The Use of d2 and Benton Tests for Assessment of Attention Deficits and Visual Memory in Teenagers with Phenylketonuria

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Abstract Hyperphenylalaninemia-related, subtle deficits of attention and of working memory are often reported in adolescents with phenylketonuria. Focused neuropsychological tests can be used to detect such deficits and to confirm the presence of poor metabolic control in the periods between routine blood phenylalanine tests, which are rarely performed in many patients from this age group due to their low treatment adherence.

We assessed the practical value of the d2 test of attention and of the Benton visual retention test for identification of teenagers, who have a high risk of brain dysfunction due to hyperphenylalaninemia. We analyzed the correlation between neuropsychological test scores achieved by 30 patients and their blood phenylalanine profiles since the neonatal period.

We observed strong correlation between the Concentration Performance scores on the d2 test and the quality of metabolic control within last month prior to the follow-up visit in the outpatient clinic ($r = -0.72$; $p = 0.0003$). The mean z-score was significantly higher in patients with good metabolic control than in those with poorly controlled hyperphenylalaninemia (0.44 vs. -1.12 ; $p = 0.00002$). On contrary, the results of the Benton visual retention test did not correlate significantly with the individual blood phenylalanine profiles.

We believe that neuropsychological assessment should be used in adolescents with phenylketonuria on a regular basis in order to increase the self-awareness in these patients and, consequently, to increase their treatment adherence and safety. The d2 test can be effectively used for detection of attention deficits and seems to be a valuable supplementary procedure for routine follow-up.

Abbreviations

Phe Phenylalanine
PKU Phenylketonuria
SD Standard deviation

Introduction

Phenylketonuria (PKU, OMIM 261600) is the most common inborn error of metabolism in man. If untreated, the disease manifests as severe brain damage resulting from chronic hyperphenylalaninemia. Treatment using a low-phenylalanine diet should start in the neonatal period (ideally before the tenth day of life) and should be continued for the rest of the patient's life. If properly treated, patients usually show normal development (Blau et al. 2010) although discrete neuropsychological abnormalities have also been reported (Enns et al. 2010).

Metabolic control is usually sufficient in prepubertal patients. Unfortunately, adolescents with PKU typically relax the dietary regimen, resulting in insufficient treatment adherence (Walter et al. 2002). Poorly controlled hyperphenylalaninemia often leads to high brain phenylalanine concentrations, with a resulting imbalance in production of neurotransmitters and eventually dysmyelination (Feillet et al. 2010). Neuropsychological deficits are often observed

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in teenagers and adults without proper metabolic control of hyperphenylalaninemia. These include typical deficits in executive function, attention problems, and decreased working memory (Feillet et al. 2010; Bik-Multanowski et al. 2011).

Current guidelines for PKU include frequent monitoring of blood phenylalanine (Phe) concentration with subsequent adjustment of the dietary treatment if blood levels exceed the maximal recommended range (0.36 mmol/L in patients younger than 12 and 0.6 mmol/L thereafter). In addition, the cognitive status should be assessed twice, at approximately 12 and 18 years of age (van Spronsen et al. 2017). However, other than the classic Wechsler Intelligence Tests, no specific methods for cognitive assessment are recommended. Previous studies show that overall intelligence in PKU patients correlates with the extent of life-long hyperphenylalaninemia (Waisbren et al. 2007). Thus, the sporadic use of Wechsler tests has little practical value for early detection and quantitative measurement of PKU-specific cognitive impairment. The use of focused tests that allow assessment of subtle neuropsychological deficits (e.g., in the areas of attention and working memory) could increase the chance for early detection of the above deficits and, consequently, for timely adjustment of the patient's dietary therapeutic regimen. Therefore, establishment of a robust set of neuropsychological tests, suitable for repeated assessment of teenagers with PKU would be useful.

