

# Introduction

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For over 40 years, as a scientist and clinician, I have been working on research to understand and cure a blood disease that kills thousands of children and young adults worldwide every year, and affects millions of people around the world. It is called Cooley's anemia, after Dr. Thomas Cooley, a physician who first described it in 1925. Children with the disease used to die in their teens and 20s. Now they live into their 30s and 40s and beyond as progress has been made in treatment. Although there is currently no reliable cure for the disease, there are realistic hopes and dreams that one will be discovered in the not too distant future.

This book is about Cooley's anemia: the patients; the families; the research to understand the disease; the ways to treat it; and the search for a cure.

Life is a struggle for all of us. We must survive disease, disappointment, adversity in the world around us, and the cruel blows of fate. This book is about people with a terrible blood disease that haunts their whole life and the struggle that entails. It is also the struggle of physicians caring for them, as well as the struggle of scientists trying to find a cure for the disease.

Linda De Pasquale has lived with Cooley's anemia for 45 years. She is a rare long-term survivor of the disease. But to keep herself alive and well, she has to stick herself with a needle and bear its

## 2 Turning Blood Red: The Fight for Life

discomfort for eight to twelve hours almost every day to receive the life-saving drug she needs. She also has to receive blood transfusions every two or three weeks.

After so many years with a disease in which bone erosion and cardiac complications are common, who knows when her bones will break or when her heart will truly fail? But for now, she is well. Her body and spirit survive, and she embraces every day as “the newest day” of the rest of her life. She enjoys her daily life on the combined fuels of medicine, friendship, love, and belief. What does she believe in? Herself, God, Buddha, her doctors, her friends. She believes in them all and is committed to life.

Who can tell her anything about her future that she doesn't already know? Looking out her window onto the waters off Sandy Hook, New Jersey, living her life as fully as she can, she says: “We're all going to die. It's simply a matter of when and how and how much we will suffer.

“We'll all get either cancer or heart disease or some other bad medical condition sometime. Why worry? Worrying and complaining are wasted emotions; they don't help. I just live life as it comes.”

How has Linda coped? Her mother was her savior during childhood. When she died, her aunt, her doctors, and her friends took her place. But it is Linda D., with her commitment to complying with her treatment and her mental toughness, who has willed herself to survive so much longer than most other patients with her disease. She knows that, “If I don't take care of myself, no one else can help me.

“And someday there'll be a cure. But right now, I'm doing everything I can.”

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Cooley's anemia is a disease of the blood protein, hemoglobin. Hemoglobin transports oxygen in the red blood cells that circulate in our blood. There are billions of red cells circulating in our blood every minute of our lives. And each red cell has millions of hemoglobin molecules in it to transport oxygen to all of our cells.

Cells need oxygen for their power. Oxygen allows cells to make proteins, to turn fats and sugars into energy, and to keep the organs of our body functioning properly. Without oxygen, we die; without hemoglobin, we die.

## **Hemoglobin: The magic gift**

Our red blood cells are simply porous bags of hemoglobin. Oxygen is transported through the body bound to hemoglobin. When oxygen in our lungs moves into our red cells, it is grasped by hemoglobin and then released to all of our body's cells as it is carried in our circulating blood. The blood cells are red because that is the color of hemoglobin bound to oxygen (oxygenated hemoglobin).

Hemoglobin has the unique and extremely efficient ability, developed over millennia, to pick up the oxygen we breathe as blood passes through our lungs. Just as efficiently, hemoglobin releases the oxygen as blood flows to all of our organs such as the kidneys, liver, brain, and heart.

Like a biological magic sponge, hemoglobin picks up oxygen as we breathe, and bathes our cells with it where it is needed for the cells to function well and stay alive.

## **Red cells and anemia**

Red blood cells, like all other blood cells, are made in the bone marrow cavities inside our bones. The earliest red blood cells somehow know that their main job is to make large amounts of hemoglobin. We don't know how they know, but they know.

