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# The Clinical Outcome of Hurler Syndrome after Stem Cell Transplantation

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## ABSTRACT

**Hurler syndrome (HS) is a severe inborn error of metabolism causing progressive multi-system morbidity and death in early childhood. At present, stem cell transplantation (SCT) is the only available treatment that can prevent central nervous system disease progression in HS patients. Although SCT has been shown to be effective for several important clinical outcome parameters, the reported clinical outcome after successful SCT is variable among HS patients and there are still some major limitations.**

**This review will focus on the clinical outcome of HS patients after successful SCT, with particular emphasis on the long-term outcome and complications. In addition, factors that are suggested to contribute to the variable outcome are outlined, as well as the limitations of SCT in HS patients.**



## KEY WORDS

Hurler Syndrome • Mucopolysaccharidosis I • Stem cell transplantation • Clinical outcome • Long-term outcome

## INTRODUCTION

Hurler syndrome (HS) is a severe inborn error of metabolism (IEM) causing progressive multi-system morbidity, including developmental deterioration, and death in early childhood. The first stem cell transplantation (SCT) for HS was performed more than 25 years ago [1] and SCT is still the only treatment available that can prevent disease progression of the central nervous system (CNS). Although SCT has been shown to be effective for several important clinical outcome parameters, the reported clinical outcome after successful SCT is variable among HS patients and there are still some major limitations. Although enzyme replacement therapy (ERT) has recently become available for Mucopolysaccharidosis type I (MPS I), this treatment option is not indicated for HS patients because it is currently incapable of crossing the blood-brain barrier (Figure 1).

This review will focus on the long-term clinical outcome of HS patients after successful SCT. In addition, the progress that has been made in recent years will be discussed. Furthermore, several factors that are suggested to contribute to the variable outcome are discussed, as well as the limitations of SCT in HS patients.

### Hurler Syndrome

MPS I is a rare autosomal recessive IEM caused by a deficiency of the lysosomal enzyme alpha-L-iduronidase (IDUA). Historically, MPS I has been divided into 3 clinical subtypes: Hurler (severe), Scheie (mild/attenuated), and Hurler-Scheie (intermediate). This classification is based on clinical factors, including the age of onset, the rate of functional deterioration, and the range of affected organs (eg CNS involvement). There are, however, no precisely defined clinical or biochemical criteria that can reliably distinguish among these subtypes [2,3]. It has been acknowledged that these 3 subtypes represent a broad continuous spectrum of clinical phenotypes, caused by a high degree of genotype heterogeneity and associated with various degrees of residual enzyme activity [2,4].

HS is the most severe phenotype in the spectrum of MPS I, with incidence estimates varying between 1:76,000 and 1:164,000 [5,6]. The severely deficient, or absent, IDUA enzyme activity in HS patients results in a progressive accumulation of the incompletely degraded glycosaminoglycans (GAGs) dermatan and heparan sulphate within the lysosomes. Eventually,

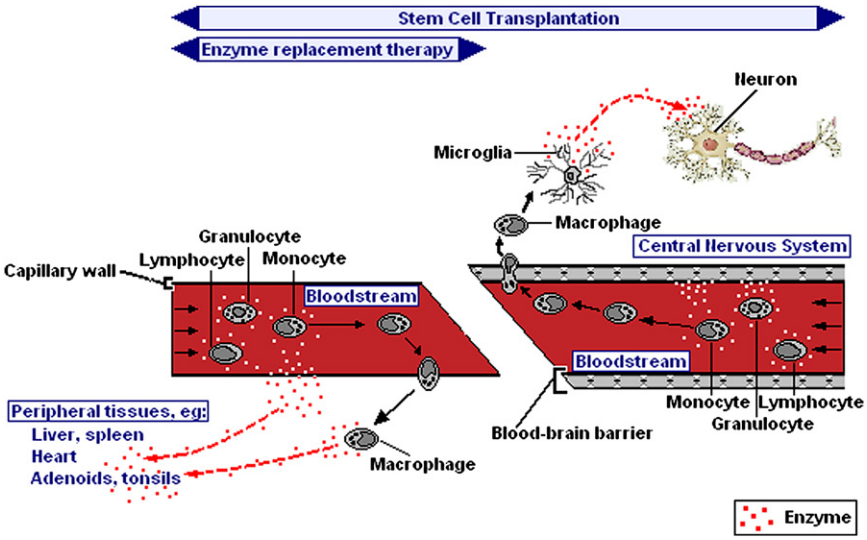


**Figure 1.** Enzyme delivery to the peripheral tissues and the central nervous system after stem cell transplantation.
In the *peripheral tissues*, the donor monocytes are able to cross the capillary wall, after which they differentiate into tissue-macrophages and secrete the deficient enzyme for delivery to the various cells. In addition, these peripheral tissues will presumably also benefit from the freely circulating enzymes, secreted by the donor leucocytes, because these enzymes are able to cross these capillary walls as well. Likewise, in enzyme replacement therapy, the intravenously administered enzymes are able to cross the capillary wall of these peripheral tissues.
In the *central nervous system* (CNS), the donor monocytes cross the blood-brain barrier and differentiate into microglia. These 'CNS macrophages' deliver the deficient enzyme to the neurons and other cells in the CNS. As freely circulating enzymes in the bloodstream are not able to cross the blood-brain barrier, the brains depend on these donor monocytes alone. This explains why enzyme replacement therapy is not beneficial for the disease manifestations involving the CNS in contrast to a successful stem cell transplantation.

widespread progressive cellular, tissue, and organ failure will originate, presenting clinically as progressive and ultimately fatal multi-system deterioration [2]. The exact pathogenesis of the tissue and organ failure in HS patients is still poorly understood. As the non-degraded GAGs accumulate, the number, as well as the volume, of the lysosomes increases, eventually causing mechanical and chemical disruption of the cell [7]. In addition to this direct (primary) effect of accumulating GAGs on cellular function, secondary pathways are thought to be triggered as well, including inflammatory processes (macrophage/cytokine release) [8] and the accumulation of GM2 and GM3 gangliosides in the brain [9-11]. The clinical phenotype and the detection of an increased amount of urinary GAGs, showing predominant excretion of heparan and dermatan sulphate, often suggest the diagnosis. Definitive diagnosis of HS is confirmed by severely decreased or absent IDUA enzyme activity in peripheral blood leukocytes, filter paper blood spots or fibroblasts, and subsequent mutation analysis [2,12].

