop secondary pulmonary hypertension. Studies have shown that approximately 6 to 11% of adults with SCD have PH confirmed by right heart catheterization, and in these patients there is a 6-year mortality of 37% (Klings & Machado et al., 2014; Mehari, Gladwin, Tian, Machado, & Kato, 2012). There is little data regarding pulmonary hypertension in pediatric patients with SCD. Although there is significant data showing that pulmonary hypertension secondary to SCD increases morbidity and mortality in adult patients, there is little information regarding how PH affects mortality in children with SCD (Klings & Machado et al., 2014).

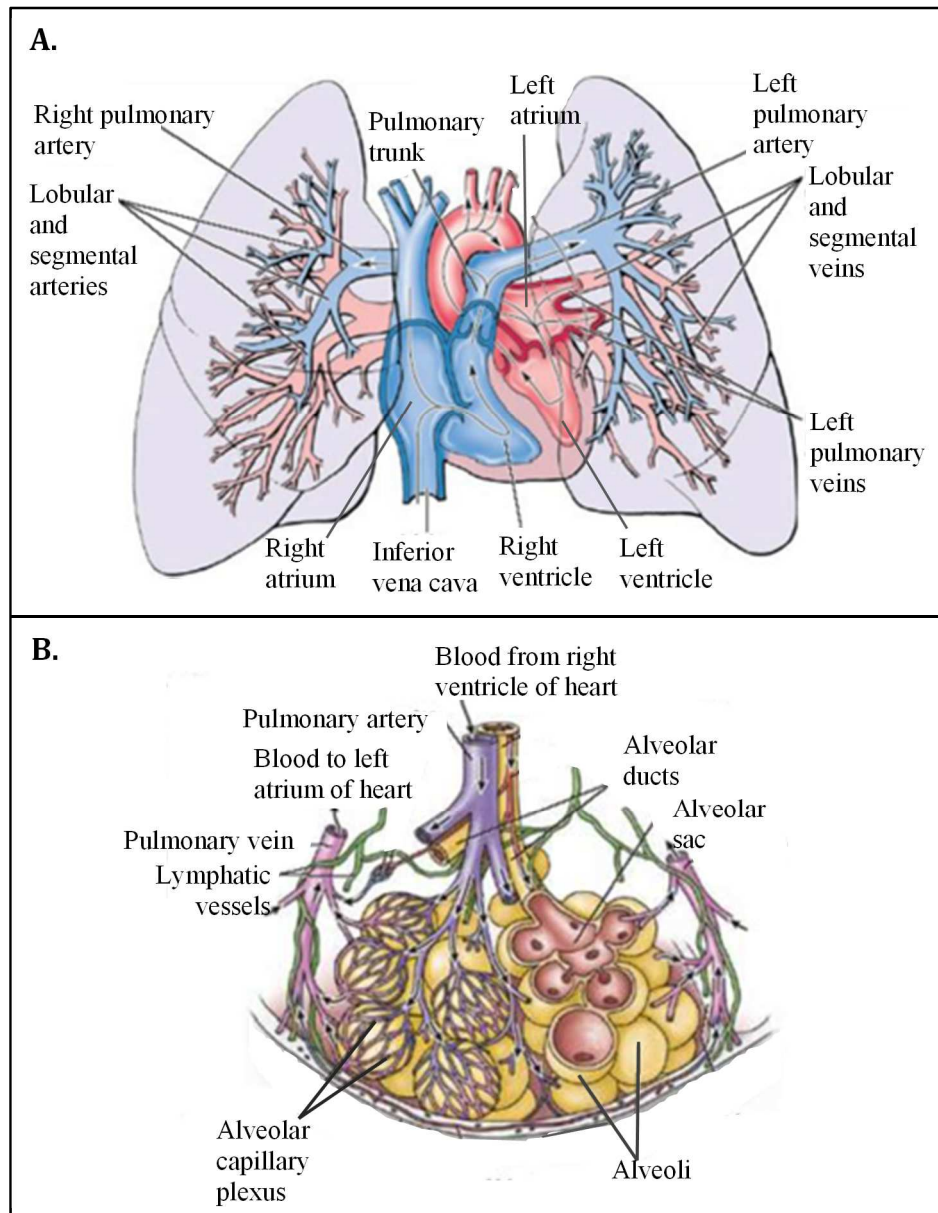
Although diagnosing PH requires right heart catheterization, the American College of Cardiology Foundation and the American Heart Association recommend using Doppler echocardiography as a screening test for PH (McLaughlin et al., 2009). A tricuspid valve regurgitant jet velocity (TRV) of 2.5 m/s or higher, measured by Doppler echocardiography, is suggestive of PH (McLaughlin et al., 2009). Because Doppler echocardiography is non-invasive, there is much more information available regarding TRV than right heart catheterization, an invasive procedure. However, several studies have shown that echocardiography has a low predictive value for PH, with only a 25% predictive value with  $TRV \geq 2.5$  m/s, and a 64% predictive value with  $TRV \geq 2.9$  m/s (Parent et al., 2011). The low availability of right heart catheterization data and the low predictive value of  $TRV \geq 2.5$  m/s makes it difficult to know the extent of PH among the SCD population.

We conducted a retrospective longitudinal cohort study chart review to examine the prevalence and incidence of elevated TRV ( $\geq 2.5$  m/s) in children with SCD as well as factors that may be associated with an elevated TRV. This study also aims to determine the change of TRV over time in response to SCD-directed therapies, including HU and chronic transfusions, in children with SCD.

### **Normal Anatomy and Function of the Heart and Pulmonary Vasculature**

The heart and lungs pump blood through the body and provide oxygen to the body's tissues (Figure 1). Deoxygenated blood collects in the right atrium via the inferior and superior venae cavae before moving through the tricuspid valve into the right

ventricle when the atrium contracts (Pappano & Wier, 2012). This period of ventricular filling is called diastole, and normal right ventricular diastolic pressure is between 3 and 8 mmHg (Pappano & Wier, 2012). When the right ventricle contracts, the pressure forces the tricuspid valve closed, preventing backflow of blood and pushing the blood through the pulmonary valve into the pulmonary artery (Pappano & Wier, 2012). This period of ventricular contraction is called systole; normal right ventricular systolic pressure is between 15 and 30 mmHg (Pappano & Wier, 2012). Blood flows from the pulmonary arteries at a normal resting pressure between 12 and 16 mmHg into the pulmonary alveolar capillary plexuses surrounding the alveoli in the lungs (West, 2012). The pulmonary capillaries is where gas exchange occurs between the blood and the inspired air, with CO<sub>2</sub> diffusing out of the blood and O<sub>2</sub> diffusing into the blood and bound by hemoglobin (West, 2012). The oxygenated blood then moves into the pulmonary veins and into the left side of the heart (Pappano & Weir, 2012).



**Figure 1.** Blood flow through heart and pulmonary vessels. (A) Blood from the body collects in the right atrium moves into the right ventricle before it is pumped into the pulmonary arteries. It then moves through the pulmonary vessels and collects in the left atrium before moving into the left ventricle where it is pumped back into the body. (B) Blood from the right ventricle is pumped into the pulmonary artery where it moves into the alveolar capillary plexus to be oxygenated. It then moves into the pulmonary veins and collects in the left atrium of the heart. Adapted from Moore, Agur, & Dalley, 2011.

## **Primary Pulmonary Hypertension**

Pulmonary hypertension (PH) is defined as a mean pulmonary arterial pressure (mPAP) that is elevated above 25 mmHg at rest (Hoeper et al., 2013). The most common cause of primary PH is left-sided heart disease, which leads to an increase in pulmonary venous pressure, and results in an increase in post-capillary PVR (Lerner and Mandel, 2014). This form of pulmonary hypertension is referred to as pulmonary venous hypertension. However, primary PH can also be caused by pathological changes in the pulmonary arteries. These arterial changes lead to an increase in pre-capillary PVR and can ultimately result in right-sided heart failure and is referred to as pulmonary arterial hypertension (PAH) (Runo & Loyd, 2003).

### *Etiology and Histology of Pulmonary Hypertension*

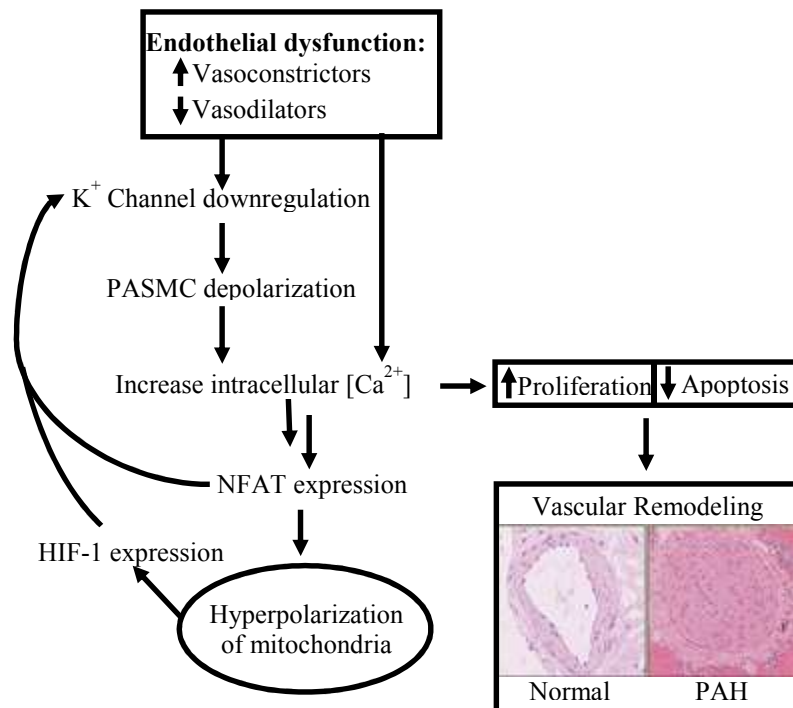
Pulmonary venous hypertension has three etiologies (Lerner and Mandel, 2014). The first is systolic heart failure caused by coronary artery disease, infiltrative disease, and congenital heart disease (Lerner and Mandel, 2014). Next is heart failure with preserved ejection fraction, meaning that the ejection fraction is normal, but there is abnormal left ventricular relaxation, filling, and distensibility (Lerner and Mandel, 2014). Finally, it can be caused by valvular disease, mainly mitral stenosis and aortic stenosis (Wilson et al., 2012; Lerner and Mandel, 2014).

All etiologies of pulmonary venous hypertension lead to passive PH (Lerner and Mandel, 2014). In passive PH the left atrial pressure is passively transmitted to the pulmonary artery system, causing the pulmonary capillary wedge pressure to increase

while the PVR remains normal (Guazzi and Borlaug, 2012). Chronic passive PH can progress to reactive PH, where both the pulmonary capillary wedge pressure and the PVR are increased (Guazzi and Borlaug, 2012). Reactive PH is characterized by congestive vasculopathy caused by cellular wall remodeling, impaired vascular reactivity, endothelial dysfunction, and abnormal smooth muscle tone (Guazzi and Borlaug, 2012; Guazzi, 2008; Delgado et al., 2005). Characteristic histological changes include medial hypertrophy, abnormalities in the elastic fibers of the arterial walls, and intimal fibrosis (Delgado et al., 2005). It is also characterized by arterialization of the pulmonary veins through growth factors, glycoproteins, and serine elastase causing smooth muscle growth (Graham et al., 2011).

