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Data Article

Data on phenylalanine-to-tyrosine ratios in assessment of tetrahydrobiopterin (BH₄)-responsiveness in patients with hyperphenylalaninemia

Barbka Repic Lampret^a, Mojca Zerjav Tansek^{b,c}, Blaz Groselj^{c,d}, Jaka Sikonja^c, Tadej Battelino^{b,c}, Urh Groselj^{b,c,1,1,*}

^a Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, UMC Ljubljana, Ljubljana 1000, Slovenia

^b Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC Ljubljana, Ljubljana 1000, Slovenia

^c Faculty of Medicine, University of Ljubljana, Ljubljana 1000, Slovenia

^d Institute of Oncology Ljubljana, Ljubljana 1000, Slovenia

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ABSTRACT

Blood phenylalanine-to-tyrosine (Phe/Tyr) ratio is an important indicator of metabolic control in phenylketonuria patients. We present the data that highlights the role of Phe/Tyr-ratio in the evaluation of tetrahydrobiopterin (BH₄)-responsiveness in patients with hyperphenylalaninemia. Our data complements the results from the original research article by Tansek et al., 2012 [1]. We performed a BH₄-loading test in 32 patients after four days of increased protein intake (2000 mg/kg body weight). Blood sampling was performed 96, 72, 48, 24, 16 h, and moments before oral administration of BH₄ in a dose of 20 mg/kg body weight. Additional

Abbreviations: BH₄, tetrahydrobiopterin; cPKU, classic phenylketonuria; F, female; M, male; MHP, mild hyperphenylalaninemia; mPKU, mild phenylketonuria; NI, not included; NR, non-responder; Phe, phenylalanine; Phe/Tyr-ratio, phenylalanine-to-tyrosine ratio; R, responder; Tyr, tyrosine.

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* Corresponding author at: Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC Ljubljana, Faculty of Medicine, University of Ljubljana, Bohoriceva 20, Ljubljana 1000, Slovenia

E-mail addresses: barbka.repic@kclj.si (B.R. Lampret), mojca.zerjav-tansek@mf.uni-lj.si (M.Z. Tansek), bgroselj@onko-i.si (B. Groselj), tadej.battelino@mf.uni-lj.si (T. Battelino), urh.groselj@kclj.si (U. Groselj).

Social media: [@JSikonja](https://twitter.com/JSikonja) (J. Sikonja), [@UrhGroselj](https://twitter.com/UrhGroselj) (U. Groselj)

¹ Present address: School of Medicine, Stanford University, California, USA

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blood samples were collected 8 and 24 h after its administration. Phenylalanine (Phe) and Tyrosine (Tyr) levels were determined from dried blood spots by tandem mass spectrometry. Phe/Tyr-ratio reached a plateau after three days of increased dietary protein intake. Fifteen patients (47%) responded to BH₄, defined as a decrease of Phe-of at least 30% after 24 h of BH₄ administration. Phe/Tyr-ratios were significantly higher in non-responders compared to responders. In the responder group, Phe/Tyr-ratios decreased in average of 67% ($p = 0.001$) and 45% ($p = 0.001$) after 8 and 24 h of BH₄ administration, respectively. Phe/Tyr-ratio decreased after 8 h of drug administration also in the non-responder group, but not 24 h after administration.

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Specifications Table

Subject	Endocrinology, Diabetes and Metabolism
Specific subject area	Inborn errors of metabolism; Hyperphenylalaninemia; Phenylketonuria; tetrahydrobiopterin responsiveness
Type of data	Table Chart Figure
How the data were acquired	Dried blood spots were analysed by Tandem Mass Spectrometry. All data was acquired from electronic medical records and analysed by Microsoft Office 365 Excel and IBM SPSS Statistics, version 26.0.
Data format	Raw
Description of data collection	To assess the response to tetrahydrobiopterin (BH ₄), patients ceased with their nutritional supplements for hyperphenylalaninemia and started receiving a protein intake of 2000 mg/kg body weight for five days. After four days on a modified diet, they received 20 mg/kg body weight of BH ₄ . Following acquisition, blood samples were smeared on a special filter paper.
Data source location	UMC - University Children's Hospital Ljubljana Ljubljana Slovenia 3G3C + MM Ljubljana, Slovenia
Data accessibility	Repository name: Mendeley Data DOI: 10.17632/hr7y3h7dsz.1 Direct URL to data: https://data.mendeley.com/datasets/hr7y3h7dsz/1
Related research article	M.Z. Tansek, U. Groselj, S. Murko, H. Kobe, B.R. Lampret, T. Battelino, Assessment of tetrahydrobiopterin (BH ₄)-responsiveness and spontaneous phenylalanine reduction in a phenylalanine hydroxylase deficiency population, <i>Mol. Genet. Metab.</i> 107(1-2) (2012) 37-42. doi: 10.1016/j.ymgme.2012.07.010.