### Natural Course without Stem Cell Transplantation

HS patients usually have a normal appearance during the first 6 months of life, although certain, mostly non-specific, symptoms are often already present in this initial period. These include mild facial dysmorphisms, persistent rhinitis, recurrent upper respiratory tract infections, hepato-splenomegaly, recurrent hernias, and mild thoracolumbar kyphoscoliosis [13,14]. After 6 months of age, HS patients will begin to develop progressive multi-system morbidity including progressive psychomotor retardation, impaired hearing and vision, organomegaly, severe musculoskeletal manifestations, and cardiovascular and pulmonary failure with death in early childhood [2]. Based on an MPS I registry, excluding patients with a known family history and prenatal diagnosis, diagnosis in HS patients (n=137) was made at a median age of 10 mos (2-82 mos) with a median interval from symptom onset until diagnosis of 4 mos (0-28 mos). This underscores the difficulties of diagnosing patients with an evolving phenotype [15].

## STEM CELL TRANSPLANTATION IN HURLER SYNDROME

Frantantoni et al [16] were the first who demonstrated the possibility of cross-correction of the defective metabolism in cultured fibroblasts derived from Hurler and Hunter syndrome patients. A similar correction was subsequently shown *in vivo* by Di Ferrante et al [17]. They demonstrated an induced degradation of GAGs in Hurler (and Hunter) syndrome patients after the administration of normal human plasma. These observations resulted in the first trial of SCT for HS, reported in 1981 by Hobbs et al [1]. Biochemical improvement as well as a dramatic reversal of the clinical manifestations in a 1 yr old boy with HS was demonstrated and this case was a landmark "proof-of-principle" experiment.

### Pathophysiology of Stem Cell Transplantation

Following successful SCT, the donor-derived stem cells provide a continuous endogenous source of the deficient enzyme. The various peripheral tissues (including the liver, spleen, lungs, and heart) presumably benefit from both enzyme secretion by infiltrated (donor) macrophages as well as enzymes secreted into the bloodstream by (donor) leukocytes. Because freely circulating enzymes are not able to cross the blood brain barrier, the CNS relies on infiltration by macrophages alone. In the CNS, these macrophages differentiate into microglia, which secrete the deficient enzyme for recapture by the surrounding neurons (Figure 1) [18].

### Natural Course of Hurler Syndrome Patients after Stem Cell Transplantation

Since the first bone marrow transplantation more than 25 yrs ago [1], over 500 SCTs have been performed in HS patients worldwide. Hence, SCT for HS accounts for the majority (approximately 40%) of SCTs performed in lysosomal storage disorders [19,20]. After engraftment, significant improvement in the clinical course of HS patients has been observed, although there is wide variation among the various tissues and organ systems, as well as among HS patients. The outcome of the various tissues and organ systems after SCT is discussed below, with particular emphasis on the long-term outcome (>3 yrs after SCT).

**Biochemistry.** Within 3 mos after successful SCT, the leukocyte enzyme activity (IDUA), which is either absent or severely decreased in untreated HS patients, increases to values within the expected range of the donor (heterozygote carrier or unaffected homozygote) [21-26]. At the same time, the previously increased GAG excretion is reduced to the upper limit of age-matched control values, with a small amount of dermatan sulphate and a hardly detectable fraction of heparan sulphate at electrophoresis [21,24-26]. The obtained enzyme activity and GAG excretion remained stable during long-term follow-up, provided that donor chimerism remained stable [21-23;25-27].

**Viscera.** All untreated HS patients suffer from hepato-splenomegaly with preserved hepatic function [2]. In addition, a combination of respiratory manifestations is usually present. This includes a reduced lung capacity, caused by interstitial pulmonary deposition of GAGs and restricted diaphragmatic excursions, as well as upper respiratory obstruction [28-30]. These manifestations contribute to the severe obstructive sleep apnea syndrome, commonly reported in untreated HS patients [28,31]. In addition, recurrent upper and lower respiratory tract infections often occur [31]. Eventually, pulmonary hypertension and cor pulmonale may develop, contributing to cardiopulmonary failure and early death in untreated HS patients [29].

In the cardiovascular system, left-sided cardiac valve abnormalities (especially the mitral valve), progressive GAG deposition in the coronary arteries, thoraco-abdominal aorta and systemic vessels with subsequent stenosis, as well as primary myocardial involvement are reported in untreated HS patients. These manifestations will eventually evolve into a dilated cardiomyopathy with congestive heart failure, largely contributing to the early death [3].

After successful engraftment, the metabolism and clearance of GAGs is dramatically improved in the highly perfused visceral organs, such as the liver, spleen, adenoid, tonsils, heart, and lungs. A major reduction of the hepato-splenomegaly as well as relief of the obstructive airway symptoms, including persistent rhinorrhea and obstruction of the upper airways, is seen in all patients within the first 3-6 mos after SCT [21-22,25-26,32,33]. The occurrence of obstructive sleep apnea is markedly decreased after SCT and pulmonary insufficiency is no longer observed, except for those cases caused by transplantation-related pulmonary complications [21,26,34-35]. Assessed by echocardiography, myocardial function is preserved and hypertrophy regressed after successful SCT and remains so in long-term survivors [25,26,36-38]. Post-mortem examination of a 17 yr old HS patient 14 yrs after SCT showed only minimally affected coronary arteries [39], which is in sharp contrast to the progressive coronary artery disease observed in untreated children [40,41]. These improvements have made death resulting from coronary occlusion or severe congestive heart failure exceptionally uncommon in HS patients after successful SCT [38]. In contrast to these beneficial effects on the myocardium, long-term follow-up revealed, however, that the mitral and aortic valve deformities persisted and often progressed [38,42]. Although valvular prolapse, insufficiency, or stenosis did evolve in some cases, surgical intervention was only rarely required. In addition, a case of persisting coarctation of the aorta, probably because of progressive GAG deposition, has been reported in one HS patient, despite successful SCT [43].