The increased PVR seen in PAH is caused by a decrease in the lumen cross-sectional area of the pulmonary vasculature (McLaughlin et al., 2009). In the majority of cases, this is caused by excessive pulmonary arterial smooth muscle cells (PASMC) and endothelial cell proliferation and a decreased rate of apoptosis, which in turn leads to vascular remodeling (Figure 2) (McLaughlin et al., 2009). Studies have shown that abnormal hyperpolarization of mitochondria leads to the inappropriate activation of transcription factor HIF-1 while activated T-cells cause activation of transcription factor NFAT (Bonnet et al., 2006; Bonnet et al., 2007). The inappropriate activation of these transcription factors leads to the down-regulation of the voltage gated potassium channel, Kv1.5, in PASMC, which in turn causes depolarization of the PASMC and a subsequent influx of free  $Ca^{2+}$  through voltage gated calcium channels (Bonnet et al., 2006; Bonnet et al., 2007). The increased intracellular free calcium stimulates pulmonary

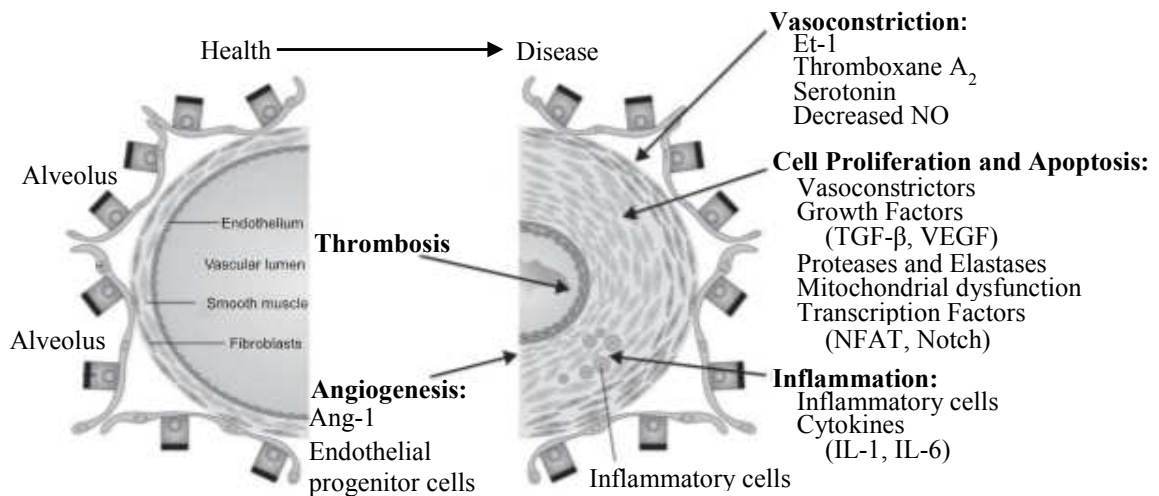
vasoconstriction and PASMC proliferation (Platoshyn et al., 2000). Apoptosis is also suppressed by de novo expression of the anti-apoptotic protein survivin in PASMC (McMurtry et al., 2005). Endothelial injury and inflammation may also play a role in vessel wall remodeling and cell proliferation through the activation of pro-inflammatory cytokines IL-1 $\beta$  and IL-6, which promote thrombosis and are potent mitogens (McLaughlin et al., 2009; Eddahibi, Morrell, d'Ortho, Mæijje, & Adnot 2002).



**Figure 2.** Pathways leading to vascular remodeling in PH. Activated T-cells cause the increased expression of NFAT while abnormal hyperpolarization of mitochondria causes an increase in expression of HIF-1. NFAT and HIF-1 expression cause downregulation of potassium channels and subsequently increased PASMC depolarization and increased intracellular free-calcium that increases proliferation and decreases apoptosis of endothelial cells and smooth muscle cells. Adapted from Bonnet et al., 2006; Bonnet et al., 2007; and Mclaughlin et al., 2009.

In approximately 20% of PAH patients, excessive vasoconstriction significantly contributes to the decreased luminal cross-sectional area (Figure 3) (McLaughlin et al., 2009). There are 4 mechanisms that may contribute to this phenomenon. First, is a shift in balance has been found from prostacyclin, a vasodilator that inhibits platelet activation and has antiproliferative properties, towards thromboxane A<sub>2</sub>, a vasoconstrictor that promotes platelet activation and proliferation (Christman et al., 1992). This is supported by the decrease in prostacyclin synthase in the endothelial cells of small and medium sized pulmonary arteries of PH patients (Tuder et al., 1999). Second, reduced amounts of nitric oxide (NO), a vasodilator that contributes to basal pulmonary vascular tone, may also contribute to the excessive vasoconstriction in PAH (Stamler, Loh, Roddy, Currie, & Creager 1994; Celermajer, Dollery, Burch, & Deanfield. 1994). It has been found that PAH patients have reduced amounts of NO synthase (NOS) in their lungs, and NOS knockout mice have substantially increased PVR that causes PH (Giaid & Saleh, 1995; Steudel et al., 1997). Third, PAH patients have elevated plasma levels of Endothelin-1 (ET-1), a vasoconstrictor that also stimulates smooth muscle cell proliferation, and plasma levels of ET-1 correlate with severity and prognosis of PAH (Rubens et al., 2001). Fourth, plasma levels of serotonin, a vasoconstrictor that promotes PASMHC hypertrophy and hyperplasia, are increased in PAH patients (McLaughlin et al., 2009). A polymorphism in the gene for the serotonin transporter is present in approximate 70–80% of PH patients as opposed to 20–30% of the general population (Eddahibi et al., 2001). This polymorphism causes an increase in expression of the serotonin transporter in

platelets and lung tissue, though this likely indicates susceptibility to PH as opposed to causation (Eddahibi et al., 2001).



**Figure 3.** Effect of excessive vasoconstriction in PH. An imbalance in vasoconstrictors and vasodilators causes sustained and excessive constriction of pulmonary blood vessel, resulting in a decrease in luminal cross-sectional area. Adapted from Wilkins, 2012.

PAH patients have characteristic histological findings in the pulmonary arteries. These findings include intimal fibrosis, medial hypertrophy, and adventitial proliferation (Archer & Rich, 2000). These changes to the vasculature frequently lead to the obliteration of small arteries, and occasionally vasculitis or changes in walls of pulmonary veins (Archer & Rich, 2000). In many cases, there are plexiform lesions found distal to vascular obstructive lesions (Archer & Rich, 2000). Plexiform lesions are complex vascular structures containing many vascular channels remodeled from pulmonary arteries and composed of apoptosis-resistant myofibroblasts, PASMC, and possibly undifferentiated mesenchymal cells, lined with endothelial cells, and they are

considered to be a hallmark of PAH (Archer & Rich, 2000; Jonigk et al., 2011). These plexiform lesions show up-regulation of remodeling associated genes including HIF-1 $\alpha$ , TGF- $\beta$ , vascular endothelial growth factor- $\alpha$  (VEGF- $\alpha$ ), VEGF receptors, Ang-1, Tie-2, and THBS1 (Archer & Rich, 2000; Jonigk et al., 2011). They also show markers for vascular sprouting, a mechanism for creating new vasculature, including NOTCH and matrix metalloproteinases (Archer & Rich, 2000; Jonigk et al., 2011).

### *Diagnosis of Pulmonary Hypertension*

Symptoms of PH include: exertional dyspnea, fatigue, weakness, dyspnea at rest, edema, ascites, anorexia, chest pain, palpitations, or syncope (McGoon et al., 2004; Lourenço, Fontoura, Henriques-Coelho, & Leite-Moreira, 2012). The definitive diagnosis of PH requires the finding of mPAP above 25 mmHg at rest during right heart catheterization (McGoon et al., 2004). Given the cost and risks associated with right heart catheterization, non-invasive screening methods, namely transthoracic Doppler echocardiography, are used to screen for PH when it is suspected from clinical presentation. If PH is suspected, the American College of Cardiology Foundation and the American Heart Association recommend screening for PH with echocardiography (McLaughlin et al., 2009).

Doppler echocardiography can be used to approximate the mPAP. This is done through the use of the modified Bernoulli's formula:

$$sPAP = 4(TRV)^2 + RAP$$

where sPAP is the systolic pulmonary artery pressure, TRV is the tricuspid valve regurgitation velocity, and RAP is the right atrial pressure (Pyxaras et al., 2011). RAP is estimated through the use of inferior vena cava diameter and inferior vena cava collapsibility index (Beigel, Cercek, Luo, & Siegel, 2013). After the sPAP is estimated, it can be used to estimate the mPAP with the following equation:

$$\text{mPAP} = 0.61(\text{sPAP}) + 2 \text{ mmHg}$$

Other findings in echocardiography that may be suggestive of PH include right atrial enlargement, right ventricular dilation or hypertrophy, a right-to-left septum shift, and a TRV elevated to  $\geq 2.5$  m/s (Klings & Machado et al., 2014; McLaughlin et al., 2004).

Although echocardiography can be a useful screening tool for PH, it is imperfect. One study found that the positive predictive value for PH with TRV elevated to 2.5 m/s or above on Doppler echocardiography was only 25%, and improved to 64% when the TRV threshold was raised to 2.9 m/s or higher (Parent et al., 2011). Another study found that Doppler echocardiography was inaccurate in the estimation of sPAP when compared to right heart catheterization in 48% of cases (Fisher et al., 2009). Because of these imperfections, Doppler echocardiography remains useful for screening, but right heart catheterization remains the gold standard for diagnosis.

### *Classification of Pulmonary Hypertension*

The Fifth World Symposium on Pulmonary Hypertension established clinical classifications to categorize PH into five groups that share similar pathological findings, hemodynamic characteristics, and management (Table 1) (Simonneau et al., 2013). In

Group 1 PH, individuals have pulmonary arterial hypertension (PAH) in the setting of a normal left ventricular filling pressure; they often have a transpulmonary gradient elevated above 12 mmHg (Simonneau et al., 2013; Lourenço et al., 2012; Klings & Machado et al., 2014). The incidence of PAH is 2.4 to 7.6 cases per million per year, with a prevalence of 15 to 26 cases per million (Lourenço et al., 2012). Group 2 PH contains PH due to left-sided heart disease (Simonneau et al., 2013). This is also known as pulmonary venous hypertension and is characterized by elevated pulmonary venous pressures secondary to left atrial hypertension (Lerner & Mandel, 2014). It has the additional characteristic of a pulmonary capillary wedge pressure greater than 15 mmHg (Lerner & Mandel, 2014; Mehari et al., 2013; Klings & Machado et al., 2014). Group 3 contains pulmonary hypertension associated with lung disease or hypoxia, but the Fifth World Symposium on Pulmonary Hypertension did not distinguish if these were pulmonary venous hypertension or pulmonary arterial hypertension (Simonneau et al., 2013). Group 4 includes chronic thromboembolic pulmonary hypertension, which is pulmonary hypertension caused by pulmonary thromboemboli (Lang and Madani, 2014). Finally, Group 5 contains pulmonary hypertension with unclear multifactorial mechanisms including hematological disorders, systemic disorders, metabolic disorders, and other disorders (Simonneau et al., 2013). Pulmonary hypertension due to left heart disease (Group 2) is the most common of the different classes of pulmonary hypertension (Lerner & Mandel., 2014). However, there is little data regarding the incidence and prevalence for classes of pulmonary hypertension other than Group 1.

