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Dried Blood Spot Analysis: An Easy and Reliable Tool to Monitor the Biochemical Effect of Hematopoietic Stem Cell Transplantation in Hurler Syndrome Patients

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Hurler syndrome (HS), the most severe phenotype in the spectrum of mucopolysaccharidosis type I, is caused by a deficiency of the lysosomal enzyme alpha-L-iduronidase (IDUA). At present, hematopoietic stem cell transplantation (HSCT) is the only treatment able to prevent disease progression in the central nervous system, and therefore considered the treatment of choice in HS patients. Because IDUA enzyme activities after HSCT have been suggested to influence the prognosis of HS patients, monitoring these activities after HSCT remains highly important. The use of dried blood spots (DBS) for enzyme analysis can be a useful alternative to the conventional leukocyte assay. Importantly, this method allows for convenient worldwide shipment, and can therefore be applied to monitor patients from larger areas of the world, or during large-scale international studies. Furthermore, this method requires only a minimal amount of blood. From 13 HS patients receiving HSCT, 36 paired whole blood and DBS samples were analyzed to assess leukocyte and DBS IDUA activities, respectively. To correct for potential interfering factors, simultaneous assay of the alpha-Galactosidase-A (AGA) activity was performed in the DBS samples and an IDUA/AGA ratio was calculated. A strong linear correlation was demonstrated between the DBS IDUA/AGA ratio and the leukocyte IDUA activity ($r^2 = .875$, $P < .001$). This correlation was applicable to all enzyme activities, including the activities measured early after HSCT as well as heterozygous activities because of mixed chimerism or the use of a carrier donor. These results demonstrate that the DBS method is reliable to monitor the biochemical effect of HSCT in HS patients.

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INTRODUCTION

Hurler syndrome (HS) is the most severe phenotype in the spectrum of mucopolysaccharidosis type I, an autosomal recessive inborn error of metabolism. Because of a severe deficiency of the lysosomal enzyme α-L-iduronidase (IDUA), HS patients suffer from a progressive and ultimately fatal multisystem disease. At present, hematopoietic stem cell transplantation (HSCT) is the only treatment able to prevent deterioration of the central nervous system in these patients. Successful donor engraftment in HS patients has shown to be highly effective, evident by an increased life expectancy and significant improvement of biochemical as well as clinical outcome parameters [1]. At present, over 500 HSCTs have been performed in HS patients worldwide, making HS the most frequently transplanted inborn error of metabolism and prototype for HSCT in these disorders [2,3].

The current standard for the definite diagnosis of HS as well as the biochemical monitoring after

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Table 1. Patient Characteristics and Results

	Symbol	No. of Patients	No. of Samples	Age at Sampling (Months) Median (Range)	Interval Post-HSCT (Months) Median (range)	DBS IDUA/AGA Ratio Median (Range)	Leukocyte IDUA Median (Range)
All patients		13	36	40.5 (12.5-96.5)	22.1 (0.4-74.8)	0.67 (0.13-1.60)	38.5 (6.5-88.0)
Unaffected/full	■	11	26	43.5 (14.9-96.5)	22.2 (2.2-.59.5)	0.80 (0.36-1.60)	47.0 (19.0-88.0)
Affected/full	◇	1[a]	1	63.6	34.1	0.45	30.0
Unaffected/mixed	△	1[b]	3	74.0 (58.5-82.6)	66.3 (50.7-74.8)	0.20 (0.19-0.22)	11.0 (10.0-11.0)
Early post-HSCT	○	5[c]	6	16.4 (12.5-24.6)	0.7 (0.4-0.9)	0.29 (0.13-0.48)	19.3 (6.5.39.0)

Affected/full indicates heterozygous donor/full donor chimerism; DBS, dried blood spot; early post-HSCT, samples were collected early after HSCT; No: number, unaffected/full: unaffected donor/full donor chimerism; unaffected/mixed, unaffected donor/mixed donor chimerism.
[a]HLA-haploidentical mother.
[b]Stable donor chimerism of 25%.
[c]All 5 patients were transplanted using an unaffected donor and achieved full donor chimerism. Subsequent samples of these patients are represented in the group unaffected/full.

