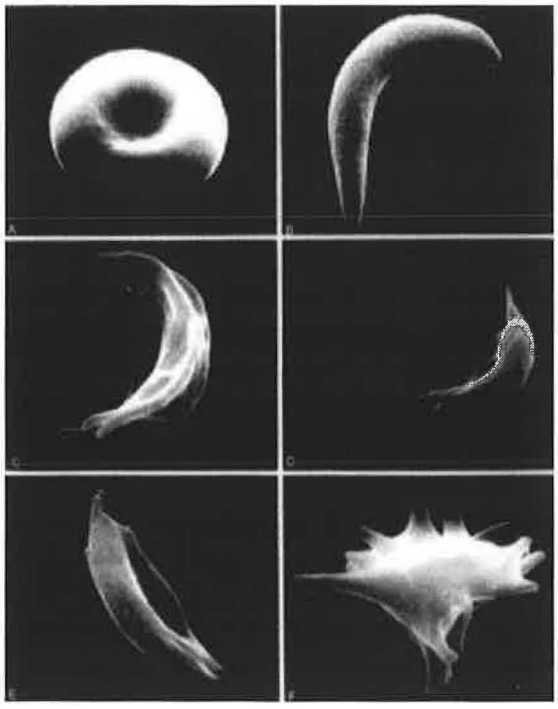
Sickle Cell Anemia: New Insights into Vascular Occlusion



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Dr. Rutherford's interests are primarily in "benign" Hematology. Her clinical practice includes disorders of hemostasis, sickle cell anemia and other hemoglobinpathies. Her research interests are in novel immunotherapy for immune thrombocytopenic purpura (ITP) and in clinical outcomes in patients with hemophilia.

This is to acknowledge that Cynthia Rutherford, M.B.,Ch.B. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Rutherford will be discussing off-label uses in her presentation.

Almost exactly 100 years ago a young black dental student from Grenada was admitted to Presbyterian Hospital in Chicago, with jaundice, pain, cough and fever. His case was subsequently reported by James Herrick in a paper entitled "Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia", in *Archives in Internal Medicine* in 1910, and was the first description of a case of sickle cell anemia (SCA) in the Western literature. [1] Medical historians have identified this patient as Walter Clement Noel, who returned to Granada to practice dentistry and subsequently died there at the age of 32, from an illness which was probably acute sickle chest syndrome. [2]

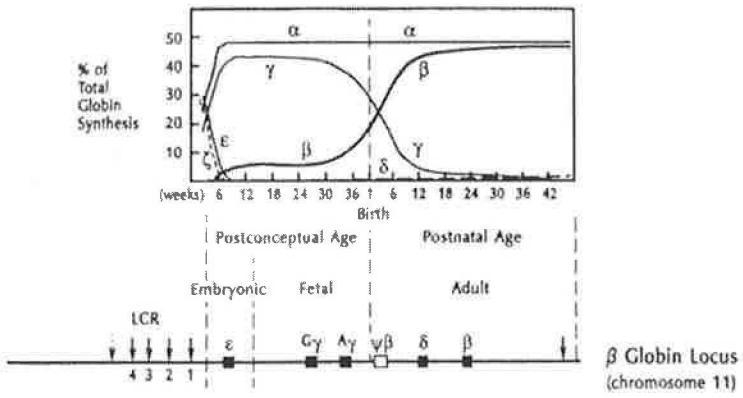
The condition we now know as sickle cell anemia (SCA) from these early descriptions of its characteristic sickle-shaped red blood cells has been known in Africa for many centuries, for its dreaded painful crises and early death. A remarkable mythology arose around SCA in Africa, where many believed the children who died young from the disease were reincarnated, the so-called "repeater children". These children were healthy during their first months, and became increasingly ill and lethargic, usually dying of infection before the age of five. The reincarnation myth likely arose because of the physical similarities noted in subsequent affected children. [3]

Although SCA is a serious, life-threatening illness for the homozygote who carries two copies of the sickle cell gene, the heterozygote was at a biological advantage prior to antimalarial therapy, as the single sickle gene protected against the dire consequences of cerebral *Plasmodium falciparum* malaria. This resulted in a balanced polymorphism, which ensured the gene would persist. [4]

In the 100 years since this first case of SCA was documented, there has been an remarkable expansion of knowledge of this condition, including Linus Pauling's description of the abnormal hemoglobin electrophoresis of in SCA, designating it the first molecular disease, Ingram's discovery that the fundamental defect was caused by a single amino acid substitution in the sickle hemoglobin and the localization of this defect to the β-globin chain of hemoglobin. [5-7]

Genetics of sickle cell disease [4]

Figure 1: β-globin Gene Locus: pattern of switching during fetal and adult life



The hemoglobin molecule consists of the heme porphyrin moiety, which carries oxygen, and two pairs of globin chains. Production of α-globin chains, controlled on chromosome 16, begins in early fetal life, and a pair of α-globin chains is found in all hemoglobin molecules. The principle mutation responsible for sickle cell disease is in the β-globin gene, which is found on the 11^{th} chromosome, in an area known as the β globin gene locus. This locus includes a series of genes, which control synthesis of the various globin chains that sequentially combine with α-globin during embryonic, fetal and adult life. For most of fetal life two α-globin molecules combine with a pair of γ-globin molecules forming fetal hemoglobin (HbF); β-globin secretion does not begin until late fetal life and only reaches adult levels about 9 months of age.

It is on this specific β-globin gene that the sickle cell disease mutation occurs, where a single nucleotide switch results in the single amino acid substitution of valine for glutamic acid at the 6^{th} position. This profoundly alters the properties of the hemoglobin thus formed, named sickle hemoglobin (HbS). The substituted amino acid forms a hydrophilic bond with adjacent globin chains, and forms insoluble polymers of sickle hemoglobin under conditions of low oxygen tension. These polymers disrupt the normally fluid contents of the usually flexible red blood cells, making it difficult for them to negotiate the tiny, 3-micron diameter of the capillaries of the microcirculation. Although the HbS polymers dissolve once oxygen tensions are restored, their repeated formation-dissolution damages the red cell membrane, eventually rendering it rigid, and unable to control cell hydration and electrolyte balance.