In our previous paper, we reported using the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB) tests to measure attention span and working memory in patients with PKU (Bik-Multanowski et al. 2011). Here, we evaluate the practical value of two similar, classic paper-and-pencil tests: the d2 test of attention and the Benton visual retention test.

Material and Methods

A group of 30 PKU patients who were treated early and continuously, aged 13–18 years and with normal intellectual development, participated in the study. All patients had been followed up in our clinic since infancy, and dietary treatment was initiated in their first month of life. Table 1 presents details on the studied population, including the treatment initiation time and IQ results at study entry.

Study participants took the d2 test of attention and the Benton visual retention test. In the d2 test the individual examined is asked to cross out any letter “d” with two marks above it or below it in any order. The surrounding distractors are usually similar to the target stimulus, for example a “p” with two marks or a “d” with one or three marks (Semrud-Clikeman and Teeter Ellison 2009; Leclercq and Zimmermann 2002).

Six parameters of the d2 test were assessed: total number of items processed (TN), raw score of errors (E), percentage of errors (E%), total number of items minus error scores (TN-E), the fluctuation rate (FR), and concentration performance (CP; the number of correct d2 items minus commission errors). The performance of the above-listed measures was referenced to normative data to control for the effect of age (Dajek and Brickenkamp 2010).

In the Benton test, the person examined is shown ten designs one at a time and asked to reproduce each one as exactly as possible on plain paper from memory (Benton 1992).

One parameter was assessed with regard to the Benton visual retention test: the number error score. The type A method of test administration was used (viewing each design for 10 s before reproducing them).

We analyzed 5,378 results of blood phenylalanine concentration to evaluate the influence of the most recent and historical Phe fluctuations on the attention capacity and visual working memory in teenagers with PKU. Correlations of the test results with the medium- and long-term blood Phe dynamics were analyzed to assess the usefulness of the d2 and the Benton tests in assessing the quality of metabolic control in PKU patients.

The one-sided Pearson's correlation statistic was used and Sidak's correction for multiple comparisons was applied to calculate the statistical significance of the correlation coefficient, r . The level of $r = 0.52$ was considered significant (corrected $p < 0.003$; $N = 30$; number of comparisons = 42). Student's t -test was used for comparison of test scores between patients with good and insufficient metabolic control.

The local ethics committee approved the study and the patients provided informed consent for participation.

Results

Only three out of six parameters assessed in the d2 test significantly correlated with blood Phe levels (TN, TN-E, and CP). The strongest correlations were observed for the period 1 month prior to neuropsychological testing. However, the highest values of the r coefficient were noted for the CP value ($r = -0.72$). The mean z -score for CP was significantly higher in patients with good metabolic control (0.44 vs. -1.12 ; $p = 0.00002$ in t -test). The correlation between neuropsychological score and blood phenylalanine concentration was weaker for longer observational periods (1 year, 2 years, first 12 years of life) and for the life-long changeability of Phe (standard deviation of Phe values in the first 12 years of life). The details on neuropsychological examinations and Phe dynamics are presented in Table 2.

Table 1 The PKU patients

Patient	Gender	Treatment start (day of life)	Age at psychological assessment (years)	IQ (Wechsler scale)	Mutations of the <i>PAH</i> gene
1	F	31	14	93	p.R408W/p.R408W
2	M	26	13	124	p.R408W/p.R408W
3	F	27	13	110	p.R408W/p.R408W
4	F	12	13	123	p.R408W/p.R408W
5	M	20	14	115	p.R408W/p.R243Q
6	F	31	15	113	p.R408W/p.I283F
7	F	13	15	99	p.[T63P;H64N]/N
8	F	11	13	141	p.R408W/p.R408W
9	M	28	17	96	p.R408W/p.R408W
10	F	20	15	132	p.R408W/N
11	F	19	14	97	p.R408W/p.R408W
12	F	16	14	116	p.R408W/N
13	M	10	18	117	p.R408W/p.R408W
14	F	8	12	124	p.R408W/p.R408W
15	F	19	13	106	p.R408W/p.R408W
16	M	11	14	112	p.R408W/p.R408W
17	F	13	12	107	p.R408W/p.E183Q
18	F	9	14	119	p.R408W/p.R408W
19	M	11	12	122	p.R408W/p.R408W
20	F	13	14	87	p.R408W/p.I283F
21	F	14	17	105	p.R408W/p.I283F
22	M	13	15	110	p.R408W/IVS2+5G>C
23	F	10	14	107	p.R408W/p.R408W
24	M	27	15	101	p.R408W/p.R408W
25	M	14	14	122	p.R408W/IVS9-2C>A
26	F	31	18	90	p.R408W/p.R408W
27	F	29	14	108	p.R408W/p.R408W
28	F	14	17	106	p.R408W/p.R408W
29	F	27	14	107	p.R408W/p.R243X
30	M	14	15	104	p.R408W/p.R408W

