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Evaluation of three biochemical markers in the monitoring of Gaucher disease

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Summary: Several markers have been developed for the biochemical monitoring of Gaucher disease. Three of the most commonly used markers are acid phosphatase, angiotensin-converting enzyme (ACE) and chitotriosidase. The rationale for using all three concurrently is not clear. A retrospective study was therefore carried out on data collected from 28 paediatric patients treated with enzyme replacement therapy. All three markers fell with time. However, chitotriosidase showed the steepest time trend, the largest trend by case interaction, and the lowest residual variance, making it the most reliable of the three. ACE correlated strongly with chitotriosidase, but acid phosphatase did not correlate well with either and also had the largest residual variance, indicating that it was too 'noisy' to be informative. The absence of a 'gold standard' for assessing Gaucher disease complicates the interpretation of these findings, but they suggest that acid phosphatase be dropped from routine clinical practice, and that chitriosidase be used in preference to ACE.

Gaucher disease is an inherited lysosomal disorder. It is characterized biochemically by a deficiency of β-glucosidase (glucocerebrosidase), and the consequent accumulation of its substrate, glucocerebroside (EC 3.2.1.21). Affected individuals develop varying degrees of hepatosplenomegaly, bone disease and, occasionally, central nervous system involvement (Beutler and Grabowski 2001).

Enzyme replacement therapy (ERT) is currently the treatment of choice (Barton et al 1991; Weinreb et al 2002). There is little doubt that it results in significant amelioration of the clinical signs and symptoms, although no formal measure of clinical status exists to confirm this.

A number of biochemical abnormalities have been described in Gaucher disease (Aerts and Hollak 1997), and it is therefore not surprising that several markers have been developed in an attempt to evaluate response to treatment. It is important

to continually review the situation, as some of these markers are likely to be less useful than others and such markers could be dropped from routine management protocols. In our department, three markers are measured routinely, chitotriosidase (EC 3.2.1.14), angiotensin-converting enzyme (ACE; EC 3.4.15.1) and total acid phosphatase (EC 3.1.3.2).

The purpose of the present study was to compare the information content of the three markers measured serially, in order to rank them in terms of clinical usefulness.

PATIENTS AND METHODS

Patients

Our hospital is one of two centres recognized in the United Kingdom for the management of paediatric Gaucher disease. Guidelines for the diagnosis, evaluation and management of paediatric Gaucher disease, developed in the two centres, have been in place since 1997.

Routine biochemical monitoring is carried out as part of the evaluation. The three enzyme markers chitotriosidase, angiotensin-converting enzyme and total acid phosphatase are used specifically to monitor the response to treatment.

Data were collected on 31 paediatric patients in whom the diagnosis of Gaucher disease had been biochemically confirmed by demonstration of deficiency of β-glucosidase, either in peripheral blood leukocytes or in cultured fibroblasts. All were being treated with ERT. A total of 321 blood samples had been collected at regular intervals from the children. While some had been assayed for all three parameters, others had been analysed for only one or two.

Methods

Chitotriosidase and angiotensin converting enzyme were assayed in heparinized plasma that had been separated within 18 h of the blood sample being taken and stored at −20°C until assay. Blood for acid phosphatase was collected into plain tubes and serum was separated immediately, acidified to pH 4−5 with 3 mmol/L acetic acid and stored at −20°C until assay.

Angiotensin-converting enzyme (ACE): ACE was assayed on a Cobas Mira analyser by a method based on that of Holmquist and colleagues (1979) using Sigma reagent (catalogue no. 305−10). Hydrolysis of a synthetic tripeptide, furylacryloylphenylalanylglycylglycine, by ACE to furylacryloylphenylalanine and glycine resulted in a decrease in absorbance at 340 nm, which was directly proportional to the ACE activity. The between-batch coefficients of variation (CVs) at mean activity of 30 and 96 U/L ACE were 12.4% and 8.7%, respectively.