**Neurocognitive function.** In untreated HS patients, normal cognitive and motor developmental milestones are usually achieved until the age of 6 mos. Thereafter, psychomotor development slows down, plateaus, and, eventually, progressive cognitive and neurological deterioration takes place [3,44]. The pathophysiology of mental deterioration in HS patients is not completely clarified. Both the primary effect of accumulating GAGs on cellular function as well as the secondary inhibition of ganglioside-degradative lysosomal enzymes, causing accumulation of the gangliosides GM2 and GM3 in the CNS, are thought to play an important role [9]. This secondary

ganglioside accumulation has been shown to cause ectopic dendritogenesis and cholesterol accumulation in MPS mice, resulting in neuronal dysfunction [9].

Studies in transplanted MPS I dogs and cats showed relative low levels of IDUA enzyme activity in brain tissue and cerebrospinal fluid (CSF), compared to the enzyme levels in the donor [45-47]. Despite these low enzyme levels, statistically significant reductions in lysosomal GAG storage in brain tissue and CSF of transplanted MPS I animals were reported, compared to nontransplanted MPS I animals [45-47]. These GAG reductions were comparable to those seen in the liver and the acquired GAG content in the brain tissue and CSF appeared to be close to the levels measured in the donors [45,47]. Additionally, biochemical analysis demonstrated decreased CNS ganglioside (GM2 and GM3) storage after transplantation [47]. Furthermore, histopathologic analysis showed a clear therapeutic response in the meninges, choroid plexus, and perivascular lesions, although the improvement of the neuronal lesions appeared to be only limited [45-48].

Long-term follow-up of HS patients after SCT showed that when SCT is performed early enough in the disease process, ie before extensive cerebral damage has occurred, the neurocognitive development improved or stabilized in most children, preventing progressive mental deterioration. During longitudinal evaluation, many patients have continued to gain skills, although often at a slightly slower rate as compared to unaffected children [21-23,25-27,49-51]. The neurocognitive measurements in HS patients after SCT are likely to be negatively affected by the associated motor difficulties as well as the vision, hearing, and speech impairment [22]. Furthermore, learning difficulties, especially language problems, are frequently reported later in the course of the disease, often causing special educational needs [21,22,25,27,50,51].

The neurocognitive outcome after SCT has been shown to vary widely among HS patients. The variation is theorized to depend on multiple factors, including genotype, age at transplantation, cognitive situation at transplantation, donor chimerism, donor status, and enzyme activity after SCT [22,23,25,26,49,50]. The influence of these factors on the neurocognitive outcome is discussed further in this article, although the small study populations of current studies often limit significancy.

Another CNS manifestation, which probably contributes to neurocognitive dysfunction in untreated HS patients, is communicating hydrocephalus. The hydrocephalus is thought to be caused by a defective reabsorption of CSF by the arachnoid villi as well as thickened meninges, both resulting from GAG accumulation [3,26,35]. After successful SCT, hydrocephalus is either prevented (when not present before SCT) or stabilized, according to long-term follow-up; successfully engrafted patients no longer required ventriculo-peritoneal shunting after transplantation [21,22,25,26,33,51]. In addition to the hydrocephalus, the progressive cerebral atrophy and massive accumulation of GAGs in the Virchow-Robin spaces, which can be demonstrated by magnetic resonance imaging and computed tomography in all untreated HS patients, was arrested and subsequently reduced in several patients after SCT [21,25,26,52].

In addition to the biochemical correction within the brain and the prevention of elevated intracranial pressure, the prevention of severe obstructive sleep apneas, as well as improvements in visual acuity and hearing, are thought to contribute to the preservation of neurocognitive development in HS patients after SCT as well [26,49].

**Vision.** The predominant ocular manifestation in HS patients is progressive corneal clouding from dermatan sulphate deposition in the cornea [53]. This is associated with photophobia and causes a progressively decreasing visual acuity in untreated patients. After successful SCT, the reported outcome of corneal clouding varies among HS patients, probably also from difficulties in the objective grading of the corneal opacities. Clinical improvement was seen in 20 to 100% of the patients, whereas only a few patients showed progression of corneal clouding. Corneal transplantation was therefore rarely required after SCT [21,22]. However, in most patients mild residual corneal clouding existed after SCT [1,32,54-56] and long-term follow-up showed that this residual corneal clouding persisted unchanged [21,22,26,27].

In addition to the corneal clouding, retinal degeneration is a commonly found ocular complication in untreated HS patients and it contributes to the decreased visual acuity as well. Despite the stabilization or improvement of the retinal function observed soon after successful SCT [54], progressive retinal degeneration did occur and it is likely to cause a decreased visual acuity in the long-term survivors [55,57]. Optic atrophy, caused by optic nerve edema secondary to elevated intracranial pressure, has been documented in untreated HS patients and may contribute to visual impairment as well [55,58]. Along with the increased intracranial pressure, the optic nerve edema resolved after successful SCT and atrophy was not longer observed [55].

**Hearing.** In untreated HS patients, severe and progressive hearing loss, around 70 decibels or more, is a universal problem. This is usually caused by a combination of conductive as well as neurosensory components, including Eustachian tube dysfunction because of GAG depositions with subsequent otitis media, dysostosis of the auditory bones, damage resulting from recurrent ear infections, and nerve damage [2,3,59].