N not identified

Figure 1 shows the relationship between CP and the mean blood Phe concentrations during a period of 1 month before neuropsychological testing. The average CP decreases below the 50th percentile in patients in whom blood Phe exceeds approximately 0.6 mmol/L (10 mg/dL).

In contrast to the d2 test of attention, the results of the Benton visual retention test did not correlate significantly with the assessed Phe values.

Table 3 presents details of the statistical analysis.

Discussion

In our study, we assessed the practical value of two classic neuropsychological tests to assess the treatment effectiveness in adolescents with PKU. Our results show that the

extent of hyperphenylalaninemia exerts mainly short-term and medium-term effects on the neuropsychological capacity of teenage patients in the field of attention. This is consistent with previous findings in patients with phenylketonuria (Schmidt et al. 1996) and it may correspond with the postulated effect of fluctuations in dopamine concentration in the prefrontal cortex because of brain tyrosine deficits secondary to hyperphenylalaninemia (De Groot et al. 2010). The neurotransmitter-related prefrontal cortex dysfunction in patients with phenylketonuria seems to be largely reversible, in contrast to dysmyelination, which was reported after several years of poor metabolic control in PKU patients.

The frequency of blood phenylalanine monitoring, which is high in young children, becomes low and

Table 2 Blood phenylalanine (Phe) and the results of neuropsychological tests

Patient	The d2 test of attention (z-score)						Benton test Number error score (sten)	Mean Phe prior to neuropsychological assessment (mmol/L)				
	TN	E	E%	TN – E	FR	CP		Last month	Last year	Last 2 years	First 12 years of life	Standard deviation in first 12 years
1	1.75	−0.55	0	1.55	1.03	1.64	6	0.16	0.29	0.22	0.31	0.24
2	1.12	−0.70	−0.22	1.12	0.20	1.55	5	0.19	0.29	0.25	0.28	0.24
3	0.91	−0.80	−0.33	0.87	1.64	0.52	NA	0.24	0.45	0.53	0.47	0.47
4	−0.95	−1.55	0.25	−0.95	1.40	−0.49	7	0.32	0.5	0.49	0.35	0.14
5	−0.25	0.67	1.12	−0.1	0.91	0.1	7	0.36	0.37	0.41	0.32	0.38
6	−0.27	−0.12	0.25	−0.12	1.22	−0.07	6	0.37	0.31	0.28	0.49	0.33
7	0.17	−0.25	0	0.33	1.75	0.12	6	0.39	0.22	0.16	0.32	0.24
8	0.84	0.17	0.73	1.12	0.64	1.22	6	0.4	0.4	0.42	0.3	0.27
9	−0.1	0.49	0.61	0.07	1.64	0.12	10	0.52	0.51	0.49	0.64	0.33
10	1.75	−1.40	−1.34	1.40	0.91	−0.15	4	0.58	0.63	0.59	0.42	0.26
11	−0.33	0.61	1.12	−0.12	−0.35	0.07	10	0.6	0.35	0.29	0.41	0.3
12	0.22	1.88	2.32	0.35	1.64	0.61	5	0.6	0.55	0.53	0.43	0.26
13	−2.32	1.28	1.12	−1.88	0.91	−1.28	6	0.62	0.49	0.5	0.49	0.31
14	−0.67	0.77	1.12	−0.67	−0.30	−0.30	NA	0.7	0.67	0.61	0.69	0.52
