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Haematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium

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Summary:

Fifty patients affected by sickle cell anaemia underwent transplantation of HLA-identical haematopoietic stem cells (bone marrow, 48; cord blood, 2). Two groups of patients were considered for transplantation. Group 1 included 36 permanent residents of a European country who, retrospectively, met the inclusion criteria accepted at a consensus conference held in Seattle in 1990, wherein children were selected because they already had evidence of a morbid course. Group 2 included 14 patients who were transplanted earlier, had not received more than three blood transfusions and were transplanted because they had decided to return to their country of origin. Kaplan–Meier estimates of overall survival, event-free survival and disease-free survival at 11 years of the whole grafted population are 93, 82 and 85%, respectively. In group 1, overall survival, EFS and DFS were 88, 76 and 80% and in group 2, 100, 93 and 93%, respectively. Clinical manifestations of the disease, as well as disease associated haemolytic anaemia, disappeared in all successfully treated patients. Recovery of spleen function was present in seven out of 10 evaluated patients. Adverse events (death, absence of engraftment, mixed chimerism and relapse) occurred more frequently in group 1 than in group 2 (25% *vs* 7%, $P < 0.001$). Acute graft-versus-host disease (GVHD) was present in 20 patients (grade I or II, 19; grade III, 1), chronic GVHD in 10 (limited, 7; extensive, 3). One patient developed an acute myeloid leukaemia. Gonadal dysfunction was present in all patients (six boys and eight girls) transplanted close to or after puberty, although transient in one adolescent girl.

Keywords: sickle cell anaemia; stem cell transplantation

In a prospective study of the Cooperative Study of Sickle Cell Disease based on 3764 patients, it was shown that life expectancy in patients suffering from sickle cell disease, was decreased by 25 to 30 years, as compared with the black American population in general.[1] These results applied to a population with optimal health care. However the morbidity remained severe and increased with age.

Preventive and supportive measures were the main therapeutic tools available until the last decade. Recently, hydroxyurea has been shown to improve the clinical course in both adults and children.[2,3] However, bone marrow transplantation remains the definitive treatment for cure and eradication of the disease. The first patient cured of sickle cell anaemia had been transplanted for an acute myeloid leukaemia.[4] Our group was the first to propose bone marrow transplantation for sickle cell anaemia *per se*.[5,6] However the unpredictable course of the disease has led to the establishment of selection criteria to avoid submitting a mildly affected child to a potentially dangerous procedure.[7] In the present study, we compare a population of severely affected patients with a population of children mildly affected by the disease, in whom transplantation was done before the occurrence of severe symptoms.

Patients, donors and methods

Fifty patients (27 females, 23 males) with homozygous sickle cell anaemia (haemoglobin genotype S/S) were transplanted in four Belgian centres between April 1986 and January 1997. Informed consent of the parents and/or the patients was obtained. The countries of origin were Democratic Republic Congo (formerly Zaire), 43; Cameroon, 3; Nigeria, 2; Burundi, 1; and Central Republic of Africa, 1. Age at the time of transplantation ranged from 0.9 to 23 years (median, 7.5) and the follow up from 0.3 to 11 years (median, 5).

Group 1 included 36 patients (18 females, 18 males, age range, 1.7 to 23 years) with a median age of 8.6 years. They were transplanted because of the severity of their disease. Retrospectively, they indeed fulfilled the inclusion criteria[7] of severity (frequent painful crises, 36; seizures or strokes, 6; acute chest syndrome, 20; osteonecrosis, 3). Two patients did not fulfill the criteria of age (less than 16 years). They were two female students (17 and 23 years old), who decided to undergo transplantation, well aware of the inherent risks of the procedure, but unable to accept the morbidity of the disease.

Group 2 included 14 patients (nine females, five males, age range, 0.9 to 15 years) with a median age of 2 years. This group included asymptomatic patients, transplanted at

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a young age since their family had to return to Africa where medical care would not be optimal. The patients in this group had had minimal symptoms (painful episodes but no acute chest syndrome, no seizure nor stroke, no priapism, no chronic organ damage) and no more than three blood transfusions before transplantation.

Donors were HLA-genoidentical siblings in 49 patients. For one patient, the donor was the HLA-phenoidentical father. The source of stem cells was bone marrow in 48 patients, cord blood in two.

