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Bone marrow transplantation for Maroteaux–Lamy syndrome (MPS VI): Long-term follow-up

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Summary: We describe the results of bone marrow transplantation (BMT) in four patients with mucopolysaccharidosis type VI (MPS VI, McKusick 253200)—Maroteaux–Lamy disease. The indications for transplantation were cardiomyopathy in three patients and severe obstructive sleep apnoea in one. The follow-up period ranges between 1 and 9 years, and three of the patients are at mainstream schools. In all of the patients the facial features have become less coarse and the cardiac manifestations have improved or remained stable. However, skeletal changes have persisted or even progressed, although posture and joint mobility have improved and all the patients have remained ambulatory and active. BMT appears to prolong survival and improve the quality of life in MPS VI patients, but careful selection of patients is essential.

Mucopolysaccharidosis type VI (MPS VI, McKusick 253200), or Maroteaux–Lamy disease, is characterized biochemically by a deficiency of the lysosomal enzyme arylsulphatase B (ASB, EC 3.1.6.12), resulting in the accumulation of dermatan sulphate in various tissues and fluids. Clinically, the disorder is characterized by coarse facies, dysostosis multiplex, short stature, corneal opacities, obstructive sleep apnoea (OSA) and cardiomyopathy. Mental development is usually normal (Neufeld and Muenzer 1995). Bone marrow transplantation (BMT) has been attempted in a small number of patients (Hite et al 1997; Hoogerbrugge et al 1995; Imaizumi et al 1994; Krivit 1992; Krivit et al 1984; McGovern et al 1986; Resnick et al 1992). However, detailed follow-up reports are available on only two (Imaizumi et al 1994; McGovern et al 1986).

We describe the results of BMT in four patients with MPS VI, including long-term follow-up in two patients.

PATIENTS AND METHODS

Patients: All four patients (2 males and 2 females) were born to unrelated parents and were diagnosed during the third year of life. In each case urine glycosaminoglycan (GAG) electrophoresis showed excessive excretion of dermatan sulphate and a diagnosis of MPS VI was confirmed following the demonstration of a deficiency of ASB in their leukocytes. Coarse features, corneal clouding and dysostosis multiplex were present in all four patients. In addition, patients 1, 2 and 3 had echocardiographic evidence of cardiomyopathy, which in patient 1 was also clinically evident. Patient 3 had hydrocephalus, which did not require intervention. A younger sister of patient 3 is similarly affected.

Transplantation details: The transplants were performed at the Westminster Children's Hospital and Great Ormond Street Hospital (two at each centre) between 1988 and 1996. The indications for BMT were cardiomyopathy in patients 1, 2 and 3 and OSA not resolving after tonsillectomy in patient 4. The donors were HLA-identical siblings in patients 1, 2 and 3 and an unrelated, HLA-identical donor in patient 4. All the patients received busulphan and cyclophosphamide conditioning. Patients 1 and 2 received a lower dose of busulphan and a higher dose of cyclophosphamide. Prophylaxis against graft-versus-host disease (GVHD) was with cyclosporin alone in patients 1–3. Patient 4 received cyclosporin and Campath.

Monitoring of engraftment: Engraftment was monitored using quantitative urinary GAG excretion and electrophoresis; measurement of leukocyte arylsulphatase B activity; determination of blood group where appropriate; restriction fragment length polymorphism.

Psychological assessment: Pre-BMT assessment was carried out between 12 months and a few days before BMT using the following methods according to the developmental level of each child: British Ability Scale (BAS) in patients 1 and 2, the Bayley Scales of Infant Development in patient 3, and the Merrill–Palmer scale for mental age in patient 4. Post-BMT assessment was carried out every 12–24 months. The following methods were used: Wechsler intelligence scales for children (WISC) in patient 1, the BAS in patient 2, and the Wechsler preschool and primary intelligence scales (WPPSI-R) in patients 3 and 4.

Laboratory methods

Leukocyte arylsulphatase activity: Leukocyte ASB activity was measured following the separation of ASB and arylsulphatase A (ASA) on DEAE-cellulose columns and assay of the enzyme using *p*-nitrocatechol sulphate as substrate.

Sample preparation: Leukocytes were prepared and stored at $-20^{\circ}C$ until assay. The enzymes were extracted as described by Vellodi et al (1992) except that the final concentration of the Triton X-100 in the supernatant was 0.2%.