HSCT is the measurement of the IDUA enzyme activity in leukocytes [4]. It has already been demonstrated that the leukocyte enzyme activity, absent or severely decreased before HSCT, significantly increases to normal or heterozygous activities after HSCT. A significantly lower leukocyte activity is observed when a heterozygous carrier donor is used or when only mixed chimerism is achieved [1]. Because the IDUA enzyme activity after HSCT has been suggested to influence the prognosis of HS patients [1], monitoring IDUA activity after HSCT is highly important.

Measuring lysosomal enzyme activities using dried blood spots (DBS) has already been demonstrated to be an easy method that can be a useful alternative to the conventional leukocyte assays [5-7]. Importantly, this method requires only a minimal amount of blood and allows for convenient worldwide shipment, allowing enzyme analysis from larger areas of the world and making international comparison studies easier to accomplish. Whether this method is reliable to monitor the biochemical effect of HSCT in HS patients, however, is at present unknown.

MATERIALS AND METHODS

Samples

After informed consent of the patients and families, paired DBS and whole blood samples from HS patients receiving HSCT at the University Medical Center Utrecht, were collected during clinical follow-up. HSCT donors were either unrelated donors, homozygous unaffected family donors, or heterozygote family donors. The stem cell source consisted of a bone marrow or cord blood graft. Donor chimerism was determined by analysis of polymorphic DNA sequences by variable number of tandem repeats (VNTR), as previously described [8].

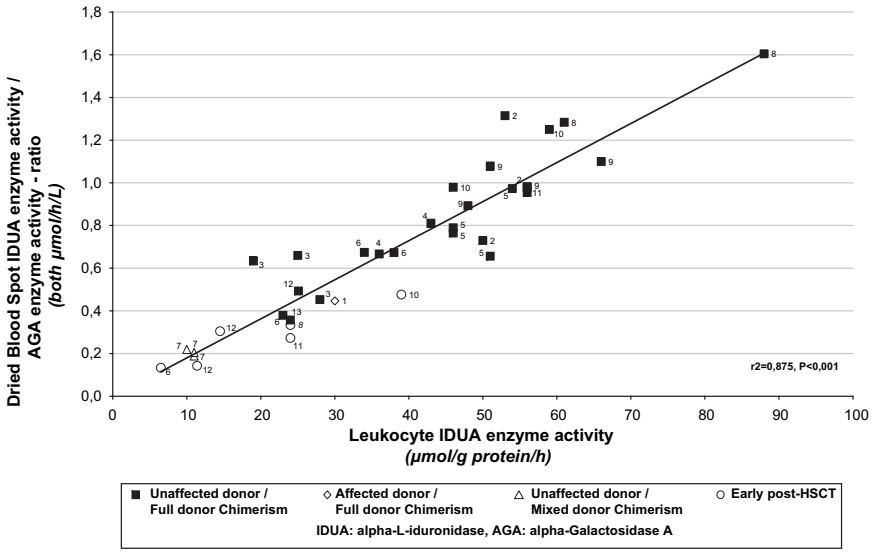

Figure 1. Dried blood spot IDUA/AGA ratio versus leukocyte IDUA activity in Hurler syndrome patients receiving hematopoietic stem cell transplantation. A linear correlation is shown between the leukocyte IDUA enzyme activity (µmol/g protein/h) on the horizontal axis and the corresponding dried blood spot IDUA/AGA ratio (both µmol/h/L) on the vertical axis.

DBS IDUA Activity

IDUA enzyme activity in DBS samples was measured at the University Medical Center Utrecht, as previously described by Chamoles et al. [5]. DBS were prepared by dispensing whole blood onto Guthrie cards. Samples were collected at different medical wards, obtained by various staff members. The DBS were subsequently dried for at least 4 hours at room temperature and stored at −20°C in a plastic bag until analysis.

To assess the quality of the bloodspot, the lysosomal enzyme α-Galactosidase A (AGA), deficient in Fabry disease, served as a control in all analyzed DBS samples, and an IDUA/AGA ratio was calculated. All measurements were performed in duplicate. Both IDUA and AGA activity were expressed as μmol/h/L blood.