After successful SCT, hearing is either gradually normalized, improved, or stabilized in most HS

patients [21,22,25,27,33,60]. Next to the rapidly improving conductive component, a late improvement (5-6 yrs after SCT) of the neurosensory component has been observed as well [25]. Only a small number (0-20%) of successfully engrafted patients required hearing aids [22,25,27]. In contrast, repeated grommet insertions due to chronic recurrent middle ear infections are still commonly required after SCT [22,61].

**Linear growth.** Untreated HS patients suffer from severe and progressive growth failure, achieving a maximal height of approximately 110 cm even when longer survival is achieved [2]. This growth failure is characterized by a disproportionately short trunk. After SCT, linear growth is often maintained for several years. However, long-term follow-up results (3-16 yrs post-SCT) showed that this growth gradually fell to -1 standard deviation in most patients (67-71%) and even below -2 standard deviations in several patients (29-40%; all >6 yrs post-SCT) [21;22;25]. This reduced linear growth could mainly be attributed to a disproportionately short trunk, evidence by a reduced sitting height, independent of the presence or absence of kyphoscoliosis [21,22,25]. In a recent study using cord blood as a stem cell source, Staba et al [51] documented normal growth velocities within 1 yr after SCT in the majority of HS patients. These growth velocities remained normal during their follow-up (maximum of 6 yrs after SCT). Longer follow-up is needed to determine the effect of cord blood transplantation on the linear growth of long-term survivors.

**Skeletal outcome.** Due to failure of ossification and abnormal bone modeling, several orthopedic complications will progressively evolve in untreated HS patients, leading to severe disability and discomfort. These include thoraco-lumbar kyphosis resulting in a typical gibbus deformity with subsequent spinal cord compression; odontoid dysplasia at risk of developing cervical spine injury; acetabular dysplasia with subsequent hip (sub)luxation; and genu valgum resulting in significant deformities [62]. In addition, carpal tunnel syndrome (CTS) and associated trigger digits, causing difficulties in fine motor tasks, are also frequently reported in untreated HS patients [63,64].

Except for odontoid dysplasia, which showed progressive improvement after successful SCT [65,66], increasing genu valgum, progressing acetabular dysplasia and the development of CTS were not prevented by SCT, despite continued engraftment [21,22,25,62,66]. In addition, HS patients often showed progression of the thoracolumbar kyphosis, although the rate of progression varied among patients [21,22,25,62,66]. As a consequence, orthopedic and neurosurgical interventions were still necessary in several HS patients after successful SCT, although these interventions might have been postponed to a later age. The fact that the musculoskeletal manifestations can deteriorate after successful SCT is presumably related to the relative avascularity of the ground substance of the musculoskeletal tissues, precluding enzyme penetration into these tissues [62]. Although some authors were hopeful, speculating that the musculoskeletal complications after SCT were less severe or did progress less rapidly compared to untreated patients [22,71], other authors were less optimistic [62,67,72]. Careful systematic long-term follow-up and documentation of orthopedic complications after SCT is warranted.

The patient's age at transplantation might be an important variable in the skeletal outcome after SCT. Age at SCT was documented to significantly influence the age at the time of CTS surgery in HS patients ($P$=.007). In addition, there was a trend towards increased time to onset of CTS in HS patients with higher enzyme levels after SCT, although the cohort was too small to determine an absolute enzyme level providing maximal benefit [71].

**Joint mobility and functional performance.** Stiffness and contractures of the joints are very often reported in untreated HS patients [2]. The restricted joint mobility preferentially affects the hands, leading to characteristic claw hand deformities [73], as well as the shoulders. However, the typical restrictions of these joints are often combined with a limited range of motion in the elbows, wrists, hips, knees, ankles, and/or spine [2].

After successful SCT, improvements in the upper extremity joint mobility have been documented in most HS patients [21,22,25,33,62,67]. Predominantly, the shoulder and elbow movements showed a dramatic improvement in range of motion measurements. The development of clawing of the hands was limited compared to untreated cases. Parental reports confirmed these improvements, reporting increased daily activities that require upper extremity mobility [67]. These improvements of the upper extremity joint mobility were maintained during the follow-up of long-term survivors (n=44, ranging 3.2-15 yrs post-SCT) [21,25,62,67]. Unfortunately, the lower extremities showed a less successful outcome. A combination of fixed flexion deformities of the lower limbs, progressive hip (sub)luxation, limited hip abduction, genu valgum, and generalized muscle weakness did frequently lead to a severely reduced walking ability or even complete wheelchair dependency in long-term survivors [22,27,62,69,74]. To maintain function and improve the quality of life of the long-term survivors, a more active approach to the expected orthopedic manifestations has been adopted, including intensive physical therapy [62,75,76]. The benefits of early surgical intervention, such as innominate osteotomy for hip dysplasia, physeal stapling for genu valgum, and spinal fusion for thoracolumbar kyphosis, for functional performance in long-term survivors needs to be established during longer follow-up [66,68,69].

**Sexual development and fertility.** Since the life expectancy of HS patients after SCT has only recently reached pubertal ages, little has been reported regarding the sexual development of these patients. Vellodi et al [22] reported primary ovarian failure with subsequent delayed puberty in 2 of 5, and sexual infantilism in 1 of 5 pubertal age HS patients after SCT. The other 2 patients were thought to develop normally. The ovarian failure was probably related to the chemotherapy received before transplantation, although large amounts of lysosomal storage have been observed in the ovary of untreated female MPS I mice, consistent with a reduced fertility [77]. There are, however, no studies that have evaluated lysosomal storage in the reproductive system of human patients. Pregnancy in a 21-yr old female HS patient (severe genotype), 20 yrs after SCT, has been reported by Hendriksz et al [78]. Due to concerns regarding her own health, she decided to terminate the pregnancy.