Total acid phosphatase (ACP): Total ACP was assayed on a Cobas Bio analyser using Randox reagent (AC1011) based on the method of Hillmann (1971). ACP hydrolysed the chromogenic substrate α-naphthyl phosphate at pH 5.2 to phosphate and α-naphthol. The latter was coupled to Fast Red TR salt to yield a coloured azo dye. The absorbance at 405 nm was directly proportional to ACP activity.

The between-batch CVs at mean activity of 12.3 and 23.1 were 5.1% and 5.5%, respectively.

Chitotriosidase: Chitotriosidase was assayed as described by Young and colleagues (1997) using 4-methylumbelliferyl-β-D-*N*′,*N*″-triacetylchitotriose fluorescent substrate (Sigma). The between-batch CV at the upper reference limit of 150 nmol/h per ml was 5%.

Statistical methods

In the absence of a direct 'gold standard' measure of clinical status, it was necessary to rely on indirect measures. The markers all declined over time, initially very steeply but less so subsequently, so they could be compared in terms of their rates of decline and how the rates differed between cases. The first step was to transform the data so that the time trend was essentially linear. This was achieved by logarithmically transforming the three markers and plotting them on a log-transformed timescale, adding one day to the time since start of treatment. This means that the percentage change in each marker is broadly constant over constant multiples of time, for example from 1 to 2 years or from 2 to 4 years or from 4 to 8 years, etc.

A 'useful' marker should show relatively wide variability in the rate of decline from one case to another, reflecting underlying differences in the rate of clinical improvement. A 'useful' marker should also be relatively free of measurement error, with serial measurements for each case tending to follow the overall trend. These two indicators of performance can be compared using repeated measures analysis of variance, with each marker in turn as the dependent variable, case as a fixed effect, and log time since start of treatment as a continuous variable. The interaction between cases and log time indicates the heterogeneity in the time trend from one case to another, and the residual term gives the variability about the trend within cases. The interaction term is compared from one marker to another in terms of its statistical significance (measured by the *F*-ratio), while the within-case variability is compared directly, being in the same natural log units for all three markers.

The correlations between markers were investigated by analysis of covariance, with each pair of markers as dependent variable and covariate, respectively. For the overall correlation, an adjustment for log time was included. The *t*-value for the covariate marker's level of significance then gave the correlation between the two markers adjusted for time trends. The within-subject correlations between markers were investigated similarly, but also including a fixed effect adjustment for case.

RESULTS

The data consisted of 31 cases and 321 measurement occasions, of which 28 cases had at least two post-treatment measurements, a total of 293 occasions. Table 1 summarizes the numbers, means and standard deviations (SD) of the three markers.

Figure 1 shows a plot of log chitotriosidase activity against log time, indicating the linearity of the time trends for each case after natural log transformation. Note that, with one exception, chitotriosidase fell with time in all cases.

Table 1 Summary statistics of the three markers post-treatment in 28 cases

Variable	*N*	*Mean*	*SD*
Years on treatment	293	3.1	2.5
ACE	253	136	116
ACP	231	12.2	10.6
Chitotriosidase	275	3369	4042

Table 2 gives summary statistics for the three markers from the analysis of variance. The mean time trend, i.e. the slope of the plot for the average case (e.g. in Figure 1) is highly significantly negative for all markers, confirming that they fall steeply with time. The trend for chitotriosidase is appreciably greater than for ACE or acid phosphatase. The time trend also varies highly significantly between cases, as measured by the trend by case interaction *F*-ratio. Here again, chitotriosidase has appreciably the largest value, indicating that it distinguishes between cases better than the other markers. Finally, the residual variance is smallest for chitotriosidase, which shows that it is relatively the least noisy of the three markers.

