

And what I'll try to do today is provide a brief overview of the state of sickle cell disease and sickle cell anemia as we're seeing patients survive longer and longer, really living sometimes into the seventh decade. And what are the new clinical manifestations that we're seeing of sickle cell disease as the population is aging, with a particular focus on the development of what we think is a very important complication pulmonary hypertension. I'll briefly touch on why they may be developing pulmonary hypertension in the relationship to chronic hemolytic anemia. But then I'll talk a bit more about pulmonary hypertension itself and how we diagnose it by right heart catheterization, what are the diagnostic criteria, and what is the impact of having pulmonary arterial hypertension in patients with sickle cell disease. And finally I'll just briefly touch on how all of this may relate to an emerging understanding of complications of stored red cells, or stored blood. So I think they'll be a little bit for everybody in this talk.

So as you all know sickle cell disease is an autosomal recessive disorder of the gene that codes for hemoglobin specifically the beta globin subunit. And so one point mutation that's inherited you need two copies of this, results in a substitution at position six of the beta globin of the glutamic acid which is converted to a valine. And what happens is the hemoglobin is normal when the red cells oxygenated but as you know when it's deoxygenated the two hydrophobic contact site that develops around that valine on the beta change results in one hemoglobin tetramer binding to another. And it actually crystalizes and it forms sort of a liquid crystal kinetic within the red cell and it forms these crystals which are long, polymer bundles that within the red cell. And these bundles form large spheres that you can see here within the red cell, they ultimately distort the shape sometimes manifest as the classic sickle shape but they make the red cells very stiff and rigid. And this would

be what it would look like if on electron microscopy you were to break open and look at these long, polymer bundles or crystals within the sickled red cell.

And so what happens is as blood goes through the microcirculation, these rigid cells are almost like a half frozen bag, or a half frozen water balloon. You know if you have a wet water balloon you can grab it, it slips through your hands, that's what a red cell is like. Red cells actually squeeze and deform and slip through very small blood vessels. But if you take that same water balloon and you freeze it halfway in the refrigerator it's kind of crunchy inside, it won't fit through a narrow vessel and in fact it tends to break or shear with those ice crystals shearing through that water balloon. So what happens is that leads to hemolysis but it also entraps these red cells within the microcirculation so you block blood flow. And as you know any time you block blood flow to an organ it produces ischemia, reperfusion injury and cell death and infarction. And sickle patients can infarct any organ, you know typically we get bone marrow infarctions which create ischemia and edema of the bone marrow and swelling of the bone marrow which causes intense, severe pain and that's often the presentation you see with acute pain crisis. It's really pain in the bones from bone marrow infarctions. But they infarct any organ, a patient with sickle cell will present with a liver infarction with elevated transaminases, lumps or bumps on the skin with subcutaneous infarctions, muscle infarctions, stroke, many, manifestations of acute organ infarction.

Now what's sickle cell is also called sickle cell anemia. And for many years the anemia of sickle cell was considered to just lead to fatigue and gall stones from the accumulation of bilirubin. But we now think that the hemolytic anemia of sickle cell is really more ominous. Now if you look at a

normal red blood cell, it will survive 90-120 days. But if you have homozygous SS disease that red cell will only survive 15-30 days. In fact this is one of the highest rates of hemolytic anemia of any human disease, only a few are worse, like paroxysmal nocturnal hemoglobinuria. So the average patient with homozygous SS disease will hemolyze one unit of blood a day. So if you think about that they'll live with a steady state hemoglobin of 7 grams per deciliter, they utilize about 20% of total metabolic energy just making new red blood cells. So if you see a homozygous SS patient they're always going to be very skinny and that's because they're using so much energy to make new blood. If you see a sickle cell patient in your clinic and they're not skinny that means they don't have SS, they have SC or an S beta plus thalassemia, or they've inherited some sort of metabolic syndrome mutations on top of those sickle cell disease. They're usually very skinny and almost like marathon runners with all that energy making new red cells.

The other thing this leads to is if you get an acute Parvovirus B19 infection which causes transient red cell aplasia, normally children that get that, you won't even know they've had it. They'll have a viral illness, they'll become anemic but you won't know they had that condition. But a sickle patient it's a gray of complication. They stop making red cells for 3-5 days, everyday they're hemolyzing one unit of blood and they're starting at a hemoglobin of 7, within about 2 days they've got a hemoglobin of 3 and it's critical. So that's another way that the high rate of hemolytic anemia is manifest.