**Survival.** Life expectancy has been significantly improved after successful SCT [21,22,26,27,79]. This is illustrated by survival, extending into the third decade of life after successful SCT [1,27,80], compared to a median survival of less than 5 years in untreated patients and only rare survivors beyond 10 years [2,79]. This greater lifespan of HS patients after SCT is mainly the result of the prevention of cardio-pulmonary complications as well as the prevention of severe developmental deterioration [38].

### Factors influencing the Natural Course after Stem Cell Transplantation

Interestingly, the natural course of clinical manifestations after successful SCT has been shown to be highly variable among HS patients. This variability has been suggested to depend on multifarious factors, including:

**Genotype.** The gene coding for the IDUA enzyme, has been localized to chromosome 4 at location 4p16.3 [81]. At present, 95 disease-causing mutations of the IDUA gene have been identified, including missense, nonsense, and splice site mutations, deletions, and insertions [82].

Although genotype-phenotype correlations do not always hold, the presence of a nonsense mutation on both IDUA alleles causes severe HS in all cases [83]. These nonsense mutations are believed to result in a complete lack of residual enzyme activity, causing rapid accumulation of lysosomal GAG storage with early onset of clinical signs and rapid disease progression. Two of these mutations, W402X and Q70X, are particularly common in patients of European ancestry, accounting for up to 70% of MPS I disease alleles, although considerable differences in frequency are seen across the various countries [84,85].

However, many patients have at least 1 private mutation, restricted to a certain family pedigree, making phenotype prediction difficult [86]. In addition, many of the biochemical tests, used in a clinical setting to diagnose IDUA enzyme deficiency, are not able to discriminate between severe and intermediate phenotypes; therefore, these tests are not helpful in predicting the clinical phenotype.

**Age at transplantation, neurocognitive development at transplantation, and neurocognitive outcome.** Various reports have shown a favorable and stable neurocognitive function in HS patients with a relatively low risk of progressive cognitive deterioration, but only when SCT is performed early in life and before cerebral damage has occurred [21-23,25,26,49,50]. Significant correlations, between the age at SCT and the slope of the cognitive development after SCT (-0.64; $P<.03$ and -0.51; $P=.01$), as well as the baseline Mental Development Index (MDI) and slope of the cognitive development after SCT (0.76; $P<.05$ and 0.77; $P=.0002$), have been documented in HS patients and support this finding [23,50]. Thus, both a younger age at SCT and a higher baseline MDI are associated with better cognitive trajectories after SCT. Hence, some have suggested that SCT should only be indicated in HS patients under the age of 2 yrs and with no or only minimal cognitive impairment (MDI >70). However, as the neurocognitive outcome of HS patients older than 2 yrs at SCT or with a baseline MDI below 70 did vary considerably [22,23,49,50], these contraindications must be used with caution [22,25]. The neurocognitive situation before SCT might be difficult to evaluate, especially in younger patients or when hearing loss is present. In addition, SCT might still be indicated after 2 yrs of age in those patients who present with a slightly milder phenotype. Therefore, it is important to realize that a decision about SCT must be made on an individual basis.

**Donor status, donor chimerism, and enzyme activity.** A significantly higher leukocyte enzyme level was accomplished in HS patients when an unaffected homozygous donor was used and full donor chimerism was achieved, compared to the situation where a heterozygous carrier donor was used or only mixed chimerism was achieved [21,23-26,87]. This higher enzyme level after SCT was associated with a significantly greater reduction of the urinary GAG excretion in 39 HS patients, studied by Church et al [24], although this latter effect was not observed in smaller series by other authors (in 15, 9, and 11 HS patients, respectively) [21,25,26].

**Donor status, donor chimerism, enzyme activity, and neurocognitive outcome.** The question remains whether the level of leukocyte enzyme activity actually influences the long-term neurocognitive outcome. Additionally, it is unknown whether this leukocyte enzyme activity is a direct reflection of the enzyme level delivered to the CNS.

The parents of HS patients, heterozygote carriers with enzyme levels of approximately 50% of normal, are completely asymptomatic. In addition, patients with the Scheie phenotype, presenting with only trace amounts of residual IDUA enzyme activity, have an attenuated clinical course without CNS deterioration. Furthermore, a few HS patients with a confirmed severe genotype were reported to have a normal neurocognitive development (MDI >80) after SCT despite of mixed donor chimerism or a heterozygous donor and low enzyme levels [21,25,26,50,88]. The acquired enzyme activity in most of these patients was, however, still above 50% of normal. These observations demonstrate that low enzyme levels, although often at least in the heterozygous range, are not necessarily associated with poor neurocognitive outcome.

On the other hand, Peters et al [23] observed a correlation between the donor enzyme status (homozygous unaffected versus heterozygous carrier) and the MDI at follow-up, in HS children with a baseline MDI above 70. However, the children with homozygous donors had a significantly higher baseline MDI and were significantly younger at SCT. As both age and MDI at SCT have been shown to be crucial for the neurocognitive outcome after SCT, the correlation between donor enzyme status and MDI at follow-up is confounded [23]. In the same study, all children who retained normal mental functioning (MDI >80) at follow-up, received a homozygous donor transplant and showed full donor chimerism with normal enzyme activity, and not one child whose acquired enzyme activity was low (from a heterozygous carrier donor of mixed chimerism) showed normal mental functioning [23]. Therefore, the enzyme level still seems to be an important influencing factor. A slightly lower neurocognitive score after SCT in HS patients with subnormal enzyme levels was also seen by Guffon et al [25] although the study population was too small to draw conclusions.

Whether normal leukocyte enzyme activity, achieved when using a homozygous unaffected donor and with full donor chimerism, favorably contributes to the cognitive development of HS patients should be analyzed in future studies with larger study populations [23]. Nevertheless, we are aware that various pediatric transplantation centers now prefer an unrelated donor rather than a related family donor with a high a-priori risk of being a carrier.

