

# Therapeutic Potential of Valproic Acid for Postviral Olfactory Dysfunction: A Single-Arm Pilot Study.

著者	OGAWA Takao, MATSUMOTO Koji, TOJIMA Ichiro, KIKUOKA Hirotaka, HIRA Daiki, SHIMIZU Takeshi
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## ABSTRACT

1  
2 **Objectives:** Although some patients with postviral olfactory dysfunction (PVOD)  
3 recover spontaneously, many others are left with the degree of smell loss and  
4 there are no established drugs for the treatment of patients with PVOD. Valproic  
5 acid (VPA) has been widely used for the treatment of epilepsy. Its potential  
6 neuroregenerative effects have been shown via animal studies. This is the first  
7 study to treat PVOD patients with VPA. This open-label, single-arm, phase II  
8 study was conducted to investigate the effects of VPA in patients with PVOD.

9 **Methods:** The patients received oral tablets of VPA 200 mg twice a day for 24  
10 weeks. In total, 11 patients with PVOD were recruited. Odor scores of  
11 recognition and detection threshold (measured with a T&T olfactometer), and  
12 visual analog scale were examined during the treatment.

13 **Results:** All odor scores significantly improved over time. Although the mean  
14 duration of olfactory dysfunction in this study was 11.5 months, both odor  
15 recognition threshold and odor detection threshold scores significantly improved  
16 4 weeks after treatment initiation compared to the pre-treatment threshold  
17 scores. The olfactory recovery rates in patients treated with VPA were clearly  
18 better than those we previously reported in PVOD patients who received

1 Toki-shakuyaku-san, the traditional treatment in Japan. The olfactory recovery  
2 rates of patients with PVOD at 12 weeks and 24 weeks of VPA treatment were  
3 both 77.8%, and the olfactory cure rates at 12 weeks and 24 weeks of VPA  
4 treatment were 33.3% and 44.4%, respectively. No serious adverse events were  
5 observed.

6 **Conclusions:** VPA seems to be a safe treatment option in patients with PVOD.  
7 The effects of VPA treatment for PVOD patients should be studied with a  
8 controlled study design in the future.

9

10 **Keywords:** postviral; olfactory dysfunction; valproic acid; treatment; recovery

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1

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## INTRODUCTION

3

4 Postviral olfactory dysfunction (PVOD) develops after an upper respiratory  
5 infection, which is one of the major causes of olfactory dysfunction. Several  
6 potential causative viruses including rhinovirus, coronavirus, influenza virus,  
7 parainfluenza virus have been reported in PVOD patients<sup>1,2</sup>. It was reported in a  
8 recent multicenter study that 85.6% of patients with severe acute respiratory  
9 syndrome coronavirus 2 (SARS-CoV-2) infection develop olfactory dysfunction  
10 and about a half of those patients develop persistent olfactory dysfunction<sup>3</sup>.  
11 Because the SARS-CoV-2 infection remains a pandemic, the proportion of  
12 patients suffering from olfactory dysfunction may increase. The sense of smell is  
13 important not only for perceiving the flavors of foods and beverages but also for  
14 detecting olfactory cues that could be construed as environmental dangers, such  
15 as a leaky cooking gas pipeline, toxic levels of ammonia or sulfur dioxide in the  
16 air, or decaying organic matter in the backyard. Therefore, patients with olfactory  
17 dysfunction have a markedly impaired quality of life.

18 Olfactory training is the recommended treatment for PVOD<sup>4,5</sup>. The efficacy  
19 of olfactory training, which is safe and non-invasive, has been demonstrated

1 through randomized controlled trials in patients with PVOD<sup>6</sup>. However, there are  
2 currently no established drugs proven efficacy in a randomized control trial for  
3 the treatment of PVOD<sup>4,5</sup>. A systematic review revealed that oral and intranasal  
4 steroids are the most frequent treatment strategies but need to be administered  
5 with caution because of the potential risks of steroids. Toki-shakuyaku-san  
6 (Tsumura, Tokyo, Japan) and zinc sulfate have been traditionally used for the  
7 treatment of PVOD in Japan. However, there is little evidence for the  
8 effectiveness of these drugs.