Value of the Data

- The data supports the use of phenylalanine-to-tyrosine (Phe/Tyr) ratio in the assessment of tetrahydrobiopterin (BH₄)-responsiveness.
- The data could help physicians in predicting the response to BH₄ in patients with hyperphenylalaninemia.
- The data could be used by field experts for the development of personalized treatments for patients with hyperphenylalaninemia.

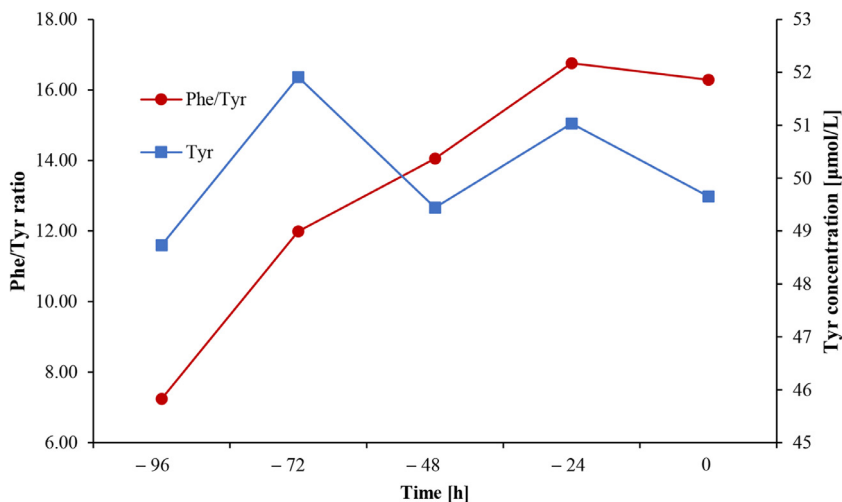


Fig. 1. Phe/Tyr-ratios and Tyr-concentrations prior to BH₄ administration.

Phe/Tyr-ratio and Tyr-concentrations were evaluated separately for nine patients: 4 responders and 5 non-responders. The marks on the x-axis of the figure represent the time prior to BH₄ administration and dots represent the average Tyr-concentrations (blue) and Phe/Tyr-ratios (red). As Phe/Tyr-ratio steadily rose and reached a plateau after three days of increased dietary protein uptake, Tyr-concentration remained stable. Abbreviations: Phe/Tyr-ratio – phenylalanine-to-tyrosine ratio; Tyr – tyrosine.

- Further studies of BH₄-responsiveness could benefit from the addition of Phe/Tyr-ratio in metabolic monitoring.
- Due to the rarity of hyperphenylalaninemia-causing errors of metabolism, additional data on treatment selection is beneficial.

1. Data Description

1.1. Patients' characteristics

We included 32 patients (17 females) with a median age of 5.7 years. The youngest patients was 1 year old and the oldest 22 years. According to BH₄-responsiveness criteria, 15 patients were responders (9 females) and 17 non-responders (8 females). Age difference between the two groups was not statistically significant ($p = 0.355$), although metabolic phenotypes were differently distributed between the responder and non-responder groups. Nine patients with classic phenylketonuria (cPKU), 4 with mild phenylketonuria (mPKU) and 4 with mild hyperphenylalaninemia (MHP) were in the non-responder group, while in the responder group none had cPKU, 8 had mPKU and 7 MHP. No side effects were observed during the BH₄-loading test. Detailed patients' characteristics are presented in [Table 1](#).