Remarkable electron micrographs have shown these rope-like sickle hemoglobin polymers, and the resultant bizarre shapes the red cell assume under low oxygen tensions. We see these same cells in the peripheral blood smear that Dr. Irons drew in his patient's chart 100 years ago.

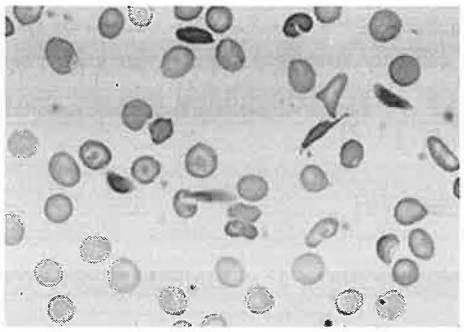


Figure 2: Blood smear in SCA: presence of dense and irreversibly sickled cells

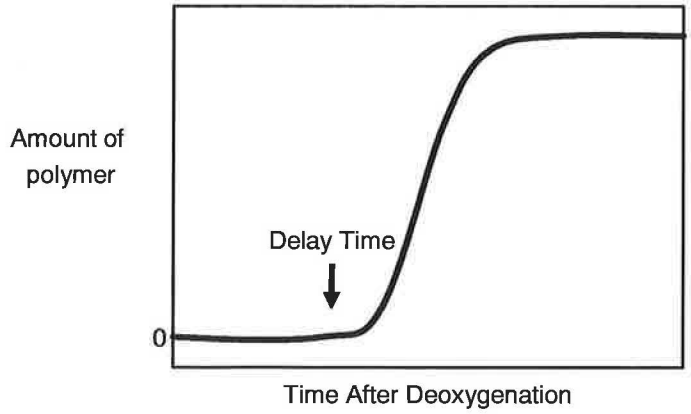
These characteristic cells are known as irreversibly sickled cells; they are dehydrated, dense, and their membranes are rigid. The demise of these cells is imminent; red cell survival may be dramatically shortened from its normal 120 days to 10 to 20 days in sickle cell anemia.

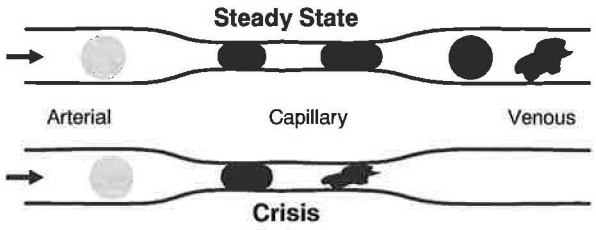
Although the same fundamental mutation, the genotype, is present in all patients with sickle cell anemia, many factors modify the phenotypic manifestations and severity of the disease, so called pleiotropic or secondary factors. (Table 1)

Table 1: Genetic factors that modify expression of sickle cell anemia
o Increased concentration of fetal hemoglobin, HbF, including 0.158C→T mutation: hereditary persistence of fetal hemoglobin
o Presence of concurrent α-thalassemia
o Polymorphisms elsewhere in the hemoglobin molecule
o Polymorphisms in endothelial markers [8]

Formation of the sickle polymer [4]

Formation of sickle polymers does not occur immediately upon deoxygenation. There is a well recognized "delay time" before they develop. During many trips around the circulation this polymer formation does not occur until after the red cell has negotiated the stringent environment of the microcirculation, and is in larger bore vessels on the venous side, where its rigidity and bizarre shape do not cause obstruction. Understanding the factors that influence this "delay time" in polymer formation and the red cell transit time in the microcirculation are central to understanding the pathophysiology of sickle cell disease and the vascular occlusion responsible for almost all its serious manifestations. These influences turn out to be much more complex than was originally suspected.





The tendency for the sickle polymers to form is dependent on many features, which are listed in Table 2.

Table 2: Factors promoting sickle polymer formation

- Low oxygen tension of the tissues
- Low pH of the tissues
- High concentration of intracorpuscular sickle hemoglobin, HbS:
  - Low levels of protective hemoglobins, such as fetal hemoglobin (HbF), HbA2
  - Dehydration of the red cells

The concentration of sickle hemoglobin in red cells is affected by the presence of other hemoglobins, principally fetal hemoglobin. Fetal hemoglobin has a remarkable mitigating effect on the formation of sickle cell polymers, proportional to its concentration. Neonates, with their high percentage of HbF, and individuals with a second β-globin gene cluster mutation, known as hereditary persistence of fetal hemoglobin, have very mild, if any, manifestations of sickling. The amount of fetal hemoglobin relates to overall survival and frequency and severity of pain crises. Individuals with concurrent α-thalassemia are also protected, because of generally low concentrations of intracellular hemoglobin (low mean cell hemoglobin concentration, MCHC).

The presence of the sickle polymer significantly affects the red cell membrane in several ways. Oxidative damage of the membrane components, actin and spectrin, leads to a more rigid membrane structure. [9] Changes in red cell membrane phospholipids, with resultant appearance of phosphatidylserine on the red cell surface causes several deleterious effects, including increased adhesion to the endothelial cell, enhanced phagocytic removal of the erythrocyte and promotion of coagulation by providing a surface where coagulation components can be assembled. [10-12]

Other vital functions dependent on an intact membrane are also affected, and as the membrane becomes progressively damaged the complex controls of cation homeostasis are disrupted. The red cell membrane becomes abnormally permeable to cations, including sodium, potassium, magnesium and calcium. Calcium entry into the red cell activates the Gardos channel in the red cell membrane, by which potassium is actively transported out of the red cell. [13] This potassium loss contributes to its dehydration, which is further exacerbated by concurrent activation of the potassium chloride co-transporter, especially under conditions of low pH. Dehydration of the cell leads to higher concentrations of the sickle hemoglobin that enhance polymer formation further, with the formation of increasingly dense red cells, which eventually become irreversibly sickled cells. The Gardos channel may also be activated by cytokines, which may partially account for the association of sickle cell vascular occlusive crises with infective

episodes. Therapy to block the Gardos channel, and thus prevent red cell dehydration, is being actively studied in animal models and in patients. [4, 14]