9 The pathophysiology of PVOD is not fully understood. Histological analysis  
10 of the olfactory epithelium in patients with PVOD showed reduced numbers of  
11 olfactory receptor cells and nerve bundles<sup>7</sup>, and the degree of degeneration of  
12 the olfactory epithelium was correlated with the degree of olfactory dysfunction<sup>8</sup>.  
13 These results indicate that failure of regeneration of the olfactory epithelium after  
14 viral injury could be one potential mechanism for olfactory dysfunction in patients  
15 with PVOD. Therefore, treatment strategies for PVOD should focus on the  
16 regeneration of surviving olfactory epithelium neurons.

17 Valproic acid (VPA) has been widely used for the treatment of epilepsy.  
18 Recent studies have demonstrated that VPA acts as a histone deacetylase

1 inhibitor. VPA promotes the differentiation of cultured neural stem cells and  
2 neurite outgrowth<sup>9</sup> and its potential neuroregenerative effects were reported in  
3 animal models of spinal cord<sup>10</sup> and optic nerve injury<sup>11</sup>. Basal cells of the  
4 olfactory epithelium include neural stem cells, which proliferate and differentiate  
5 into mature olfactory sensory neurons and serve to replace neurons lost during  
6 injury<sup>12</sup>. These results indicate that VPA could be useful in the treatment of  
7 PVOD. We previously reported that oral VPA administration promotes the  
8 regeneration of olfactory sensory neurons in the damaged olfactory  
9 neuroepithelium of mice<sup>13</sup>. In the present study, we investigated the effects of  
10 VPA in patients with PVOD.

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12

## METHODS

13

14 This single-center, open-label, single-arm, phase II study was conducted from  
15 January 2016 to August 2017 on 11 patients with PVOD who were enrolled at  
16 Shiga University of Medical Science in Japan. The efficacy and safety of valproic  
17 acid in patients with PVOD were assessed. All participants gave written informed  
18 consent. The study protocol was approved by the Ethics Committee of the

1 Faculty of Medicine at Shiga University of Medical Science (Ethics number  
2 27-67). The trial was performed according to the tenets of the Declaration of  
3 Helsinki. All patients signed informed consent and the study was conducted  
4 according to clinical practice guidelines. This study was registered at University  
5 Hospital Medical Information Network (no. 000019966).

6

### 7 **Patient eligibility**

8

9 The inclusion criteria were the following: age from 20 to 65 years at the initiation  
10 of the study and diagnosis of PVOD. The exclusion criteria were the following:  
11 premenopausal female patients; patients taking carbapenem, barbituric acid,  
12 phenytoin, carbamazepine, ethosuximide, amitriptyline, clobazam, lamotrigine,  
13 salicylic acid, benzodiazepine, warfarin, erythromycin, cimetidine, or  
14 clonazepam; patients who had drug hypersensitivity to VPA; patients with severe  
15 depression; patients who had attempted suicide; patients with liver dysfunction;  
16 patients with renal dysfunction; patients with urea cycle abnormality; patients  
17 with a history of an encephalopathy or a coma due to an unknown cause;

1 patients having a family member with urea cycle abnormality; and patients with  
2 subjective olfactory loss before onset of PVOD.

3

#### 4 **Diagnosis**

5

6 PVOD was diagnosed by a questionnaire and a clinical examination.

7 All the patients were examined by computed tomography (CT) of the sinus, and

8 nasal endoscopy. The diagnostic criteria were as follows: (1) history of upper

9 respiratory infection before the olfactory loss; (2) sudden onset of olfactory loss;

10 and (3) no evidence of conductive olfactory loss, such as rhinosinusitis, nasal

11 polyps, mucosal edema of the olfactory fissure, deformation of the nasal septum,

12 or neoplastic lesions, on examination by nasal endoscopy and sinus CT scan.