**Stem cell source: cord blood.** In various studies, the use of cord blood (CB) as the stem cell source in HS patients was associated with higher rates of successful sustained engraftment, compared to bone marrow and peripheral blood (84-85% vs 26-75%) (Table 1) [21,23,50,51,89]. However, the results obtained with SCTs using CB mainly involved SCTs performed during the last decade, whereas many of the studies using bone marrow (BM) or peripheral blood (PB) have been accomplished over the last several decades. Therefore, these higher rates of successful engraftment in CB recipients might have been caused by other factors, including changes in conditioning regimen. This is supported by a recent retrospective European group for Bone and Marrow Transplantation (EBMT) study, analyzing risk factors for graft failure in 146 HS patients. This study showed no significant difference in engraftment between the use of CB, BM and PB stem cells [87].

Although no difference in survival or graft failure could be demonstrated between the recipients of the various stem cell sources, the authors did show that significantly more patients receiving a CB transplant achieved full donor chimerism (93%), compared to other stem cell sources (BM and PB, 66%: OR 9.31; $P=.044$). Furthermore, all patients receiving a CB transplant obtained normal enzyme levels, compared to 60% in the other group [87]. Other studies that used CB as a stem cell source, also showed high incidences of full donor chimerism and normal enzyme levels in HS patients (Table 1) [24,51,89,90], as well as in other IEM [90-92]. Because full donor chimerism and higher enzyme levels after SCT have been suggested to positively influence the neurocognitive outcome after SCT, CB might be a preferential stem cell source for HS patients. Whether the stem cell source could influence the long-term outcome, including the neurocognitive development after SCT, should be further clarified according to systematic long-term follow-up.

Furthermore, the use of CB for SCT has several other important advantages compared to other stem cell sources (BM, PB), including: 1) Cord blood is readily available (often within a month), significantly reducing the time between diagnosis and SCT [51,91,93]. This is particularly important for the rapidly progressive CNS involvement in HS patients; 2) Donors with a larger number of human leukocyte antigen disparities can be used, increasing the possibility of finding a suitable donor [51,94]; 3) Despite the higher level of human leukocyte antigen incompatibility, lower rates of acute and chronic graft-versus-host disease (aGVHD, cGVHD) are reported [51,90,94]; 4) The likelihood of transmitting (viral) infections is reduced [94,95]; and 5) Although highly speculative, there are suggestions of trans-differentiation into cell types such as osteoblasts, chondroblasts, and neurons, because of the more primitive stem cell population in CB [96-98].

Disadvantages that are reported using CB as a stem cell source are: 1) An increased aplasia duration, due to the lesser amount of stem cells in CB; 2) The availability of only one donation per donor; and 3) Less adoptive immunity, which might increase the likelihood of life-threatening viral reactivations [93]. These disadvantages are, however, less applicable to HS patients.

**Table I.** *Results of engraftment and enzyme activity after stem cell transplantation in Hurler syndrome patients*

| | n | Stem cell source | Conditioning regimen | Median age at SCT (mos) | Median follow-up (mos) | Successful SCT[1] (%) | Full donor chimerism[2] (%) | Normal enzyme level[3] (%) | GvHD Acute[4] (%) | GvHD Chronic[5] (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Peters et al.* | 21 | BM[6] | ↔[8] | 20 | 34 | 43 | 87 | 56 | 30 | 30/18 |
| *1996* [50] | 19 | BM[7] | ↔[9] | | | 26 | | 67 | | |
| *Peters et al.* | 28 | BM[10] | ↔[12] | 22 | 88 | 75 | 71 | 30 | 32 | 0/0 |
| *1998* [23] | 26 | BM[11] | ↔[13] | 23 | 55 | 35 | 89 | 0 | 55 | 41/23 |
| *Souillet et al.* | 27 | BM | ↔[14] | 25 | 72 | 70 | 52 | 53 | 9 | 0/0 |
| *2003* [21] | | | | | | | | | | |
| *Staba et al.* | 20 | CB | Bu*Cy/ATG[15] | 16 | 30 | 85 | 100 | 100 | 25 | 12/0 |
| *2004* [51] | | | | | | | | | | |
| *Boelens et al.* | 146 | ↔[16] | ↔[17] | 18 | 44 | 56 | 71 | 60 | 16 | 7/2 |
| *2007* [87] | | | | | | | | | | |
| *Boelens et al.* | 15[18] | CB | ↔[20] | 19 | 18 | 40 | 100 | 100 | 15 | 19/8 |
| *2007* [89] | 25[19] | | ↔[21] | | | 84 | 90[22] | 100 | | |

Ara-C indicates cytarabine; ATG, anti-thymocyte globulin; BM, bone marrow; Bu, busulphan; Bu*, busulphan (targeting), CB: Cord blood, Cy: cyclophosphamide; Cy*, cyclophosphamide (high dose); Flud-Based, Fludurabine based myeloablative regimen; GVHD, Graft-versus-Host Disease; n, number of patients; PB, peripheral blood; RIC, reduced intensity conditioning; SCT, stem cell transplantation; TBI, total body irradiation; TLI, total lymphoid irradiation; VP-16, etoposide; ↔, various.