Separation of ASB and ASA: The columns (4×20 mm) were prepared as described by Ellis et al 1975 (the Technicon T-piece and rubber septum were not used) and

filled with DEAE-cellulose equilibrated with 50 mmol/L Tris–acetic acid buffer, pH 7.5. The eluate was collected into tubes which contained 10 μl 5% human serum albumin. A 40 μl sample in 0.2% Triton X-100 (containing 100–150 μg protein) was loaded on to the column which was then eluted with 50 mmol/L Tris–acetic acid buffer, pH 7.5. Three 500 μl fractions were collected. These fractions contained the ASB. The column was then eluted with 50 mmol/L Tris–acetic acid buffer containing 1 mol/L NaCl, pH 7.5, and three further fractions were collected that contained the ASA.

Assay of ASB: *p*-Nitrocatechol sulphate (88 mmol/L) in 0.6 mol/L acetate buffer pH 5.28 (125 μl) was added to the three ASB fractions. Samples were incubated at 37°C for 60 min. The reaction was stopped by the addition of 4 mol/L NaOH (250 μl) and the absorbance was read at 514 nm.

Assay of ASA: *p*-Nitrocatechol sulphate (183.3 mmol/L) in water (125 μl) and 1.2 mol/L acetate buffer pH 5.7 (250 μl) were added to the ASA fractions. Samples were incubated at 15°C for 60 min. The reaction was stopped and the absorbance was read as described for ASB.

Urinary glycosaminoglycans: Assay of total GAG excretion and electrophoresis of urinary GAGs was as described by Whiteman and Young (1977).

RESULTS

All four patients fully engrafted following BMT, and have remained so for the period of follow-up. Patient 1 had severe acute graft-versus-host disease (GVHD), and patients 2 and 3 had grade 2 acute GVHD. Patient 2 also developed chronic mild hepatic GVHD which resolved by the end of the first year post BMT. Patient 3 had transient renal impairment 5 months after BMT due to cyclosporin toxicity.

Patients 1 and 2 have been followed up for 6 and 9 years respectively post BMT and patients 3 and 4 for only 1 and 2 years respectively (see Table 1). The period of follow-up has therefore been too short in patients 3 and 4 to comment on many aspects of follow-up.

Table 1 Baseline data

Patient no.	*Sex*	*Age at diagnosis* (years)	*Indication for BMT*[a]	*Age at BMT* (years)	*Follow-up period* (years)
1	F	3	CM	9.5	6
2	M	2	CM	8.75	9
3	M	2.4	CM	3	1
4	F	2.75	OSA	4.9	2

[a] CM, cardiomyopathy; OSA, obstructive sleep apnoea

External characteristics: The facial features have been modified, becoming less coarse (Figure 1). The corneal clouding has remained unchanged. Three of the patients need glasses.

Growth and development: All patients remained very short (<3rd centile) after BMT mostly owing to poor spinal growth. Of the two patients who are of pubertal age, patient 1 developed ovarian failure, probably secondary to chemotherapy, and patient 2 developed hypogonadotrophic hypogonadism, the cause of which is unclear. Both have required hormone replacement therapy with oestrogen and testosterone respectively.

Psychological assessment: From the available post-BMT data, patient 1's overall IQ fell in the low-average range. The other three patients had overall IQ in the borderline range. However, patient 2 has completed his school final examination. In comparing pre- and post-BMT scores, there appears to be a drop in the score of patient 1 (from 98 to 85). However, this drop has to be taken in the context of the correlation between the two tests used, the BAS and the WISC full-scale IQ, which is poor. Patient 2 is the only one who was tested using the same test (BAS) on both occasions. His results indicate no change (72 and 74). Patient 3 was tested before BMT on the Bayley Scale of Infant Development, and most recently on the WPPSI-R. Similarly, patient 4 was tested before BMT on the Merril–Palmer Scale, and following BMT on the WPPSI-R. Both the Bayley Scale of Infant Development and the Merril–Palmer Scale are testing development and hence are difficult to compare with the WPPSI-R which tests intelligence. It is therefore very difficult to draw any firm conclusions about patients 3 and 4. Their latest WPPSI-R test results are 77.

ENT: Conductive hearing loss due to middle ear effusion, necessitating insertion of grommets, was present in all four patients before BMT.

Patient 1 was found to have a right-sided conductive hearing loss 4 years post BMT. There was no evidence of effusion, and it was therefore felt that the hearing loss might have resulted from ossicular fusion. However, the following year she developed an unexplained severe (70 dB) unilateral sensorineural loss, which has persisted. Patient 2 has normal hearing. The other two patients have mild to moderate conductive hearing loss.

Dysphonia and hoarseness were present in patients 1 and 2 prior to BMT. This has now resolved. Prior to BMT patient 3 had severe OSA not responding to tonsillectomy (enlarged adenoids were not found). Two years after BMT, only rare OSA during active sleep was observed.