Passage of sickle red cells through the microvasculature

Several factors affect the time the red cell spends traversing the microcirculation, as shown in Table 3. The sickled erythrocytes show increased adherence to endothelial cells, both *in vivo* and *in vitro*. [15] There is a complex series of interactions between receptors on the endothelial cell and the red cell, and even between sickle cells and the extracellular matrix. [16, 17] Some of the implicated receptors on the red cell include the integrin, $\alpha_4\beta_1$, and CD36, both of which have increased expression on newly-produced red cells, the reticulocytes (which are increased as part of the compensatory process for the vigorous hemolysis in SCA) and in sickle red cells. Integrin $\alpha_4\beta_1$ interacts with VCAM-1 (vascular cell adhesion molecule-1), which is expressed on the endothelial surface after exposure to cytokines and hypoxia; the strength of the reaction between VCAM-1 and $\alpha_4\beta_1$ is further enhanced by hypoxia. The ligand, thrombospondin has an important bridging function between erythrocyte CD36 and several endothelial components, including CD36, $\alpha_2\beta_1$ and heparan sulphate proteoglycans. Laminin on endothelial cells bind strongly to red cells through the B-CAM/Lu protein, which carries blood group antigens of the Lutheran blood group; this adhesion is increased by epinephrine. [18] Epinephrine also enhances erythrocyte binding via ICAM-4 and αVß3. [19] These findings are intriguing, in that many sickle cells patients believe stress increases their episodes of sickle vaso-occlusive crisis.

Table 3: Factors which prolong transit time of sickle red cells in the microcirculation

- Enhanced adhesion of red cells to the endothelium
  - Activation of endothelial cells
  - Increased expression of pro-adhesive markers on red cells
- Increased vasomotor tone, enhancing vasoconstriction
  - Nitric oxide, endothelin-1 and eiconasoids
- Activation of coagulation
- Release of free radicals associated with tissue damage

Solovey *et al* showed an increased number of circulating endothelial cells in patients with sickle cell disease, especially around the time of a sickle cell crisis. The majority of these endothelial cells had the CD36 marker, implying they were of microvascular origin, and most showed the presence of activation markers, including VCAM-1, ICAM-1 (intercellular adhesion molecule-1), P-selectin and E-selectin. They concluded that the vascular endothelium is activated at all times in patients with sickle cell disease, with an apparent increase at times of vaso-occlusive crisis. [20] The amount of soluble VCAM-1 in the blood is recognized as a surrogate marker for endothelial activation and is elevated in sickle cell crisis. [21]

Patients with sickle cell anemia have long been recognized as having mild continued activation of the coagulation system, even in the steady state. [22] Supportive evidence includes consistent increases in fibrin degradation products, D-dimers, prothrombin fragment 1+2, fibrinopeptide A and thrombin-anti-thrombin complexes, in conjunction with decreases in factor V, factor XIII and plasminogen, and plasminogen activator. More recent studies have shown elevated whole blood tissue factor in SCA, and the presence of circulating endothelial cells expressing tissue factor on their surface. These findings suggest the activated endothelial cell is the likely instigator of the intravascular coagulation. It is possible that the thrombin thus generated serves to further activate endothelial cells, with the production of P-selectin. [23] Changes in the red cell surface, particularly the expression of phosphatidylserine, may both provide a surface for ongoing assembly of clotting factors, and serve as yet another factor promoting adhesion to the endothelium. Thrombin also causes endothelial cell retraction, exposing the subcellular matrix, which provides yet another site for red cell adhesion.[24]

Other extra-erythrocytic factors implicated include the leucocytes, particularly the neutrophils, levels of which are often elevated in SCA, because of hyposplenism. [25] The majority of patients with SCA have no spleen function after early childhood, because the splenic microcirculation, with its slow sinusoidal flow, proves uniquely vulnerable to vascular occlusion and early splenic infarction. Hyposplenic patients typically run mildly elevated granulocyte counts, with exaggerated increases in the setting of inflammation. An elevated white count may play a part in the etiology of the disease; it is known to predict for disease severity, even mortality. Baseline white count is an independent risk factor for acute chest syndrome and stroke. In SCA circulating granulocytes show signs of activation, including down regulation of L-selectin. The converse is that patients receiving Hydroxyurea who have lower neutrophil counts, because of myelosuppression, have had fewer episodes of vascular occlusive crisis.

Although the platelet count may also be elevated in any hyposplenic state, platelets do not seem to be especially involved in microvascular occlusion, nor have anti-platelet drugs been found ameliorate the vaso-occlusive crises of SCA.

Role of nitric oxide in Sickle Cell Anemia

Nitric oxide is a very active molecule, with several powerful biological effects relevant to the pathophysiology of SCA. [26] Nitric oxide (NO) is produced constitutively in endothelial cells, by the enzyme NO synthase, from the amino acid, Arginine. Diffusion of NO into vascular smooth muscle causes relaxation of vascular tone and resultant vasodilatation, which makes red cell transit easier. Additional NO diffuses into the blood flowing past and reacts with molecules in the plasma and with the oxyhemoglobin in red cells. The red cell membrane is relatively impervious to NO, which is rapidly destroyed by free hemoglobin. In SCA there is more free hemoglobin in the plasma because of intravascular hemolysis and NO is more rapidly destroyed. [27] These low levels of NO promote vasoconstriction and may lead to vascular occlusion. The

vasculature of the lung is particularly affected by these changes; low NO levels can lead to pulmonary vasoconstriction and likely predispose to pulmonary hypertension.

Nitric oxide also acts in a protective manner to inhibit gene transcription of pro-adhesive and pro-inflammatory molecules such as VCAM-1 and P-selectin by endothelial cells; reduction of this function of NO would promote adhesion of sickle red cells to the endothelium. Nitric oxide also has an effect on the circulating blood components, inhibiting platelet aggregation and adhesion of leucocytes. A major study of the consortium of Comprehensive Study of Sickle Cell Disease will look at L-arginine supplementation, as early studies have suggested this agent can increase NO levels.

Sickle cell disease Animal Models [28, 29]

Using transgenic technology sickle β-globin and normal human α-globin genes have been introduced into the germ lines of mice to produce several animal models of SCA, which have been extremely useful both in understanding the pathophysiology of the disease and in assessing therapy. Currently there are four different mouse models. The newest model, which has been produced by a combination of transgenic and knockout methods, has generated a mouse with only human sickle hemoglobin, which has a condition most closely resembling human SCA. These knockout mice are anemic, although not as severely so as a human patient, and their blood contains irreversibly sickled cells.