[1]alive and >10% donor chimerism

[2]% of patients with ≥95% donor chimerism from total number of successful transplanted patients

[3]% of patients with normal enzyme level (according to local reference values) from total number of successful transplanted patients

[4]% of grade II-IV (moderate to severe) acute GVHD

[5]% of total chronic GVHD / % of total extensive chronic GVHD

[6]High dose of BM (≥$3.5*10^8$ cells/kg)

[7]low dose of BM (<$3.5*10^8$ cells/kg)

[8]Bu/Cy (12), VP-16/Cy/Ara-C/TBI (3), Cy/Ara-C/TBI (2), Bu/Cy/ATG (4)

[9]Bu/Cy (8), VP-16/Cy/Ara-C/TBI (1), Cy/Ara-C/TBI (4), Bu/Cy/ATG (1), Bu/Cy/TLI (2), 0,5Bu/0,5Cy/TBI (3)

[10]HLA-genotypically identical siblings

[11]HLA-haploidentical related donors

[12]Bu/Cy (23), Bu/Cy/TBI (1), Cy/TBI (1), Bu/Cy/TBI (1), ? (2)

[13]Bu/Cy (8), Ara-C/Cy/TBI (12), Cy/TBI (3), Bu/Cy/TBI (1), VP-16/Ara-C/Cy/TBI (1), ? (1)

[14]Bu/Cy (11), Bu/Cy/ATG (5), Bu/TBI (1), Bu*/Cy/ATG (10)

[15]Bu*/Cy/ATG (20)

[16]BM, PB, or CB

[17]Bu/Cy (68), Bu/Cy* (30), Bu* (15), Flud-based (17), RIC (18)

[18]SCTs < 2001

[19]SCTs ≥ 2001

[20]Myeloablative (14), RIC (1)

[21]Myeloablative (24), RIC (1)

[22]n=2 patients with mixed chimerism; 90% and 94%, still increasing

Since these young patients require only small doses of stem cells in order to obtain successful engraftment (because of their low body weight) and as relatively low graft failure rates are reported after the use of CB, one CB donation is sufficient in most cases. Furthermore, because of their young age, HS patients have suffered from only limited viral infections before SCT, considerably reducing the probability of developing viral reactivation.

## Limitations of Stem Cell Transplantation in Hurler Syndrome

Although SCTs in HS patients have clearly shown to be effective, evident by an increased life expectancy and improvement of various important clinical outcome parameters, there are still some major limitations, including:

**Incomplete response.** Successful engraftment does not prevent disease progression in all organ systems and because the life expectancy of HS patients has been increased, several severe disease manifestations have become apparent in long-term survivors. Despite achieving successful engraftment, the following conditions have been reported by various authors: progression of retinal degeneration likely to result in visual deficiency [55-57]; continued thickening of cardiac valves leading to progressive valvular prolapse, insufficiency, or stenosis [38,42]; and musculoskeletal deterioration causing severe orthopedic complications [21,22,25,27,62,66-70].

In addition, it was noticed that the neurocognitive development continued to deteriorate during the immediate post-transplantation period [51,99]. This might be due to the delay in macrophage (microglia)

repopulation in the CNS, compared to other tissues. Donor CNS microglial engraftment in mice was demonstrated to be only 23% at 6 mos and 30% by 1 yr post-SCT, compared to nearly 90% splenic macrophage engraftment by 1 mo and 98% by 6 mos [100]. Although it is not clear what level of microglial engraftment is required to arrest CNS disease progression in HS patients, the significantly slower rate of CNS microglia repopulation (especially at parenchymal sites [100]) might account for the disease progression in the immediate post-transplantation period [8,100].

Furthermore, many of the complications associated with HS appeared to be irreversible, leading to residual disease, including mild corneal clouding [1,32,54-56] and variable neurocognitive dysfunction [21-23,25-27,49-51]. It should be clear that, in order to improve the outcome of HS patients after SCT, the SCT has to be performed as early as possible in the course of the disease; before the onset of irreversible tissue damage has occurred.

**Morbidity, mortality, and graft failure.** Other important limiting factors for the success of SCTs in HS patients are treatment related morbidity and mortality (TRM) [21,23,50,51,87]. The most frequent occurring transplantation-related complications are aGVHD and/or cGVHD, pulmonary complications (hemorrhages and infections), and (mainly viral) infections and reactivations. Survival rates of HS patients after SCT vary between 50% and 85% [21,23,50,51,87,89]. In addition to morbidity and mortality, graft failure is also frequently reported in HS patients, resulting in relative low 'alive and engraftment' rates, varying between 34% and 85% after first SCT [21-23,50,51,79,87,89]. In a retrospective EBMT study, risk factors for graft failure in HS patients (n=146) were identified. T cell depletion ($P$=.02) and reduced-intensity conditioning (RIC, P=.002) were found to be risk factors, while busulphan targeting protected against graft failure (P=.028). The results of this study have resulted in novel EBMT-guidelines for SCT in HS patients [101]. These observations might explain why the more recent studies, using protocols according to the novel EBMT-guidelines, showed better results in terms of higher 'alive and engraftment' rates [21,51,87,90].

## OTHER TREATMENT OPTIONS

### Enzyme replacement therapy

Since 2003, ERT has become commercially available for MPS type I patients. Recombinant human IDUA (laronidase) has shown to give major clinical improvements in patients with an attenuated form of MPS type I (Scheie and Hurler-Scheie) and for these patients ERT is the treatment of choice [3,102-104]. However, as the freely circulating enzymes, administered during ERT, are not capable of crossing the blood-brain barrier, this therapy is not effective in preventing or treating the neurological signs and symptoms (Figure 1). ERT is therefore not indicated for those patients suffering from a disorder with CNS involvement, including HS patients.

In an attempt to improve their quality of life, ERT has been used in transplant naïve HS patients, although only in small series [105-107]. The reasons to use ERT instead of SCT in these patients were: a delayed diagnosis; significant developmental delay at diagnosis; lack of a compatible SCT donor; and major concerns of parents about the risk of transplant-related complications. Although various clinical benefits were reported, not all affected tissues and organs did improve [105,106]. Interestingly, an increase in cognitive function at a normal rate in the patients who started ERT at a younger age (<2.5 yrs) was reported during a 1-yr treatment period by Wraith et al [105]. However, some of the developmental gains that were observed during this study could be related to the improvement in overall health status, including the prevention of severe obstructive sleep apnea and improvement of visual activity and hearing [26,49], and, in addition, follow-up was rather short. Long-term follow-up, after 3 yrs of ERT, was reported in only 1 HS patient, started with ERT at 5 yrs of age. This patient showed a continued decline is respiratory status, musculoskeletal involvement as well as developmental skills [107].