**Table 1.** Classification of Pulmonary Hypertension

<b>Classification</b>
Group 1: Pulmonary arterial hypertension 1.1 Idiopathic PAH 1.2 Heritable PAH 1.3 Drug and Toxin Induced PAH 1.4 PAH associated with: <ul style="list-style-type: none"><li>• Connective tissue disease</li><li>• HIV</li><li>• Portal hypertension</li><li>• Congenital Heart Disease</li><li>• Schistosomiasis</li></ul>
Group 1': Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomas Group 1'': Persistent pulmonary hypertension of the newborn
Group 2: Pulmonary hypertension due to left heart disease 2.1 Left ventricular systolic dysfunction 2.2 Left ventricular diastolic dysfunction 2.3 Valvular disease 2.4 Congenital or acquired left heart inflow or outflow tract obstruction and congenital cardiomyopathies
Group 3: Pulmonary hypertension due to lung disease and/or hypoxemia 3.1 Chronic obstructive pulmonary disease 3.2 Interstitial lung disease 3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern 3.4 Sleep-disordered breathing 3.5 Alveolar hypoventilation disorders 3.6 Chronic exposure to high altitudes 3.7 Developmental lung diseases
Group 4: Chronic thromboembolic pulmonary hypertension
Group 5: Pulmonary hypertension with unclear multifactorial mechanisms 5.1 Hematologic disorders: <ul style="list-style-type: none"><li>• Chronic hemolytic anemia</li><li>• Myeloproliferative disorders</li><li>• Splenectomy</li></ul> 5.2 Systemic disorders: <ul style="list-style-type: none"><li>• Sarcoidosis</li><li>• Pulmonary histiocytosis</li><li>• Lymphangioleiomyomatosis</li></ul> 5.3 Metabolic disorders: <ul style="list-style-type: none"><li>• Glycogen storage disease</li><li>• Gaucher disease</li><li>• Thyroid disorders</li></ul> 5.4 Other: <ul style="list-style-type: none"><li>• Tumoral obstruction</li><li>• Fibrosing mediastinitis</li><li>• Chronic renal failure</li><li>• Segmental PH</li></ul>

Adapted from Simonneau et al., 2013

### *Prognosis*

Prognosis after diagnosis of PH through right heart catheterization is variable based on the underlying etiology (McLaughlin et al., 2009). For primary PH of all etiologies, the survival rate is 79% one year after diagnosis, and declines to 29% four years after a diagnosis is made (McLaughlin et al., 2004). Idiopathic PAH (Group 1) and PAH related to HIV (Group 1) have similar prognoses, with one-year survival and three-year survival of 77% and 35%, respectively (McLaughlin et al., 2009). Those with PAH related to the scleroderma spectrum of diseases (Group 1) have a much worse prognosis, with only 40% survival two years after diagnosis (McLaughlin et al., 2009). However, those with PAH related to congenital heart disease (Group 1) have a much better prognosis with 77% survival after 3 years (McLaughlin et al., 2009).

There are several factors that contribute to the prediction of poor prognosis across all etiologies of PH. One of these predictors is the World Health Organization's functional classifications, where a more advanced functional class indicates higher impact on the patient's life and a worse prognosis (Table 2) (Taichman et al., 2014). Other predictors of poor prognosis include poor exercise capacity indicated by 6-minute walk distance test or cardiopulmonary exercise test, high right atrial pressure, significant right ventricular dysfunction, evidence of right ventricular failure, low cardiac index, or elevated brain natriuretic peptide (McLaughlin et al., 2009).

**Table 2.** World Health Organization Functional Classifications for Pulmonary Hypertension

Class I	PH does not limit physical activity. Ordinary physical does not cause undue dyspnea, fatigue, chest pain, or near syncope.
Class II	PH causes slight limitation of physical activity, though the patient is comfortable at rest. Ordinary physical activity may cause undue dyspnea or fatigue, chest pain or near syncope.
Class III	PH causes severe limitation of physical activity, though the patient is comfortable at rest. Less than ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope.
Class IV	PH causes the inability to carryout any physical activity, and may show signs of right heart failure. Dyspnea and/or fatigue may be present at rest, and discomfort is increased with any physical activity.

Adapted from Taichman et al., 2014

### *Treatment of Pulmonary Hypertension*

Current treatment for PH can be both for management of symptoms and for the underlying etiology. For all five groups of PH, the American College of Cardiology Foundation and the American Heart Association recommend as general measures low level graded aerobic exercise such as walking, a sodium restricted diet (less than 2,400 mg per day), obtaining routine vaccinations such as influenza and pneumococcal pneumonia, and avoiding pregnancy (McLaughlin et al., 2009). The American College of Cardiology and the American Heart Association also recommend diuretics for managing right ventricular volume overload, calcium channel blockers if the patient has a positive acute vasodilator response, and warfarin anticoagulation therapy for all patients with idiopathic PAH (Group 1) (McLaughlin et al., 2009). One study found that with pharmaceutical therapy targeted to PAH (Group 1) there was an 85% survival rate within the first year (Thenappan, Shah, Rich, & Gomberg-Maitland 2007).

For patients with Group 1 PH there are targeted PAH therapies available that include several classes of pharmaceuticals including prostanoids, endothelial receptor

antagonists, Phosphodiesterase-5 (PDE-5) inhibitors, and soluble guanylate cyclase stimulators (Figure 4) (McLaughlin et al., 2009). The first class of pharmaceuticals used for PAH targeted therapy is prostanoids. Prostanoids are synthetic analogues of prostacyclin, also called prostaglandin I<sub>2</sub> (Humbert & Ghofrani, 2015). Prostaglandin I<sub>2</sub> is naturally produced by endothelial cells and acts by binding to the IP receptors expressed in vascular smooth muscle cells, which initiates the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (Humbert & Ghofrani, 2015). The cAMP acts as a second messenger and increases protein kinase A activity, which in turn initiates downstream effects that culminate in vasodilation (Humbert & Ghofrani, 2015). Prostaglandin I<sub>2</sub> also has antithrombotic, antiproliferative, antimitogenic, and immunomodulatory properties (Humbert & Ghofrani, 2015; Clapp et al., 2002; Akagi et al., 2013). Prostanoids currently approved for use in PAH patients include epoprostenol, treprostinil, iloprost, and beraprost (Humbert & Ghofrani, 2015). Selexipag is another prostanoid, but it is in clinical trials and has not yet been approved (Humbert & Ghofrani, 2015).

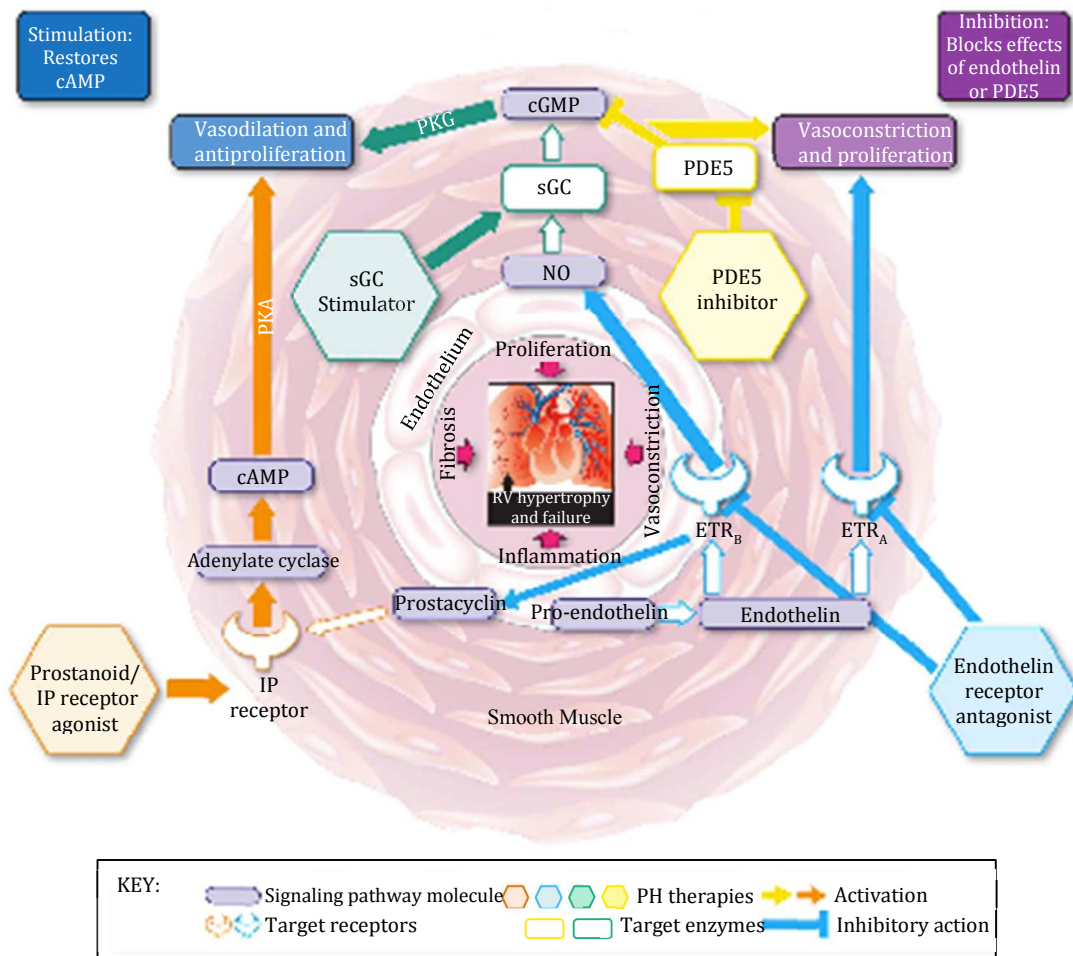
Endothelin receptor antagonists work by blocking endothelin receptor A and endothelin receptor B from being bound by endothelin-1, a potent vasoconstrictor that also promotes PASMC proliferation (Motte, McEntee & Naeije, 2006). Endothelin receptor A is mainly located in vascular smooth muscle cells, while endothelin receptor B is in both endothelial cells and vascular smooth muscle cells; both contribute to vasoconstriction and PASMC proliferation (Motte, McEntee & Naeije, 2006). Drugs in

this class of pharmaceuticals include bosentan, ambrisentan, and macitentan (Humbert & Ghofrani, 2015).

The next class of pharmaceuticals is PDE-5 inhibitors. PDE-5 is highly expressed in the pulmonary vascular smooth muscle as well as in right ventricle cardiomyocytes, and converts cyclic guanosine monophosphate (cGMP) to guanosine monophosphate (GMP), preventing cGMP's function as a second messenger to activate protein kinase G in order to trigger vasodilation and inhibit proliferation (Wilkins, Wharton, Grimminger, & Ghofrani, 2008). PDE-5 inhibitors prevent the degradation of cGMP and in turn promote vasodilation and inhibition of pulmonary vasculature smooth muscle cell proliferation (Wilkins et al., 2008). Drugs within this class of pharmaceuticals include sildenafil and tadalafil (Humbert & Ghofrani et al., 2015).

The final class of pharmaceuticals approved for treatment of PAH is soluble guanylate cyclase stimulators. Soluble guanylate cyclase is an enzyme that binds NO and then converts guanosine triphosphate (GTP) to cGMP, which then acts as a second messenger to trigger vasodilation and inhibit vascular smooth muscle cell proliferation (Wilkins et al., 2008). Soluble guanylate cyclase stimulators act in two ways, first they sensitize soluble guanylate cyclase to endogenous NO by stabilizing the soluble guanylate cyclase-NO bond, second they directly stimulate soluble guanylate cyclase independent of NO in order to increase the generation of cGMP (Humbert & Ghofrani, 2015). Currently, the only drug in this class of pharmaceuticals that has been approved for the treatment of PAH is riociguat (Humbert & Ghofrani, 2015). The World Health Organization recommends sequential combination therapy for the treatment of PAH,

meaning that a patient’s physician will add additional pharmaceuticals from other classes to the patient’s current treatment plan in order to improve their overall treatment (Humbert & Ghofrani, 2015). There have been several trials with sequential combination therapies showing varied results, these trials are summarized in Table 3.



**Figure 4.** The molecular targets of approved PAH therapies. Prostanoids act by binding the IP receptor, and ultimately results in vasodilation and antiproliferative actions. Endothelin receptor antagonists work by inhibiting endothelin receptor A (ETRA) and endothelin receptor B (ETRB), which prevents vasoconstriction and PASM proliferation. PDE5 inhibitors prevent the degradation of cGMP into GMP, and in turn prevent vasoconstriction and PASM proliferation. Soluble guanylate cyclase (sGC) stimulators promote the formation of cGMP, which in turn promotes vasodilation and prevents proliferation. Adapted from Humbert & Ghofrani, 2015.