As yet there is no perfect animal model of SCA, because the animal models mimic only the sickle hemoglobin production and lack the other epistatic or modifier genes. However, the transgenic mice have been invaluable in understanding NO reactions and led to some very interesting conclusions regarding balance of globin chains and the role of α-thalassemia in improving the overall outcome. Sickle mouse models have also revealed the nitric oxide and nitric synthase role in the kidneys and have been used to confirm the hypothesis that the sickle hemoglobin gene is protective against malaria.

Clinical Manifestations of Sickle Cell Anemia

The Cooperative Study of Sickle Cell Disease (CSSCD) is integral to clinical studies in SCA. The CSSCD is a multi-institutional investigation of the natural history of sickle cell disease from birth through adulthood, and includes data collected at 23 institutions in a uniform, standardized fashion on 3800 patients, followed longitudinally. Recruitment has included mildly affected patients, to ensure that the study would not reflect only a severely affected hospital-based population.

Table 4: Clinical manifestations of sickle cell disease	
Vascular Occlusion	
	Micro-infarcts - painful crises
	Macro-infarcts - organ damage
Anemia	
	Severe chronic hemolysis
	Aplastic crises - parvovirus B19
Constitutional Effects	
	Impaired growth and development
	Increased susceptibility to infection

Sickle cell disease has protean manifestations that affect every organ system. [30] While the primary manifestation is vascular occlusion, there are profound systemic effects otherwise, including those associated with severe anemia *per se*, with vigorous hemolysis and with constitutional effects, including those associated with increased susceptibility to infection, as delineated in Table 4. Compared with the overall African-American population, patients with SCA have shorter survival, as seen in Figure 3. [31] It is notable that hemoglobin F concentration has a profound effect on survival. These data were collected by CSSCD in the early 1990's. Very recent data from the pediatric program at UT Southwestern showed that 711 children, followed since they were identified by newborn screening, had an 86% overall survival at 18 years of age, a substantial reduction in mortality, which can be attributed to several factors, including the identification of newborns with sickle cell disease, comprehensive care by pediatricians expert in care of sickle cell disease, prophylactic penicillin and the availability of effective vaccines to prevent bacterial infection. [32, 33]

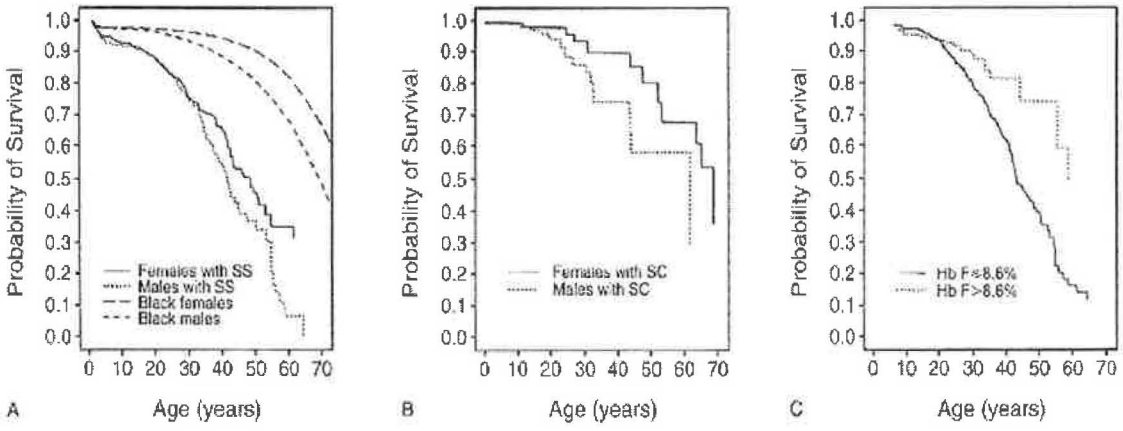


Figure 3: Survival curves in sickle cell syndromes [31]

Clinical manifestations of vascular obstruction in sickle cell disease

One of the common misconceptions about SCA is that there are acute episodes of vascular occlusion, but between these episodes the disease is relatively quiescent. It has become increasingly obvious that there is ongoing chronic organ damage. [34]

Table 5 : Clinical manifestations of sickle cell disease vaso-occlusion

Acute	Chronic
Pain crisis	**Aseptic necrosis**
Acute chest syndrome	**Pulmonary hypertension**
Hematuria	**Hyposthenuria**
Cerebral thrombosis*	**Neuropsychological dysfunction**
Hand foot syndrome*	**Ankle ulcers**
Splenic sequestration*	**Splenic atrophy**
Priapism	

* principally a pediatric problem

This protocol will discuss two important acute vaso-occlusive phenomena, sickle pain crisis and acute chest syndrome, and two chronic states of vaso-occlusion, namely pulmonary hypertension and chronic neuropsychological damage

Sickle cell pain crisis

This is the most common vaso-occlusive phenomenon, the commonest cause of recurrent morbidity in patients with SCA, and is responsible for about 90% of hospital admissions. MRI shows avascular necrosis of bone marrow at sites of pain. The relatively slow flow in the bone marrow sinusoids makes this organ very sensitive to vascular occlusion. The pain is attributed to the inflammatory response, with resultant increase in intramedullary pressure, and it can be very severe. The occurrence of sickle pain crises is very variable. Figure 4. [35] Some patients have frequent severe crises, others have them rarely. Platt *et al* found that the incidence of pain crises was directly proportional to the hemoglobin level, and inversely proportion to the square root of the fetal hemoglobin level. Obviously the other genetic factors which modify disease severity are implicated in this very variable disease frequency. Patients with multiple vaso-occlusive crises have a significantly higher mortality. [36]

The factors which precipitate a pain crisis can be predicted from knowledge of the pathophysiologic process – the same factors which either shorten the "delay time" in sickle polymer formation or increase the transit time in the microvasculature - hypoxia, acidosis, cold, dehydration, infection, and psychological stress.

A sickle pain crisis is not associated with any increase in hemolysis, so hemoglobin, reticulocyte count and hemolytic parameters are usually baseline. Highly specialized

studies show an increase in the number of "dense cells", but changes in these and in the number of irreversibly sickle cells cannot be determined from the blood smear appearances or other routinely available studies.