Cardiac status (*Table 2*): Patient 1 had clinical evidence of cardiomyopathy at presentation. Pre- and post-BMT echocardiograms were available for all four patients. Thickening of the mitral and tricuspid valves was evident before BMT in all the patients and the aortic and pulmonary valves were also thickened in patients 2 and 4 (Table 2). Myocardial involvement was noticed in the older patients only (Table 2). In one it developed in less than 2 years, leading to severe infiltrative cardiomyopathy. Cardiac involvement was the indication for BMT in three patients. Following BMT significant improvement was noted in patients 1 and 2. These

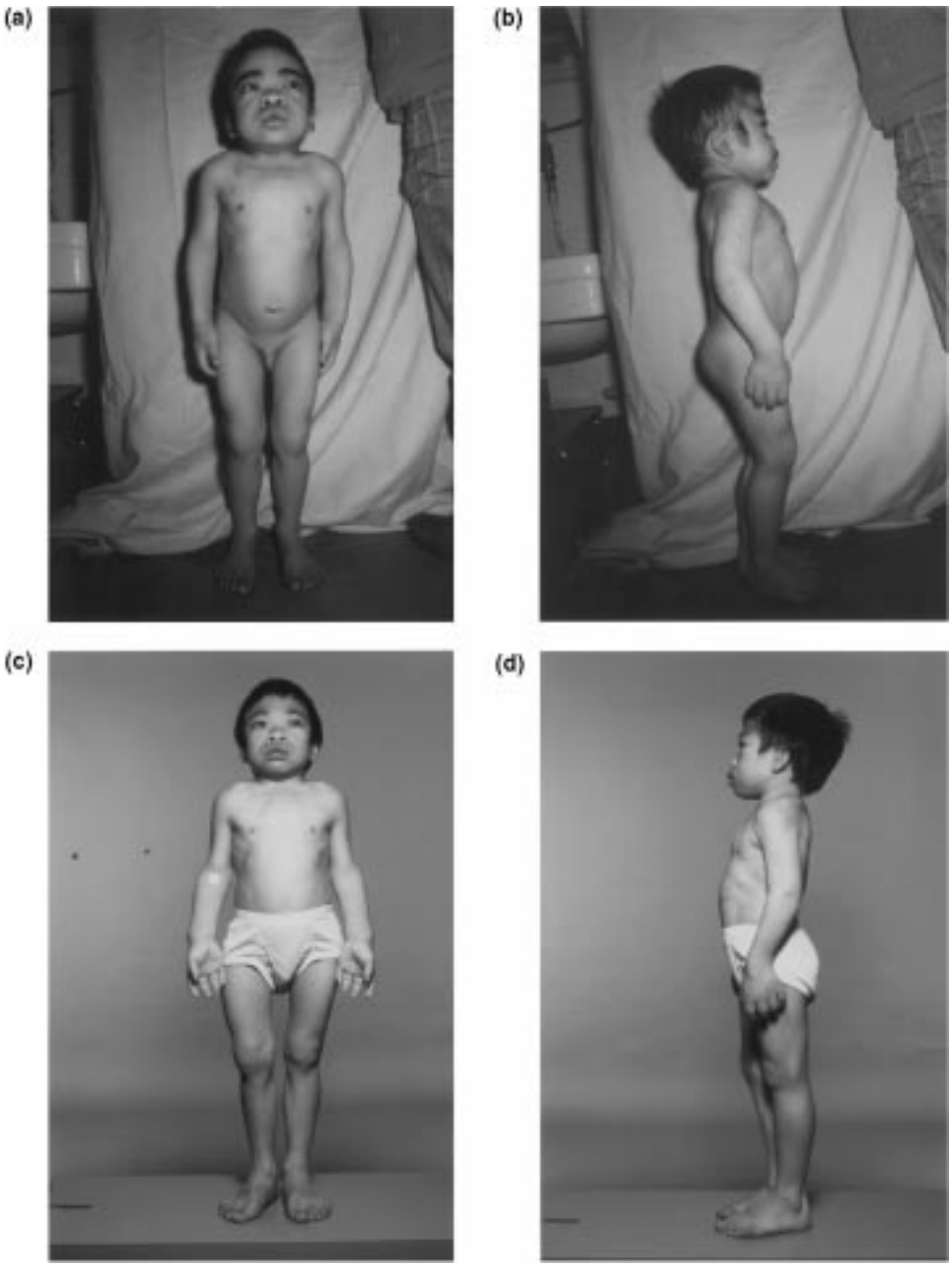

Figure 1 Patient 2 before (a and b) and after (c and d) BMT showing resolution of facial features and improved posture

