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Pathophysiology and treatment of sickle cell disease
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Chapter 1

Abstract

Sickle cell disease is a hereditary haemoglobinopathy caused by a mutation in the \( \beta \)-globin gene. The disease is characterised by recurrent vaso-occlusive crises resulting in severe organ damage and a sharply reduced life expectancy. The formation of haemoglobin-S polymers in hypoxic conditions plays a pivotal role in sickle-cell disease and produces the characteristic phenotype of sickle-shaped erythrocytes that promote vaso-occlusion. Endothelial cell activation, enhanced erythrocyte and leukocyte adhesion, vasoconstriction and coagulation activation play an important role in vaso-occlusive crises.

Treatment of pain and hydration remain the main interventions in the management of vaso-occlusive crises. Hydroxyurea has been shown to prevent vaso-occlusive crises by increasing the amount of foetal haemoglobin. Allogeneic stem-cell transplantation is the only curative therapy. However, transplantation-related mortality, graft-versus-host disease and the limited availability of HLA-identical donors restrict this therapeutic option.
Introduction

Sickle cell disease (SCD) is a recessive monogenic inherited disorder affecting the hemoglobin molecule by a mutation in the gene encoding for β-globin. In addition to homozygotic SCD (HbSS, sickle-cell anaemia), SCD also includes double heterozygous states such as hemoglobin SC disease (HbSC), hemoglobin SD or SE disease (HbSD and HbSE) and HbSβ-thalassemia (HbS/β-thal). The hallmark of SCD is the recurrent episode of severe pain caused by ischemia resulting from vaso-occlusive of bones, the so-called vaso-occlusive crisis or vaso-occlusive crisis. The first signs and symptoms may occur at the age of six months and can lead to frequent hospital admissions for the treatment of vaso-occlusive crises. On the long run, the chronic hemolytic anemia and recurrent micro and macrovascular occlusion result in extensive organ damage and subsequently in a reduced life expectancy.1 The mean life expectancy for homozygous SCD patients in North America is 42 years for males and 48 years for females.1

Epidemiology

SCD is a prevalent disease, which originates from Central and West Africa, India and Saudi Arabia. As a result of slavery and migration the disease is present throughout the entire world with an estimated prevalence of sickle cell trait (carriership of HbS) ranging from 8% in the Afro-American population of the United States of America to 40% in endemic areas in West Africa, India and Saudi Arabia.2 In the Netherlands, subjects with sickle cell trait are found to originate predominantly from Surinam and the Netherlands Antilles but recently an increasing number of HbS-carriers and sickle cell patients are immigrants from West African countries such as Ghana and Nigeria. A recent survey in the Academic Medical Center in Amsterdam among 1016 pregnant women originating from Surinam and West Africa showed a prevalence of sickle cell trait of 12% and 15% respectively.3 The total number of patients with SCD in the Netherlands is estimated to be at least 600.4

Clinical presentation

Sickle cell disease is characterized by recurrent vaso-occlusive crises triggered by infection, heavy physical exertion, high altitude, dehydration, exposition to cold or mental stress, but may also develop spontaneously. More than 50% of the patients with SCD experience more than one vaso-occlusive crisis per year for which hospital admission and treatment with opiates is required. However, most episodes of vaso-occlusive crises do not require hospitalization and are treated at home. Chronic hemolytic anemia and recurrent vaso-occlusion of the microvasculature are responsible for the progressive development of disabling organ damage such as, splenic infarction, avascular osteonecrosis, cardiomyopathy, renal failure, leg ulcers and priapism.5 In particular,
splenic dysfunction is responsible for an increased risk of infection with encapsulated bacteria, such as Streptococcus pneumoniae. Other complications that are associated with a strongly reduced life expectancy are stroke, acute chest syndrome, pulmonary hypertension, and acute splenic sequestration (in children). As much as 10% of children with SCD are diagnosed with acute stroke and in at least 20% of these children signs of cerebral infarction can be found when magnetic resonance imaging (MRI) of the brain is used to screen for this complication. Acute chest syndrome is defined as a syndrome of pulmonary complaints (dyspnea, thoracic pain and fever) in combination with new infiltrate on chest X-ray and is an important cause of death in sickle cell disease. Acute chest syndrome may be caused by airway infection, fat emboli or pulmonary infarction but most cases develop during vaso-occlusive crises without a clear explanation. Recently, it was shown that 30% of adult patients with SCD have pulmonary hypertension (PHT) which is correlated with early death. The pathogenesis of PHT has not fully been elucidated but nitric oxide (NO) scavenging by free circulating hemoglobin due to chronic intra-vascular hemolysis appears to play an important role. Acute splenic sequestration is an acute complication in young sickle cell patients characterized by a rapid enlargement of the spleen due to massive erythrocyte sequestration resulting in a life-threatening anemia.