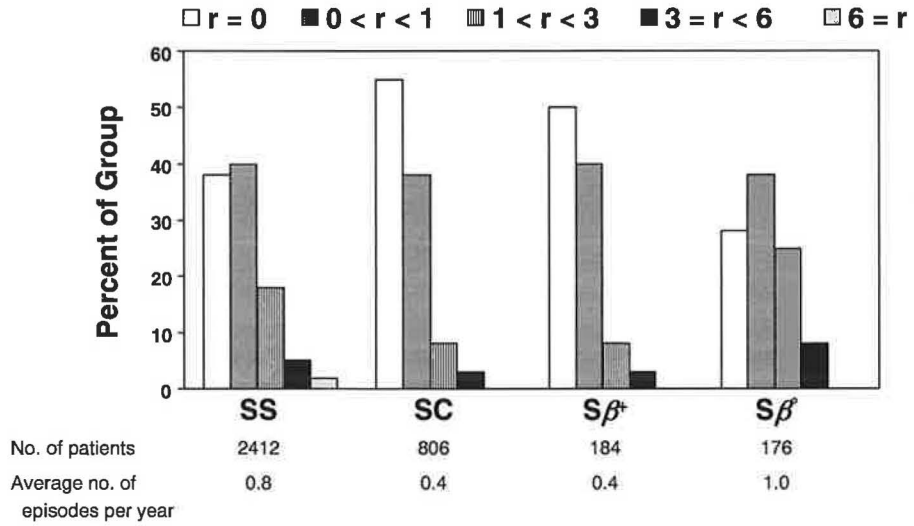


Figure 4: Frequency of severe pain crisis in patients with sickle cell disease [35]

The inflammatory response to marrow necrosis may be associated with an increase in the white count. Other than MRI studies there are no objective measures routinely available which confirm a sickle cell vaso-occlusive crisis, and the physician must believe the patient.

Management involves supportive care, with early adequate pain relief, hydration with hypotonic solutions, treatment of underlying infections, and oxygenation. [37] Incentive spirometry has been shown to be protective of more serious pulmonary problems. [38] A patient with a severe pain crisis must be assessed carefully, as this may be a harbinger of sickle chest syndrome or systemic fat emboli. [39]

Acute Chest Syndrome

The acute chest syndrome is the leading cause of death in patients with sickle cell disease, in both pediatric and adult populations. [40] There is no single cause recognized, and thus the optimal treatment is uncertain. For the purpose of large multi-center studies carried out by the CSSCD, acute chest syndrome has been defined as a new pulmonary infiltrate, involving at least one complete lung segment, consistent with the presence of alveolar consolidation, with an associated clinical syndrome including chest pain, fever and tachypnea, wheezing or cough. Recently the CSSCD published results of evaluation of diagnostic testing and therapeutic outcomes on 538 patients, who had 671 episodes of acute chest syndrome. [41] Interestingly, nearly half the patients were admitted to hospital with other problems, usually a vaso-occlusive crisis. Specific causes for acute chest syndrome were identified in 38% of the patients, including pulmonary fat

embolism and 27 different infectious pathogens; sometimes embolism and infection were identified in the same patient. Leading the list of infectious organisms were community-acquired pneumonias such as *Chlamydia pneumoniae, Mycoplasma* and a variety of viral pneumonias, but there was a wide range of pathogens identified.

Acute chest syndrome is a dramatic event, and patients can become hypoxic very quickly, as a vicious cycle of hypoxia→occlusion of pulmonary microvasculature→worsening hypoxia can ensue. Thirteen percent of patients in this study required mechanical ventilation, and 3% of patients died. Treatment must be aggressive; in addition to intensive supportive care and antibiotics, which all patients received, the most successful treatment strategies were bronchodilators and transfusion. In this study both simple and exchange transfusions were used, without either method demonstrating definite superiority.

Pulmonary Hypertension

Pulmonary hypertension is seen in many types of inherited and acquired hemolytic anemia. [42] In addition to SCA, it is also seen in such hemolytic anemias as thalassemia, hereditary spherocytosis and paroxysmal nocturnal hemoglobinuria. The common thread seems to be nitric oxide (NO) depletion, as this molecule is scavenged by free hemoglobin released during hemolysis. Arginase, released by hemolyzed red cells, depletes Arginine, the substrate for NO, worsening the lack of NO. Without the important vasodilatory function of NO, especially in the pulmonary vasculature, and lacking its role in suppressing endothelial activation, the stage is set for pulmonary vascular occlusion and reperfusion injury, with progressive lung damage. Autopsy data has shown unsuspected obliterative pulmonary vasculopathy in a third of patients with SCA.

A recent prospective study recruited 195 SCA patients from the community and assessed them with Doppler echocardiography; 32% met the authors' criteria for pulmonary hypertension, namely a tricuspid regurgitant jet velocity of at least 2.5 meters per second. [43] Univariate analysis revealed that increasing age, oxyhemoglobin desaturation, impaired renal function and increase in direct bilirubin were predictors for a high regurgitant jet velocity. Pulmonary hypertension conferred a ten-fold increase in mortality on patients, including from sudden death. Since so many patients were asymptomatic at the time of their positive study, or had been misdiagnosed with congestive heart failure, both the authors and the accompanying editorial strongly support screening SCA patients with echocardiography, so that patients with pulmonary hypertension can be treated aggressively before it becomes an end-stage condition. [44]

Neuropsychiatric dysfunction in sickle cell anemia

Children with sickle cell anemia are at high risk for thrombotic stroke from overt cerebral infarcts. [45] With improved neuro-imaging techniques, even neurologically intact children have been found to have had silent cerebral infarcts, which can be correlated with neuropsychometric deficits. [46-48] Autopsy studies on adult patients suggest that

many have significant ischemic brain damage. Koshy *et al* found that brain histopathology showed diffuse chronic gliotic scarring from prior microinfarcts, both in gray matter and deep white matter, even in individuals not recognized as having had stroke or other CNS problems. [49] There is little clinical data in adult patients, but a small pilot study of 33 patients from the NHLBI (unpublished data) suggests at least 50% of adult SCA patients have neuro-imaging and neuro-cognitive defects. MRIs showed bilateral, multifocal lesions, some involving watershed areas, with frontal and parietal regions most affected. While these patients had normal neurological examinations, they did complain of non-focal symptoms, particularly memory loss and headache. Even some patients with normal MRI demonstrated abnormalities on formal neurocognitive testing.