13 Patients were excluded if they had a history of head trauma.

14

#### 15 **Olfactory assessment**

16

17 Olfactory function was evaluated using a T&T olfactometer (Daiichi Yakuhin

18 Sangyo Inc., Tokyo, Japan), which is the standard test for measuring the



1 threshold score of odor detection and recognition in Japan<sup>4</sup>. The normal odor  
2 recognition threshold score is 1.0 or less. Patients were diagnosed with anosmia  
3 when the odor recognition threshold score was 5.6 or greater. According to the  
4 criteria proposed by the Japan Rhinology Society, the degree of recovery is  
5 classified into four groups based on the odor recognition threshold score after  
6 treatment: 1) 'cured,' when the odor score was restored to 2 or less; 2)  
7 'improved,' when the score was decreased by  $\geq 1$  from the pre-treatment score;  
8 3) 'no change,' when the score remained within 1 point of the pre-treatment  
9 score; and 4) 'worsened,' when the score was increased by  $\geq 1$  from the  
10 pre-treatment score. An assessment of 'cured' or 'improved' was defined as  
11 recovery. The visual analog scale (VAS) on a 0–100 mm scale (0 = anosmia,  
12 100 = normosmia) was also used to assess subjective olfactory function. All  
13 tests were administered by otolaryngologists blinded to the patient's treatment.

14

## 15 **Study design**

16

17 Before enrollment, all patients underwent a questionnaire interview, regular  
18 physical examination, olfactory assessments, blood test (complete blood count,

1 liver and renal function tests, creatine phosphokinase, amylase, ammonia), and  
2 urinalysis. Each patient was instructed to take valproic acid sodium tablets  
3 (Depakene-R®; Kyowa Hakko Kirin, Tokyo, Japan), such that 200 mg of VPA  
4 was administered twice a day (total daily dose, 400 mg) for 24 weeks. Follow-up  
5 visits were scheduled at 1, 4, 8, 12, 18, and 24 weeks after initiation of VPA  
6 treatment. Olfactory assessment, blood test, and urinalysis similar to those  
7 performed before treatment were conducted at each follow-up visit except at the  
8 1-week visit. Plasma levels of VPA were also measured at each follow-up visit.  
9 The VPA treatment was stopped if any abnormalities on blood test or urinalysis  
10 were found, if the olfactory dysfunction was fully resolved, if there were any  
11 serious adverse effects attributable to VPA use, or if the patient refused to  
12 continue treatment for any reason.

13 The primary endpoint was improvement from baseline in the odor  
14 recognition threshold score after treatment with VPA. The secondary endpoints  
15 were recovery rate, improvement from baseline in the odor detection threshold  
16 score, and the occurrence of adverse events after VPA treatment.

17

18

## 1 **Statistical analysis**

2

3 Statistical comparisons between pre- and post-treatment periods were  
4 conducted by using univariate generalized estimating equations with adjustment  
5 for repeated measurements. Values of  $P < 0.01$  were considered to indicate  
6 statistical significance. All statistical analyses were performed with R version  
7 3.3.1<sup>14</sup>. Data are shown as the mean  $\pm$  standard deviation.

8

9

## **RESULTS**

10

### 11 **Patient characteristics**

12

13 A total of 11 patients (10 female and 1 male) with PVOD were enrolled in the  
14 study. The patient characteristics are shown in Table 1. Their age was  $54.9 \pm 8.0$   
15 years. The duration of disease until the first visit was  $11.5 \pm 13.3$  months. The  
16 odor detection thresholds, odor recognition thresholds, and VAS were  $2.6 \pm 1.4$ ,  
17  $4.6 \pm 1.2$ ,  $19.2 \pm 13.6$ , respectively. Of the enrolled 11 patients, 2 (18.2%)  
18 patients had no history of treatment for POID, 9 (81.8%) patients were previously

1 treated for PVOD with either intranasal steroids (n = 3), TSS (n = 2), or a  
2 combination of TSS with zinc sulfate (n = 3). The majority of patients (8/11,  
3 72.7%) had severe hyposmia or anosmia. Two patients (patient No. 10 and No.  
4 11) were withdrawn from the study because of abnormal blood test results  
5 (explained in the section "Adverse events") during the treatment period; the  
6 remaining 9 patients completed the treatment, and their data were included in  
7 the analysis. One patient (No. 3) stopped the VPA treatment at the 18-weeks  
8 follow-up visit because the degree of recovery was assessed to be 'cured'; this  
9 patient's data were included in the analysis.