**Table 3.** Summary of sequential combination therapy trials

Successful Trials	Unsuccessful Trials
PACES: epoprostenol+ sildenafil (Simonneau et al., 2008)	FREEDOM-C: ERAs or PDE-5 inhibitors + treprostinil (Tapson et al., 2012)
TRIUMPH: bosentan + treprostinil (McLaughlin et al., 2010)	PHIRST: bosentan + tadalafil (Galie et al., 2009)
PATENT: ERAs or non-IV prostanoids + riociguat (Ghofrani et al., 2013)	COMPASS-2: sildenafil + bosentan (Humbert & Ghofrani, 2015)
SERAPHIN: PDE-5 inhibitor or non-IV prostanoid + macitentan (Pulido et al., 2013)	NCT00323297: bosentan + sildenafil (Pfizer, 2014)
STEP: bosentan + iloprost (McLaughlin et al., 2006)	PATENT PLUS: sildenafil + riociguat (Galie et al., 2015)
GRIPHON: PDE-5 inhibitors or ERAs + selexipag (McLaughlin et al., 2015)	

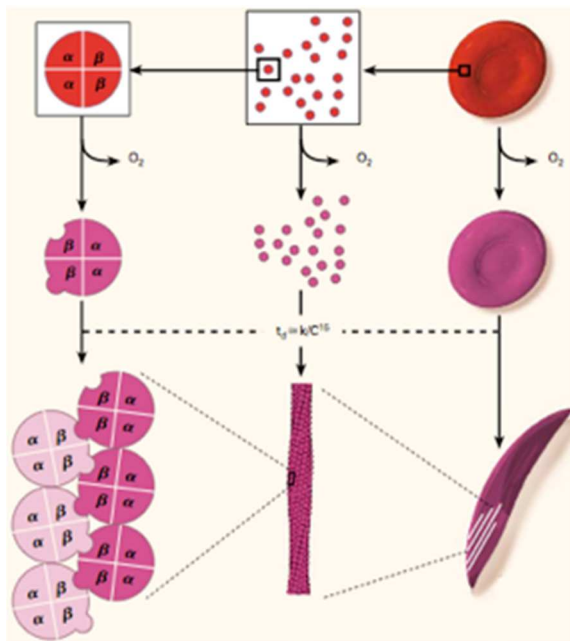
Adapted from Humbert & Ghofrani et al., 2015

For patients with Group 2 PH it is recommended to treat the underlying left heart disease (McLaughlin et al., 2009). Patients with Group 3 PH are recommended to treat the underlying chronic lung disease and hypoxemia (McLaughlin et al., 2009). Patients with Group 4 PH should be treated with anticoagulation therapy and may be considered for treatment with pulmonary thromboendarterectomy, a surgical procedure that removes thrombemboli from the pulmonary arteries in order to substantially lower the PVR and improve cardiac output (Hoeper, Mayer, Simonneau, & Rubin, 2006). Treatment for group 5 PH varies based on the underlying cause. For example, patients with SCD are recommended to use SCD specific therapies such as hydroxyurea (HU) and chronic blood transfusions, and PAH targeted therapy is only recommended for individuals whose symptoms suggest that PAH-targeted therapies may have clinical benefits (Klings & Machado et al., 2014).

## Sickle Cell Disease

### *Genetics and Pathology*

Normal adult hemoglobin (Hb A) protein is composed of four globular subunits, two  $\alpha$ -chains and two  $\beta$ -chains. Each subunit is composed of a protein chain and a heme group; the heme group contains an iron ion that reversibly binds oxygen, allowing oxygen to be transported from the lungs to the rest of the body (Marengo-Rowe, 2006). There are many variants of hemoglobin, though most are clinically insignificant and rare (Thom, Dickson, Gell, & Weiss, 2013).



**Figure 5.** Hemoglobin S. S causes red blood cells to change from the normal biconcave disk to a sickle shape when oxygen is lost. Adapted from Bunn, 1997.

SCD is a term used to describe a group of genetic diseases that affect the hemoglobin within red blood cells. In SCD, at least one copy of the gene for the  $\beta$ -chain contains a mutation that causes glutamic acid, the amino acid in the sixth position, to be replaced with valine (Bunn, 1997), and is referred to as sickle hemoglobin (Hb S) (Stuart & Nagel, 2004). This mutation causes a hydrophobic motif that allows the  $\beta$ 1 chain from one hemoglobin molecule to bind

with a  $\beta$ 2 chain from another hemoglobin molecule when they are in a deoxygenated state, forming long rods within the red blood cell (Figure 5) (Stuart & Nagel, 2004). This

causes the red blood cell to change from its normal biconcave disc into a sickle-shaped cell (Stuart & Nagel, 2004). These sickled cells have a decreased oxygen carrying capacity and can occlude blood vessels by adhering to endothelial cells in post-capillary venules as well as leukocytes causing the formation of a heterocellular aggregate, resulting in tissue hypoxia (Stuart & Nagel, 2004). This occlusion of the microvasculature (i.e., vaso-occlusion) can occur anywhere in the body and can affect any organ (Stuart & Nagel, 2004). The combination of Hb S with any variant that is not Hb A results in SCD (Driscoll, 2007).

Patients that are homozygous for the Hb S gene (Hb SS genotype) typically have the most severe form of the disease. Red blood cells exclusively contain Hb S and have the potential to polymerize and cause the cell to form the sickle shape, and the rate and extent of polymerization is proportional to the duration and extent of deoxygenation (Rees, Williams, & Gladwin, 2010). The Hb SS genotype reduces the lifespan of a red blood cell from 100 - 120 days to 7 – 12 days (Bender & Hobbs, 2012).

The Hb SC genotype tends to be clinically milder than the Hb SS genotype. In this form of SCD, the patient is a double heterozygote for Hb S and Hb C, meaning that they inherit the Hb S gene from one parent and the Hb C gene from the other parent (Stuart & Nagel, 2004). In the Hb C mutation, the amino acid lysine replaces glutamic acid at the sixth position in the  $\beta$ -chain (Nagel, Fabry, & Steinberg, 2003). Hb C causes the red blood cell to lose water and potassium ions ( $K^+$ ) due to high potassium-chloride co-transport activity, which causes dehydration of the red blood cell and concentration of the hemoglobin (Nagel, Fabry, & Steinberg, 2003). This dehydration and subsequent

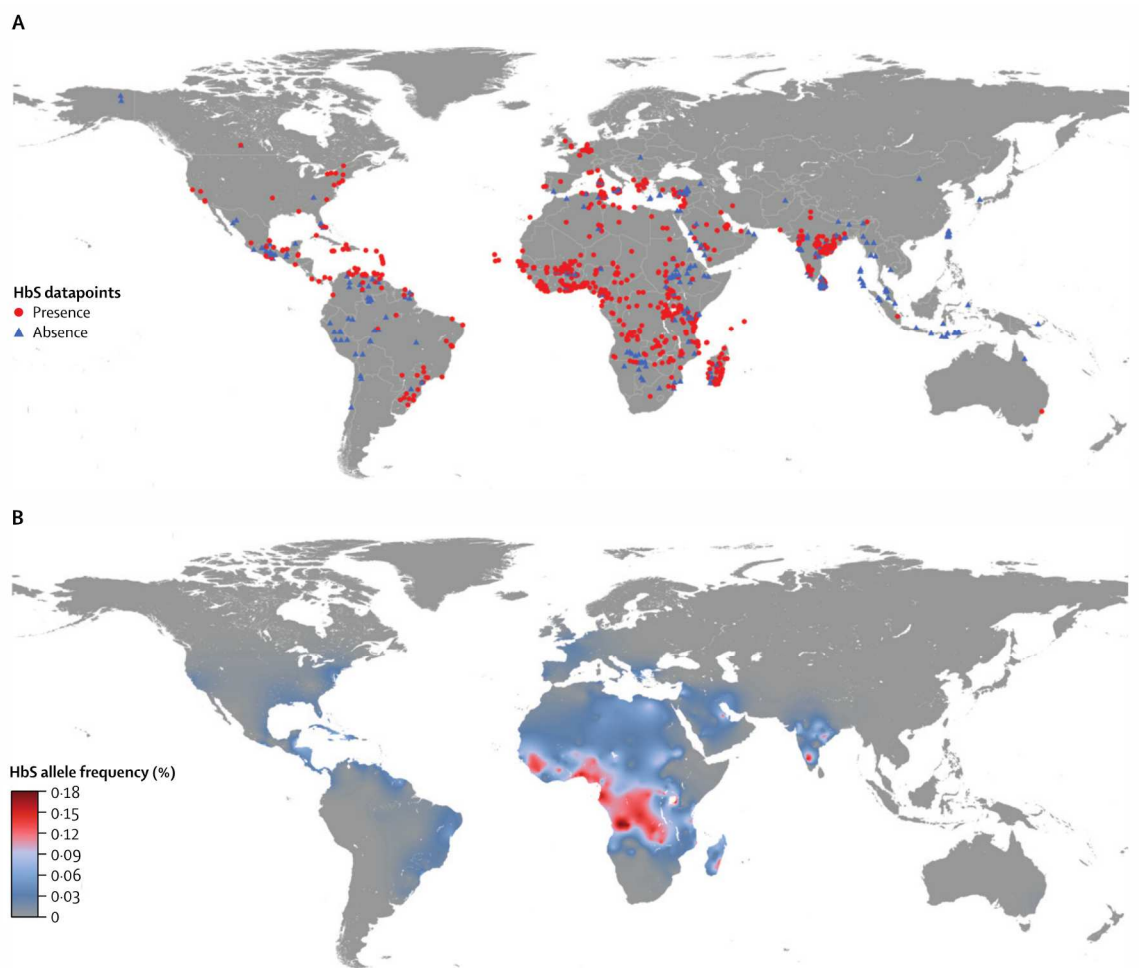
concentration of hemoglobin increases the tendency of Hb S to polymerize and the red blood cells to sickle (Nagel, Fabry, & Steinberg, 2003).

In the Hb S $\beta$ -thalassemia genotypes, the patient is a double heterozygote for HbS and  $\beta$ -thalassemia.  $\beta$ -thalassemia is a disease that causes either decreased or no production of the  $\beta$ -chain, resulting in either a reduced number of normal hemoglobin ( $\beta^+$ -thalassemia) or no normal hemoglobin ( $\beta^0$ -thalassemia) (Galanello & Origa, 2010). Patients with the Hb S $\beta^+$ -thalassemia genotype will have a reduction of normal  $\beta$ -chains in their hemoglobin, which results in a clinical course similar to that of the Hb SC genotype (Clarke & Higgins, 2000; Galanello & Origa, 2010). Those with the Hb S $\beta^0$ -thalassemia genotype produce no normal hemoglobin, resulting in a more severe clinical course that mimics the Hb SS genotype (Clarke & Higgins, 2000; Galanello & Origa, 2010).

### *Prevalence*

SCD is the most common monogenic disease worldwide (Weatehrall, Hofman, Rodgers, Ruffin, & Hrynkow, 2005). It is widespread in sub-Saharan Africa, and in the Middle East and India, with Hb S carrier frequencies ranging from 5–40% of the population in those regions (Figure 6) (Weatherall & Clegg, 2001). SCD is also seen in variable frequencies in countries such as the Brazil, Caribbean countries, and Central American countries where there have been major population movements from African countries, with an estimated frequency of heterozygotes between 5 and 25% (Weatherall et al., 2005). In the United States, approximately 89,000 individuals are affected

(Brousseau, Panepinto, Nimmer, & Hoffmann, 2010). In the United States of America, SCD mainly affects individuals of African and Hispanic descent, with an incidence of 73.1 per 1,000 African American newborns and 6.9 per 1,000 Hispanic newborns, compared to 3.0 per 1,000 white newborns (Ojodu, Hulihan, Pope, & Grant, 2014).



**Figure 6.** Hb S allele frequency worldwide. The allele frequency is highest in sub-Saharan Africa, but varies in areas where there have been large populations movements from African countries. Adapted from Piel et al., 2013.

### *Complications*

SCD is a progressive, debilitating disease with many complications (Table 4). Complications of SCD arise in infancy, as levels of fetal hemoglobin decline and the levels of Hb S increase, between 6 and 9 months of age (Oneal et al., 2006). When cells containing Hb S enter into areas of the body with lower blood oxygen levels they can take the sickle shape, adhere to blood vessel as well as to leukocytes, and create an occlusion of the microvasculature called vaso-occlusive events (VOEs) (Stuart & Nagel, 2004; Steinberg, 1999). These VOEs are responsible for acute episodes of severe bony pain, commonly located in the extremities, back, and chest (Steinberg, 1999). Vaso-occlusion in other organs can lead to severe, life-threatening complications, including acute chest syndrome (ACS), stroke, and splenic sequestration (Steinberg, 1999). Other vaso-occlusive complications include priapism which often leads to impotence; chronic kidney damage or nephropathy; microvascular eye disease or retinopathy; leg ulcers, and avascular necrosis of the hips or shoulders (Bender & Hobbs, 2012; Rees et al., 2010; Gladwin & Vichinsky, 2008; Steinberg, 1999).