The conditioning regimen consisted of busulfan, 16 mg/kg, and cyclophosphamide, 200 mg/kg (Bu16/Cy200) in 22 patients, Bu16/Cy200/total lymphoid irradiation (TLI) in six, Bu16/Cy200/anti-thymocyte globulins (ATG) in 14, Bu14/Cy200 in six, and Bu 500 mg/m^2/Cy200 in two patients. The dose of TLI was 750 cGy in one fraction over 6 h with 18 MV X-rays produced by linear acceleration (the lungs being shielded). TLI was given in patients heavily transfused to reduce the risk of rejection. Later, TLI was replaced by ATG. The dose of ATG (Fresenius; Hoechst Marion Roussel, Belgium) was 15 to 90 mg/kg divided in three doses before transplantation.

The patient who underwent a second bone marrow transplantation was conditioned by a regimen previously described by Storb *et al*[8] containing cyclophosphamide, ATG and a thoraco-abdominal irradiation.

Following the observation of a high incidence of neurological events post-transplantation,[9] 13 patients received a neurological prophylaxis consisting of anticonvulsant drugs (phenytoin or carbamazepine). In addition, these patients received blood transfusions to maintain the platelet count above 50×10^9/l and Hb levels between 90 and 110 g/l. Strict control of blood pressure values, cyclosporin A and magnesium levels were reinforced.[9,10]

To prevent GVHD, 25 patients were given cyclosporin A alone for a period of 6 to 18 months and 25 cyclosporin A together with a short course of methotrexate.

Acute GVHD was treated by steroids. Chronic GVHD required the use of azathioprine (four patients) and thalidomide (four patients).

Prophylaxis of infections included gut decontamination and infusions of immune globulins during the first year post-transplantation. It was suggested that penicillin should be continued until recovery of spleen function.

Chimerism studies were performed by at least one of the following methods: cytogenetics when donor and recipient were of the opposite sex, residual levels of HbS when the donor had a A/A genotype of the Hb and studies of variable numbers of tandem repeats in DNA if donor and recipient were of the same sex.

The Lansky scale was used to measure the functional status of the patients instead of the Karnofsky score, because it gives a better description of children's activities (100–80, able to carry on normal activities, no special care is needed; 70–50, mild to moderate restriction; 40–10, moderate to severe restriction).

Statistical analysis

The Kaplan–Meier method was used to calculate overall survival, event-free survival and disease-free survival. Events included deaths, absence of engraftment, rejection and recurrence of the disease. Disease-free survival was defined by the absence of clinical and biological signs and symptoms of sickle cell anaemia. Comparison of the percentage of events was done using the Fisher's exact test. The Wilcoxon rank-sum test was used to assess the significance of the difference of age in groups 1 and 2.

Results

Engraftment and survival

Among the 50 patients, engraftment occurred in 47. The median time to obtain an ANC $>0.5 \times 10^9$/l was 19 days (range, 11–32); the median time to achieve a platelet count $>50 \times 10^9$/l, without platelet support, was 20 days (range, 10–108). Three patients never presented any sign of engraftment (Table 1). They were 6, 7 and 14 years old at the time of transplantation; two of them were on a chronic transfusion program for acute chest syndrome or strokes; the third patient had suffered vaso-occlusive crises and had been transfused more than three times. The number of nucleated cells infused in these three patients was two (one patient), and 4×10^8 cells/kg (two patients). The conditioning regimen consisted of Bu14/Cy200 in one patient and Bu16/Cy200/ATG in two patients. These patients remained free of symptoms for several years after autologous recovery, due to a persistent high level of HbF.[11]

Rejection occurred in two patients: the first patient rejected the graft, between day 30 and 60, although initial engraftment had been confirmed by cytogenetic studies. The donor was the father and a second successful bone marrow transplantation was performed on day 60, from the same donor. This patient was in group 1.

The second rejection occurred 18 months after bone

Table 1 Results of transplantation in sickle cell anaemia according to the patient's status at the time of the procedure

	Group 1[a]	*Group 2*[b]	*P value*
No. patients	36	14	
Sex: F/M	18/18	9/5	
Age (years), median (range)	8.6 (1.7–23)	2.0 (0.9–15)	0.0016
No. RBC transfusions	>3	⩽3	
Survival	34	14	
Deaths	2	0	
Absence of engraftment	3	0	
Relapse	1	1	
Mixed chimerism (>30% recipient cells)	3	0	
Total (%)	9 (25)	1 (7)	<0.001
Acute GVHD grade I–II	14	5	
Actue GVHD grade III–IV	1	0	
Chronic GVHD limited	5	2	
Chronic GVHD extensive	3	0	

[a]Group 1, patients severely affected by sickle cell anaemia at the time of transplantation.
[b]Group 2, patients transplanted at an early stage of the disease.
GVHD = graft-versus-host disease.

marrow transplantion in a 4-year-old boy, conditioned with Bu14/Cy200. This patient was in group 2.