The earliest red blood cells made in our bone marrow have nuclei that carry our chromosomes and DNA. After dividing a few times, these nucleated red blood cells in the bone marrow, bursting with hemoglobin, become smaller, lose their nuclei, and leave the marrow to enter our circulating blood. Once in our blood stream, they become mature red blood cells that float around and

## 4 Turning Blood Red: The Fight for Life

live for months, providing us with the oxygen-carrying hemoglobin we need.

If there are too few red blood cells in our blood, or too little hemoglobin in each of the red blood cells, we have anemia. In either case, we have too little total hemoglobin and not enough oxygen delivery to our tissues.

A little anemia is tolerable; too much anemia is not. If the anemia is severe enough, we feel weak because our cells do not get enough oxygen, and eventually we die.

We can get anemia by bleeding, say from a stomach ulcer or a gunshot wound. We can get anemia because our red blood cells burst prematurely when attacked, for example, by viruses or drugs. Or we can get anemia because we simply don't make enough normal red blood cells because of troubles in our bone marrow.

One of those bone marrow troubles can be in making hemoglobin. Cooley's anemia is a result of that kind of trouble.

### **Cooley's anemia: too little $\beta$ globin**

Normal hemoglobin holds oxygen the way a baseball pitcher's fingers hold a baseball. Two kinds of hemoglobin fingers (or globin protein chains) called by the Greek letters alpha ( $\alpha$ ) and beta ( $\beta$ ) globin protein chains, and another part of hemoglobin, called heme, acting as a thumb, hold the oxygen in hemoglobin in place, and release the oxygen to the cells in the body as the red blood cells circulate.

Just as an expert pitcher's fingers have been educated over time and through experience to deliver the ball precisely, the structure of the  $\alpha$  and  $\beta$  globin chains of human hemoglobin have evolved to interact optimally and precisely with oxygen. But while a pitcher only has to throw 100 or so pitches to the right spots in each game, hemoglobin grasps oxygen from the lungs and delivers it to tissues with perfection, billions of times a day.

Neither  $\alpha$  nor  $\beta$  globin chains alone can grip the oxygen. In fact, it takes two  $\alpha$  and two  $\beta$  globin chains, together with heme, to hold

and release oxygen properly. The normal hemoglobin we have circulating in our blood cells is called adult hemoglobin or hemoglobin A (HbA). Cooley's anemia is caused by a problem in making one key part of HbA, the  $\beta$  globin chains. Without the  $\beta$  globin chains, hemoglobin cannot bind or release oxygen.

The human  $\beta$  globin is a protein like all other proteins, composed of specific chemical building blocks called amino acids. The precise instructions for the amino acid sequence of  $\beta$  globin, again like that of all other proteins, are encoded in our chromosomes by the genes in our DNA. The human  $\beta$  globin gene is located on chromosome 11, at a place called the human  $\beta$  globin gene locus. We carry one  $\beta$  globin gene at this locus on each of the pair of chromosomes that we inherit, one from each parent, so that we have a total of two  $\beta$  genes per individual.

Our two human  $\beta$  globin genes determine the amount of human  $\beta$  globin we produce. Normally, these two genes allow us to produce enough  $\beta$  globin to fill our red cells with normal amounts of normal hemoglobin. However, in Cooley's anemia, little or no  $\beta$  globin is made. A defect in both of our two human  $\beta$  globin genes leads to either too little or no  $\beta$  globin chain production from these genes, and thus to Cooley's anemia. These gene defects in our  $\beta$  DNA are called  $\beta$  thalassemia mutations. If we inherit a  $\beta$  thalassemia gene from both parents, and we have no normal  $\beta$  globin genes, we will end up having little or no HbA. As a result, we will suffer from a severe anemia, Cooley's anemia.

Cooley's anemia is also called homozygous  $\beta$  thalassemia, Mediterranean anemia or thalassemia major. In this book, I will use Cooley's anemia and thalassemia interchangeably to signify the severe form of the disease. This inherited condition affects families in all parts of the world, although it is most prevalent among people of Greek and Italian ancestry who live near the Mediterranean Sea. That is why it is also called thalassemia: "thalasso" is the Greek word for the sea.