Table 2 Cardiac changes pre and post BMT

	Pre-BMT assessment				*Most recent assessment*			
Patient no.	*Age at test* (years)	*Valvular disease*[a]	*Myocardial involvement*	*Clinical signs*	*Age at test* (years)	*Valvular disease*[a]	*Myocardial involvement*	*Clinical signs*
1	9.2	MV and TV thickening	Mild post BMT wall thickening	Mild MR	15.75	MV thickening	No	Mild MR
2	8.33	Abnormal thickening of all valves	Rapidly progressive cardiomyopathy	No	17.3	Mild MR and AS; mild MV thickening	No	No
3	2.4	MV and TV thickening	No	No	4.2	Mild MV and AV thickening; trivial TR and MR	No	No
4	4.9	Slightly thickened valves Trivial MR	No	No	7	Mild MR, MV thickening	No	No

[a] AV, aortic valve; AS: aortic stenosis; MV, mitral valve; MR, mitral regurgitation; TV, tricuspid valve; TR, tricuspid regurgitation

favourable effects were noticed during the first 2 years after BMT, followed by stabilization. The results in patients 1 and 2 have been described in a previous paper reviewing the effects of BMT on the cardiac manifestations of the MPS disorders (Gatzoulis et al 1995). The latest echocardiographic data indicate that there is no myocardial involvement in any of the patients, although valve disease persists.

Musculoskeletal status: In patients 1 and 2 there has been a significant improvement in posture, with a less lordotic stance, and in the range of movements at most of the major joints. However, the dysostosis multiplex has persisted (Figures 2–4).

Progressive hip changes in two patients required surgical correction by shelf acetabuloplasty and bilateral varus osteotomy to relieve hip stiffness (Figure 2). The spinal curvature has worsened in patients 2, 3 and 4. All patients have had MRI scans and flexion-extension radiographs of the cervical spine. Although odontoid hypoplasia was an invariable finding, and patient 2 has developed some spinal canal stenosis (Figure 5), there was no evidence of cervical cord compression or atlantoaxial instability during the follow-up period. Carpal tunnel syndrome was noticed in all four patients. Patients 3 and 4 had bilateral decompression prior to BMT. Patients 1 and 2 were not tested prior to BMT, but were found to have carpal tunnel syndrome following BMT, requiring decompression 3.5 and 4.5 years after BMT respectively. Patient 2 required a second decompression 7.5 years following BMT.

Biochemistry (*Table 3*): Prior to BMT the leukocyte ASB activity was very low but post transplantation the activity increased and has remained within the normal reference range. The urinary GAG/creatinine ratio is now only slightly elevated in all four patients and electrophoresis of the urinary GAGs shows that dermatan sulphate is still being excreted. However, the ratio of dermatan sulphate to chondroitin sulphate is much lower than is found in untreated patients.

DISCUSSION

The long-term results of BMT in Hurler disease (MPS IH) are now known (Hoogerbrugge et al 1995: Peters et al 1996: Vellodi et al 1997). However, owing to the very small number of MPS VI patients who have undergone transplantation, and the slow progression of the disorder when compared with Hurler disease, evaluation of the results of BMT has been more difficult. Two other MPS VI patients have been described in detail. The first patient was a 13-year-old girl with biventricular heart failure and severe obstructive sleep apnoea (McGovern et al 1986). Forty months after BMT she had only right ventricular dilatation with normal left ventricular function. Her OSA had disappeared and subjective improvement in the range of joint movement was reported, although no improvement could be demonstrated radiologically. The second patient underwent transplantation at the age of 13.75 years because of progressive cardiac dysfunction and skeletal deformities (Imaizumi et al 1994). Her cardiac status did not deteriorate further and a slight improvement in the range of joint movements was noted 47 months after BMT. The skeletal changes did not improve radiologically.