Figure. The many sickled erythrocytes are clearly visible when viewed under a microscope. (peripheral blood smear, Jenner-Giemsa stain, x12,500)
Pathophysiology

The formation of polymers of sickle hemoglobin (HbS) is responsible for the characteristic ‘sickle’ phenotype of erythrocytes in SCD. (figure) In contrast to the simple mechanical obstruction of the microvasculature by sickled erythrocytes, it has now become clear that etiology of vaso-occlusion in sickle cell disease is the result of a complex interplay between endothelial activation, cytokine release, intercellular adhesion, vasoconstriction, and coagulation activation.

Polymerization of HbS

SCD is caused by a single nucleotide substitution in the gene encoding the β-globin protein. This nucleotide substitution results in the production of less water-soluble hemoglobin which polymerizes upon deoxygenation. The polymerization of HbS causes the characteristic phenotype of the sickled erythrocyte. The extent of polymerization is defined not only by deoxygenation but also by the intracellular HbS and fetal hemoglobin (HbF) content. Until birth the β-globin gene is inactive and the γ-globin genes are responsible for the production of HbF. High HbF and low HbS concentrations in the erythrocytes of newborns with SCD result in an almost asymptomatic state during the first six months of life. In comparison, cellular dehydration of erythrocytes increases the HbS concentration and causes increased polymerization of HbS and formation of sickled cells. Cellular dehydration is caused by the loss of intracellular water through cytokine activated Gardos-potassium channels.

Endothelial activation and increased adhesion

Recent observations have revealed that the pathophysiology of SCD is not limited to the occlusion of the microcirculation by the entrapment of sickled cells. A chronic inflammatory reaction characterized by cytokine production, endothelial activation, coagulation activation and transmigration of monocytes constitutes another important pathophysiological mechanism in SCD. Under normal circumstances the duration of the exposure of erythrocytes to hypoxic and acidic conditions in the microvasculature is too short to induce local polymerization of HbS. However, due to local adhesive interactions of erythrocytes, leukocytes and endothelial cells the blood flow velocity in the microvasculature decreases. Subsequently, local polymerization of HbS and formation of sickled cells occur, which ultimately leads to complete vaso-occlusion. The resulting ischemia is responsible for subsequent inflammatory reactions that cause further activation of the endothelium. Thus, vaso-occlusion in SCD seems to start with adhesion of erythrocytes and leukocytes to adhesion molecules on activated endothelial cells in predominantly post-capillary venules. The role of leukocytes in this cascade is illustrated by the fact that leukocytosis is a risk factor for the onset of the acute chest syndrome and vaso-occlusive crisis in
asymptomatic sickle cell patients. Both during vaso-occlusive crises and asymptomatic periods, sickle cell patients have a high number of circulating endothelial cells with increased expression of adhesion molecules such as ICAM-1, VCAM-1, P- en E-selectin, reflecting ongoing endothelial activation and damage. In murine models of SCD, P-selectin appeared to play a pivotal role in experimental vaso-occlusion and inhibition of P-selectin either by neutralizing antibodies or the use of P-selectin knock-out mice prevented experimentally induced vaso-occlusion. These observations suggest a prominent role of adhesion molecules, such as P-selectin, in the pathophysiology of SCD.

Vasoconstriction
Nitric oxide (NO), produced by the endothelial cells, has a strong vasodilatory and blood flow regulatory effect. Due to chronic hemolysis, high concentrations of extra-cellular hemoglobin can be found in the circulation of patients with SCD. This free hemoglobin scavenges NO resulting in a compensatory increased production of NO, and subsequently a lower concentration of L-arginine, the substrate for NO-synthase.

Increased production of NO during low substrate availability results in the production of superoxides instead of NO. This results in less NO bioavailability and more oxidative stress and endothelial damage caused by the superoxides. Decreased bioavailability of NO will result in vaso-occlusion partly due to an increase in the production of the vasoconstrictor peptide endothelin-1. Hemolysis induced scavenging of NO is an important etiological mechanism in the development of sickle cell related pulmonary hypertension.