15	0.52	−0.80	−0.84	−0.49	−0.38	0.05	6	0.73	0.88	0.83	0.63	0.42
16	−0.15	−1.08	−1.17	−0.46	1.28	−1.34	6	0.73	0.64	0.64	0.7	0.38
17	1.03	−0.22	0.20	1.08	0.52	−2.32	6	0.8	1.02	0.71	0.46	0.41
18	−0.64	0.49	0.55	−0.33	2.32	−0.33	4	0.85	0.77	0.73	0.68	0.24
19	−0.27	0.33	0.25	−0.1	−0.30	0.15	7	0.9	1.1	0.97	0.49	0.36
20	0.41	−0.27	−0.02	0.46	0.12	0.35	7	0.94	0.91	0.86	0.46	0.31
21	−0.91	0.05	0.25	−0.84	−0.07	−0.35	6	0.98	0.53	0.56	0.39	0.32
22	−1.03	−1.08	−1.34	−1.55	0.44	−2.32	5	1.0	1.01	0.92	0.45	0.28
23	−1.40	0.49	0.25	−1.34	0.91	−1.03	6	1.06	1.21	1.12	0.52	0.27
24	−0.35	0.61	0.61	−0.22	2.32	0.02	5	1.07	0.91	0.8	0.65	0.33
25	−1.88	−0.74	−1.22	−2.05	0.25	−2.32	5	1.13	1.02	0.79	0.52	0.38
26	−1.55	−0.52	−0.67	−1.75	1.28	−1.64	6	1.13	0.82	0.64	0.34	0.26
27	0.15	−1.34	−1.40	−0.30	1.03	−1.64	5	1.18	1.14	1.07	0.61	0.49
28	−1.88	0.05	−0.22	−1.75	−0.07	−1.88	6	1.19	1.14	1.07	0.41	0.26
29	−1.40	−0.61	−1.17	−1.75	0.77	−1.75	6	1.31	0.86	0.86	0.76	0.38
30	−0.70	−1.28	−1.75	−1.75	0.05	−2.32	4	1.37	1.3	0.73	0.7	0.33

NA not assessed

insufficient in many teenagers with PKU because of low treatment adherence (Walter et al. 2002). Thus, measurement of Phe concentration might be not adequate to assess short-term metabolic control. In our study, analysis of the d2 test of attention scores revealed a strong inverse correlation of hyperphenylalaninemia and concentration performance in teenagers with PKU. Assessment of this parameter could be helpful in monitoring the quality of metabolic control in the last period preceding the follow-up visit at a metabolic clinic. However, repeated use of the d2

test of attention requires further study to determine if it consistently reflects treatment effectiveness, especially because of the possible learning effect in tested individuals. Other tests, such as computerized tests, e.g. CANTAB or Amsterdam Neuropsychological Tasks (Bik-Multanowski et al. 2011; De Sonnevile 1999), could be used alternately with classic paper-and-pencil methods. It should also be noted that a baseline level of attention should be measured in every patient to enable further monitoring of concentration performance. We believe that such complex neuropsychy-

Fig. 1 The relationship between concentration performance and blood phenylalanine

chological assessments could be routinely performed at 12 years of age, as was recently suggested (van Spronsen et al. 2017), and then could be repeated every 1–2 years.

In addition, careful analysis of Fig. 1 leads to conclusions supporting the current recommendations on maintenance of blood phenylalanine below 0.6 mmol/L in patients over 12 years of age. Hyperphenylalaninemia exceeding this threshold can result in worsening concentration performance.