If we inherit one  $\beta$  thalassemia gene from one parent, and a normal  $\beta$  globin gene from the other, we have what is called

“ $\beta$  thalassemia trait” or thalassemia minor, a harmless condition with little or no anemia. With the trait, increased production of  $\beta$  globin by the one normal  $\beta$  globin gene compensates for the low or absent activity of the defective  $\beta$  gene.

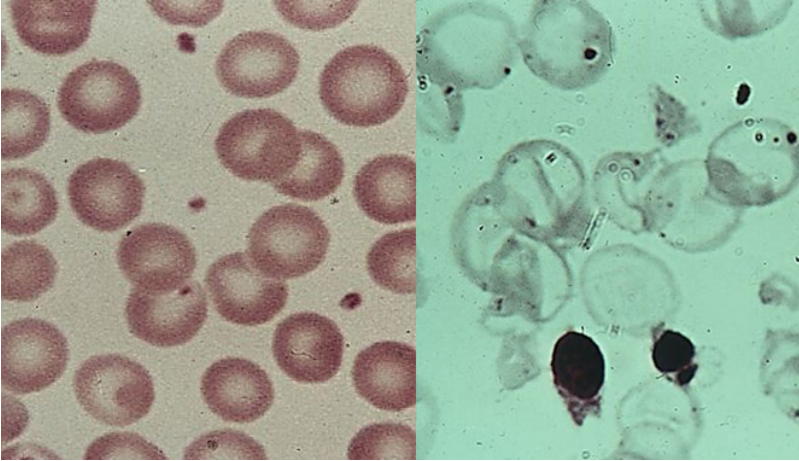
People with thalassemia trait are not easily diagnosed without special blood tests. They appear normal, and usually don't have anemia or other problems. That is why it is so difficult to know before birth whether a new infant of two parents who each have the trait is at risk for having Cooley's anemia, unless thalassemia trait has been recognized in the parents beforehand, or they already have a child with the disease.

## And too much $\alpha$ globin

The low or absent  $\beta$  globin and HbA in Cooley's anemia is bad enough, but there is a lot worse that follows. Despite too little or no  $\beta$  globin,  $\alpha$  globin, the other type of globin chain in HbA, continues to be made at its usual normal high level in red cells, and piles up in the cells.

In HbA, the two  $\alpha$  and two  $\beta$  globin fingers and the thumb, heme, are all required to hold oxygen in place and release it to the body's cells appropriately. In contrast, free  $\alpha$  globin chains alone, without  $\beta$  partners, are useless and harmful. These  $\alpha$  globin chains cannot fold properly to grip or hold the oxygen. Instead, the free  $\alpha$  globin becomes a tangle of proteins, aggregating as undesirable globs, eventually destroying most of the red cells in which they accumulate.

Our bodies recognize cells with the misshapen  $\alpha$  tangles as abnormal, and use scavenger cells to rapidly destroy them. In this process of abnormal red cell production in the bone marrow of Cooley's anemia patients, called “ineffective erythropoiesis,” most of the diseased red blood cells, with their  $\alpha$  globin tangles in the bone marrow, do not survive.



Red blood cells on a blood smear from a normal subject (left), and from a patient with Cooley's anemia (right). The thalassemia red cells are deformed, and contain too little hemoglobin.

Similarly, the circulating red blood cells of Cooley's patients are also destroyed quickly after they leave the bone marrow since they too carry an excess of  $\alpha$  globin. The circulating red blood cells in thalassemia appear very abnormal; they are misshapen and have too little hemoglobin.

Too little  $\beta$  globin, too much  $\alpha$  globin, thalassemia.