Red blood cells containing sickle hemoglobin are abnormally fragile, lasting 7-14 days compared to the 120-day lifespan of a normal red blood cell (Steinberg, 1999). This high rate of red blood cell breakdown, or hemolysis, is also associated with complications of SCD, including anemia; cholelithiasis, due to the excess bilirubin released from the red blood cells; and acute aplastic episodes, a form of anemia where the bone marrow is not producing enough red blood cells (Steinberg, 1999).

**Table 4.** Common complications of SCD

<b>Complication</b>	<b>Features</b>	<b>Frequency</b>
<b>Vaso-occlusive complications</b>		
Vaso-occlusive events	Pain caused by entrapment of red blood cells and leukocytes in the microcirculation resulting in vascular obstruction and ischemia.	More than 70% of all patients with SCD
Acute chest syndrome	A new alveolar pulmonary infiltrate that is caused by a combination of infection, fat embolism from the bone marrow, and vaso-occlusion of the pulmonary vasculature.	40% of all patients with SCD
Stroke	Death of brain cells caused by ischemia in the brain.	10 % of children with SCD
Splenic sequestration	An acutely enlarged spleen with hemoglobin more than 2g/dL below the baseline. Can rapidly progress to shock and death	Occurs in 10 – 30% of children with SCD
Priapism	Painful and unwanted erections that occur spontaneously, during nocturnal erections, or with fever and dehydration	10 – 40% of men with SCD
Nephropathy	Damage to the kidneys or kidney disease	5 – 20% of adults with SCD
Retinopathy	Damage to the retina, the light sensing portion of the eye	Rare in Hb SS, but occurs in 50% of adults with Hb SC
Leg ulcers	A slow-healing break in the skin of the leg	20% of adults with Hb SS
Avascular necrosis	Death of bone tissue due to ischemia	10 – 50% of adults with SCD
<b>Complications of Hemolysis</b>		
Anemia	A temporary interruption of red blood cell production.	
Cholelithiasis	Gallstones, hardened deposits in the biliary tract.	Present in most adults, though often asymptomatic.
Acute Aplastic Episodes	Rapidly occurring severe anemia caused by infection from parovirus B19	
<b>Infectious Complications</b>		
Streptococcus pneumoniae sepsis	An overwhelming immune response to a bacterial infection, can be life threatening	10% of children younger than 5 years old with Hb SS

Bender & Hobbs, 2012; Rees et al., 2010; Gladwin & Vichinsky, 2008; Vichinsky et al., 2000; Ohene-Frempong et al., 1998; Steinberg, 1999

### *Mortality*

Patients with SCD often have an early mortality; the median life expectancy is 42 years for males and 48 years for women with the Hb SS genotype, and the medium life expectancy is 60 years for males and 68 years for females with the Hb SC genotype (Platt et al., 1994). Approximately 19% of these deaths occur during a pain episode, 28% were related to pulmonary complications, and 16% were caused by kidney failure (Hamideh & Alvarez, 2013). SCD also leaves patients susceptible to infection and related complications, including sepsis and bacteremia, which was a leading cause of death in infants with Hb SS prior to the introduction of more effective vaccines over the past 15 years (Steinberg, 1999).

### *Treatments for SCD*

Current treatments for SCD include HU and chronic blood transfusions. HU has multiple mechanisms that contribute to its therapeutic effects for SCD, including increasing the production of fetal hemoglobin (Hb F) (Green & Barral, 2014). Hb F is composed of two  $\alpha$ -chains and two  $\gamma$ -chains rather than  $\beta$ -chains (Platt, 2008). In fetuses, Hb F is expressed in order to deliver oxygen to the tissues of the unborn child; but toward the end of fetal development the adult form of hemoglobin is expressed and the amount of Hb F declines (Oneal et al., 2006). By the time the child is six months old, little Hb F is found in the blood (Oneal et al., 2006). However, some individuals have mutations that cause the expression of Hb F to continue into adulthood (Bank, 2006; Akinsheye et al., 2011). The Cooperative Study of Sickle Cell Disease, the largest cohort study of SCD in

the US which followed 3751 individuals from 1979 to 1988, found that higher Hb F levels were inversely related to the incidence of VOE related pain crises and ACS (Vichinsky et al., 1997; Castro et al., 1994). It is hypothesized that the presence of fetal hemoglobin helps to reduce the concentration of Hb S and makes polymerization of the hemoglobin molecules less likely to occur (Rees et al., 2010). These findings led to the use of HU for the treatment of SCD (Platt et al., 1984). HU is a cytotoxic drug that was initially used to treat polycythemia vera by inhibiting cell division and therefore reducing the abnormally high hematocrit and platelet count (Platt, 2008). The benefit of HU use in SCD is threefold. First, it decreases the levels of Hb S containing red blood cells because they are produced by rapidly dividing progenitor cells, resulting in the increased production of Hb F-containing red blood cells, since they arise from progenitor cells that divide more slowly (Platt, 2008; Green & Barral, 2014). Second, as a cytotoxic agent, HU decreases the production of white blood cells and platelets, which are instrumental in pro-inflammatory cellular adhesion and vascular injury (Platt, 2008; Green & Barral, 2014). Third, as HU is metabolized, it stimulates the production of NO, which in turn binds soluble guanylate cyclase to stimulate both vasodilation and Hb F production (Platt, 2008). Through all of these mechanisms of action, HU therapy decreases the number of VOEs, decreases the number of ACS events, and reduces mortality associated with SCD (Charache et al., 1995; Steinberg et al., 2003).

Chronic blood transfusions have been shown to reduce the risk of stroke, reduce the number of ACS events, and reduce the frequency of VOEs (Hirst & Wang, 2002; Miller et al., 2001). The STOP I and STOP II trials showed that regularly performed

blood transfusions every three to four weeks maintained levels of Hb S below 30% of total circulating blood and was able to reduce the risk of first stroke occurring by 70% (Adams, et al., 1998). However, chronic blood transfusions carry the risk of iron overload, serious allergic reactions, hemolytic reaction, volume overload, and alloimmunization (Klings & Machado et al., 2014).

### **Pulmonary Hypertension Secondary to SCD**

SCD-associated cor pulmonale, or right-sided heart enlargement caused by disease in the lungs or pulmonary vasculature, was first described in literature in 1936 (Yater & Hansmann, 1936). However, the first cases of PH in SCD confirmed by right heart catheterization was not published until 1966 (Rubler & Fleisher, 1966). This report found biventricular hypertrophy, elevated pulmonary vascular resistance, absence of significant valvular disease, and sudden death caused by pulmonary infarction in two patients in their 40's with SCD (Rubler & Fleisher, 1966). More recent studies have found that 6–11% of adult patients have pulmonary hypertension confirmed with right heart catheterization, however, approximately 30% of adults with the Hb SS genotype and 10–25% of adults with the Hb SC genotype have a TRV  $\geq 2.5$  m/s which is suggestive of PH but is not adequate to diagnose PH (Klings & Machado et al., 2014).

PH secondary to SCD has characteristics of both PAH and pulmonary venous hypertension, and is part of Group 5 PH (Table 1) (Simonneau et al., 2013, Klings & Machado, et al., 2014; Mehari et al., 2013). Hemodynamic features include a mPAP  $\geq 25$  mmHg, pulmonary artery wedge pressure  $\geq 15$  mmHg, an increased PVR due to the

lower baseline produced by the anemia induced cardiac output and low blood viscosity, and a TPG > 12 mmHg (Klings & Machado et al., 2014; Mehari et al., 2013). There are many proposed etiologies, including: 1) a sickle cell related vasculopathy resulting in hyperplasia of the intima and in situ thrombosis; 2) chronic hypoxemia that is exacerbated by sleep hypoventilation; 3) parenchymal and vascular injury resulting from repeated episodes of ACS; 4) systemic scavenging of NO by plasma free hemoglobin as a result of hemolysis; 5) elevated arginase activity causing a decrease in the arginase/ornithine ratio, resulting in a reduced amount of NO production (Oppenheimer & Esterly, 1971; Adedeji, Cespedes, Allen, Subramony, & Hughson, 2001; Castro, 1996; Ataga et al., 2004; Reiter et al., 2002; Jison & Gladwin, 2003; Morris et al., 2003). It is very likely that PH in SCD is caused by combinations of these proposed mechanisms (Oppenheimer et al., 1971; Adedeji et al., 2001; Castro, 1996; Ataga et al., 2004; Reiter et al., 2002; Jison & Gladwin, 2003; Morris et al., 2003).

There are many findings associated with secondary PH with SCD. There is an associated decrease in prostacyclins and NO (vasodilators) and an increase in vasoconstrictors (prostaglandins and endothelins), which is also typical of primary PH (Christman et al., 1992). Laboratory markers and clinical measures associated with PH in SCD are summarized in Table 5.

**Table 5.** Laboratory markers and clinical values associated with secondary PH with SCD

Increased laboratory values	Blood urea nitrogen
	Lactate dehydrogenase (LDH)
	Uric acid
	$\gamma$ -glutamyltransferase
	Direct bilirubin
	Indirect bilirubin
	Aspartate aminotransferase
	Iron
	Ferritin
	Reticulocyte count
Decreased laboratory values	Platelet count
	Hemoglobin level
	Hematocrit
	transferritin
Clinical values	Proteinuria
	Shorter six-minute walk distance
	$\geq 10$ blood transfusions
	Older age
	History of sepsis/bacteremia
	History of ACS
	History of asthma

(Fonesca, Souza, Salemi, Jardim, & Gualandro, 2012; Ataga et al., 2006; Gladwin et al., 2004; Hagar et al., 2007; Minniti et al., 2009)

There is an increased morbidity and mortality for SCD patients with PH. Factors associated with increased mortality include a mPAP  $\geq 25$  mmHg, transpulmonary gradient  $\geq 12$  mmHg, 6 minute walk distance  $< 400$  meters, WHO functional class of III or IV, and a TRV  $\geq 2.5$  m/s (Mehari et al., 2013; Gladwin et al., 2004). With a TRV elevated to between 2.5 and 2.9 m/s there is a rate ratio of mortality of 4.4, and that raises to 10.6 when TRV is elevated above 2.9 m/s (Gladwin & Vichinsky, 2008; Klings & Machado et al., 2014).

There are several treatment options for PH associated with SCD. The first treatment option is HU. In small studies, HU was found to decrease TRV as well as a decrease baseline LDH and reticulocyte count (Olmes et al., 2009). In addition, HU has an NO donor effect, enabling vasodilation (Cokic et al., 2006; Cocik, Andric, Stojilkovic, Noguchi, & Schechter, 2008; Huang, Kim-Shapiro, & King, 2004). An *in vitro* study showed that HU enhances phosphorylation of endothelial cell NOS, resulting in increased NOS activity and increased NO production (Cokic et al., 2006). Another study found *in vivo* evidence that HU induces the NO/cGMP pathway in endothelial progenitor cells (Cokic et al., 2008). HU has also been found to interact with catalase in the presence of H<sub>2</sub>O<sub>2</sub> to produce a ferrous-NO catalase complex that subsequently releases NO (Huang et al., 2004). There has been shown to be a lower prevalence of PH in SCD patients that are taking HU (Ataga et al., 2004).

Chronic transfusion therapy is another treatment option. It has been shown that chronic transfusion therapy reduces Hb S to less than 50% within weeks (Klings & Machado et al., 2014). The American Thoracic Society extrapolates that chronic transfusion therapy will reduce mortality from PH by reducing ACS, VOE, and hemolysis (Klings & Machado et al., 2014).