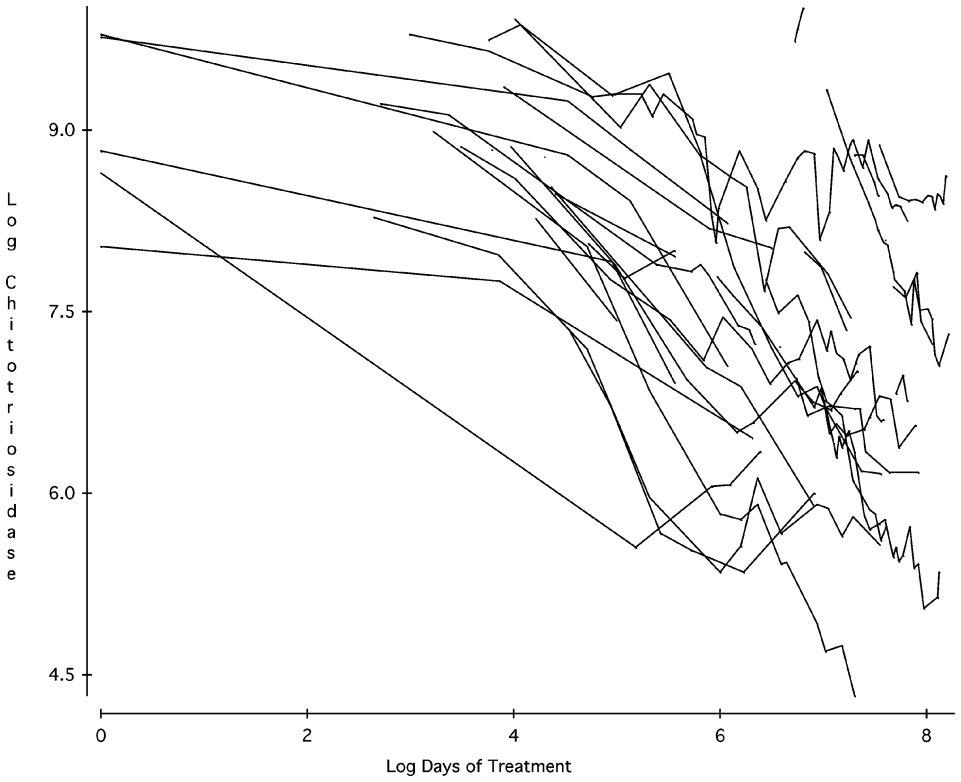

Figure 1 Plot of chitotriosidase activity versus time by case, both axes on natural log scales

Table 2 Summary statistics from the repeated measures analysis of variance

Marker	*Mean time trend*	*p-Value*	*Time trend × Case (F- ratio)*	*p-Value*	*Residual variance*
ACE	−0.32	<0.0001	4.1	<0.0001	0.098
ACP	−0.22	<0.0001	2.5	<0.0001	0.123
Chitotriosidase	−0.52	<0.0001	16.2	<0.0001	0.080

Tables 3 and 4 give the overall and within-case correlations between markers. Overall, there are strong and highly significant correlations, indicating that cases with relatively high values at any one time on one marker are likely also to be high on the other markers. This suggests that all three markers are proxies for underlying clinical status. But within cases there is little association between acid phosphatase and either ACE or chitotriosidase, whereas there remains a strong correlation between ACE and chitotriosidase. This suggests that within-cases acid phosphatase is fairly noisy and uninformative, whereas ACE and chitotriosidase both detect case-specific trends over time.

DISCUSSION

Each of the three markers that we studied is thought to reflect disease severity in Gaucher disease, but in the absence of a gold standard measure of clinical status the findings are tricky to interpret. Chitotriosidase falls the fastest of the three markers over time, it shows the greatest differences between cases in terms of the rate of fall, and it is the least noisy in terms of residual variation. This is consistent with the idea that chitotriosidase declines linearly over time at a rate that varies from patient to patient (see Figure 1).

Table 3 Overall correlations between the three markers adjusted for time trends

	ACE	*ACP*	*Chitotriosidase*
ACE	1		
ACP	0.55***	1	
Chitotriosidase	0.71***	0.55***	1

***$p < 0.0001$

Table 4 Within-case correlations between the three markers adjusted for time trends

	ACE	*ACP*	*Chitotriosidase*
ACE	1		
ACP	0.08	1	
Chitotriosidase	0.58***	0.24**	1

**$p < 0.01$; ***$p < 0.0001$

The markers are highly correlated, reflecting the fact that a case with a high value of one marker at a point in time is likely also to have high values of the other markers. However, after adjusting for differences between cases, acid phosphatase is no longer correlated with the other markers, so that the information it contains differs from that for chitotriosidase and ACE. It may be uninformative, owing to its large measurement error (it has the largest variability in Table 2), or it may be informative in a different way. The second interpretation is not well supported by the data, since the marker falls relatively slowly with age, and the rate of fall is similar across cases (Table 2).