So all one really needs to know about Cooley's anemia is:

- (1) The normal hemoglobin molecule is composed of two kinds of protein chains called  $\alpha$  and  $\beta$  globin chains, which, together with heme, are necessary to bind and release oxygen.
- (2) For hemoglobin to work properly, the two  $\alpha$  and two  $\beta$  globin chains have to interact with each other and fold together with heme to form a complete and functional normal hemoglobin molecule, called adult hemoglobin or hemoglobin A (HbA).
- (3) In Cooley's anemia, there are too few or no  $\beta$  globin chains produced; so there is not enough HbA. Because of this lack of  $\beta$  globin and the low level of hemoglobin in their blood, the patients have low blood counts or anemia.

- (4) In Cooley's anemia, the  $\alpha$  globin chains continue to be made in normal amounts, but unattached to  $\beta$  pieces. The free  $\alpha$  globin chains aggregate, form tangles, and thus are toxic to the red blood cells in both marrow and circulating blood, resulting in increased red cell destruction and worsening of the anemia.

To repeat: Too little  $\beta$  globin, too much  $\alpha$  globin, too little hemoglobin, abnormal red cells, disease.

### Surviving to have the disease

Infants destined to have Cooley's anemia survive at birth because they, like all of us, produce another type of oxygen-carrying hemoglobin in fetal life called fetal hemoglobin (HbF). HbF ( $\alpha_2\gamma_2$ ), contains so called gamma ( $\gamma$ ) globin protein chains instead of the  $\beta$  globin ones. HbF is the essential and major hemoglobin of all human fetuses. Around birth, the production of HbF is essentially switched off and the production of HbA is switched on. The small amounts of HbF made after birth and into adult life provide only limited oxygen carrying capacity for thalassemia patients.

Why do we need HbF as fetuses? The best explanation for the presence of HbF in fetal life is that it helps the fetus survive, especially when the oxygen supply is limited. HbF binds oxygen more avidly than HbA, like a pitcher holding the ball more tightly. This preferentially allows the fetal red cells with HbF to grab and hold oxygen more tightly than the mother's red cells with HbA, as blood travels in the placenta between mother and fetus.

Infants with Cooley's anemia would die *in utero* if HbA were the only hemoglobin available. The existence of HbF allows their survival.

HbF production is normally turned off around birth in all of us, as  $\beta$  globin and HbA production is turned on, presumably, again, to maintain the fetal oxygen advantage for future progeny. Cooley's anemia results as  $\beta$  globin and HbA production become necessary for oxygen transport after birth as HbF production declines.

We know that patients with Cooley's anemia have normal  $\gamma$  globin genes and normal amounts of HbF *in utero*, and that they survive fetal life normally. So why don't they just revert to making adequate amounts of fetal hemoglobin when they can't make HbA? We don't know why but they can't.

If we could find ways to allow HbF production to persist after birth and into adult life at the high levels it is produced in the fetus, Cooley's anemia patients would be cured. In fact, there are some rare humans who have only fetal hemoglobin in their blood as adults and are healthy; this is a phenomenon that tells us that there is a way to survive without  $\beta$  globin.

The molecular events involved in the switch from human fetal to adult hemoglobin around birth are being better understood and could eventually lead to new therapies. There are already several drugs that are known to increase fetal hemoglobin production. These insights and approaches to regulating and increasing fetal hemoglobin will be discussed later in the book.

## Treating the disease

Patients with Cooley's anemia require blood transfusions to combat their anemia in order to survive. When we become anemic from any cause, we have the capacity to spontaneously increase the number of red blood cells that we produce in our bone marrow several-fold to compensate for the anemia.

Similarly, Cooley's anemia patients have this capacity and do increase the number of red cells they make as they try to compensate for their anemia but to no avail. This is because the premature destruction by scavenger cells of most of the defective nucleated red cells with their excess toxic  $\alpha$  globin tangles leads to the production of more defective red cells in a vicious cycle that results in a mass of accumulated cells. This mass expands the bone marrow cavity abnormally, and erodes and deforms the bones. The end result of this process in Cooley's anemia is that abnormal red cells fill the

bone marrow, but are ineffective in producing useful blood cells with useful hemoglobin.