Mixed chimerism was present in six patients. In three, mixed chimerism was stable with less than 10% of recipient cells. In three patients, the percentage of recipient cells was respectively 35, 30 and 50%, 6, 13 and 60 months after transplantation. Those three patients belong to group 1.

Forty-eight patients are alive 4 months to 11 years after transplantation with a median follow up of 5 years. Two patients died: one patient died 3 months after bone marrow transplantation of acute GVHD complicated by CMV and *Aspergillus* infections; the other patient died 6 years after transplantation of a sudden death at the age of 16. The patient had been asymptomatic since transplantation with a normal level of Hb and a complete chimerism (100% donor cells).

Overall, the survival probability is 93%. In groups 1 and 2, overall survival probabilities are respectively 88 and 100% (Figure 1).

The event-free survival for the 50 patients is 82%. In groups 1 and 2, EFS are 76 and 93%, respectively (Figure 1). The disease-free survival for the 50 patients is 85%. In groups 1 and 2, DFS are 80 and 93%, respectively (Figure 1).

Cord blood transplantation was performed in two patients aged 5 and 3 years. Engraftment was slow but present. Growth factors (filgrastim; Amgen Roche, Belgium) were used from day 23 and 17, respectively. Clinical data, number of cells infused and time for recovery are summarised in Table 2. Acute GVHD was not present but the 5-year-old girl developed *de novo* chronic GVHD 18 months after cord blood transplantation, when cyclosporin A was stopped. It was a lichenoid type of chronic GVHD affecting only the skin and confirmed by a biopsy. PUVA were not effective. These two patients had a level of fetal hemoglobin decreasing slowly during the first months, in accordance with the intrinsic properties of stem cells of cord blood origin.[12]

Transplant-related morbidity

Graft-versus-host disease: Grade I acute GVHD was detected in 10 patients, grade II in nine and grade III in one. Chronic GVHD was diagnosed in 10 patients, being limited in seven patients and extensive in three patients. Two patients with chronic GVHD developed mild dyspnea related to *Bronchiolitis obliterans*. This was confirmed by CT scan and pulmonary function tests. Four patients developed severe GVHD (one acute grade III and three chronic and extensive). The four patients were in group 1.

Neurological complications: Eighteen patients had seizures after transplantation, five occurred in 13 patients receiving anticonvulsant therapy, while 13 occurred in the 37 not receiving prophylaxis (38 *vs* 35%). Among the 18 patients, four presented with a history of seizures or strokes before transplantation. In the group of 32 patients who did not present neurological complications, two had a history of seizures before transplantation. There was no recurrence of strokes among the four patients who had suffered from stroke before transplantation.