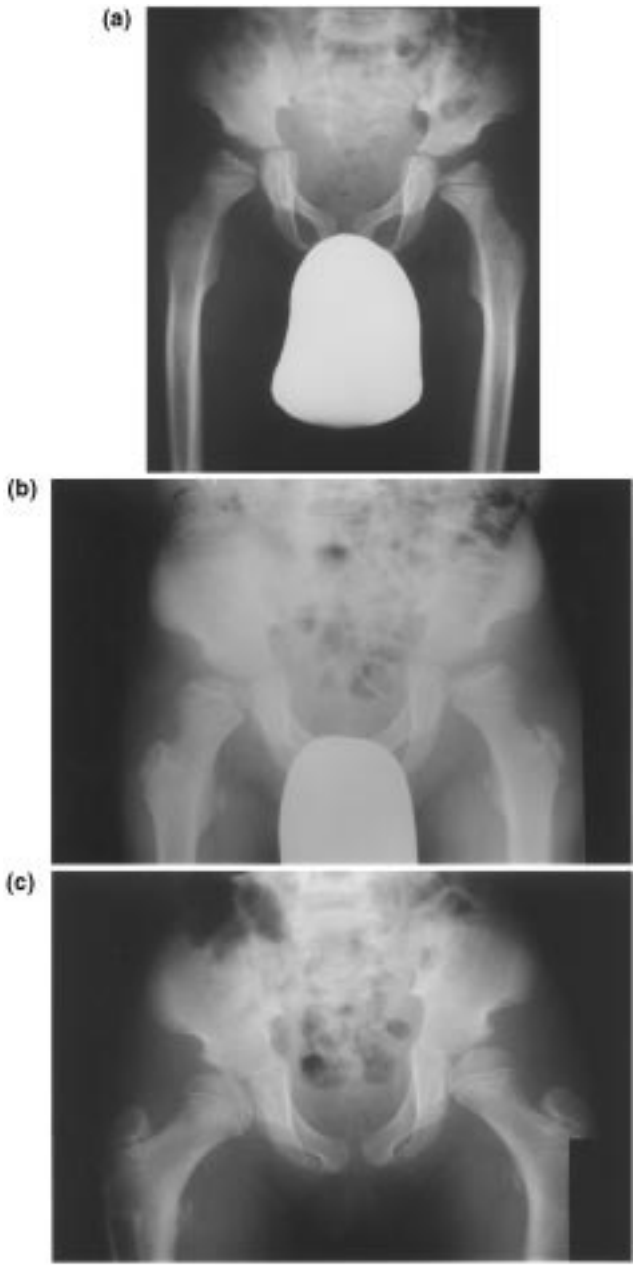

Figure 2 Pelvic radiography of patient 2. (a) Pre BMT: flared iliac wings with shallow acetabula. Mild hip subluxation. Flattened irregular capital femoral epiphyses. (b) Four years post BMT (aged 12.75 years): further subluxation with fragmentation of the capital femoral epiphyses. (c) Seven years post BMT (aged 15.75 years): following bilaterial varus osteotomies, reasonable cover of the capital femoral epiphyses with good reconstitution