The combination of ERT and SCT has been evaluated in only a limited number of studies. The outcome of 7 HS patients receiving ERT in combination with SCT was reported by Tolar et al [108]; a larger series of 22 HS patients receiving ERT and SCT was compared to a historic cohort in a multicenter study by Cox-Brinkman et al [109]. In both studies, ERT was started after confirmation of the diagnosis and its administration was continued until successful engraftment was achieved. Although ERT was well tolerated in both studies, also previously shown by Grewal et al [110], the combination of ERT and SCT did not significantly influence the 'alive and engraftment' rate, nor did it influence the SCT-associated morbidity rate in the study of Cox-Brinkman et al [109]. Therefore, these authors concluded that only for HS patients in a poor clinical condition, making them ineligible for SCT, ERT is indicated and only when one expects that ERT can improve the clinical condition in a short period of time. In HS patients in a moderate/good clinical condition, early diagnosis and subsequent timely SCT were considered to be more important than the use of ERT prior to SCT [109]. Tolar et al [108], on the other hand, reported a high survival rate and acceptable morbidity after the use of ERT in combination with SCT, despite the fact these patients were at risk for pulmonary complications. Although based on

a small study population, they recommended ERT in the pre-transplantation period in all severe HS patients in order to improve morbidity and mortality. It is evident that further research in this area is warranted before a definitive statement can be made on the use of ERT in SCT procedures.

### Mesenchymal Stromal Cell Therapy

Mesenchymal stromal cells (MSCs) are non-hematopoietic pluripotent stem cells with the potential to differentiate into various cells of mesenchymal origin (eg osteoblasts, chondrocytes, and astrocytes). These MSCs could therefore be valuable for HS patients, improving or preventing some of the residual disease, including the musculoskeletal and neurocognitive manifestations.

Although BM contains both hematopoietic and non-hematopoietic progenitors, Koc et al [111] were the first to demonstrate that allogeneic bone marrow transplantations in HS patients do not result in the replacement of the non-hematopoietic progenitors, including MSCs. This could have been caused by the small number of non-hematopoietic progenitors in the BM. Therefore, some years later, Koc et al [112] infused MSCs, isolated and expanded from the bone marrow of the original donor, in HS (and metachromatic leukodystrophy) patients (n=5 and n=6, respectively) who were successfully engrafted. Although the procedure appeared to be safe and the bone mineral density was either maintained or slightly improved in all patients, there was no change in the overall health and no detectable influence on the neurocognitive and physical development of HS patients. Therefore, further studies are needed to evaluate the potential role of MSCs in the management of HS patients.

### Gene Therapy

Gene therapy represents a potential alternative therapy by supplying a functional copy of the gene. This could result in a constant delivery of the missing enzyme to all tissues and organs or to targeted organs, for example the musculoskeletal system or CNS [113]. Although gene therapy performed in MPS I animal models did seem encouraging, further research is needed before it could be clinically useful for HS patients [113,114].

## NEONATAL SCREENING

The achievement of an optimal clinical outcome in HS patients after successful SCT, especially a favorable neurocognitive outcome, has been shown to depend on the age at transplantation [23,49,50]. Hence, early diagnosis as well as subsequent timely SCT, ideally before the onset of irreversible pathology, has become highly important for a successful outcome. Because unrelated CB is suggested as a preferential stem cell source and this source is readily available, timely transplantation shortly after diagnosis has become possible. In addition, early detection of HS, ideally in the presymptomatic stage, could be made possible by implementing a neonatal screening program. In addition to a better outcome of clinical manifestations after early SCT, there are several other advantages of early diagnosis effected by neonatal screening in HS patients, including: 1) a higher rate of success with less TRM when SCT is performed in the neonatal period [91]. This is probably due to reduced organ damage pre-SCT and fewer cases of viral reactivations after transplantation; 2) The possibility of genetic counseling for the parents of an affected child, to provide the option of prenatal diagnosis or pre-implantation genetic diagnosis for subsequent pregnancies; and 3) Avoidance of the prolonged and stressful process of diagnosis [115]. Possible disadvantages of neonatal screening for HS include: 1) The costs of a neonatal screening program; and 2) The false positive findings that could generate anxiety in parents [115]. However, because early diagnosis and subsequent early SCT appear to be associated with a better clinical outcome, the overall healthcare costs of HS patients could potentially be decreased, reducing the overall financial burden. Furthermore, since lysosomal storage disorders have a combined incidence of around 1:7700 (even up to 1:5000 when all lysosomal storage disorders are considered) [116], (which is twice as prevalent as phenylketonuria), the simultaneous detection of multiple, treatable lysosomal storage disorders combined in one neonatal screening method could be economically feasible.

Although it is already technically possible to diagnose HS using dried blood spots [12,117,118], demonstration of a significant medical benefit due to early diagnosis is mandatory before such a newborn screening for HS can be implemented [119]. To meet this criterion, an international observational study evaluating the long-term outcome after successful SCT in HS patients, including the influence of early diagnosis, has been initiated.

## CONCLUSION

SCT is the preferred treatment for HS patients and the long-term outcome of clinical manifestations in HS patients after successful SCT appears to be promising. There are, however, some major limitations, including high graft failure and TRM and morbidity rates, although the more recent studies show better results. Furthermore, some clinical manifestations appear to be irreversible or continue to progress despite successful SCT, causing residual disease burden in long-term survivors. Interestingly, the long-term

clinical outcome appears to be highly variable among HS patients, presumably caused by various factors. Genotype, age at SCT, clinical situation at SCT, donor status, donor chimerism, stem cell source, and enzyme activity have all been suggested to influence the long-term outcome, although this conclusion was based primarily on small study populations. Unfortunately, standardized long-term follow-up studies performed in HS patients are lacking to confirm these suggestions. To evaluate the natural course and the influence of various patient and donor characteristics on the long-term outcome of HS patients after successful SCT, an international long-term follow-up study has been proposed and initiated, recently.

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