Well this is the only thing I'll tell you about sort of scientific mechanisms, but there's an emerging appreciation that hemolysis inside blood vessels, damages blood vessels and activates the clotting

system. So any form of hemolytic anemia where there's a lot of hemolysis in the vasculature like PNH like sickle cell disease, like thalassemia intermedia, they get more clots, and over time they will develop pulmonary hypertension. Now why is that? Well normally here's a red cell, I don't know if you can see it from the back of the room there, but when a red cell hemolyzes it releases hemoglobin. Now that hemoglobin dimerizes and it scavenged by half the globin, half the globin mops up that hemoglobin. The problem is if you hemolyze everyday like a sickle patient, your body can't keep up with the synthesis of haptoglobin so the haptoglobin levels go to zero. And in fact the sine qua non of a hemolytic anemia with intravascular hemolysis is undetectable haptoglobin. If you think someone has HUS, if you think they have TTP, if you think they have active PNH or active sickle cell disease, and you send the haptoglobin it will be less than 5 the limits of detection.

So what happens is you get this hemoglobin now spilling into the plasma, that doesn't bind to haptoglobin, and that hemoglobin reacts with the molecule called nitric oxide very fast and it scavenges nitric oxide. Nitric oxide is a very important molecule made by our blood vessels that dilates, that opens up blood vessels. Nitric oxide also inhibits clotting, so you scavenge the nitric oxide. It turns out the red cell also has an enzyme called arginase in it. And arginase is an enzyme that chews up arginine and arginine is converted by the nitric oxide synthase enzyme to make nitric oxide. So it turns out not only do you scavenge the NO by releasing hemoglobin but you scavenge the substrate needed to make more NO by releasing arginase. And that depletes nitric oxide, that leads to blocking of vasodilation so you get constriction of blood vessels and ultimately it leads to activation of platelets and clots. So the message that I want to share with you is that any disease with a high level of hemolysis will scavenge nitric oxide, it will cause vasoconstriction and it will

activate platelets. And what we see in sickle cell disease is over decades of hemolyzing just everyday hemolyzing, it wears out the blood vessels and ultimately leads to a vasculopathy.

So what's the clinical relevance of having sickle cell disease for decades and decades and living to adulthood, or having hemolysis for decades and decades. Well it's sort of a good news, bad news story that's going on in the world of sickle cell disease and that is since the 1970's the good news is survival is improving dramatically and why is this happening. Well first of all it's the most important thing is screening. We know who has sickle cell and we take better care of those babies. The other thing is sanitation. In sub-Saharan Africa, 98% of babies born with homozygous SS disease die within the first 2 years. And they're dying of rotavirus infection, they're dying of malaria, they're dying of bacterial infections, so they can't tolerate that infectious hit. So just in northern Europe and the U.S. by having appropriate sanitation, we see in eliminating these common bacteria and viruses, we're seeing improved survival. The other thing is all children get penicillin for 5 years after birth and the reason is that sickle patients auto infarct their spleen. So they're susceptible to encapsulated bacteria like salmonella. We don't see much salmonella but if you're a pediatrician taking care of sickle patients you'll see salmonella. So we put them on penicillin everyday of their life for the first 5 years and that helps. The other thing is Hydroxyurea which is a drug that induces fetal hemoglobin and blocks sickling, that's FDA approved, that's improving outcomes. And finally there's modern transfusion, modern blood banking. If someone has early signs of pneumonia and sickle cell, we transfuse them and that's improving survival. All of these things are letting our patients live longer and the last this was studied in 1984 in the cooperative study of sickle cell disease, the median age of survival for men was 42 and the median for women

was 48. You know we need to do better than that but you see this is improving. And we will now see patients in our clinic sometimes 70 years of age.

Now what's the bad news part of it? Well the bad news part of it is their entire lives they're still sickling, they're still having organ injury, they're still hemolyzing and they're wearing out their organs and they're developing chronic complications of sickle cell disease. And this is a list you know delayed puberty, erectile dysfunction because of priapism which is sickling in the veins of the penis, skin ulcers, chronic skin ulcers that result in scars and chronic pain around the ankles, avascular necrosis of the hip, really big problem as our patients are aging they're needing hip replacement surgeries. Chronic renal failure, this is one of the top risks for death in patients with sickle cell disease. Functional asplenia, they've infarcted their spleen, chronic anemia, now interestingly this chronic anemia has now been associated with cognitive dysfunction in studies that have looked at this in the aging population. Obstructive sleep apnea, cardiomegaly and I'm going to talk about pulmonary hypertension.