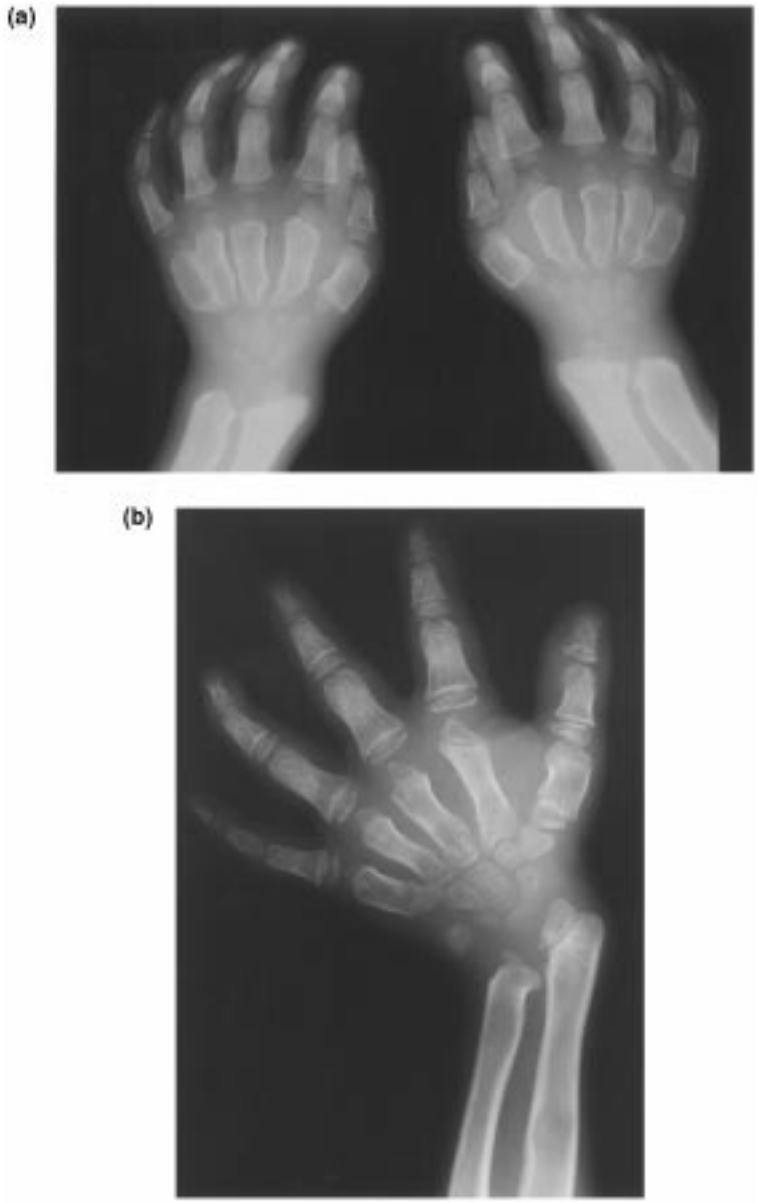

Figure 3 Hand radiography of patient 2. (a) Pre BMT: claw deformity. Broad phalanges with proximal pointing of the metacarpals. V-shaped deformity of the distal radius and ulna. Small carpal bones. (b) Several years post BMT: failure of growth of the distal ulna resulting in ulnar deviation of the hand. The radial growth plate is almost in line with the radial shaft. Improvement in the claw deformity

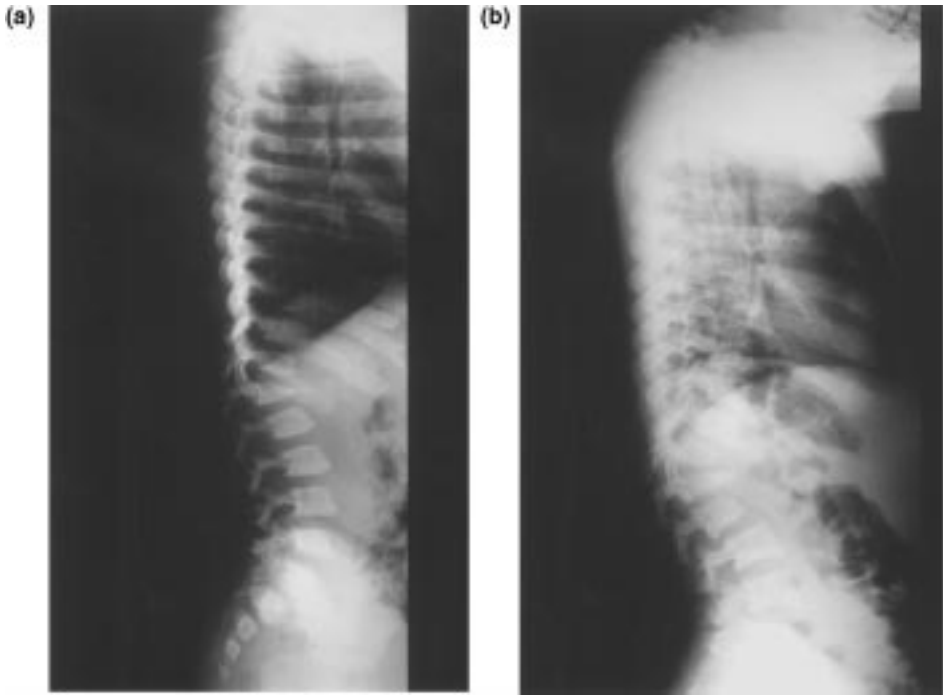

Figure 4 Lateral spine radiography of patient 2. (a) Pre BMT: mild thoraco-lumbar kyphosis associated with hypoplasia and anterior wedging of L1. Anterior and inferior 'hooks' are present on the lumbar vertebral bodies with posterior scalloping and slender pedicles. (b) Nine years post BMT (aged 17.75 years): increased kyphosis with anterior wedging of D12 and L1. Irregularity of the vertebral end-plates with disc narrowing

Table 3 Biochemical data

	Before BMT			*After BMT (most recent results)*		
Patient	*GAG/Cr ratio*	*WBC ASB*	*WBC ASA*	*GAG/Cr ratio*	*WBC ASB*	*WBC ASA*
1	ND	0.7[a]	ND	7[d]	179[b]	62[c]
2	ND	1.4[a]	60[c]	8[d]	107[b]	66[c]
3	107[e]	10[b]	54[c]	22[e]	120[b]	65[c]
4	56[e]	10[b]	146[c]	15[e]	478[b]	89[c]

WBC, white blood cells; ASB, arylsulphatase B; ASA, arylsulphatase A; GAG/Cr, glycosaminoglycan : creatinine ratio; ND, not done