Coagulation activation
Patients with SCD have chronically activated coagulation. Factors promoting this hypercoagulable state are increased expression of tissue factor on circulating monocytes and endothelial cells and increased expression of procoagulant phospholipids such as phosphatidylserine on erythrocytes. Coagulation activation may affect vaso-occlusion in sickle cell by numerous mechanisms. Importantly, thrombin is one of the strongest activators of endothelial cells and is responsible for the increased expression of P-selectin on endothelial cells and monocytes in SCD thereby promoting cellular adhesion. Despite this, to date no studies have provided convincing evidence that anti-coagulant treatment may decrease the frequency of vaso-occlusive crises in patients with SCD.
Treatment

Pain
Acute microvascular vaso-occlusion results in ischemia of the involved tissue and induces severe pain. Pain is therefore a very common symptom of sickle cell disease which strongly impairs quality of life of patients with SCD. Personalized treatment protocols of pain consisting of acetaminophen, codeine and NSAIDs, will assist patients in treating mild and intermediate vaso-occlusive crises at home. Unbearable pain is the most frequent reason why patients with SCD visit the emergency department. Usually treatment with intravenous opioids, such as morphine, is necessary. However, despite the powerful analgetic effect of morphine, optimal pain relief is often hampered because of physicians’ fear for addiction and side-effects and lack of objective and clear parameters to assess the severity of the vaso-occlusive crisis. The use of pethidin is discouraged because of the short half-time, toxic metabolites and the increased risk of addiction.

Transfusion therapy
Blood transfusion increases oxygen carrying capacity and decreases HbS percentage and therefore has several specific indications in SCD. (table) However, transfusions have considerable side effects including transfusion related infections, iron overload and allo-immunisation despite the transfusion of Kell and Rhesus C and e matched blood. Because of these complications, the indications for blood transfusion in patients with sickle cell disease are limited. Important indications for exchange transfusion in order to reduce the HbS% <30% are acute neurological complications, severe acute chest syndrome and sepsis with multiorgan failure. Chronic transfusion has shown to be a successful strategy to prevent stroke in sickle cell patients with previous stroke or children at high risk for stroke identified by an increased cerebral blood flow measured by transcranial Doppler. Transfusion up to a hemoglobin level of 6.0-6.5 mmol/l prior to surgical interventions has demonstrated to be as effective in the prevention of postoperative complications as exchange transfusion. Prophylactic transfusion during pregnancy is controversial since no effect on pregnancy outcome was demonstrated despite the reduced incidence of vaso-occlusive crises and acute chest syndrome.20
Table. Indications for transfusion therapy in sickle cell disease.3

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<thead>
<tr>
<th>Type</th>
<th>Indications</th>
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<tr>
<td>Acute</td>
<td>• Acute anaemia: splenic and/or liver sequestration and severe aplastic crisis&lt;br&gt;• Acute stroke&lt;br&gt;• Acute Chest Syndrome&lt;br&gt;• Preoperative&lt;br&gt;• Sepsis&lt;br&gt;• Refractory priapism&lt;br&gt;• SCD-multi-organ failure syndrome</td>
</tr>
<tr>
<td>Chronic</td>
<td>• Prophylaxis against recurrent stroke&lt;br&gt;• For stroke prevention when transcranial doppler velocities are abnormal in children&lt;br&gt;• (Severe) pulmonary hypertension&lt;br&gt;• Refractory congestive heart failure&lt;br&gt;• Symptomatic anemia</td>
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Screening, prevention, vaccination and antibiotics

Annually 40 to 60 children with SCD are born in the Netherlands and introduction of a nationwide screening program for neonates and primary prevention for SCD has been introduced successfully since January 2007. In other countries, screening programs to diagnose children with SCD at early age have proven to be effective in the prevention of fatal infections due to splenic dysfunction.20 Active immunisation of young children against H. Influenzae and S. Pneumoniae combined with antibiotic prophylaxis until the age of 5 has dramatically decreased mortality among children with SCD.

Hydroxyurea

A landmark randomized controlled trial showed that hydroxyurea reduces the frequency of vaso-occlusive crises in patients with SCD.22 Next to the induction of HbF, hydroxyurea also reduces adhesive interactions between sickle cells and the activated endothelium and seems to improve production of NO. Furthermore, recent reports have shown that hydroxyurea therapy also prevents sickle cell related organ damage.23 Yet, 40 % of patients does not respond to hydroxyurea therapy.23 Furthermore, hydroxyurea may be toxic or oncogenic after long term use despite the fact that so far no increased incidence of leukemia or myelodysplasia has been reported in SCD patients with prolonged use of hydroxyurea. Hypo-methylating agents, such as 5-azacytidine and decitabine (5-azadeoxycytidine), have an even higher potency than hydroxyurea to increase HbF. To date, toxicity and the need for parenteral administration prevent use of these drugs in daily practice.