The American Thoracic Society suggests the use PAH specific therapies when the patient's symptoms suggest that these pharmaceuticals may yield clinical benefit (Klings & Machado et al., 2014). A prostacyclin agonist or an endothelin receptor antagonist are recommended for SCD patients with elevated PVR and normal pulmonary artery wedge pressure (Klings & Machado et al., 2014). In 2009 the ASSET trials studied the use of

bosentan, and endothelin-1 receptor antagonist, but stopped the trial early due to low enrollment (n = 26) (Barst et al., 2008). However, they did find a non-significant increase in cardiac output on bosentan compared to placebo, and that the drug was well tolerated at standard dose without an increase in VOE or other events (Barst et al., 2008). A small retrospective study (n = 14) done after the ASSET trials showed that there was a statistically significant increase in 6 minute walk distance and a trend of decreased TRV with the use of either bosentan or ambrisentan, both endothelin receptor antagonists (Minniti et al., 2009). However, the American Thoracic Society recommends against the use of Sildenafil, a PDE-5 inhibitor (Klings & Machado et al., 2014). Although small studies showed that sildenafil was able to improve exercise capacity, lower systolic pulmonary artery pressure, lower TRV, and improve functional class, the Walk-PHaSST trial was ended early because there was an increase in hospitalization rates for pain in patients taking sildenafil compared to those on the placebo (Machado et al., 2005; Derchi et al., 2005; Machado et al., 2011).

There are conflicting data regarding the prevalence of PH in children with SCD. The American Thoracic Society reports that 10–20% of pediatric SCD patients have a TRV  $\geq 2.5$  m/s (Klings & Machado et al., 2014). However, a meta-analysis combining data from 11 small studies reports that 30% of pediatric patients with SCD have a TRV  $\geq 2.5$  m/s (Kato et al., 2007). Notably, many of these studies were done retrospectively with little information indicating why patients were screened, therefore subject to screening bias toward more clinically severe cases (Hagar, Michlitsch, Gardner, Vichinsky, & Morris, 2007; Kato et al., 2007).

Children with SCD and an elevated TRV of 2.5 m/s or higher are 13 years old on average and more likely to have the Hb SS genotype (Hagar et al., 2007; Kato et al., 2007; Gordeuk et al., 2011). It appears that TRV elevated to 2.5 m/s or above does not have the same impact on mortality in pediatric patients as it does in adults (Hagar et al., 2007; Kato et al., 2007; Gordeuk et al., 2011). It has been suggested that there is a different mechanism leading to an elevated TRV in children with SCD than there is in adults with SCD (Hagar et al., 2007; Kato et al., 2007; Gordeuk et al., 2011). When compared to adult populations, children with SCD and TRV  $\geq$  2.5 m/s are more likely to have had a history of sepsis or bacteremia, ACS, asthma or and other obstructive pulmonary disorders (Hagar et al., 2007; Kato et al., 2007; Gordeuk et al., 2011). In adults with SCD, TRV  $\geq$  2.5 m/s is associated with age, end organ disease, and hepatitis C (Hagar et al., 2007). However, both populations have been found to have an associated decrease in hemoglobin levels, increase in reticulocyte count, increase in bilirubin, and increase in LDH levels, indicating that hemolysis likely plays a role in both children and adults (Kato et al., 2007; Pashankar et al., 2008; Minniti et al., 2009). Treatment of PH in children has not been well studied. One small study has shown that TRV may be able to be lowered to below 2.5 m/s with early detection and treatment with HU (Klings & Machado et al., 2014; Hagar et al., 2007; Pashankar, Carbonella, Bazy-Asaad, & Friedman, 2008).

### **Aims of This Study**

Little information is available regarding PH in pediatric patients with SCD. Although right heart catheterization is required to diagnose PH, Doppler echocardiography is a screening tool used to identify individuals with TRV  $\geq 2.5$  m/s, a risk factor for PH (Klings & Machado et al., 2014). Because it is a non-invasive procedure, there is much more TRV data available than right heart catheterization data. This study is a longitudinal cohort study chart review looking at pediatric and adult echocardiograms, as well as laboratory values and clinical values, in children and young adults with SCD.

The first specific aim of this study is to determine the prevalence and incidence of elevated TRV ( $\geq 2.5$  m/s). Next, this study aims to determine demographic and clinical factors associated with an elevated TRV. Finally, this study aims to determine the change of TRV over time in response to SCD-directed therapies, including HU and chronic transfusions, in children with SCD.

## **MATERIALS AND METHODS**

### **Study design**

We conducted a retrospective longitudinal cohort study chart review to examine the prevalence and incidence of elevated TRV ( $\geq 2.5$  m/s) in children with SCD as well as factors that may be associated with an elevated TRV.

### **Setting**

This study was performed at Boston Medical Center (BMC), an urban safety net institution caring for an underserved population, of which more than 80% are eligible for public insurance. Approximately 200 children and 250 adults with SCD are cared for by pediatric and adult hematologists, respectively, in our institution.

### **Research participants**

Eligible subjects were identified using the registry of children with SCD that has been in place at Boston Medical Center since 2000. We included individuals if they were between the ages of 8 and 17 between July 31, 2003 and June 30, 2015. We excluded those who did not have any echocardiograms in this time period. In addition, those with comorbidities that may predispose that individual to an early onset pulmonary hypertension were excluded from our study, including: congenital heart disease (McLaughlin et al., 2009), chronic lung disease (e.g. bronchopulmonary dysplasia) (Khemani et al., 2007), connective tissue disease (e.g. systemic lupus erythematosus)

(McLaughlin et al., 2009), vascular abnormalities (e.g. moya moya syndrome) (Scott & Smith, 2009, Dobson et al., 2002), and premature birth (<35 weeks gestation) (Khemani et al., 2007). For individuals with a history of pulmonary embolism, data collection was censored at the date of the first pulmonary embolism (Kasper et al., 1980; Becattini et al., 2006). We also excluded echocardiograms performed during an emergency department visit or as an inpatient stay because VOs and ACS have been shown to transiently elevate TRV (Machado et al., 2007).

### **Data collection**

A chart review was performed to examine the prevalence and incidence of elevated TRV measured by echocardiography in individuals with SCD. The electronic health record system (Centricity®, GE Systems) was adopted in 2002, and was used to collect outpatient data including demographic data and baseline data. Inpatient data was available in a separate electronic health record, Sunrise Clinical Manager® (Eclipsis).

Several types of data were collected from chart review. First, demographic data were collected, including: date of birth, gender, race, ethnicity, and SCD genotype. Second, data were collected on the presence and dates of sickle cell-related complications previously shown to be associated with elevated TRV, including: stroke, splenic sequestration and/or splenectomy, nephropathy, retinopathy, priapism, sepsis or bacteremia, iron overload, leg ulcers, asthma, obstructive sleep apnea, tonsillectomy and/or adenoidectomy, deep vein thrombosis, pulmonary embolism, microalbuminuria, and proteinuria (Gladwin et al., 2004; De Castro et al., 2008). Third, information was

gathered on therapies with the potential to lower TRV, namely hydroxyurea and chronic blood transfusions, and the dates they were initiated (Pashankar, Carbonella, Bazy-Asaad, & Friedman, 2008; Pashankar, Manwani, Lee, & Green, 2015; Detterich et al., 2015). Fourth, laboratory data associated with elevated TRV were collected, including: baseline hemoglobin levels and baseline reticulocyte count (Pashankar, Carbonella, Bazy-Asaad, & Friedman, 2008; Pashankar, Manwani, Lee, & Green, 2015; Detterich et al., 2015). Finally, data was collected on acute care utilization, including number of emergency department visits for VOEs, number of emergency department visits for ACS, number of hospital admissions for VOE, and number of hospital admissions for ACS because a history of VOE related crises has been shown to be related to PH (Hagar et al., 2007).

For each pediatric echocardiogram, data was collected from reports stored on the electronic health record system, PWF. For each adult echocardiogram, data was collected from reports on Logician. Data collected for pediatric and adult echocardiograms are summarized in Table 6.

### **Statistics**

For all categorical data, including demographic data, SCD related complications, history of proteinuria, and history of microalbuminuria the 2-tailed Fisher's exact test was used. For hemoglobin levels and reticulocyte count the 2-tailed t-test assuming equal variances was used.

**Table 6.** Data collected from pediatric and adult echocardiogram reports

	Pediatric Echocardiograms	Adult Echocardiograms
Left heart	Atrial dilation	Atrial size
	Ventricular dilation	Aortic root size
	Ventricular hypertrophy	Ventricular wall motion (normal vs. abnormal)
	Ventricular dysfunction	Ventricular diastolic function (normal vs. abnormal)
	Ventricular systolic function (normal vs. abnormal)	Ventricular internal diameter at end systole
	Ventricular end diastolic volume Z-score	Ventricular internal diameter at end diastole
	Ventricular mass Z-score	Ventricular mass index
	Ventricular mass to volume ratio	Ventricular size
	Ventricular mass to volume Z-score	Fractional shortening
Ventricular ejection fraction	Ventricular ejection fraction	
Right heart	Atrial dilation	Atrium size
	Gradient between right ventricle and right atrium	Estimation of atrial pressure
	Does septal position predict right ventricular pressure more than half of the systemic blood pressure	Atrial function (normal vs. abnormal)
	Ventricular hypertrophy	Ventricular size
	Presence of ventricular systolic dysfunction	Ventricular function (normal vs. abnormal)
	--	Estimation of ventricular systolic pressure
	Pulmonary artery size Z-score	Pulmonary artery (normal vs. abnormal)
	--	Description of inferior vena cava (normal vs. abnormal)
Valves	Presence of aortic regurgitation	Presence/severity of aortic valve regurgitation
	Presence of pulmonary regurgitation	Presence/severity of pulmonary valve regurgitation
	Presence of tricuspid regurgitation	Presence/severity of tricuspid valve regurgitation
	TRV	Presence/severity of mitral valve regurgitation
	--	Presence/severity of aortic valve stenosis
	--	Presence/severity of pulmonary valve stenosis
	--	Presence/severity of tricuspid valve stenosis
	--	Presence/severity of mitral valve stenosis

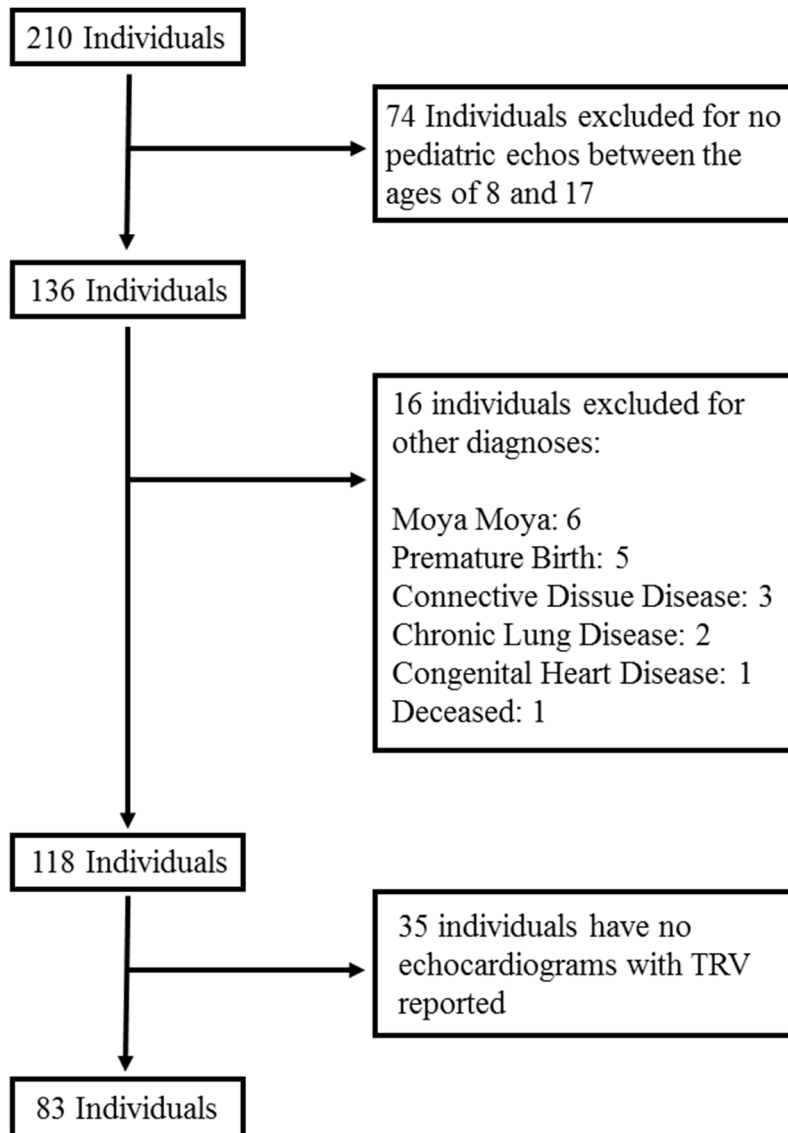
Table 6. continued

Other	Pediatric echocardiograms (continued)	Adult Echocardiograms (continued)
	Date of echocardiogram	Date of echocardiogram
	Calculated age of individual at time of echocardiogram	Calculated age of individual at time of echocardiogram
	Location of echocardiogram (inpatient, outpatient, emergency department)	Location of echocardiogram (inpatient, outpatient, emergency department)
	Diagnosis	Indication of echocardiogram
	Height	--
	Weight	--
	Body mass index	--
	Body surface area	--
	Presence of pericardial effusion	Presence/severity of pericardial effusion
	Presence of trabeculations	--
	Systemic systolic blood pressure	--
	Systemic diastolic blood pressure	--
	Report author	--