So here's a patient that was referred to my clinic from Ohio; eighteen year old young man with sickle cell disease, homozygous SS disease so he has two of those substituted valines in position 6 of the beta globin gene. This is about, just so you know, homozygous SS is about 75% of the population. About 25% have SC where there's another mutation on one copy of the beta globin. Now interestingly he's not the typical patient you think about, he has rare pain, rare acute chest syndrome and he's been getting simple blood transfusions since he was 9 because he had a stroke. And this is another emerging thing that you're going start seeing in your practices is these children that have been transfused for much of their life, because 30% of children are at risk of stroke and are

put in chronic transfusion programs. So these patients are now growing up with a different phenotype of sickle cell disease, they've been transfused, partly protected.

The other thing I want to point out to you is if you work in the emergency room you think everybody with sickle cell is always coming in pain and always using narcotics and suffering and 70% of sickle patients hardly ever come to the hospital. They're managing their pain at home, they're coming to the hospital none or less than 1 time per year. Thirty percent are having chronic, severe pain and those 30% of people aren't making it up, they also have the highest mortality. So look at this guy, he's on chronic simple transfusion, he gets about 2 units a month, he's on no narcotics, he was never admitted for pain. Things seem pretty good for this guy right? He's got a white count of 15,000 that's typical, SS patients have white counts of 10,000-20,000 without acute infection. It's an inflammatory condition from all of these little ischemia injuries that are happening to him all the time. His LDH is very high, about 3 times normal which is consistent with hemolysis, because there's LDH in the red cells, his haptoglobin is undetectable. Now here's what's interesting, he's transfused so his hemoglobin A is 51% and his S is only 30 and we're taught that with a hemoglobin S of 30 or less you should be protected. So we're doing everything here by guidelines but notice this strange thing. His reticulocytes are really high, 695,000 absolute retics yours would be 30,000 absolute. So this guy is someone who despite being transfused, he hyper-hemolyzes. Now why does he hyper-hemolyze we don't know. Does he happen to have a G6 PD deficiency or some other inherited mutation that was protective from endemic malaria? We don't know. But he hyper-hemolyzes even with transfusions and even though he doesn't have pain there's a problem now because of this hemolysis. We did a blood gas and his blood gas was reasonable. His

carboxyhemoglobin is 4%. Does anybody know why his carboxyhemoglobin is high? You might think he's a smoker, he doesn't smoke. It turns out that when heme is broken down it's metabolized into carbon monoxide by an enzyme called heme oxygenase. So if you hemolyze a lot your carbon monoxide will rise and you'll have a high CO hemoglobin. So this is a measure of having a high rate of hemolysis. We did a bunch of tests, his autoimmune profile is negative, HIV profile is negative, HEP serology is negative. We're doing these because we're looking for other reasons why this young gentleman may have really bad pulmonary hypertension that I'm about to show you.

So this is his CAT scan, now this is a nice pearl. What you see here is this is a thin cut high resolution CAT scan and you see these areas of white and then areas of dark. And you would think the abnormal area is the white area, that would be a ground glass infiltrate, maybe there's edema or there's infection there. Right? But in truth this is a classic presentation of a patient with severe, pulmonary arterial hypertension and this is called a mosaic perfusion pattern. And actually the abnormal part is not the white, it's the dark. It's sort of like a Westermark x-ray for a pulmonary embolism. What happens is there's no blood flow going to the darker areas of the lungs so there's less blood volume there. The blood is being directed to the areas with good blood vessels so there's more water density in the blood vessels making it look more white. So if you have an inspiratory CAT scan in someone with pulmonary arterial hypertension, or chronic thromboembolic pulmonary hypertension you will often see this mosaic perfusion set pattern. And this is a really nice example of that. You can sort of see it, but here's his aorta and here's his pulmonary artery trunk, you can certainly see that left main pulmonary artery. The main pulmonary artery here should be less diameter than the aorta and certainly that left main should be much smaller than the aorta. You can

see he has a big pulmonary artery. And these are just some other cuts showing this mosaic profusion pattern. And that's sort of a classic.

Now notice this abdominal cut, I don't know if you can see this white area projecting, that's his spleen. It's a brick of calcium. Ninety-eight percent of homozygous SS patients when they're adults just have a small, calcified, splenic remnant because they've had so many infarctions to that spleen by age two they are functionally asplenic and that's why they're at increased risk of infection with these encapsulated organisms.