It is well established that anemia can affect both neurocognitive testing and imaging studies; improving hematocrit can reverse this trend in dialysis patients receiving erythropoietin. [50]. Functional brain studies such as PET scanning in patients with SCA show reversible ischemic dysfunction and frontal lobe hypometabolism. [51] Powars showed that transfusion improved PET abnormalities in 4 children. [52] Now patients with SCA are living longer it is imperative to document the problems associated with cerebral vasculopathy and anemia, and develop strategies to prevent and treat them

Our own center is poised to participate in a NHLBI-sponsored protocol, where several Comprehensive Sickle Cell Centers will study this issue in depth. This study will evaluate the hypotheses that neurocognitive testing in neurologically asymptomatic adult SCA patients will be abnormal when compared with healthy community controls, and that a larger percentage of adult patients with abnormal MRI will have abnormal testing than those with normal MRI. It is also proposed that volumetric MRI will be more sensitive than conventional MRI to predict patients with neurocognitive defects. The study also has a treatment arm, with the hypothesis that correction of anemia with transfusion will improve cognitive function, when compared with patients who receive standard care.

Treatment of Sickle cell disease

Increasing understanding of the pathophysiology of the sickle cell vaso-occlusive process helped bring Hydroxyurea into active clinical use to raise fetal hemoglobin levels, and helpful animal models for pre-clinical trials have enabled several other treatments to be brought to clinical trial. The sickling process can be interrupted in several ways – fundamentally by reducing sickle hemoglobin polymer formation and by counteracting the factors that lead to red cell adhesion to the microvascular endothelium.

Hydroxyurea therapy

Hydroxyurea is a ribonucleotide reductase inhibitor that increases red cell fetal hemoglobin, in association with myelosuppression. In 1995, a phase III, randomized placebo-controlled double blind trial of Hydroxyurea in severely-affected SCA patients

(three major crises in the previous year) was halted early, because the treated group had such a dramatic reduction in morbidity – the incidence of vaso-occlusive crisis, acute chest syndrome and transfusion were halved. [53] The drug was FDA-approved for SCA, and has been the treatment mainstay for the severely affected patient, although not all benefit. Its effects are multiple: in addition to increasing the fetal hemoglobin level, it alters red cell rheology, red cell adhesion to endothelium, reduces endothelial activation as measured by soluble VCAM-1 levels and reduces neutrophil number. Hydroxyurea is administered orally and has few significant side effects, other than myelosuppression, in the short term. It requires regular monitoring of blood counts, and long term safety has not been established. Follow-up of the initial study population 9 years later still showed reduction in mortality in the Hydroxyurea-treated cohort. [54]

Table 6 : Emerging treatment strategies in sickle cell anemia [4, 55]

Pathophysiologic process targeted	**Mechanism**	**Therapy**
Sickle polymer production	Increase fetal hemoglobin	Hydroxyurea Decitibine [56] Short chain fatty acids [57, 58]
Red cell dehydration and enhanced polymer formation	Block Gardos channel Inhibit KCl Co-transport	Clotrimazole (toxic) [14] Mg pidolate [14, 59]
Vascular tone and endothelial activation	Increase NO availability	NO inhalation Oral Arginine therapy
Endothelial activation	Anti NFK-B	Sulphasalazine [60]
Coagulation	Decrease thrombin generation	Warfarin [61, 62] Heparin
Red cell flow in microvasculature	Decreases viscosity and adhesion	Poloxamer 188

Other emerging treatments [55]

Inhalation of nitric oxide ameliorates acute chest syndrome in transgenic mice, [63] and has been helpful in a pilot study of children with vaso-occlusive crisis, reducing their opioid use and pain scores. [64] It is also being used in patients with pulmonary hypertension, although it is less useful in this chronic situation. [65] Arginine is the precursor of NO; its levels are low in SCA. Oral administration of L-arginine has been

shown to reduce endothelial markers and some benefit in pulmonary hypertension. [66, 67]. An NHLBI-sponsored trial of arginine in SCA is in the final stages of planning, and both adult and pediatric programs of the UT Southwestern Comprehensive Sickle Cell program will participate.

Purified poloxamer 188 is a non-ionic block copolymer surfactant. As such it has some qualities that make it very attractive as a therapeutic agent in SCA. It appears to block red cell-endothelial adhesion and to reduce blood viscosity, resulting in improved microvascular blood flow. It is safe and well tolerated at the doses required in vivo to produce these effects. [68] Preliminary studies were sufficiently encouraging that a randomized trial was undertaken. [69] Patients receiving the poloxamer 188 had a statistically significant, albeit small, benefit in the duration of sickle cell crisis (133 hours versus 141 hours). However, subset analysis showed a much more dramatic benefit in younger patients (21 hours) and in patients receiving hydroxyurea (16 hours). This agent is not yet licensed, and more trials are underway.

Hemopoietic cell transplantation

This is the only curative treatment in SCA, and to date about 200 transplants have been performed, essentially all in children, in a research setting, with the rate of event-free survival about 80%. There are many barriers to transplant application, starting with availability of a related HLA-matched donor. The immediate post-transplant period can be particularly difficult, both from graft-versus-host disease and from neurologic problems, as their abnormal vasculature makes SCA patients uniquely vulnerable to cerebral hemorrhage when thrombocytopenic. Ideally transplant should be undertaken before there are too many complications, but predicting a child's future clinical course is fraught with difficulty. A few patients have developed stable donor-host chimerism after transplant, raising hopes for non-myeloablative transplant in the future. Alternative sources of stem cells are also being evaluated, including umbilical vein stem cells and unrelated matched donors. [70]

Conclusion

For much of the hundred years since SCA was first recognized in Western medicine, knowledge of the pathophysiology of this complex disease has outstripped our ability to treat patients. With increasing understanding of the mechanisms of endothelial activation and adhesion, with animal models for pre-clinical assessment and with some promising treatment leads that can be tested in national multicenter studies there is hope that the treatment of patients with SCA will finally catch up with the science.