Reference ranges. ASA and ASB activities are expressed in nmol/h per mg protein: (a) 38–193, (b) >80, (c) 48–165. Reference range (a) was determined using WBC that had been sonicated and stored at −20°C and WBC that had been stored unsonicated, prior to assay. Subsequently it was found that ASB is not stable in stored sonicates. Reference range (b) was determined using WBC which had been stored unsonicated. No difference in ASA activity was found with either method of storage. GAG/Cr ratio: (d) 1–5, (e) 2–15

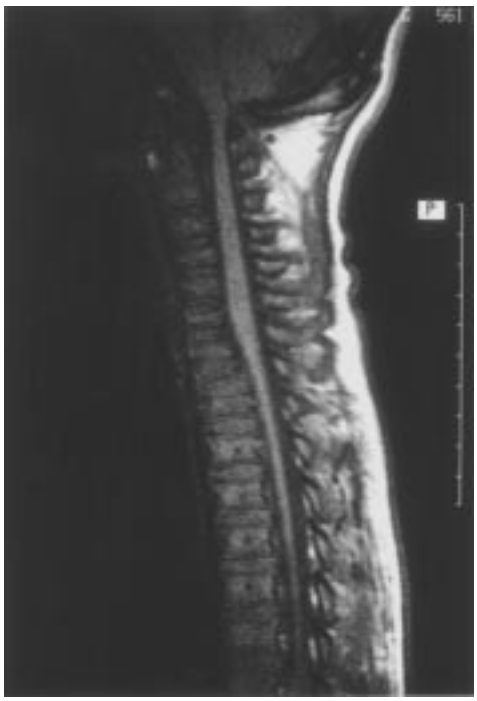

Figure 5 Patient 2, MRI scan of the spinal cord showing spinal canal stenosis, with no evidence of cord compression or atlantoaxial instability

The results of BMT in our patients suggest that quality of life has improved. All our patients are active, mobile and study in mainstream schools, mixing well with their peers. However, the clinical heterogeneity seen in this disorder precludes us from drawing definite conclusions regarding the effect of BMT on survival in all four. The effects on the cardiomyopathy and obstructive sleep apnoea have been beneficial, and this is likely to have had a favourable effect on survival. In two of the patients, one can be more specific. Patient 2 had clearly been deteriorating over the 2 years preceding BMT, with clear evidence of myocardial decompensation, and it is highly unlikely that he would have survived for more than a few years without BMT (Tan et al 1992). Fibroblasts from patient 3 analysed in Professor J. J. Hopwood's laboratory (Adelaide) were found to have very low 4-sulphatase activity by immuno-quantitation (0.23 ng/mg cell protein, normal reference range 30–200 units) that was comparable to the level in two clinically severe patients (0.07 and not detected) and contrasts with the level of 1.95 found in a clinically less severe patient. In addition, no residual activity towards a natural trisaccharide substrate could be detected. These results would be consistent with a prediction of a severe clinical phenotype (Litjens et al 1996).

BMT has not reversed the bone changes and has not prevented further skeletal deterioration in our patients. However, joint mobility has improved in all the

patients. This is most probably due to a favourable effect on soft tissues (joint capsules, ligaments, etc.) but not on bones, which are affected *in utero* by the storage pathology that rapidly increases after birth (Crawley et al 1997). Simonaro and colleagues (1997) investigated the effect of BMT on newborn MPS VI rats. Of 24 successfully engrafted MPS VI newborn rats (1–2 days old) that were monitored for 8 months after BMT, clinical (growth, facial dysmorphia) and radiographic improvements were noted in only one. Enzyme replacement therapy (ERT) from birth in MPS VI kittens was shown to be very effective at reduction of development of pathology in all tissues examined except cartilage and cornea. However, there was radiographic evidence of improved bone trabecular pattern, bone density and dimensions (Crawley et al 1997).

The poor growth observed in our patients may be attributed mainly to the severe uncorrected skeletal dysplasia, but other factors such as obstructive sleep apnoea, cardiomyopathy (prior to BMT) or the endocrine complications of BMT (ovarian failure) may contribute as well.

The cornea is probably irreversibly affected during fetal life (Crawley et al 1997). No corneal clearing was observed over a 14–30 month period when corneal buttons from MPS VI affected cats were transplanted into normal feline corneae (Aguirre et al 1992). The lack of clearing in our patients as well as others (Imaizumi et al 1994) contrasts with the findings in MPS I patients, in whom marked clearing was observed (Vellodi et al 1997). However, unlike MPS I, in MPS VI corneal clouding is mild to begin with and clearing may be difficult to demonstrate.