I'm sure some of these questions will be on the board. So we did an echo of his heart and we do something called a tricuspid regurgitant jet velocity. This is a very important test where we look at the right side of the heart, this is his right ventricle this his left, his right atrium. When you look at the leak backwards from during, when the right ventricle squeezes you look at the backwards leak going through the tricuspid valve and 87% of us have a little leak, you can quantify that leak with Doppler. And the velocity of that leak is relative to how high the pressure is in the right ventricle. And without going into detail that spot using the modified Bernoulli equation you can do something called 4 times that velocity squared. But I'll come back to that so don't remember that yet, but this is the Doppler envelope and it's 6 meters per second. The normal is less than 2.5. This is one of the highest Doppler regurgitant jet velocity that I've seen and that's consistent with him having a very high right ventricular systolic pressure, or a very high pulmonary pressure.

The other thing we can look at is the 4 chamber view of his heart. Now this is the right ventricle and this is the left ventricle. Now normally the left ventricle is big and the right ventricle is a little half crescent moon on the side. In him, his right ventricle is enormous and in fact that septum is bowing into the left ventricle and this is an example of severe right ventricular dilation and failure. And this is his right atrium, it's probably the biggest right atrium I've seen. You can't even see the left atrium it's almost obliterated so you can imagine how hard it is for him to pump blood out of that left ventricle. Not only is the right ventricle dilated and failing and not pumping blood, but that left ventricle can't even fill up with blood because of the pressure on it. And if you look here that when during diastole that septum moves in and that's called paradoxical septal motion. So the things you see with right heart failure are dilated right ventricle, and a dilated right atrium, you see paradoxical septal motion where during relaxation that right ventricle wall, pre-wall goes into the ventricle so it bows in like this and some people will describe a D shaped left ventricle. But instead of being round as the left ventricle should, you can kind of see that it's D shaped. So we did a right heart catheterization in him. Now normal right atrial pressure is 5, his is 40. One of the biggest determinates of poor survival is a high right atrial pressure because that means that you really have right heart failure. He has a right ventricular pressure of 144/9, the normal is 25/5. His pulmonary pressure 147/49, the normal for pulmonary is about 25-30/15, 10-15. His PA mean which should be less than 25 is 82, his wedge is relatively normal at 17, it should be less than 15 but probably that pressure on that left ventricle is making it go up a little bit, his transpulmonary gradient which is the pulmonary mean pressure minus that wedge pressure, that's the gradient across the pulmonary vasculature is very high at 65, that should be less than 12. His cardiac index is relatively low but not terrible he does still have cardiac output.

So unfortunately that gentleman we actually cleared him for a lung transplant at UPMC, he would've been the second sickle cell patient ever transplanted before, but we put him on Prostacyclin as an IV drug to improve his pulmonary pressures, we put him on Sildenafil a PBA 5 inhibitor to try to improve his pulmonary vasculature and we put him on an endothelin receptor blocker. He ended up being on 3 drugs and he did feel better, he gained 10 kilograms of weight, he really had cardiac cachexia when we saw him from heart failure, but his pressure really didn't improve. So we cleared him for lung transplant and offered it to him but he was feeling better and he decided that he wanted to try to go to college and not, you know which is a reasonable decision because he would only be the second patient in history at age 18 to have a lung transplant and unfortunately he died about 4 months later. He came to the emergency room on Thursday in Ohio probably with a viral illness or something and had a decompensation and heart failure and died. The outcome for these patients is very bad when they develop severe pulmonary hypertension so it's increasingly clear that there's this syndrome of hemolytic anemia associated with pulmonary hypertension. It's not just sickle cell. Thalassemia intermedia, paroxysmal and nocturnal hemoglobinuria, all these diseases are diseases where you have chronic hemolytic anemia and the last I checked on the literature there was 730 references for different causes of pulmonary hypertension associated with hemolytic anemia. Now in sickle cell disease the cases usually aren't like the one I showed you. Usually it's a mild to moderate increase in pulmonary pressure but despite it being a mild increase in pulmonary hypertension, they suffer a very high prospective mortality that I'm going to tell you about. And that's probably because sickle cell and pulmonary hypertension just don't mix.

You know people with pulmonary hypertension rarely tolerate additional stress for example, pregnancy can be lethal in someone with pulmonary hypertension. I think having sickle cell and pulmonary hypertension is just too much stress on the body so they don't live long enough for those pressures to usually get as high as they did in this case. We're also seeing an increasing rate of sudden death in our adults with sickle cell. Patients are being admitted to the hospital they have a pain crisis, 4 days later things are resolving, they're looking pretty good, you're doing your discharge planning and boom they have asystolic arrest, and that's increasingly reported and increasingly seen and we think that relates to the development of pulmonary hypertension in this aging population.