1.2. Tyrosine (Tyr) concentration and Phe/Tyr-ratio pre-administration of BH₄

We monitored blood Phe, Tyr-and Phe/Tyr-ratios in nine patients (5 females) at t_{-96} , t_{-72} , t_{-48} , t_{-24} and t_0 . After 3 days of increased dietary intake of protein, blood phenylalanine (Phe) concentrations reached a plateau. As observed with Phe, Phe/Tyr-ratio rose steadily for 3 days and also reached a plateau at a mean of 16.53 ([Fig. 1](#)). Tyr-concentrations stayed relatively stable during

Table 1

Patient characteristics and tetrahydrobiopterin loading results.

# ^a	# ^b	Gender	Age [year]	Metabolic Phenotype ^c	BH ₄ - resp. ^d	Phe-red. 24 h [%] ^e	Phe/Tyr 0 h	Phe/Tyr 8 h	Phe/Tyr 24 h	Phe/Tyr-red. 8 h [%]	Phe/Tyr-red. 24 h [%]
1	27	M	8	cPKU	NR	6.9	56.90	30.51	51.90	46.4	8.8
2	33	F	7	cPKU	NR	-1.5	44.35	21.82	40.94	50.8	7.7
3	34	F	2	cPKU	NR	-7.3	43.82	18.42	36.12	58.0	17.6
4	30	M	4	MHP	NR	-14.3	6.04	4.34	4.78	28.1	20.8
5	15	M	1	cPKU	NR	9.9	37.90	21.79	37.24	42.5	1.7
6	17	F	2	MHP	NR	-10.6	7.10	6.01	7.03	15.4	1.0
7	23	F	3	cPKU	NR	-15.6	36.01	18.80	33.21	47.8	7.8
8	28	M	7	cPKU	NR	12.4	61.04	28.69	69.96	53.0	-14.6
9	35	M	3	cPKU	NR	15.6	56.81	25.54	61.95	55.0	-9.0
10	26	F	8	cPKU	NR	-9.5	55.13	22.64	56.30	58.9	-2.1
11*	16	M	2	cPKU	NR	-1.9	44.48	24.84	37.57	44.2	15.5
12*	20	F	18	mPKU	NR	-12.7	27.76	10.47	29.17	62.3	-5.1
13*	NI	M	6	mPKU	NR	9.9	3.21	0.68	3.82	78.8	-18.8
14*	36	F	15	mPKU	NR	-13.6	36.46	21.51	38.10	41.0	-4.5
15	11	M	4	MHP	NR	27.6	2.51	0.79	1.56	68.7	37.7
16	13	M	4	MHP	NR	29.1	5.15	4.37	4.40	15.2	14.6
17*	19	F	12	mPKU	NR	22.2	5.29	2.86	3.84	45.9	27.3
18	9	M	8	mPKU	R	66.8	4.71	1.03	2.24	78.1	52.5
19	21	F	4	MHP	R	67.9	5.79	2.12	1.57	63.4	72.9
20	7	M	2	mPKU	R	48.7	8.50	2.40	5.37	71.7	36.9
21	10	F	8	MHP	R	72.5	3.38	1.03	1.40	69.6	58.5
22	3	F	17	mPKU	R	43.0	9.93	2.60	6.11	73.8	38.5
23	4	F	14	mPKU	R	54.3	8.64	1.90	4.51	77.9	47.8
24	NI	M	4	MHP	R	37.1	1.84	1.10	1.52	40.1	17.3
25	NI	F	2	MHP	R	30.7	7.13	3.04	6.05	57.4	15.2
26	12	F	2	mPKU	R	46.6	6.84	1.09	3.33	84.1	51.3
27	2	M	5	MHP	R	45.3	6.76	2.78	4.92	58.8	27.1
28	14	F	9	MHP	R	44.5	4.15	0.59	2.94	85.8	29.0
29*	5	M	8	mPKU	R	44.8	7.90	2.83	3.87	64.2	51.0
30*	18	F	7	mPKU	R	43.1	3.99	2.88	2.23	27.7	44.0
31*	6	M	4	MHP	R	71.1	7.18	2.11	1.69	70.6	76.5
32*	8	F	22	mPKU	R	65.9	10.36	1.82	4.59	82.4	55.7

^a Patient number. Patients 22 and 23, and patients 17 and 30 are siblings. * - Patients with all pre-BH₄-loading amino acid measurements.