Anti-inflammatory and anti-adhesive drugs

As discussed earlier, pro-inflammatory stimuli cause endothelial activation and subsequent increased expression of adhesion molecules which in turn induce leukocytes and erythrocyte
adherence to the vascular wall, which ultimately leads to vaso-occlusion. Consequently, inhibition of the inflammation, endothelial activation, or blockade of adhesion molecules could be of value in the treatment of SCD. Sulfasalazine, an anti-inflammatory drug, decreased expression of adhesion molecules on circulating endothelial cells and improved microvascular blood circulation in a murine model of SCD.24 A small study in humans confirmed these effects of sulfalazine on adhesion molecule expression.25 Treatment of vaso-occlusive crises in children with SCD with the anti-inflammatory methylprednisolone resulted in a shorter duration of admission and decreased analgesics use. However, in this study many patients were readmitted because of rebound vaso-occlusive crises after withdrawal of the methylprednisolon.26 Specific interventions that blocking adhesion molecules such as integrin αVβ3 and P-selectin, have only been tested in animal models of SCD but seem promising.13,27 Another important factor in the perseverance of chronic inflammation in patients with SCD are oxygen free radicals which are produced during reperfusion of ischemic tissue. Administration of acetylcytstein, a potent antioxidant, to patients with SCD resulted in a decrease of circulating sickled cells and a non-significant decrease in the number of vaso-occlusive crises.28

**Stem cell transplantation and gene therapy**

To date, the only available curative treatment option for patients with SCD is allogeneic stem cell transplantation. Despite improved techniques and the development of reduced intensity stem cell transplantation (RIST) without the use of toxic myeloablative agents, treatment related mortality (5-10%) and graft-versus-host disease remain important problems.31 Given the risks of stem cell transplantation only a subgroup of strictly selected young patients should be considered for this treatment. Another potential curative treatment option that may become available in the future, is gene therapy. However, expectations of gene therapy are low for the near future, as it is still in a very experimental stage of development.
Chapter 1

Conclusion

SCD is a severe systemic disease with a strongly reduced life expectancy. Endothelial dysfunction plays a central role in the pathophysiology of SCD. Although the knowledge about the pathophysiology of SCD has been rapidly growing, only hydroxyurea has been added to the compendium of treatments so far. We expect allogeneic stem cell transplantation to obtain a more prominent role in the treatment of young children with SCD and a high risk of severe organ damage. However, it is important to realize that most SCD patients live in the part of the world with relatively limited medical resources and thus stem cell transplantation will not be a feasible treatment option. For current practice best medical and social support of patients remain vitally important to help them fight against their unpredictable illness and to prevent early complications and death.
This thesis

The aim of the present thesis is three-fold:
1. To gain more information on the prevalence of clinical complications and silent organ damage (chapter 3), to optimize treatment of vaso-occlusive crisis (chapter 2) and to explore potential new endpoints in the assessment of vaso-occlusive crisis (chapter 4). 2. To increase insight into the pathophysiology of SCD on a microvascular level (chapters 5-6). 3. To elucidate the prevalence, clinical presentation and pathophysiology of sickle cell related pulmonary hypertension (chapters 7-11).

In the first part of this thesis, we present the results of a randomized controlled trial to study the use of patient controlled anaesthesia (PCA) versus continuous infusion of morphine for the treatment of the vaso-occlusive crisis in SCD (chapter 2). Next, we present the results of a systematic evaluation of a large cohort of sickle cell patients in order to assess the relation between the frequency of vaso-occlusive crises and sickle cell related organ damage and clinical complications (chapter 3). Thirdly, we evaluated the use of real-time microvascular blood flow measurement with a new intravital microscopic technique of side dark field imaging in patients with SCD during vaso-occlusive crisis and steady state in comparison with healthy controls. (chapter 4)

In the second part of this thesis, the etiological role of microparticles in the procoagulant nature of SCD during vaso-occlusive crisis and steady state was studied in chapter five. In the sixth chapter, we performed a study to assess the volume of the anti-adhesive and anti-coagulant endothelial glycocalyx in both patients with SCD and controls.

In the third part of this thesis, research focuses on pulmonary hypertension. Pulmonary hypertension is a recently recognized complication which occurs in approximately 30% of adult patients with SCD and is associated with an increased risk of early death. In chapter 7, we present a large study in which the role of macrovascular occlusion of large and medium sized pulmonary arteries is evaluated by ventilation/perfusion scanning in a group of consecutive sickle cell patients. In chapters 8 and 9, we further try to elucidate the etiology of pulmonary hypertension in SCD by exploring the role of coagulation activation and plasma levels of inhibitors of nitric oxide-synthase in sickle cell patients with and without PHT. Chapter 10 describes discriminative value of an array of diagnostic tests that are frequently used for the evaluation of patients with PHT. In chapter 11, we explored the impact of pulmonary hypertension in patients with SCD on exercise intolerance.

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References

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