SUPPLEMENTAL METHODS

Blood sample processing guidelines

Blood samples were drawn from the central venous line within one hour before rASNase infusion number 1, 2, 4, and 6. The infusion line was flushed with 2 mL normal saline to avoid drug remnants. After 3 seconds, 2 mL blood was taken from the infusion line and discarded. The next 1 mL of blood was collected into a tube that contained no additives.

The tubes with the blood samples were immediately put on ice and transferred to the laboratory for serum processing. The blood samples were centrifuged as soon as possible at 600-800 g and 4°C for 10 min. Serum was harvested and divided into three aliquots (at least 100 µL each) for determination of ASNase activity, amino acid concentrations, and anti-ASNase-antibodies. The samples for amino acid determination were then deproteinated (to avoid further enzymatic activity) by adding 1 part of 10% sulphosalicylic acid to 4 parts of serum, thoroughly mixed and centrifuged for 5 minutes at 10 000-13 000 rpm and the supernatant harvested.

The samples were frozen at -80°C and stored at this temperature not longer than seven months until analysis. In preliminary experiments, the stability of the analytes (asparaginase, amino acids) in serum and CSF samples had been demonstrated for at least 12 months.

Analytical assays

The analytical assays of the serum and CSF samples were carried out by CRS Clinical Research Services Mannheim GmbH, Grünstadt, Germany. Before starting measurements, all analytical methods had been validated and documented in a validation report.

Serum levels of asparaginase were determined with a sensitive microplate reader-based method (AHA-assay).[1] The assay is more sensitive than the usually used Nessler assay and allows determining ASNase trough levels as low as 2.5 U/L.

Serum levels of the amino acids asparagine (ASN), aspartic acid (ASP), glutamine (GLN), and glutamic acid (GLU) were analyzed by use of a RP-HPLC method. The lower limit of quantification of this assay for asparagine in serum and CSF samples was 0.5 µM.

Anti-ASNase-antibodies were measured according to an ELISA method developed and validated by medac. The assay used detects ASNase-binding antibodies of IgG/IgM type. Previous results of this test in children aged 1-18 years have demonstrated the usefulness of this assay.[2] In these investigations, appearance of anti-ASNase antibodies mostly correlated with a decline of ASNase activity and clinical allergic reactions.

REFERENCES

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