10

### 11 **Olfactory outcomes**

12

13 Fig.1 shows odor scores for each patient at the different timepoints during VPA  
14 treatment. The odor recognition threshold scores (P <0.001, 95% confidence  
15 interval [CI] -0.13 to -0.05), odor detection threshold scores (P <0.001, 95%  
16 confidence interval [CI] -0.11 to -0.05), and the VAS scores (P <0.001, 95%  
17 confidence interval [CI] 0.35 to 1.35) significantly improved over time. In addition,  
18 there was a significant improvement in the odor recognition threshold scores (P

1 <0.001, 95% CI -1.83 to -0.81) and the odor detection threshold scores (P  
2 <0.001, 95% confidence interval [CI] -0.11 to -0.05) at 4 weeks after treatment  
3 initiation compared to the pre-treatment threshold scores. The olfactory recovery  
4 rates of patients with PVOD at 12 weeks and 24 weeks of VPA treatment were  
5 both 77.8% (7/9). The olfactory cure rates at 12 weeks and 24 weeks of VPA  
6 treatment were 33.3% (3/9) and 44.4% (4/9), respectively. Only 2 patients  
7 (patient No. 5 and No. 6) did not reach the criteria for recovery after 24 weeks of  
8 treatment with VPA. Patient No. 9 developed parosmia, and the odor recognition  
9 threshold score worsened from the 18-weeks follow-up visit to the 24-weeks  
10 follow-up visit.

11

## 12 **Adverse events**

13

14 During the period of VPA treatment, no drug-related serious adverse events  
15 were observed in the study participants. Mild daytime sleepiness was reported in  
16 1 patient (patient No. 3). Two patients (patient No. 10 and No. 11) were  
17 withdrawn from this study due to blood test abnormalities. In patient No. 10, mild  
18 elevation of liver enzymes (glutamic oxaloacetic transaminase [GOT] 57 IU/L,

1 glutamic pyruvic transaminase [GPT] 69 IU/L) was observed at the 4-weeks  
2 follow-up visit, which returned to normal levels 4 weeks after stopping the VPA  
3 treatment. The plasma level of VPA was within a safe range (47.3 µg/mL) at the  
4 4-weeks follow-up visit. The odor recognition threshold, odor detection threshold,  
5 and VAS at the 4-weeks follow-up visit were 2.2, 0.6, and 42, respectively, and  
6 the degree of olfactory recovery was assessed as 'no change.' Patient No. 11  
7 had a marked elevation of creatine phosphokinase (100081 IU/L), an elevation  
8 of GOT (138 IU/L), GPT (67 IU/L), and lactate dehydrogenase (401 U/L), and  
9 proteinuria at the 8-weeks follow-up visit. The patient was undergoing  
10 high-intensity strength training 1 week before the visit. Seeing the abnormal  
11 values in blood tests, he was instructed to stop strength training. The blood test  
12 results improved within 1 week after stopping the VPA treatment and strength  
13 training and returned to normal after 4 weeks. The plasma levels of VPA and  
14 ammonia were within a safe range (33.9 µg/mL and 53 µg/dL, respectively) at  
15 the 8-weeks follow-up visit. It was determined that these abnormalities were  
16 induced by strength training, and VPA treatment was identified to be negatively  
17 associated with the elevation of these enzymes. The odor recognition threshold,

1 odor detection threshold, and VAS at the 8-weeks follow-up visit were 2.0, -0.2,  
2 and 42, respectively, and the degree of olfactory recovery was 'cured.'

3 The plasma level of VPA increased to  $46.0 \pm 10.1 \mu\text{g/mL}$  after 1 week of  
4 VPA treatment. During the treatment period, the plasma level of VPA was stable  
5 within the range of 29.2-69.7  $\mu\text{g/mL}$  in each patient.