Interestingly, the mean IQ score in our patients was relatively high. The learning effect (most of the participants of this study were assessed with use of the same version of the WISC-R test in primary school), or the so-called Flynn effect, referring to “ageing” of the test, which can result in overestimation of IQ scores in a population over time (Flynn 1987), could explain this finding.

In conclusion, we believe that assessment of attention using the d2 test in teenagers with PKU can be a valuable supplement to the standard biochemical monitoring of PKU treatment effectiveness. In our opinion the d2 test can be helpful in selection of those teenagers with PKU, who underperform at school due to attention deficits and who could increase their cognitive potential in case of intensification of the every-day dietary, psychological, and social support.

Take-Home Message

The d2 test, a classic neuropsychological paper-and-pencil diagnostic tool, can be used for assessment of dynamics of attention deficits, which are typically observed in patients with phenylketonuria.

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Compliance with Ethics Guidelines

Conflict of Interest

Bożena Didycz, Magdalena Nitecka and Mirosław Bik-Multanowski declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Contributions of Individual Authors

BD designed the study, collected and analyzed data, and wrote the manuscript, MN performed the psychological tests, contributed to data analysis and consulted the manuscript, MBM helped to design and supervised the study, consulted and partially modified the methodology, contributed to data analysis and to drafting of the manuscript.

Table 3 Statistical analysis of the results

The assessed periods	The tests used in the study						The Benton visual retention test (sten) Number error score
	The d2 test of attention (z-score)						
	TN (total number of items processed)	<i>E</i> (raw score of errors)	<i>E</i> % (percentage of errors)	TN – <i>E</i> (total number of items – error score)	FR (the fluctuation rate)	CP (concentration performance)	
<i>Correlations between the results of neuropsychological tests and blood phenylalanine parameters (Pearson correlation)</i>							
Mean Phe in the last month before tests	<i>r</i> = −0.58, <i>p</i> = 0.028	<i>r</i> = −0.09	<i>r</i> = −0.42	<i>r</i> = −0.66, <i>p</i> = 0.003	<i>r</i> = −0.3	<i>r</i> = −0.72, <i>p</i> = 0.0003	−0.28
Mean Phe 1 year before tests	<i>r</i> = −0.41	<i>r</i> = −0.18	<i>r</i> = −0.47	<i>r</i> = −0.51	<i>r</i> = −0.37	<i>r</i> = −0.61, <i>p</i> = 0.01	−0.32
Mean Phe 2 years before tests	<i>r</i> = −0.38	<i>r</i> = −0.1	<i>r</i> = −0.38	<i>r</i> = −0.46	<i>r</i> = −0.31	<i>r</i> = −0.56	−0.25
Mean of yearly Phe means (first 12 years of life)	<i>r</i> = −0.29	<i>r</i> = −0.01	<i>r</i> = −0.23	<i>r</i> = −0.37	<i>r</i> = −0.05	<i>r</i> = −0.44	−0.12
Standard deviation of Phe (first 12 years of life)	<i>r</i> = 0.03	<i>r</i> = −0.01	<i>r</i> = −0.14	<i>r</i> = −0.01	<i>r</i> = −0.29	<i>r</i> = −0.1	−0.02
<i>Mean results of neuropsychological tests vs. metabolic control in the last month before psychological assessment</i>							
Patients with Phe within the recommended limit (0.6 mmol/L)	0.40	−0.13	0.38	0.46	1.05	0.44	6.55
Patients with Phe above the recommended limit	−0.72	−0.21	−0.3	−0.87	0.62	−1.12	5.65
Comparison of both groups (t-test)	<i>p</i> = 0.002	<i>p</i> = 0.8	<i>p</i> = 0.06	<i>p</i> = 0.0002	<i>p</i> = 0.11	<i>p</i> = 0.00002	<i>p</i> = 0.17

Statistically significant values are presented in bold

Ethics Approval

The study was approved by the regional Ethics Committee (29/KBL/OIL/2011).

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