The abnormal thalassemia red cells that do reach the circulating blood are prematurely destroyed in the spleen, the normal site of circulating red cell destruction. The spleen is the organ that is most sensitive to the presence of abnormal red blood cells. It essentially eats and digests these abnormal red blood cells, engorging and enlarging itself as it does so. The volume of red blood cells consumed increases with the increasing size and capacity of the spleen. This increasing capacity leads in time to the destruction of even transfused normal red cells in these patients. Early removal of the spleen (splenectomy) due to an enlarged spleen (splenomegaly) is common in thalassemia.

In addition, the much more active destruction of thalassemia red cells compared to normal red blood cells leads to an increased release of heme and globin. While the excess globin is broken down into its amino acids and re-used, the excess heme can lead to the deposition in the gall bladder of excess bilirubin which is a breakdown product of heme. This process causes gallstones and gall bladder disease, and may eventually necessitate gall bladder removal.

## **Transfusions and iron overload**

Blood transfusions are required to solve the problem of anemia. New normal blood cells provide new normal hemoglobin capable of normal oxygen binding and delivery. They also suppress the production and the expansion of the bad thalassemia blood cells, and, therefore, can forestall the development of the bone deformities.

If adequate blood transfusions are not available, most patients with Cooley's anemia do not survive past the age of five. Blood transfusions are required for life.

But the life-saving blood transfusions alone do not end the problems of patients with Cooley's anemia. Iron overload and toxicity is another problem.

The metal iron is a component of heme, a part of hemoglobin mentioned previously. It is the thumb in the pitcher's hand that, in addition to the globin fingers, is required to grip and release oxygen from hemoglobin optimally.

Normally, our iron intake is relatively low. As our red cells, the largest source of free iron, break down and release their heme iron, this free iron is either re-utilized to make new hemoglobin or excreted in our stool and urine. These relatively small amounts of free iron are easily bound by special proteins in our blood stream. Whatever free iron there is outside of red cells is kept in check by these regulatory proteins. The deposition of iron in our body's tissues and organs is normally avoided by this iron binding and excretion process.

However, in Cooley's anemia patients, the large amounts of iron in transfused blood overwhelm these normal mechanisms of iron binding and excretion, resulting in much of the excess iron being deposited in almost all organs, most dangerously, the liver and the heart. Sadly, the human body has no good way of ridding itself of this vast excess of free iron. Thus, too many blood transfusions can result in iron toxicity and death. Iron accumulated in the pancreas causes diabetes; in the liver, it leads to fibrosis and cirrhosis; in the heart, it causes scarring which leads to heart arrhythmias, heart damage, heart failure and death.

So patients with Cooley's anemia can die, either early in life from the lack of normal hemoglobin (HbA) in blood, or later in life from too much iron from blood transfusions. Until the 1970s, the treatment of Cooley's anemia was shadowed by the dilemma of a tragic choice: Too little hemoglobin, or too much iron.

## **A true miracle: controlling the iron**

In 1974, a unique new therapy was introduced to rid the body of the excess iron accumulated in Cooley's anemia patients as a result of their blood transfusions. The drug is Desferal (deferroxamine), and it binds to (or chelates) iron. The iron, chelated and bound by

Desferal, is excreted in large quantities in urine and stool and can result in “negative iron balance” i.e., more iron leaving the body than is being taken in.

Desferal is magic: it has dramatically changed and saved the lives of many, many thalassemia patients worldwide. Patients can now be adequately transfused and have enough normal hemoglobin to live normal lives, without having to worry nearly as much about dying from iron toxicity as was the case in the past. This extraordinary drug has both enhanced the lives and increased the lifespan of most patients.

However, Desferal has a major problem of its own: it cannot be taken orally. It can be given intravenously, intramuscularly, or subcutaneously.

Routine intravenous Desferal is impractical, and intramuscular use is painful. Therefore, the drug is routinely given subcutaneously. It has also been learned that to be most effective, Desferal must be given continuously over eight to twelve hours a day, almost every day. This subcutaneous administration, which requires a needle and syringe attached to a mechanical pump, is a delicate and complex procedure.