Leukocyte IDUA Activity

The leukocyte IDUA enzyme activity was fluorimetrically measured at the Erasmus University, Rotterdam, in leukocytes isolated from peripheral blood, as described previously [9]. Leukocyte IDUA activity was expressed as μmol/g protein/h.

Statistical Analysis

DBS IDUA/AGA ratios were compared with leukocyte IDUA activities using least-squares linear regression analysis.

RESULTS

Thirty-six paired whole blood and DBS samples from 13 HS patients, receiving HSCT at the University Medical Center Utrecht, between April 2003 and June 2009, were available for leukocyte IDUA and DBS IDUA/AGA analysis, respectively (Table 1). The median age at sampling was 40.5 (12.5-96.5) months, with a median interval from HSCT until sampling of 22.1 (0.4-74.8) months. Eleven patients were transplanted using an unrelated-assumed to be unaffected (n = 9) or unaffected family (n = 2) donor, and achieved full donor chimerism. One patient was transplanted using a heterozygous carrier donor (HLA-haploidentical mother) and achieved full donor chimerism, whereas in 1 patient, transplanted with an unaffected donor, a stable donor chimerism of only 25% was achieved. In 5 patients, samples were collected shortly after HSCT, ranging from 0.4 to 0.9 months post-HSCT. These patients were transplanted using an unaffected donor and achieved full donor chimerism.

As depicted in Figure 1, comparison of DBS IDUA/AGA ratios and leukocyte IDUA activities by linear regression analysis confirmed a strong correlation ($r^2 = .875$, $P < .001$). This linear relation was applicable to all enzyme activities, including normal enzyme activities (depicted as ■), heterozygous activities because of the use of a carrier donor (depicted as ◇), or mixed donor chimerism (depicted as △), as well as samples obtained early after HSCT (depicted as ○).

DISCUSSION AND CONCLUSION

This, to our knowledge, is the first study comparing DBS and leukocyte assays in HSCT treated patients for IDUA activity. A strong correlation was observed between the leukocyte IDUA enzyme activity—the current standard—and the DBS IDUA/AGA ratio in HS patients receiving HSCT.

DBS samples represent a mixture of plasma and variable types of blood cells with variable expression of lysosomal enzymes. Importantly, the number of leukocytes in peripheral blood can be significantly reduced early after HSCT, considerably lowering the absolute DBS enzyme activity. Additionally, results can be influenced by external factors, including medication or incorrect sampling, transport, or storage [5]. To correct for these interfering factors, it is therefore recommended to simultaneously measure the activity of a control enzyme in the DBS, in this study AGA, and calculate the ratio.

The use of DBS to monitor the biochemical effect of HSCT in HS patients would offer several advantages over leukocyte enzyme analysis, including (1) lysosomal enzymes, including IDUA, have been demonstrated to be highly stable in DBS for weeks at room temperature and even months when stored at lower temperatures [5]. This high stability allows for worldwide shipment of DBS samples by regular mail. The DBS method could therefore be applied to monitor patients from larger areas of the world, lacking a specialized laboratory for enzyme analysis or during large-scale international studies. (2) Because DBS can be sent by mail, transportation of samples is considerably less expensive and logistically more convenient. (3) A significantly lower sample volume of only a few drops is required, an advantage in cases where obtaining larger blood samples is not convenient or impossible.

The DBS method is already implemented at the University Medical Center Utrecht for early diagnosis of patients clinically suspected of having MPS I. The continued use of DBS during the follow-up of these patients after HSCT would therefore be a convenient and logical option.

In conclusion, this study demonstrates that the DBS method is reliable to monitor the biochemical effect of HSCT in HS patients. In the near future, the DBS method might be evaluated for monitoring the biochemical effect of enzyme replacement therapy

on milder phenotypes of MPS I (Hurler-Scheie or Scheie) and the effect of HSCT, enzyme replacement therapy, or any future therapy (eg, gene therapy) on other lysosomal storage disorders.

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