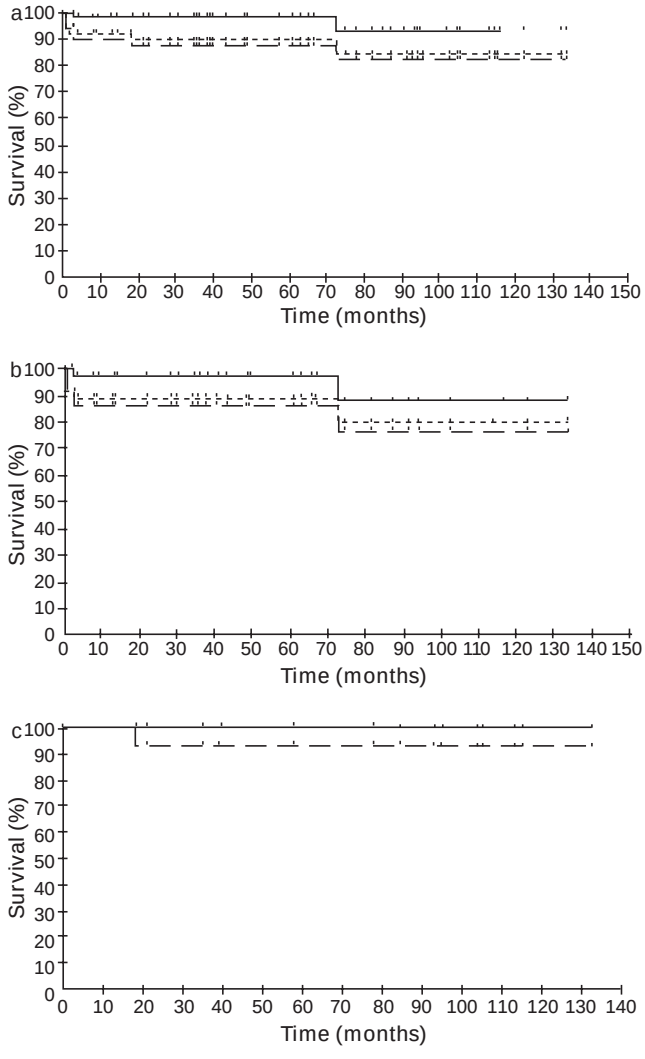

Figure 1 (**a**) Overall survival (——), EFS (– – –) and DFS (. . . .) in 50 patients transplanted in Belgium for sickle cell anaemia. The results were respectively 93, 82 and 85%. (**b**) Overall survival (——), EFS (– – –) and DFS (. . . .) in group 1 (36 patients transplanted in Belgium for sickle cell anaemia and severely affected). The results were respectively 88, 76 and 80%. (**c**) Overall survival (——), EFS (– – –) and DFS (. . . .) in group 2 (14 patients transplanted in Belgium for sickle cell anaemia early in the course of their disease). The results were respectively 100, 93 and 93%.

Long-term benefits and side-effects

Forty-five patients had stable engraftment. Disease associated manifestations as well as haemolytic anaemia disappeared in all of them. The Lansky scale was 100 for all patients, it was 90 in two patients with *Bronchiolitis obliterans* and 60 in one patient with chronic GVHD and acute myeloid leukemia.

Fourteen patients (28%) returned to their country of origin, 12 months to 5 years after transplantation (median, 21 months).

Growth was normal or even improved after transplantation except in two patients who received intensive immune suppression including steroids for chronic GVHD.

Thyroid function was normal in 27 of the 28 evaluated patients. One patient had normal levels of thyroid hormones with an elevated TSH level. This patient received a con-

Table 2 Cord blood transplantations in sickle cell anaemia

Recipient	*UPN 38*	*UPN 43*
Sex	F	F
Age (years)	5.5	3.1
Weight (kg)	23.5	16.1
Cord blood		
origin	HLA-id sister	HLA-id sister
volume (ml)	141	100
nucleated cells ($\times 10^7$/kg)	4.6	4.7
CD34 ($\times 10^5$/kg)	0.9	0.9
CFU-GM ($\times 10^4$/kg)	3.3	3.6
Engraftment		
ANC ($>0.5 \times 10^9$/l)	day 32	day 31
Platelets ($>50 \times 10^9$/l)	day 48	day 54
Chimerism	100% donor	100% donor
GVHD		
acute	0	0
chronic	0	skin (lichenoid)

UPN = unique patient number; GVHD = graft-versus-host disease.

ditioning regimen including Bu16/Cy200, without TLI. He developed chronic GVHD with *Bronchiolitis obliterans.*

The two girls transplanted after puberty, at the ages of 17 and 23, developed secondary amenorrhoea. Levels of LH, FSH and oestradiol were measured in one (UPN 22) and are shown in Table 3. If we consider that the menarche occurs around the age of 15 in sickle cell patients, six other girls were evaluable: five had primary amenorrhoea and delayed sexual maturation. All these patients required hormonal substitution. One patient had a spontaneous menarche at the age of 15. The levels of LH and FSH were increased at that time. Later, at the age of 17, she had normal levels of LH, FSH and estradiol (Table 3).

The six adolescent boys evaluated had normal sexual development, but four had decreased levels of testosterone, four increased levels of FSH and one an increased level of LH (Table 4). Fertility was not tested.

Osteonecrosis of hips and shoulders was present in three patients aged 15, 17 and 23 years at the time of transplantation. There was no improvement on X-rays, 3 years after bone marrow transplantation.

Spleen function was prospectively studied by isotopic studies, using Tc-99m-RBC and Cr-51 heat-damaged red blood cells, in 10 patients. Their ages ranged from 2.4 to 17 years at the time of transplantation (median, 13 years). In seven patients, an increase of the splenic red cell pool was seen between 3 months and 3 years after transplantation. Two of these seven patients also had an improvement of the phagocytosis of heat-damaged red blood cells. Three patients did not improve their splenic function: the first one was transplanted at the age of 17 years and the last test was done at the age of 21; the second patient had a decrease in his splenic function. He was transplanted at the age of 2.4 years and still had some residual function at that time. Later he developed severe chronic GVHD, received intensive immune suppression and developed a myelodysplasia and acute myeloid leukaemia. When the spleen function was tested 3 years after transplantation, there was an absence of splenic tissue on the isotopic studies. The third patient was transplanted at the age of 5 years. He developed a localised chronic GVHD and had no detectable spleen function 2 years after transplantation.