Now anemia per se without hemolysis doesn't actually cause pulmonary hypertension, it's just, it's not just the high cardiac output. This is a study from 1963 where they looked at people with a range of anemias as low as 2 grams per deciliter and you could see the pulmonary artery mean pressure never rose above 25. The other thing is pulmonary vascular resistance actually drops as you get anemic and the reason for that is the viscosity of blood drops and cardiac output goes up when you're anemic and pulmonary vascular resistance is calculated by the change in pressures provided by the cardiac output, so pulmonary vascular resistance actually drops with anemia. So when we see sickle cell patients that have a high pulmonary pressure and have a high relative pulmonary vascular resistance they're probably more severe than we realize.

So we looked at this when I was in the National Institute of Health and we screened initially it was 195 patients with sickle cell disease but you'll see later we've now reported data on 533 patients

screened over 10 years, and the way we screened was with echo. So this is the same thing, we're looking at the regurgitation of blood from the right ventricle into the left atrium every time that ventricle contracts. And you get this nice Doppler window and you can quantify that tricuspid regurgitant jet velocity. Now when you order an echo and you ask your doctors to quantify the pulmonary pressure or I want an echo that looks at the right ventricle and quantifies the pulmonary pressure, this is what they're going to do. They're going to look in 3 different views for the TR jet velocity and they're going to report a number in meters per second. And abnormal is anything above 2.5 meters per second and this quantification will correlate with the pulmonary pressure. Now the way it works if you want to know is you do 4 times that velocity squared. So in this case let's say this gentleman here is 4 meters per second that would be 4 squared, $4 \times 4 = 16$, four velocity squared and that gives you the systolic pressure in the right ventricle. Now this is an estimate it's not a perfect test but it's the way we screen for pulmonary hypertension. So you're looking for 2 things, that jet velocity, that calculation of the pulmonary systolic pressure, and you're looking for what the right ventricle looks like. Now 2.5 or above is abnormal but we really usually only do a right heart catheterization if that value is 3 or higher and that's because of false positives at lower levels. So if you, 3 meters per second or higher which will give you a calculated number of about 45 you're going to do a right heart cath.

So here's what we've found 67% of sickle cell patients had a normal TR jet, there was this 24% that had this intermediate range, 2.5-2.9 and 9% had this level over 3 where you would say I think they really might have pulmonary hypertension I want to do a right heart cath. And at first we thought well you know 9% is pretty high but this middle group we probably don't have to worry about them

because the pressures aren't that high. And then this is what we saw, the group less than 2.5 did relatively well. Now this is the Kaplan-Meier survival curve. What we're looking at the people alive this is 100% and 50% alive over months. So for example at 40 months 85% of people are alive. Now we wouldn't want that curve but that just shows you that having sickle cell is, it's a terrible disease and the mortality is high even without pulmonary hypertension. But look what happened if they had that borderline elevation in pulmonary pressure, they had 40% of the people were dead at 40 months. And look what the value over 3, 40% were dead at 30 months so this ended up being a relative risk of death of 10 fold and one of the biggest risk factors for death in this population. This has now been confirmed in 3 major trials, Ataga et al., a relative risk of death of 9 this is out of Duke, De Castro out of UNC, 15 and we did a study called Walk-PHASST United Kingdom in Europe where again the risk of death was 11 fold.

So what things correlate with having pulmonary hypertension in populations with patients with sickle cell? This gets us back to hemolysis. A high pulmonary estimated pulmonary pressure by the tricuspid regurgitant jet velocity correlated with all markers of hemolysis and surprisingly at the time not with inflammation, not with fetal hemoglobin which were the traditional risk factors in sickle cell patients. And here's a little one that no correlation with episodes of chest syndrome or vaso-occlusive pain crisis. And in the old days we used to think that repeated attacks of acute chest syndrome and pneumonia caused lung scarring, caused hypoxia and led to pulmonary hypertension. It turned out that the most common cause of pulmonary hypertension was not chest syndrome but rather chronic hemolysis kind of eating up those blood vessels as I showed you earlier.