^b Patient number from the original research article. NI - not included.

^c Phenotype as determined by their pre-treatment plasma phenylalanine concentrations: cPKU - classic phenylketonuria; mPKU - mild phenylketonuria; MHP - mild hyperphenylalaninemia.

^d R - responder (phenylalanine reduction \geq 30% after 24 h of BH₄ administration); NR - non-responder.

^e Measured by tandem mass spectrometry from dried blood spots. Abbreviations: BH₄ - tetrahydrobiopterin; F - female; M - Male; Phe - phenylalanine; Phe/Tyr - phenylalanine-to-tyrosine ratio; red. - reduction; resp. - responsiveness.

the test. The average of mean Tyr-concentrations from different time points was 50.16 μ mol/L (standard deviation = 1.29 μ mol/L).

1.3. Phe/Tyr-ratios regarding to phenotype

We compared Phe/Tyr-ratios in patients with different metabolic phenotypes. The highest ratios were observed in cPKU patients compared to mPKU and MHP ($p < 0.001$ for both comparisons). Phe/Tyr-ratios were comparable between mPKU and MHP whereas mPKU patients had slightly higher ratios (Fig. 2). This difference was statistically significant only for Phe/Tyr-ratios at t_{-24} and t_0 ($p = 0.019$ and $p = 0.042$, respectively). One day after receiving BH₄, there was a significant fall of Phe/Tyr-ratio in both mPKU and MHP patients relative to baseline levels (t_0), however this finding was not observed in cPKU patients ($p = 0.012$, $p = 0.003$ and $p = 0.515$,

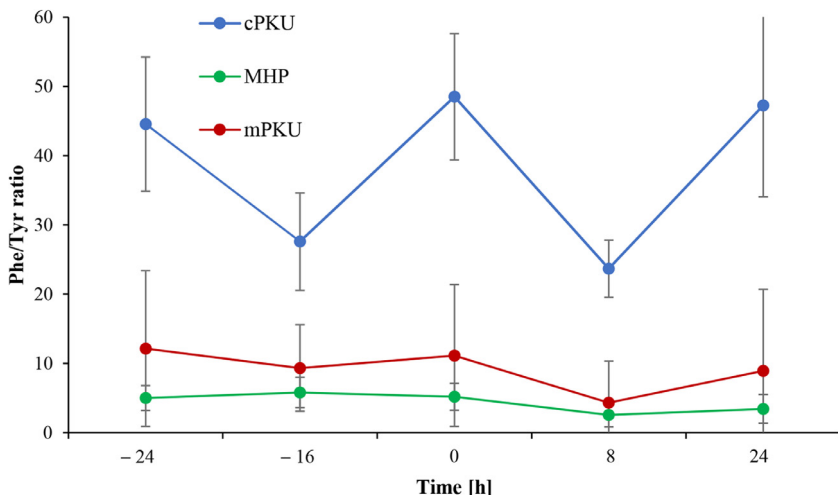


Fig. 2. Phe/Tyr-ratios and Tyr-concentrations across different metabolic phenotypes.

The figure shows average Phe/Tyr-ratios with standard deviations in patients from three phenotype groups: cPKU (blue), mPKU (red) and MHP (green). Patients with cPKU had higher Phe/Tyr-ratios throughout the observation period compared to the other two groups. mPKU and MHP patients experienced a decrease of Phe/Tyr-ratio after 8 and 24 h from BH₄ administration. Abbreviation: cPKU – classic phenylketonuria; MHP – mild hyperphenylalaninemia; mPKU – mild phenylketonuria; Phe/Tyr-ratio – phenylalanine-to-tyrosine ratio.

respectively). In the latter group, we can see an initial decrease of Phe/Tyr-ratios after 8 h of BH₄ administration which was followed by an increase to baseline concentrations 24 h after loading.