The clinical impression of the cases is that they improve steadily over time, so that markers showing a linear decline are consistent with clinical improvement. It is for this reason that we judge chitotriosidase to be the most informative marker, with ACE second and acid phosphatase a distant third.

Angiotensin-converting enzyme is an integral membrane-bound protein whose active sites are directed to the extracellular spaces (Coates 2003). In healthy subjects, plasma ACE essentially arises from the endothelium. Circulating levels are normally very low, and there is considerable inter-individual variation and stable intra-individual variation. Elevation of serum ACE in Gaucher disease was first described in 1976 (Lieberman and Beutler 1976). It is thought to result from excessive production of ACE by Gaucher cells, since immunofluorescent studies have shown that splenic Gaucher cells contain abundant quantities of the enzyme (Silverstein et al 1980).

Chitotriosidase is a lysosomal β-glucosaminidase found in about 85% of healthy subjects. Like ACE, it is normally present in very low quantities. However, strikingly elevated plasma levels are seen in Gaucher disease (Hollak et al 1994) and originate from activated macrophages.

Acid phosphatase is a lysosomal enzyme. Elevation of serum tartrate-resistant acid phosphatase (TRAP) in Gaucher disease was first described by Tuchman and colleagues in 1956. In fact, this is the predominant isoform seen in Gaucher disease (Li et al 1973). It is probably not derived from Gaucher cell lysosomal storage deposits, which contain tartrate-sensitive acid phosphatase (Chambers et al 1978). Acid phosphatase levels are also affected by osteoclast function. The isoenzyme 5b is secreted by osteoclasts and is a specific marker of bone resorption (Halleen et al 2000, 2002). Excessive bone resorption, with resulting lytic lesions, is a prominent feature of Gaucher disease (Fiore et al 2002; Wenstrup et al 2002). Although the precise mechanism for this is unknown, plasma levels of cathepsin K, a protease responsible for bone resorption, have been found to be elevated in Gaucher disease (Moran et al 2000) and it is possible that elevated ACP levels in Gaucher disease are at least partly of osteoclast origin.

Thus, the origin of ACP in Gaucher disease is likely to be multifocal, in contrast to that of ACE or chitotriosidase. There are also significant technical difficulties with measurement (Gundlach and Luttermann-Semmer 1987). The activity of ACP decreases by up to 20% within 3 h. With acidification, samples are stable for 24 h at room temperature and for 7 days at 4°C. Together with the technical difficulties with measurement, this is probably the reason for the considerable variation seen in our study. This may be a reflection of the stringent sample handling required to preserve the stability of ACP and the measurement of the small absorbance change.

Another issue is the fact that total ACP was measured and not TRAP. The performance may be enhanced by TRAP. Thus ACE is to be preferred over acid phosphatase.

The multifocal origin of ACP in Gaucher disease makes it likely to be affected by more than one factor, which accords with its being the least consistent marker. Conversely, ACE and chitotriosidase are both formed by Gaucher cells, so they would be expected to show a stronger correlation, which is what we found.

Casal and colleagues (2002) studied the relationship between various markers of Gaucher disease in 44 patients. A strong correlation was found between chitotriosidase and ACE, among other markers of macrophage activation. However, only one set of values was measured in each patient. Poll and colleagues (2002) compared MRI responses to various laboratory parameters in 30 adult patients but did not compare the laboratory parameters with each other. As far as we are aware, ours is the only study directly comparing these variables with each other over a period of time.

Our conclusions are that, in the routine monitoring of Gaucher disease, chitotriosidase is more informative than ACE, and total acid phosphatase is of little value.

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