One patient with extensive chronic GVHD required steroids, cyclosporin A and azathioprine for 4 months, and thalidomide for 30 months. He developed a myelodysplasia 35 months after bone marrow transplantation and 18 months later an acute myeloid leukaemia. The marrow cells were

Table 4 Gonadal function in adolescent boys after bone marrow transplantation for sickle cell anaemia

	Age at BMT (years)	*Preparative regimen*[a]	*Age at evaluation (years)*	*LH (mU/ml) N, 1–10*	*FSH (mU/ml) N, 1–8*	*Testo (nm/l) N, 13–35*
UPN6	7	BuCy	16	4.6	4.4	10.7
UPN23	13	BuCyTLI	17	2.4	11.6	6.6
UPN24	11	BuCyTLI	16	2.8	10.9	9.1
UPN29	14	BuCyATG	18	11	13	ND
UPN32	15	BuCyATG	17	5.4	10.9	16
UPN39	15	BuCyATG	17	2.3	7.9	14.2

[a]Bu, busulfan; Cy, cyclophosphamide; TLI, total lymphoid irradiation; ATG, anti-thymocyte globulins; UPN = unique patient number; ND = not determined.

Table 3 Gonadal function in adolescent girls after bone marrow transplantation for sickle cell anaemia

	Age at BMT (years)	*Preparative regimen*[a]	*Age at evaluation (years)*	*Amenorrhoea*	*LH (mU/ml) N, 1–13*	*FSH (mU/ml) N, 1–24*	*Estr (pg/ml N, >35*
UPN4	9	BuCyTLI	18	primary secondary	37.2	105	<10
UPN12	23	BuCy	ND	primary	ND	ND	ND
UPN14	12	BuCyTLI	19	spont men[b]	23.5	97.7	<10
UPN15	10	BuCy	15		49	51	15
			17[c]		5.9	5.1	121
UPN21	13	ByCyATG	18	primary	75	94	<10
UPN22	17	ByCyTLI	22	secondary	88	160	<10
UPN28	15	ByCyATG	17	primary	21	110	<10
UPN40	12	ByCyATG	15	primary	24	33	<10

[a]Bu, busulfan; Cy, cyclophosphamide; TLI, total lymphoid irradiation; ATG, anti-thymocyte globulins.
[b]Spontaneous menarche.
[c]UPN15, measurements done at the age of 17 giving normal values.
UPN = unique patient number.

all of donor origin. He first responded to low-dose Ara-C but now has refractory leukaemia.

Discussion

This series summarises the experience of haematopoietic stem cell transplantation for sickle cell anaemia in Belgium. One centre elected to transplant children early during the course of their disease, before their return to Africa (group 2). The other centres elected to graft more severely affected patients (group 1). The study showed the advantage of an early intervention. The rate of non-engraftment, mixed chimerism (more than 30% recipient cells), and rejection was higher in group 1 as compared to group 2 (25 *vs* 7%, $P < 0.001$). GVHD was also more frequently diagnosed in group 1. This might be associated with the fact that the patients in this group were older than in group 2 (median age, 8.6 *vs* 2.0 years, *P* value, 0.0016). Other studies have also found that the incidence of acute and chronic GVHD was remarkably low in the youngest recipients.[13]

A total of 102 patients with sickle cell disease has been transplanted in Belgium, France,[14] the United Kingdom (personal communication), Italy,[15] Germany (personal communication) and Switzerland (personal communication) (Table 5). Current Kaplan–Meier estimates of overall survival, EFS and DFS are 90, 79 and 81% respectively (Figure 2). In the USA, 32 children were transplanted for sickle cell anaemia, with estimates of overall survival and EFS of 92% and 76%, respectively.[16]

For the 45 patients transplanted in Belgium, with stable engraftment, disease-related clinical symptoms disappeared totally in all of them. For the four patients who had an autologous recovery, an increase in foetal haemoglobin was seen in three out of four, which lasted for several years after transplantation and resulted in the absence of symptoms.[11] Hydroxyurea was started when symptoms recurred, between 3 and 4 years after transplantation.