REFERENCES

1. Herrick, J.B., *Peculiar elongated and sickle-shaped red blood cells in a case of severe anemia.* Archives in Internal Medicine, 1910. **6**: p. 517-21.
2. Serjeant, G.R., *Historical review - the emerging understanding of sickle cell disease.* British Journal of Haematology, 2001. **112**: p. 3-18.
3. Onwubalili, J.K., *Sickle-cell anemia: an explanation for the ancient myth of reincarnation in Nigeria.* Lancet, 1983. **322**: p. 503-05.
4. Stuart, M.J. and R. Nagel, *Sickle-cell disease.* Lancet, 2004. **364**: p. 1343-60.
5. Pauling, L., et al., *Sickle cell anemia: a molecular disease.* Science, 1949. **110**: p. 543-48.
6. Feldman, S.C. and A.I. Tauber, *Sickle Cell Anemia: Reexamining the first "molecular disease".* Bulletin of the History of Medicine, 1997. **71**: p. 623-50.
7. Ingram, V.M., *Gene mutations in human haemoglobin: the chemical difference between normal and sickle haemoglobin.* Nature, 1957. **180**: p. 326-28.
8. Hoppe, C., et al., *Gene interactions and stroke risk in children with sickle cell anemia.* Blood, 2004. **103**(6): p. 2392-96.
9. Goodman, S.R., *The irreversibly sickled cell: a perspective.* Cellular & Molecular Biology, 2004. **50**(1): p. 53-58.
10. Yasin, Z., et al., *Phosphatidylserine externalization in sickle red blood cells: associations with cell age, density, and hemoglobin F.* Blood, 2003. **102**(1): p. 365-70.
11. Franck, P.F., et al., *Uncoupling of the membrane skeleton from the lipid bilayer. The cause of accelerated phospholipid flip-flop leading to an enhanced procoagulant activity of sickled cells.* J Clin Invest, 1985. **75**(1): p. 183-90.
12. Setty, B.N.Y., S. Kulkarni, and M.J. Stuart, *Role of erythrocyte phosphatidylserine in sickle red cell-endothelial adhesion.* Blood, 2002. **99**: p. 1564-71.
13. Brugnara, C., *Sickle cell disease: from membrane pathophysiology to novel therapies for prevention of erythrocyte dehydration.* Journal of Pediatric Hematology/Oncology, 2003. **25**(12): p. 927-33.
14. Brugnara, C., *Therapeutic strategies for prevention of sickle cell dehydration.* Blood Cells Mol Dis, 2001. **27**(1): p. 71-80.
15. Hebbel, R.P., et al., *Erythrocyte adherence of endothelium in sickle cell anemia: a possible determinant of disease severity.* The New England Journal of Medicine, 1980. **302**: p. 992-95.
16. Gupta, K., et al., *Mechanism of interaction of thrombospondin with human endothelium and inhibition of sickle erythrocyte adhesion to human endothelial cells by heparin.* Biochim Biophys Acta, 1999. **1453**(1): p. 63-73.
17. Setty, B.N. and M.J. Stuart, *Vascular cell adhesion molecule-1 is involved in mediating hypoxia-induced sickle red blood cell adherence to endothelium: potential role in sickle cell disease.* Blood, 1996. **88**(6): p. 2311-20.
18. Hines, P.C., et al., *Novel epinephrine and cyclic AMP-mediated activation of BCAM/Lu-dependent sickle (SS) RBC adhesion.* Blood, 2003. **101**(8): p. 3281-7.
19. Kaul, D.K., *"Stress" and sickle red cell adhesion.* Blood, 2004. **104**: p. 3425-26.
20. Solovey, A., et al., *Circulating activated endothelial cells in sickle cell anemia.* The New England Journal of Medicine, 1997. **337**: p. 1584-90.
21. Duits, A.J., et al., *Enhanced levels of soluble VCAM-1 in sickle cell patients and their specific increment during vaso-occlusive crisis.* Clin Immunol Immunopathol, 1996. **81**(1): p. 96-8.
22. Stuart, M.J. and B.Y. Setty, *Hemostatic alterations in sickle cell disease: relationships to disease pathophysiology.* Pediatric Pathology of Molecular Medicine, 2001. **20**: p. 27-46.

23. Matsui, N.M., et al., *P-selectin mediates the adhesion of sickle erythrocytes to the endothelium.* Blood, 2001. **98**: p. 1955-62.
24. Manodori, A.B., et al., *Enhanced adherence of sickle erythrocytes to thrombin-treated endothelial cells involves interendothelial cell gap formation.* Blood, 1998. **92**(9): p. 3445-54.
25. Okpala, I., *The intriguing contribution of white blood cells to sickle cell disease - a red cell disorder.* Blood Reviews, 2004. **18**(1): p. 65-73.
26. Schechter, A.N. and M.T. Galdwin, *Hemoglobin and the paracrine and endocrine functions of nitric oxide.* The New England Journal of Medicine, 2003. **348**(15): p. 1483-85.
27. Reiter, C.D., et al., *Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease.* Nat Med, 2002. **8**(12): p. 1383-9.
28. Nagel, R.L. and M.E. Fabry, *The panoply of animal models for sickle cell anemia.* British Journal of Haematology, 2001. **112**: p. 19-25.
29. Nagel, R.L., *A knockout of a transgenic mouse - animal models of sickle cell anemia.* The New England Journal of Medicine, 1998. **339**: p. 194-95.
30. Manci, E.A., et al., *Causes of death in sickle cell disease: an autopsy study.* British Journal of Haematology, 2003. **123**(2): p. 359-65.
31. Platt, O.S., et al., *Mortality in sickle cell disease -- life expectancy and risk factors for early death.* The New England Journal of Medicine, 1994. **330**: p. 1639-44.
32. Quinn, C.T., Z.R. Rogers, and G.R. Buchanan, *Survival of children with sickle cell disease.* Blood, 2004. **103**(11): p. 4023-27.
33. Cohen, A.R., *Returns on long-term investments in sickle cell disease.* Blood, 2004. **103**: p. 11.
34. Powars, D.R., *Sickle cell anemia and major organ failure.* Hemoglobin, 1990. **14**(6): p. 573-98.
35. Platt, O.S., et al., *Pain in sickle cell disease. Rates and risk factors.* The New England Journal of Medicine, 1991. **325**: p. 11-16.
36. Prasad, R., et al., *Long-term outcomes in patients with sickle cell disease and frequent vaso-occlusive crises.* Am J Med Sci, 2003. **325**(3): p. 107-9.
37. Rees, D.C., et al., *Guidelines for management of the acute painful crisis in sickle cell disease.* British Journal of Haematology, 2003. **120**: p. 744-52.
38. Bellet, P.S., et al., *Incentive spirometry to prevent acute pulmonary complications in sickle cell disease.* The New England Journal of Medicine, 1995. **333**: p. 699-703.
39. Vichinsky, E., et al., *Pulmonary fat embolism: a distinct cause of severe acute chest syndrome in sickle cell anemia.* Blood, 1994. **83**(11): p. 3107-12.
40. Vichinsky, E., et al., *Acute chest syndrome in sickle cell disease: clinical presentation and course. Cooperative study of sickle cell disease.* Blood, 1997. **89**(5): p. 1787-92.
41. Vichinsky, E.P., et al., *Causes and outcomes of the acute chest syndrome in sickle cell disease.* The New England Journal of Medicine, 2000. **342**: p. 1855-65.
42. Farber, H.W. and J. Loscalzo, *Pulmonary arterial hypertension.* N Engl J Med, 2004. **351**(16): p. 1655-65.
43. Gladwin, M.T., et al., *Pulmonary hypertension as a risk factor for death in patient with sickle cell disease.* The New England Journal of Medicine, 2004. **359**(9): p. 886-95.
44. Vichinsky, E.P., *Pulmonary hypertension in sickle cell disease.* The New England Journal of Medicine, 2004. **350**(9): p. 857-59.
45. Merkel, K.H., et al., *Cerebrovascular disease in sickle cell anemia: a clinical, pathological, and radiological correlation.* Stroke, 1978. **9**: p. 45-52.
46. Moser, F.G., et al., *The spectrum of brain MR abnormalities in sickle-cell disease: a report from the Cooperative Study of Sickle Cell Disease.* AJNR Am J Neuroradiol, 1996. **17**(5): p. 965-72.