## RESULTS

### **Patient Population and Demographics**

Figure 7 describes the individuals that were identified as potential subjects for this study. There were 210 individuals followed by BMC hematology with SCD and were between the ages of 8 and 30 years old between July 31, 2003 and June 30, 2015. Pediatric echocardiograms were defined as echocardiograms that were performed while the patient was between the ages of 8 and 17 years old. Of the initial 210 patients identified, 74 did not have any pediatric echocardiograms and were therefore excluded from the study, leaving 136 individuals. Of these 136 individuals 16 were excluded for co-morbid diagnoses including moya moya syndrome (6 individuals), premature birth < 35 weeks gestation (5 individuals), connective tissue disease (3 individuals), chronic lung disease (2 individuals), congenital heart disease (1 individual), and death (1 individual). Of the remaining 118 individuals, only 83 had either a measured or calculated TRV value available for one or more of their echocardiograms. Not all of the screening echocardiograms for those 83 individuals had TRV documented, making many of the individual echocardiograms ineligible for the study even though the subject had one or more eligible echocardiograms overall.



**Figure 7.** Excluded individuals. Initially 210 individuals were identified as potential subjects for this study. 74 of those individuals were excluded for not having any echocardiograms between the ages of 8 and 17. An additional 16 individuals were excluded for other diagnoses including moya moya, premature birth, connective tissue diseases, chronic lung disease, congenital heart disease, and death. 35 of those individuals did not have an available TRV value.

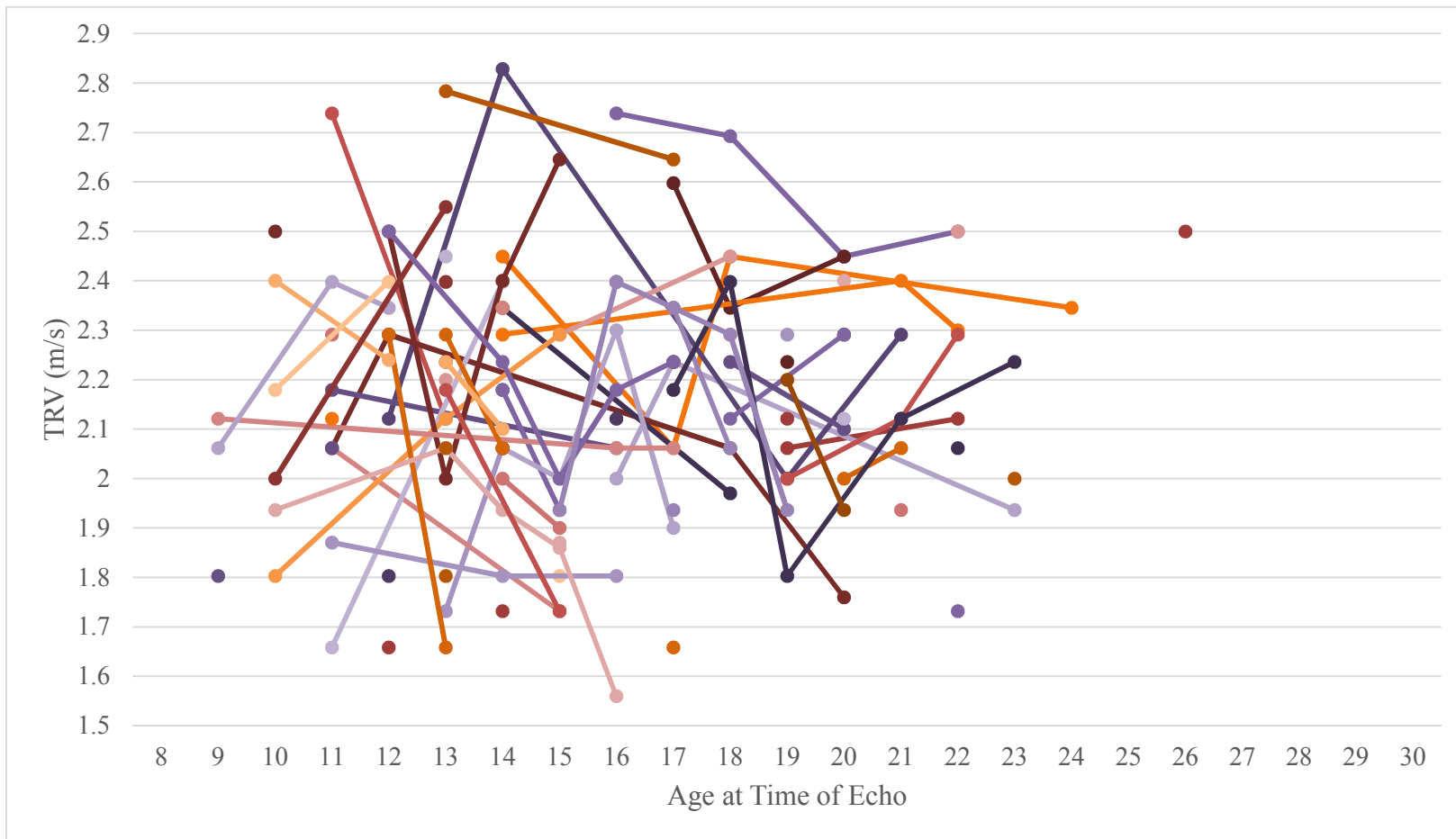
The characteristics of the study population are summarized in Table 7.

Demographic data showed that 11 (13%) subjects were born prior to 1990, 54 (65%) subjects were born between 1990 and 1999, and 18 (21%) subjects were born in or after 2000. There were 42 (51%) females; 68 (82%) subjects identified as black; and 9 (11%) identified as Hispanic or Latino. SCD history data showed that 61 (73%) subjects have the Hb SS genotype. There were 50 people with a history of ACS (59%), 18 (22%) subjects with a history of splenic sequestration and/or splenectomy, 14 (17%) people with a history of sepsis or bacteremia, 18 (22%) people with asthma. There were 53 (64%) people that have used HU at some point during our study period and 7 (8%) people that received chronic blood transfusions during our study period. Laboratory characteristics indicated that 9 (8%) people have a history of microalbuminuria and 6 (5%) people have a history of proteinuria. The average value for baseline hemoglobin level was 9.15 g/dL and the average value for baseline reticulocyte count was 8.11% of red blood cells.

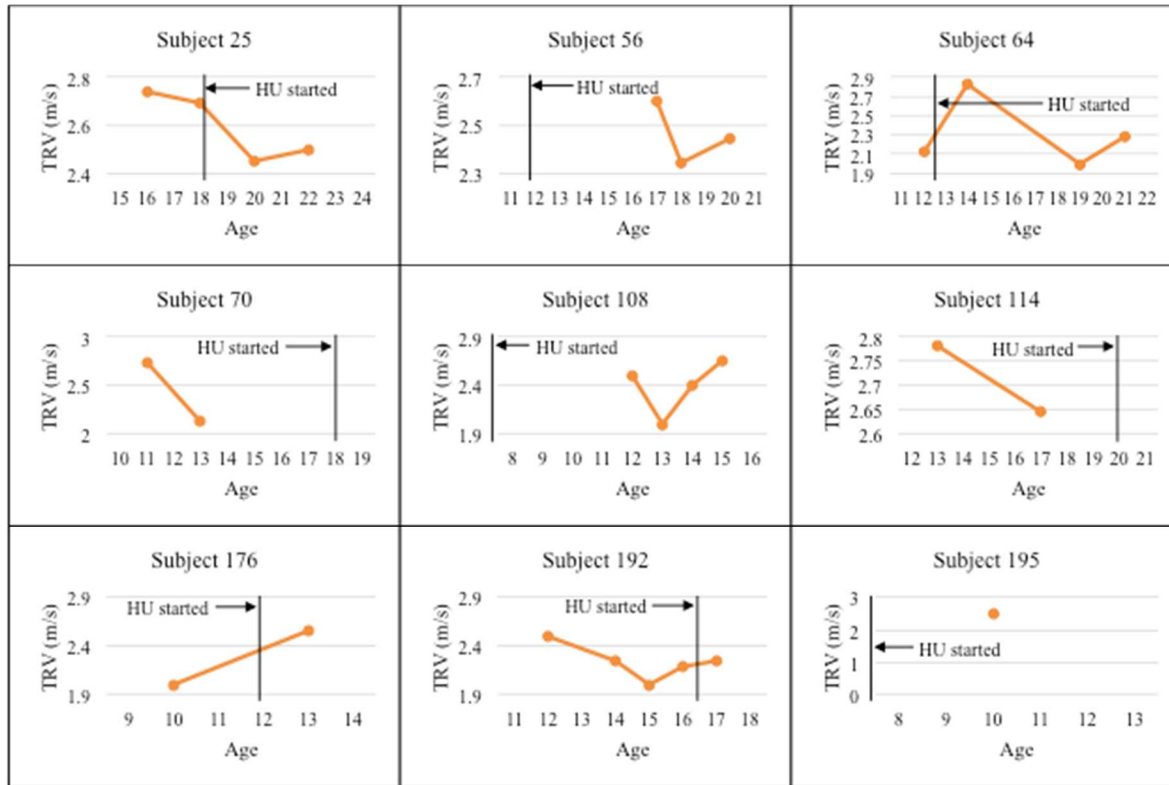
**Table 7.** Study population characteristics

		<b>Total (%) n = 83</b>
<b>Demographic variables</b>		
Year of birth	< 1990	11 (13)
	1990-1999	54 (65)
	≥ 2000	18 (22)
Gender	Female	42 (51)
	Male	41 (49)
Race	Black	68 (82)
	White	2 (2)
	Declined to Answer	11 (13)
	Answer Blank	2 (2)
Ethnicity	Not Hispanic or Latino	74 (89)
	Hispanic or Latino	9 (11)
<b>SCD history</b>		
Genotype	SS	61 (73)
	SC	21 (25)
	Sβ-Thalassemia <sup>0</sup>	1 (1)
History of:	Stroke	2 (2)
	Acute chest syndrome	50 (60)
	Splenic sequestration or splenectomy	18 (22)
	Nephropathy	3 (4)
	Retinopathy	4 (5)
	Priapism (men)	5 (12)
	Sepsis or bacteremia	14 (17)
	Iron overload	3 (4)
	Leg ulcers	1 (1)
	Asthma	18 (22)
	Obstructive sleep apnea	15 (18)
	Tonsillectomy and/or adenoidectomy	11 (13)
<b>Medications</b>		
	Hydroxyurea use	53 (64)
	Chronic blood transfusions	7 (8)
<b>Laboratory characteristics</b>		
Baseline Values:	Baseline Hb	8.95
	Baseline reticulocyte count	8.41
History of:	Microalbuminuria	6 (7)
	Proteinuria	6 (7)