Continuous infusion is necessary because stored iron appears only slowly in the blood stream over time and Desferal has to be present continuously to eliminate it. In addition, the half-life of Desferal, the time it stays in the blood stream, is relatively short.

For patients like Linda D. who can be compliant with the Desferal program, the treatment is a dream come true and has meant a long life, although at the physical and emotional expense of sticking herself under her skin for day-long infusions almost every day of her life to receive the benefits of this treatment.

But many thalassemia patients are not like Linda D. They have problems with compliance. For these patients, it is just too difficult to adhere to the almost daily regimen of subcutaneous Desferal therapy.

So Desferal along with blood transfusions is the established therapy for Cooley's anemia, but this is a demanding and difficult program. And it is not a cure. Patients on the program are still dying.

More recently, the availability of two iron chelators that are effective when taken orally, named L1 and Exjade, promise to overcome the injections and compliance problems of Desferal. No more pump, no more syringe and needles. Just a pill once or a few times a day. A true blessing, especially for patients who cannot comply with Desferal injections, or do not respond to the drug. The role of these new drugs in overall thalassemia treatment is still unfolding, but their use is already saving lives.

## The road to a cure

There should, in fact, already be a cure for Cooley's anemia. We have known so much about this disease for so long that by the 21st century, we should have already found a cure. So I thought 25 years ago, and so I think today. As mentioned, one approach would be to find a way to restore fetal hemoglobin to curatively high levels, thus ensuring effective oxygen delivery, as occurs in our fetal life. More about that later.

There are two other potentially curative approaches to the disease. We know that the only thing wrong in Cooley's anemia is that the blood cells in the patients' bone marrow have a defect in making normal human  $\beta$  globin. And that nucleated red blood cells in our bone marrow normally provide all of the  $\beta$  globin we need. One curative treatment is to transfer the normal marrow blood cells from a person with normal hemoglobin to the patient, so-called bone marrow transplantation; the other approach, human  $\beta$  globin gene therapy, is to provide normal  $\beta$  globin by either correcting the DNA defect in the patient's  $\beta$  globin gene or adding a new normal  $\beta$  globin gene to the patient's own blood-forming bone marrow cells, thus having those cells produce new normal  $\beta$  globin in normal amounts.

Bone marrow transplantation from another person, called allogeneic bone marrow transplantation (ABMT), has already been shown to cure many patients with Cooley's anemia. The new bone marrow replaces the old. Currently, it is the only treatment that can result in a cure. However, the use of ABMT is limited by the need for immunologic compatibility between the marrow blood cells of the donor and the patient. Without this compatibility, a condition called graft versus host disease can result and lead to disability or death. Because of the problem of immunologic incompatibility, there are no suitable donors for most patients with Cooley's anemia, although new *in vitro* fertilization techniques may lead to the availability of more compatible sibling donors. ABMT will be discussed in a later chapter in the book.

The other potentially curative approach, human  $\beta$  globin gene therapy, utilizes either a corrective piece of DNA or a normal  $\beta$  globin gene. In both cases, normal  $\beta$  globin DNA is added to the Cooley's patients' blood-forming marrow cells in a culture dish, and then the cells are put back into the bone marrow of the patient. Optimally, the gene altered cells will produce normal amounts of normal hemoglobin.

Human  $\beta$  globin gene therapy has cured Cooley's anemia in animal models, specifically in mice. Unlike ABMT, gene therapy has no immunological barriers to overcome, and is potentially a cure for all patients with Cooley's anemia. An early stage clinical trial using human  $\beta$  globin gene therapy in patients with Cooley's anemia is currently underway and is discussed later in the book.

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This book is about Cooley's anemia: the patients; their families; the research to understand the disease; current treatment options; and the search for a cure. All of the facts are presented as truly and accurately as I know them, and all of the material from interviews with patients, family members and doctors has been largely verified by follow-up inquiries and overlapping accounts. I alone am responsible for any errors that may be in this book.