Seizures after transplantation are a common side-effect and the use of a prophylactic scheme has been proposed to prevent these complications.[9] Recovery of the spleen function was demonstrated in seven out of 10 patients evaluated. Osteonecrosis did not improve on X-ray, although healing has been shown by others.[14] Growth and thyroid function were normal in the vast majority of patients.

Table 5 Haematopoietic stem cell transplantation in sickle cell anaemia: European data

	No. patients	*Deaths*	*No engraftment, relapse and autologous recovery*	*Second transplantation*	*Alive free of disease*
Belgium	50	2	5	1	44
France[14]	26	2	4	1	21
UK[a]	15	2	0	0	13
Italy[15]	6	3	0	0	3
Germany[b]	3	0	1	ongoing	2
Switzerland[c]	2	0	0	0	2
Total	102	9	10	2	85

Unpublished data obtained by personal communication with [a]Irene Roberts (UK), [b]Roswitha Dickerhoff (Germany) and [c]Peter Tuchschmid (Switzerland).

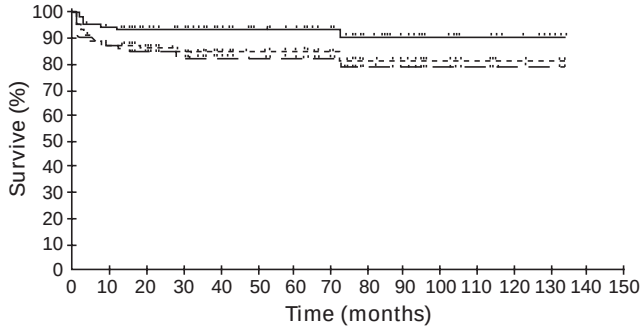

Figure 2 Overall survival (——), EFS (– – –) and DFS (. . . .) in 101 patients transplanted in Europe for sickle cell anaemia. The results were respectively 88, 76 and 80%.

Gonadal dysfunction appeared in all girls transplanted between the ages of 9 and 23 years, although it was transient in one. Boys present normal sexual maturation but most of them had abnormal LH, FSH or testosterone levels which is biochemical evidence of Leydig cell damage. None of them required hormonal replacement therapy. Busulfan is known to induce gonadal insufficiency and this should be discussed with the patient and their relatives.[17] It remains to be determined if patients transplanted early in life will also develop gonadal failure. The disorder was transient in one of our patients and a successful pregnancy after bone marrow transplantation for thalassaemia, using the same conditioning regimen, has been reported.[18]

It is now well accepted that stem cell transplantation is an approach which can eradicate the disease and cure a patient with sickle cell anaemia. However, the timing of transplantation remains a dilemma.[19,20] Unfortunately, the course of the disease is often unpredictable. The only significant prognostic factor is the level of foetal haemoglobin which is associated with a less severe outcome. Neither is an early onset of symptoms predictive of the future course of the disease, nor the absence of symptoms during the first years of life. Haplotypes and co-inheritance of alpha thalassaemia are not conclusive in the prediction of outcome. Transcranial Doppler ultrasonography might be an interesting tool to detect very early vascular damage at the cerebral level.[21]

Opponents to early transplantation argue that such a procedure might be too toxic to treat mildly affected patients who would almost never require health care. These represent probably 5% of sickle cell patients in the USA. For the remaining 95%, only a small proportion will have a HLA-identical sibling and will be candidates for transplantation. Since the best results are obtained in very young patients, we favour informing parents about the possibility of bone marrow transplantation if a familial HLA-compatible donor is available. This would give the child the best chance to be cured. In this young population, the risk of rejection is less than 5%,[22,23] while the risk of GVHD is below 20%.[24] In addition, transplant-related mortality due

to cerebral haemorrhages was shown to be less frequent in young patients without vascular damage.[9] Furthermore, if patients have been heavily transfused, alloimmunization to platelets will make the prevention and therapy of cerebral haemorrhage more difficult.[22] The use of alkylating agents, cyclophosphamide and busulfan, included in the conditioning regimen has been associated with ovarian failure, as well as testicular dysfunction. The consequences of such preparative regimens on gonads when given early in life remain to be determined. Furthermore, the social integration of sickle cell patients could be optimal since the first years of life with proper education, absence of dependence upon others as a consequence of a chronic illness, self-confidence instead of fear of unpredictable and painful problems, and later, the possibility of competing effectively for jobs.

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