And on multi varied analysis, LDH is a measure of hemolysis was an independent risk factor for developing pulmonary hypertension but note also chronic renal failure as they developed renal failure they're at risk of pulmonary hypertension. Iron overload as quantified by transferrin SAT or ferritin. Iron overload is a risk factor and they get iron overload from getting many, many transfusions. And then systolic systemic blood pressure, so this really is a systemic vasculopathy in these patients, it's not just the pulmonary vessels, it's also the other vessels, but those pulmonary vessels seem to be uniquely at risk.

So what about right heart catheterization that's the gold standard. The way we diagnose pulmonary hypertension is with a right heart catheter. This echo is a screening test, it's not 100% reliable, we need to define this. So is it common or rare when we do right heart catheterization? Is it really a risk factor for death and is it related to hemolytic anemia. And there's no been 3 major studies just published really in the last 2 years addressing this. So here's the definition of pulmonary arterial hypertension, the mean pressure during right heart cath should be greater than 25 millimeters if mercury that's 3 standard deviations above the population mean. Now we're all arguing whether we should have borderline at 20-25, whether we should do exercise provocation, but right now this remains the definition. The wedge pressure should be less than 15 to call it pulmonary arterial hypertension. The reason for that is the mean pressure in the pulmonary artery is high but after you go through the arterials, through the capillaries and hit that left atrial pressure that should be low. And the pulmonary vascular resistance traditionally we look for it to be greater than 3 Wood units. This has been dropped from the definition because there are some patients like sickle cell and portal pulmonary hypertension that have a high cardiac output and the PBR isn't that high.

So we performed a study that we published in JAMA last year, 533 patients this is the same cohort but now extended over 10 years, we have 86 patients with right heart cath, we've got hemodynamic values, we looked at things like 6 minute walk and echo. Here's what we found. First of all 56 patients out of the 533 had a mean pressure over 25. What that means is that 10.5% of all adults that we saw at the NIH had pulmonary hypertension by right heart cath. About half of them it was pulmonary arterial hypertension with a low wedge and about half of them the wedge was a little high where you might call them pulmonary venous hypertension. But 10.5% had real pulmonary hypertension. This is what their numbers looked like, mean pressures of about 36 compared to 19, they had a slightly low mixed venous SAT consistent with low cardiac output, they're TR jet of course was elevated because that estimates pulmonary pressure by echo and their 6 minute walk was low. And again they had slightly higher creatinine a little renal failure, they had more hemolysis and they had low walk, consistent with hemolysis and renal failure being risk factors. And here was the mortality, in about 30 patients we did the cath and they didn't have pulmonary hypertension and look, this is again from 100% survival to zero, more than 20% of them were alive. But look in the group that had pulmonary hypertension by cath, that 10% of the population. Sixty percent were dead by 9 years of follow up. And this is really important, here we're looking in the red at the people who had a cath that didn't have pulmonary hypertension and in green we're looking at everybody that had an echo and had a TR jet less than 2.5. So if your echo looks normal you're probably going to, you're in reasonably good shape and that's compared to the ones with pulmonary hypertension.

What predicted mortality? I'm going to go quickly here because this is just the same theme. Every measure of pulmonary hypertension; pulmonary pressure, gradients, resistance, this is the

transpulmonary gradient. If you had a transpulmonary gradient over 12 there were no survivors by 8 years, that's the pressure, the mean pressure minus that wedge. This was recently confirmed in a Brazilian study and here's the Kaplan-Meier in people with and without pulmonary hypertension, and recently confirmed in the Parents site in the New England Journal. In France they screened about 400 sickle patients, they did exclude 10% of their most severely affected patients, and they did right heart cath for everybody over 2.5. And they found that 6% of the population had pulmonary hypertension. We think that was slightly lower than our study because they excluded 10% of their sicker patients. But once again just jumping down here, 12% died in the pulmonary hypertension group versus almost none in their non pulmonary hypertension group.

So what this tells us is pulmonary hypertension and sickle cell by right heart cath occurs in about 6-11% of the population, compare that to HIV where it's only .5%, or cirrhosis .5%, or idiopathic pulmonary hypertension which is only 1 in about 2 million. And in scleroderma it's 7-10% where we universally screen. So in sickle cell disease this has about the same prevalence as what we see in a disease like systemic sclerosis.

So just to, I'll conclude here and just say that is it common or rare? Well it's common, 6-10% of the patients will have pulmonary hypertension defined by right heart cath. Is it a risk factor for death, yes and is it related to hemolytic anemia, yes. I think with that I'll stop to give a few times for questions and we can get to the next session. Thank you.