47. Armstrong, F.D., et al., *Cognitive functioning and brain magnetic resonance imaging in children with sickle Cell disease. Neuropsychology Committee of the Cooperative Study of Sickle Cell Disease.* Pediatrics, 1996. **97**(6 Pt 1): p. 864-70.
48. Miller, S.T., et al., *Silent infarction as a risk factor for overt stroke in children with sickle cell anemia: a report from the cooperative study of sickle cell disease.* Journal of Pediatr, 2001. **139**: p. 385-90.
49. Koshy, M., C. Thomas, and J. Goodwin, *Vascular lesions in the central nervous system in sickle cell disease (neuropathology).* J Assoc Acad Minor Phys, 1990. **1**(3): p. 71-8.
50. Pickett, J.L., et al., *Normalizing hematocrit in dialysis patients improves brain function.* Am J Kidney Dis, 1999. **33**(6): p. 1122-30.
51. Rodgers, G.P., et al., *Brain glucose metabolism in neurologically normal patients with sickle cell disease. Regional alterations.* Arch Neurol, 1988. **45**(1): p. 78-82.
52. Powars, D.R., et al., *Cerebral vasculopathy in sickle cell anemia: diagnostic contribution of positron emission tomography.* Blood, 1999. **93**(1): p. 71-9.
53. Charache, S., et al., *Effect of hydroxyura on the frequency of painful crises in sickle cell anemia.* The New England Journal of Medicine, 1995. **332**(20): p. 1317-22.
54. Steinberg, M.H., et al., *Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia.* JAMA, 2003. **289**: p. 1645-51.
55. Vichinsky, E., *New therapies in sickle cell disease.* Lancet, 2002. **360**(9333): p. 629-31.
56. DeSimone, J., et al., *Maintenance of elevated fetal hemoglobin levels by decitabine during dose interval treatment of sickle cell anemia.* Blood, 2002. **99**: p. 3905-08.
57. Atweh, G.F., et al., *Sustained induction of fetal hemoglobin by pulse butyrate therapy in sickle cell disease.* Blood, 1999. **93**(6): p. 1790-7.
58. Witt, O., et al., *Induction of fetal hemoglobin synthesis by valproate: modulation of MAP kinase pathways.* Am J Hematol, 2002. **71**(1): p. 45-6.
59. De Franceschi, L., et al., *Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease.* Br J Haematol, 2000. **108**(2): p. 284-9.
60. Solovey, A.A., et al., *Modulation of endothelial cell activation in sickle cell disease: a pilot study.* Blood, 2001. **97**(7): p. 1937-41.
61. Salvaggio, J.E., C.A. Arnold, and C.H. Banov, *Long-term anti-coagulation in sickle-cell disease. A clinical study.* N Engl J Med, 1963. **269**: p. 182-6.
62. Ahmed, S., et al., *Effect of low-dose warfarin on D-dimer levels during sickle cell vaso-occlusive crisis: a brief report.* Eur J Haematol, 2004. **72**(3): p. 213-6.
63. de Franceschi, L., et al., *Inhaled nitric oxide protects transgenic SAD mice from sickle cell disease-specific lung injury induced by hypoxia/reoxygenation.* Blood, 2003. **102**(3): p. 1087-96.
64. Weiner, D.L., et al., *Preliminary assessment of inhaled nitric oxide for acute vaso-occlusive crisis in pediatric patients with sickle cell disease.* JAMA, 2003. **289**(9): p. 1136-42.
65. Gladwin, M.T. and A.N. Schechter, *Nitric oxide therapy in sickle cell disease.* Semin Hematol, 2001. **38**(4): p. 333-42.
66. Morris, C.R., et al., *Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease?* Am J Respir Crit Care Med, 2003. **168**: p. 63-69.
67. Morris, C.R., et al., *Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease.* British Journal of Haematology, 2000. **111**: p. 498-500.
68. Gibbs, W.J. and T.M. Hagemann, *Purified poloxamer 188 for sickle cell vaso-occlusive crisis.* Annals of Pharmacotherapy, 2004. **38**(2): p. 320-24.
69. Orringer, E.P., et al., *Purified poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease: a randomized controlled trial.* JAMA, 2001. **286**: p. 2099-106.
70. Hoppe, C.C. and M.C. Walters, *Bone marrow transplantation in sickle cell anemia.* Curr Opin Oncol, 2001. **13**(2): p. 85-90.