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NEWBORN SCREENING – SHORT REPORT

Direct comparison of enzyme measurements from dried blood and leukocytes from male and female Fabry disease patients

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Summary Anderson–Fabry disease is an X-linked disorder that is caused by deficiency of the lysosomal enzyme α-galactosidase A. Symptoms include chronic progressive painful small-fibre neuropathy, cornea verticillata, renal failure and heart disease. Interestingly, female heterozygous patients may also show severe symptoms. After clinical suspicion, usually the determination of α-galactosidase activity in leukocytes is requested first. Alternatively, an enzymatic assay using dried blood specimens has been described. Dried blood samples require less material and are substantially more stable (several months at room temperature) than whole-blood specimens. To validate the new method and to asses its usefulness for diagnosis of female patients, enzyme activities of α-galactosidase, β-galactosidase and β-glucuronidase from 78 known Fabry patients were compared (29 males, 47 females) between both materials. In summary, the determination of α-galactosidase activity using dried blood and leukocytes as well as the ratio of α-galactosidase to β-glucuronidase in dried blood can improve the diagnostic specificity in cases of female patients who are difficult to identify when only leukocyte enzyme activities are considered.

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Introduction

Fabry disease (OMIM 301500), an autosomal inherited lysosomal storage disorder, is caused by mutations in the α-galactosidase gene that is located on the X-chromosome (Xq22.1). Surprisingly, female carriers are frequently also affected by the disease with different degrees of severity (MacDermot et al 2001). Usual symptoms include chronic progressive painful small-fibre neuropathy, cornea verticillata, renal failure and heart disease. For the diagnosis of the disorder, the enzyme activity of α-galactosidase is determined from leukocytes that are isolated from EDTA-blood. However, a certain proportion of female patients show significant residual activity so that they are not picked up by this assay alone. Recently, dried blood spots were also used for the determination of lysosomal enzyme activities. To assess the usefulness of this novel technique as well as to validate its diagnostic specificity, we compared the α-galactosidase activities from 29 male and 47 known female Fabry patients in both materials. In addition, β-galactosidase was determined to evaluate the quality of the sample and β-glucuronidase activity was measured so that the

ratio of α-galactosidase to β-glucuronidase could be calculated, which is known to be an additional indicator of Fabry disease (Lukacs et al 2005).

Results and discussion

We included a total of 76 patients with known Fabry disease in our study. Leukocytes and dried blood showed normal β-galactosidase activity, so that no pre-analytical problems were observed. Among the subjects in our study were 29 male patients who were identified equally well by the dried blood as by the traditional leukocyte assay. In four cases some negligible residual activity (<0.12nmol/mg per min) was measured with leukocytes, while two of these patients showed no measurable activity in dried blood.

Regarding female patients, 22 out of 47 were not identified using the leukocyte assay because of some significant residual enzyme activity (activity >0.23nmol/mg per min). In contrast, only seven patients would have been missed with the dried blood assay (activity >0.15nmol/spot per 45h). Interestingly, though, two patients would have been picked up by the leukocyte assay, while the dried blood test failed to do so. The increased diagnostic specificity of the dried blood assay may derive from lower α-galactosidase activity in serum of female patients, so that the disease-derived reduction of the overall activity in dried blood is more pronounced. However, it seems reasonable to test samples from suspected female patients in dried blood as well as leukocytes to improve laboratory diagnosis. In addition, the ratio of α-galactosidase to β-glucuronidase proved useful to confirm nine cases which showed only slightly lower or normal activity of α-galactosidase, so that the interpretation of enzyme activity results could not be carried out unambiguously.

Summary

In summary, dried blood has been shown to be a reliable tool for the diagnosis of Fabry patients. In certain cases it may also be helpful to use the ratio of α-galactosidase to β-glucuronidase in order to identify female patients. In addition, the combination of leukocyte and dried blood enzyme activity measurements may further improve the probability of diagnosing female Fabry patients. However, a certain number of patients cannot be picked up by either enzyme assay, so that in cases of continued suspicion further studies are required. Generally, a genetic analysis should be included in the diagnostic regimen.

References

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