Figure 8 illustrates the TRV values for all patients included in this study plotted according to age at the time of the echocardiogram. Eleven (13%) individuals had one or more TRV value at or above 2.5 m/s; three (27%) had a single elevated measurement while eight (73%) had two or more abnormal readings (Figure 9). In those with elevated TRV, values ranged from 2.50 m/s to 2.83 m/s. The average age at the time of an elevated TRV was 15.9 years. In those with elevated TRV, 9 (79%) had taken HU therapy at some point during the study period, and 6 (55%) were taking it at the time of echocardiogram reading of  $TRV \geq 2.5$  m/s. Characteristics of individuals with at least one  $TRV \geq 2.5$  m/s are summarized in Table 8. Nine out of the eleven individuals with an elevated TRV had the Hb SS genotype, which is 15% of the individuals with the Hb SS genotype within our population. One individual had the Hb SC genotype, which is 5% of the individuals with the HB SC genotype within our study population, and one person had the  $S\beta^0$ -Thalassemia genotype (the only individual with this genotype in our study population). Among these individuals the average baseline hemoglobin level was 8.3 g/dL and the average reticulocyte count was 11% of red blood cells. The reticulocyte count was significantly increased when compared to subjects with  $TRV < 2.5$  m/s ( $p = 0.04$ ). Eight (73%) of the individuals with one or more  $TRV \geq 2.5$  m/s had a history of ASC ( $p = 0.51$ ), two (18%) had a history of splenic sequestration/ splenectomy ( $p = 1.00$ ), two (18%) had a history of sepsis/bacteremia ( $p = 1.00$ ), one (9%) had a history of iron overload ( $p = 0.35$ ), and two (18%) had a history of asthma ( $p = 1.00$ ). However, none of these characteristics were found to have statistical significance when compared to subjects with  $TRV < 2.5$  m/s.



**Figure 8.** TRV in m/s at the age of each echocardiogram for all individuals included in this study. TRV over time for each patient included in this study that had TRV measured as an outpatient by echocardiography. 11 individuals had TRV elevated to 2.5 m/s or above. Of those individuals, 8 had only one TRV  $\geq$  2.5 m/s.



**Figure 9.** TRV in m/s at the age of each echocardiogram for each individual with one or more TRV  $\geq 2.5$  m/s. The start of HU therapy is indicated for each individual by a solid vertical line. Subjects 47 and 119 were not shown because each had only one TRV available, and neither has ever been on HU therapy.

**Table 8.** Individuals with TRV  $\geq$  2.5 m/s and key characteristics

Subject	Genotype	Baseline Hgb	Baseline reticulocyte count	SCD associated complications	Number of Normal Echos	Age at Abnormal Echo	TRV (m/s)	HU use at time of echo	Chronic transfusion therapy at time of echo
25	S $\beta^0$ -Thalassemia	7.0	8.0	ACS, splenic sequestration/splenectomy, sepsis/bacteremia, iron overload	1	16	2.7	No	No
						18	2.7	Yes	No
						22	2.5	Yes	No
47	SC	11.0	5.0	ACS	0	22	2.5	No	No
56	SS	7.0	8.0	ACS, asthma	2	17	2.6	Yes	No
64	SS	10.0	11.0	ACS	4	14	2.8	Yes	No
70	SS	9.0	13.0	ACS, splenic sequestration/ splenectomy	1	11	2.7	No	No
108	SS	7.0	12.0	ACS, sepsis/bacteremia	2	12	2.5	Yes	No
						15	2.6	Yes	No
114	SS	8.0	18.0	ACS	0	13	2.8	No	No
						17	2.6	No	No
119	SS	7.0	10.0	--	0	26	2.5	No	No
176	SS	9.0	7.0	--	1	13	2.5	Yes	No
192	SS	9.0	20.0	Asthma	4	12	2.5	No	No
195	SS	7.6	12.0	ACS	0	10	2.5	Yes	No

## DISCUSSION

In this 13-year retrospective longitudinal cohort study, we found that 13% of children with SCD between the ages of 8 and 17 years had at least one screening echocardiogram with TRV  $\geq 2.5$  m/s, which is in agreement with the prevalence estimates of 10-20% published by the American Thoracic Society (Klings & Machado et al., 2014).

Our study found no complications of SCD of laboratory values to be significantly associated with TRV  $\geq 2.5$  m/s. While previous studies have reported significant differences in history of sepsis and/or bacteremia, ACS, and asthma; our study found no statistical significance between subjects with TRV  $\geq 2.5$  and TRV  $< 2.5$  for these clinical histories (Hagar et al., 2007). Previous studies have also found that significant decreases in hemoglobin and increases reticulocyte counts are associated with TRV  $\geq 2.5$  m/s, our study found only an increase in reticulocyte count to be statistically significant ( $p = 0.04$ ) (Minniti et al., 2009; Pashankar et al., 2008; Amrusko et al., 2006; Kato et al., 2007).

This study did not find SCD genotype or SCD directed therapies to be associated with TRV  $\geq 2.5$  m/s. Other studies have found that all of their subjects with TRV  $\geq 2.5$  m/s had the Hb SS genotype, while their study population included other genotypes (Amrusko et al., 2006; Pashankar et al., 2008). However, our study found that 9 (82%) of our subjects with TRV  $\geq 2.5$  m/s had the Hb SS genotype, while 1 (9%) had the Hb SC genotype and 1 (9%) had the S $\beta^0$ -thalassemia genotype, and these values were not statistically different from subjects with TRV  $< 2.5$  m/s ( $p = 0.43$ ). One small study also

found that 72.7% of their subjects with  $TRV \geq 2.5$  m/s were on a chronic blood transfusion program, while none of our subjects with  $TRV \geq 2.5$  m/s were on a chronic blood transfusion program (Ambrusko et al., 2006).

Only 13% of children with SCD in our study had  $TRV \geq 2.5$  m/s, which is much lower than the 30% reported in the literature (Kato et al., 2007). One possible reason could be a screening bias in smaller studies summarized in this publication. In our institution, echocardiographic screening begins at age 8 years old and is done every 1 to 2 years, compared to other studies that had variable entry criteria that likely reflected the inclusion of more symptomatic patients, resulting in a higher prevalence of  $TRV \geq 2.5$  m/s found in these studies (Kato et al., 2007).

Differences in rate of  $TRV \geq 2.5$  m/s between this study and other studies may also be caused by HU use. Small studies have found that SCD patients taking HU have significantly lower rates of pulmonary hypertension and elevated TRV than SCD patients not taking HU; one study found that 9 (35%) of their subjects with  $TRV \geq 2.5$  m/s were on hydroxyurea while another found that 6 (33%) of their subjects with  $TRV \geq 2.5$  m/s were on hydroxyurea ( $p = 0.01$  and  $p = 0.05$  respectively) (Ataga et al., 2004; Ataga et al., 2006). Our study found that 6 (55%) of our patients with  $TRV \geq 2.5$  m/s were taking HU, and there was no statistical significance found between those with elevated TRV and those without elevated TRV. Other small studies have found a significant reduction in TRV while SCD patients were on HU therapy (Olnes et al., 2009; Pashankar et al., 2009). In this study, 53 (64%) were prescribed HU, which may partially account for the lower prevalence of  $TRV \geq 2.5$  m/s in this population.

## **Limitations**

This study is a retrospective longitudinal cohort chart review study, and therefore comes with inherent limitations. First, this study was done in a single academic center; therefore, the results may not be generalizable to the population of children with SCD. Second, not all patients had regular screening echocardiograms, which limits our ability to calculate the prevalence of TRV in this population of children. Third, not all screening echocardiograms documented TRV, making those echocardiograms ineligible for our study and making it difficult to follow the progression of TRV in individual study subjects. Finally, lab results related to elevated TRV in previous studies, such as LDH and indirect bilirubin levels, were not available for analysis in this study (Kato et al., 2007; Pashankar et al., 2008; Minniti et al., 2009).

## **Conclusion**

This study found that 13% of our study population had one or more echocardiograms with TRV  $\geq 2.5$  m/s, which is lower than other studies that have found a 30% rate of elevated TRV in children with SCD (Kato et al., 2007). While other studies have found that an Hb SS genotype, history of ACS, history of sepsis and/or bacteremia, history of asthma, history of chronic blood transfusions, low hemoglobin, and increased reticulocyte count were associated with TRV  $\geq 2.5$  m/s in children with SCD, our study found that only an increased reticulocyte count was significantly associated with TRV  $\geq 2.5$  m/s when compared to subjects with TRV  $< 2.5$  m/s (Hagar et al., 2007; Minniti et al., 2009; Pashankar et al., 2008; Ambrusko et al., 2006; Kato et al., 2007).

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## CURRICULUM VITAE

### Kristin Sheppard

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Year of Birth: 1990

### Education

#### California Polytechnic States University, San Luis Obispo, CA

B.S. Degree in Biochemistry, minors in psychology and biology (August 2012)

GPA = 3.172

M.S. Degree in Biological Sciences with a Specialization in Stem Cell Research (June 2014)

GPA = 3.721

#### Boston University School of Medicine, Division of Graduate Medical Sciences

Candidate for M.S. Degree in Medical Science (expected graduation in August 2016)

### Research Experience

#### Clinical Research in Sickle Cell Disease, with Dr. Patricia Kavanagh

Boston University School of Medicine (May 2015 – present)

I performed a longitudinal cohort study chart review examining the prevalence of indicators for pulmonary hypertension in children with sickle cell disease. I reviewed echocardiogram reports as well as history of sickle cell disease complication previously reported to be associated with pulmonary hypertension, as well as laboratory values previously reported to be associated with pulmonary hypertension.

#### Neuroscience and Stem Cell Research, with Dr. Fred Gage

Salk Institute for Biological Sciences (July 2013 – March 2014)

I utilized human induced pluripotent stem cell derived neurons and neural progenitors in conjunction with *in-vivo* imaging techniques to study the integration of these cells into a functional neural network to create a new mouse model of schizophrenia. Experience with tissue culture, viral transduction, murine survival surgery with sterile technique and nanoinjection, immunohistochemistry, fluorescence imaging, confocal imaging, and 2-photon imaging.

#### Marine Biology Research, with Dr. Elena Keeling

California Polytechnic State University (March 2014 – June 2014)

I worked with noninjection techniques, cell staining techniques, and confocal imaging to study the whole body regenerative capability of the marine invertebrate *Botrylloides violaceus*.

**Biochemistry Research**, with Dr. Lori Robins

California Polytechnic State University (September 2011 – June 2012)

I worked with molecular biology and gene expression techniques to express the Sma1 gene for a homing endonuclease in hopes to explore the interactions between the enzyme and DNA and its potential for gene therapy. Experience with bacterial culture, bacterial transformation, spectrophotometry, gel electrophoresis, SDS-PAGE, and RT-PCR.

**Organic Chemistry Research**, with Dr. Hasan Palandoken

California Polytechnic State University (January 2010 – June 2011)

I utilized organic synthesis techniques to create a new small molecule and explore the potential for a new brain cancer therapy that inhibits the ion exchangers on cellular surfaces. Experience with column chromatography, IR spectroscopy, carbon NMR, proton NMR, and gas chromatography-mass spectrometry.

**Previous Work and Volunteer Experience**

**Scribe America, ER Scribe** (March 2012 – August 2014)

I worked in the emergency rooms in two hospitals. I attended the patient interviews, physical exams, and ER procedures and recorded physician findings and procedures on the patient charts. I also worked to find old medical records and radiological exams for physician review.

**Teacher's Assistant Program** (September 2010 – June 2011)

I worked with seventh and eighth grade math and science classes at Fesler Junior High School to facilitate learning and promote individual discovery and understanding of scientific and mathematical material.

**Growing Grounds Volunteer** (April 2010 – June 2010)

This is an organization from Transitions-Mental Health Association designed to provide horticultural therapy for its employees who are all afflicted with a mental health issue. While volunteering there it was my job to work along side the employees and talk with them, empowering them to express themselves and teach me how to take care of the nursery.