1.4. Phe/Tyr-ratio regarding to BH₄-responsiveness

Phe/Tyr-ratios between BH₄-responders and non-responders were significantly different throughout the observation period ($p < 0.001$) and are shown in Fig. 3. In the responder group, Phe/Tyr-ratio decreased in average of 67% ($p = 0.001$) and 45% ($p = 0.001$) from baseline concentrations after 8 and 24 h of BH₄ administration, respectively. On the other hand, a decrease of Phe/Tyr-ratio from baseline was evident at 8 h after BH₄-loading and not at 24 h in the non-responder group as Phe/Tyr-ratio decreased in average 48% ($p < 0.001$) and 6% ($p = 0.356$) after 8 and 24 h from BH₄-loading, respectively.

Dataset title: Phenylalanine-to-tyrosine ratio in assessment of tetrahydrobiopterin responsiveness in hyperphenylalaninemia

Dataset description: Dataset includes the general patient information (gender, age) and their metabolic phenotype. Additionally, the data on Phe, Tyr-levels and Phe/Tyr-ratios at various time points is included.

2. Experimental Design, Materials and Methods

2.1. Participants of the study and collection of samples

To assess the role of Phe/Tyr-ratio in BH₄-reponsiveness assessment, we included (i) patients with at least one mutation that was BH₄-responsive, (ii) patients with at least one mutation with unclear effect on BH₄-responsiveness and (iii) patients that were tested for BH₄-responsiveness before the results of genetic analyses. The data on mutations and genotypes related BH₄-

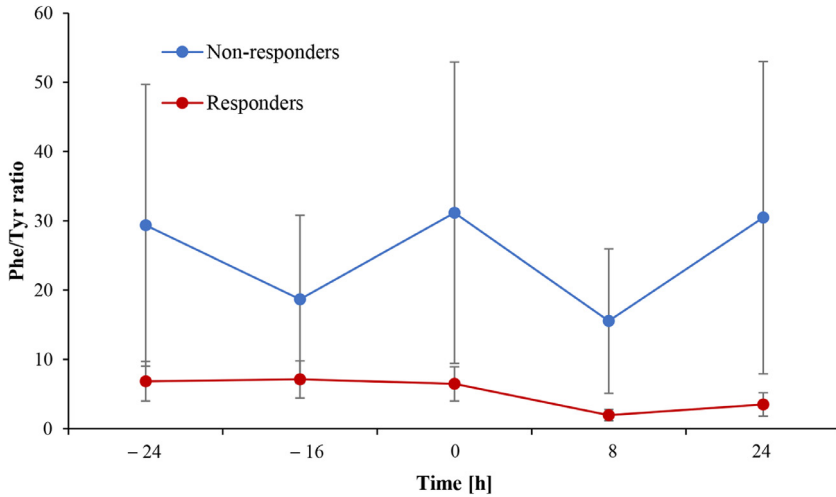


Fig. 3. Phe/Tyr-ratios and BH₄-responsiveness.

Average Phe/Tyr-ratios together with standard deviations are presented for two groups according to the response to BH₄. Non-responders (blue) had higher Phe/Tyr-ratios throughout the observation period compared to responders (red). Additionally, responders experienced a decrease of Phe/Tyr-ratios and had lower Phe/Tyr-ratios at 8 h and 24 h after BH₄-administration, while in the non-responders group Phe/Tyr-ratios were lower only 8 h after drug administration. Abbreviation: Phe/Tyr-ratio – phenylalanine-to-tyrosine ratio.

responsiveness were obtained from BIOPKU database (available at: www.biopku.org/biopku). Genetic characteristics of the included patients were previously described in the original research article by Tasek et al. [1]. Altogether, thirty-two patients (17 females) were selected for the study, were receiving dietary treatment for hyperphenylalaninemia and were regularly followed as outpatients.

2.2. Metabolic phenotype

Pre-treatment plasma Phe-levels or dietary Phe-tolerance were used to classify participants into three phenotype groups: cPKU (Phe \geq 1200 μ mol/L; Phe-tolerance \leq 350 mg/day), mPKU (Phe 600–1200 μ mol/L; Phe-tolerance 400–600 mg/day) and MHP (Phe < 600 μ mol/L on a normal diet) [1,2].