In conclusion, BMT is a useful curative measure for patients with MPS VI. It prolongs life and reverses many manifestations of the disease, especially cardiac pathology and obstructive sleep apnoea. These beneficial affects are sustained after long-term follow-up. Although BMT does not apparently change the skeletal manifestations, the patients remain active and mobile owing to the effects on soft tissue and good orthopaedic care. Since the clinical severity of MPS VI is a continuum from mildly affected to severely affected patients, early diagnosis and precise molecular and biochemical characterization are needed to identify the most suitable candidates for BMT.

REFERENCES

Aguirre G, Raber I, Yanoff M, Haskins M (1992) Reciprocal corneal transplantation fails to correct mucopolysaccharidosis VI corneal storage. *Invest Ophthalmol Vis Sci* **33**: 2702–2713.

Crawley AC, Niedzielski KH, Isaac EL et al (1997) Enzyme replacement therapy from birth in a feline model of mucopolysaccharidosis type VI. *J Clin Invest* **99**: 651–662.

Ellis RB, Ikonne U, Masson PK (1975) DEAE-cellulose microcolumn chromatography coupled with automated assay: application to the resolution of the *N*-acetyl-β-D-hexosaminidase components. *Anal Biochem* **63**: 5–11.

Gatzoulis MA, Vellodi A, Redington AN (1995) Cardiac involvement in mucopolysaccharidoses: effects of allogeneic bone marrow transplantation. *Arch Dis Child* **73**: 259–260.

Hite SH, Krivit W, Haines SJ, Whitley CB (1997) Syringomyelia in mucopolysaccharidosis type VI (Maroteaux–Lamy syndrome): imaging findings following bone marrow transplantation. *Pediatr Radiol* **27**: 736–738.

Hoogerbrugge PM, Brouwer OF, Bordigoni P et al (1995) Allogeneic bone marrow transplantation for lysosomal storage diseases. The European Group for Bone Marrow Transplantation. *Lancet* **345**: 1398–1402.

Imaizumi M, Gushi K, Kurobane I et al (1994) Long-term effects of bone marrow transplantation for inborn errors of metabolism: a study of four patients with lysosomal storage diseases. *Acta Paediatr Jpn* **36**: 30–36.

Krivit W (1992) Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI). Treatment by allogeneic bone marrow transplantation in six patients and potential for autotransplantation bone marrow gene insertion. *Int Pediatr* **7**: 47–52.

Krivit W, Pierpont ME, Ayaz K et al (1984) Bone-marrow transplantation in the Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI). Biochemical and clinical status 24 months after transplantation. *N Engl J Med* **311**: 1606–1611.

Litjens T, Brooks DA, Peters C, Gibson GJ, Hopwood JJ (1996) Identification, expression, and biochemical characterization of *N*-acetylgalactosamine-4-sulfatase mutations and relationship with clinical phenotype in MPS-VI patients. *Am J Hum Genet* **58**(6): 1127–1134.

McGovern MM, Ludman MD, Short MP et al (1986) Bone marrow transplantation in Maroteaux–Lamy syndrome (MPS type 6): status 40 months after BMT. *Birth-Defects* **22**: 41–53.

Neufeld EF, Muenzer J (1995) The mucopolysaccharidoses. In Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn. New York: McGraw-Hill, 2465–2494.

Peters C, Balthazor M, Shapiro EG et al (1996) Outcome of unrelated donor bone marrow transplantation in 40 children with Hurler syndrome. *Blood* **87**: 4894–4902.

Resnick JM, Krivit W, Snover DC et al (1992) Pathology of the liver in mucopolysaccharidosis: light and electron microscopic assessment before and after bone marrow transplantation. *Bone Marrow Transplant* **10**: 273–280.

Simonaro CM, Haskins ME, Kunieda T et al (1997) Bone marrow transplantation in newborn rats with mucopolysaccharidosis type VI: biochemical, pathological, and clinical findings. *Transplantation* **63**: 1386–1393.

Tan CTT, Scharf HV, Miller FA Jr, Edwards WD, Karnes PS (1992) Valvular heart disease in four patients with Maroteaux–Lamy syndrome. *Circulation.* **85**: 188–195.

Vellodi A, Young E, New M, Pot-Mees C, Hugh-Jones K (1992) Bone marrow transplantation for Sanfilippo disease type B. *J Inher Metab Dis* **15**: 911–918.

Vellodi A, Young EP, Cooper A et al (1997) Bone marrow transplantation for mucopolysaccharidosis type I: experience of two British centres. *Arch Dis Child* **76**: 92–99.

Whiteman P, Young E (1977) The laboratory diagnosis of Sanfilippo disease. *Clin Chim Acta* **76**: 139–147.