2.3. Dietary protein intake and BH₄-loading test

Included patients stopped taking their PKU nutritional supplements for five days during the study. At the same time, each patient received her/his own individual meal plan, prepared by a dietician based on her/his food choices. Protein intake was increased to 2000 mg/kg body weight, corresponding to an approximate Phe-intake of 100 mg/kg body weight. Adequacy of dietary intake was controlled on site.

Four days after starting the study, each patient received 20 mg/kg body weight of BH₄ (Kuvan®, sapropterin dihydrochloride, Merck Sorono, Darmstadt, Germany) in one orally-administered dose. For the determination of responsiveness, we used the reduction of blood Phe after BH₄ administration as the main criteria, while having in mind that alternative definitions of responsiveness exist [3]. A “responder” was defined when the reduction of blood Phe-level was \geq 30% after 24 h of BH₄ administration and a “non-responder” at smaller reductions [3,4].

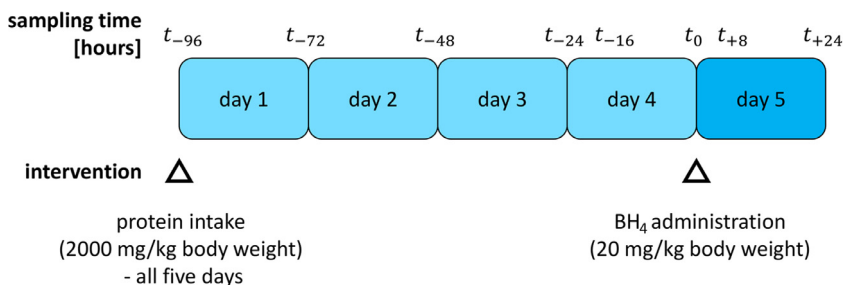


Fig. 4. Study protocol and blood samples collection.

In the first three days, blood samples were collected from only 9 patients. Day 4 (t_0) represents the day before the administration of BH_4 . Responsiveness to BH_4 was determined by the reduction from baseline of blood Phe, measured at t_{+24} . Abbreviations: BH_4 – tetrahydrobiopterin, Phe - phenylalanine.

2.4. Blood collection and amino acids measurements

Blood collection was performed four days (-96 h; t_{-96}), three days (-72 h; t_{-72}), two days (-48 h; t_{-48}), one day (-24 h; t_{-24}), 16 h (t_{-16}), and moments prior (t_0) to BH_4 administration, and 8 and 24 h (t_{+8} ; t_{+24}) later (Fig. 4). Measurements at t_{-96} , t_{-72} and t_{-48} were done only at 9 patients while all other measurements were done in all included patients. Following collection, blood was smeared on a special filter paper and dried at room temperature for approximately 3 h. Phe-and Tyr-concentrations were measured from dried blood spots by tandem mass spectrometry (Perkin Elmer PE 200 HPLC with AB Sciex 3200 QTrap).

2.5. Statistical analysis

IBM SPSS Statistics, version 26.0 (IBM, USA) was used for statistical analysis. Data was visualized in Microsoft Office 365 Excel (Microsoft Corporation, USA). The significance of Phe/Tyr-ratios change in responder and non-responder groups and in three metabolic phenotype groups was calculated with Wilcoxon matched-pairs signed-rank test. Mann-Whitney U test was used for comparison between responders and non-responders and between different phenotype groups. A p value of ≤ 0.05 was considered as statistically significant.

Ethics Statements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 2000 revision of Helsinki declaration. Written informed consent was obtained from the patients/patient's parents for inclusion into the study and for anonymized data publication and are available for review upon request. This article does not contain any studies with animal subjects performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT Author Statement

Barbka Repic Lampret: Methodology, Investigation, Writing – original draft; **Mojca Zerjav Tasek:** Investigation, Conceptualization, Writing – original draft; **Blaz Groseelj:** Investigation, Formal analysis, Writing – original draft; **Jaka Sikonja:** Investigation, Formal analysis, Visualization, Writing – original draft; **Tadej Battelino:** Investigation, Conceptualization, Supervision, Writing – review & editing; **Urh Groseelj:** Investigation, Writing – review & editing, Conceptualization.

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