6

7

## DISCUSSION

8

9 In the present study, we investigated the effects of VPA in patients with PVOD.  
10 VPA treatment significantly improved the odor recognition threshold score over  
11 time, resulting in high recovery rates. Furthermore, even though the mean of  
12 duration of olfactory dysfunction in this study was 11.5 months, a significant  
13 improvement in odor recognition and detection threshold scores was observed  
14 within a short period of 4 weeks of VPA treatment. No drug-related serious  
15 adverse events were observed. Although this was a single-arm study and had a  
16 small sample size, the results suggest that VPA could be useful in PVOD  
17 treatment. This is the first study to provide clinical evidence of the benefits of  
18 VPA in patients with PVOD.

1 TSS and zinc sulfate have been traditionally used for the treatment of  
2 PVOD in Japan<sup>4</sup> (Miwa. 2019). TSS is an herbal medicine originally used for  
3 patients with fatigue, chronic anemia, and menopausal disorders. TSS was  
4 reported to promote the neural regeneration of the olfactory epithelium after  
5 methimazole-induced injury in mice<sup>15</sup>. It was also reported to be more effective in  
6 the treatment of PVOD patients than intranasal steroids in case-control studies<sup>16</sup>.  
7 Zinc is essential for cell proliferation and differentiation. Because olfactory  
8 sensory cells are continuously regenerated, zinc is thought to be essential for  
9 the maintenance of the olfactory function. We previously reported the results of  
10 olfactory function testing in 82 PVOD patients (mean age,  $56.4 \pm 14.0$  years;  
11 mean duration of disease until the first visit,  $7.4 \pm 11.8$  months) treated with TSS  
12 and/or zinc sulfate, and the cumulative olfactory recovery and cure rates at 6  
13 months after the first visit were 47.3% and 23.6%, respectively<sup>15</sup>. In the present  
14 study treated with VPA, the olfactory recovery and cure rates at 6 months were  
15 77.8% and 44.4%, respectively, which are higher than the rates reported in our  
16 previous study with TSS and/or zinc sulfate. Although it is difficult to make a  
17 direct comparison between our present and previous studies due to the  
18 differences in the sample size and the enrolled patients, these results suggest



1 that VPA treatment is effective for PVOD patients and worthy of further  
2 investigation with controlled studies.

3 In previous studies with mean assessment intervals of 3<sup>18</sup>, 14<sup>19</sup>, and 37  
4 months<sup>20</sup>, spontaneous recovery of olfaction was observed in 6%, 32%, and  
5 66% of patients with PVOD, respectively. Based on these results, Damm et al.  
6 discussed that the degree of spontaneous recovery in PVOD patients may  
7 present a linear progression over time<sup>6</sup>. In our present study, despite the mean  
8 duration of disease being 11.5 months, significant improvements were observed  
9 in the odor recognition threshold and odor detection threshold at 4 weeks of VPA  
10 treatment. Although this study did not have a control group, these results support  
11 the therapeutic effects of VPA in PVOD patients.

12 In the present study, no recovery in the odor recognition threshold score  
13 was observed for 2 patients (patient No. 5 and No. 6). Patient No. 5 had olfactory  
14 loss for a long period of 33 months, and patient No. 6 had anosmia (both the  
15 odor recognition and detection threshold scores were 5.8). Previous studies  
16 revealed that residual olfactory function is an important prognostic factor for  
17 PVOD. It may be difficult to restore the olfactory function with VPA treatment

1 when the olfactory epithelium has lost the capacity to regenerate its neurons due  
2 to severe damage.

3 The optimal dosage of VPA in treatment of PVOD is not clearly defined.  
4 The dose of VPA we used in this study was the lowest dose used for the  
5 treatment of epilepsy in adults. In the clinical practice of epilepsy, although  
6 controversial, the therapeutic plasma level of VPA ranges 50 to 100 µg/mL with  
7 a broad recommended dose range<sup>21</sup>, and serum levels greater than 100 µg/mL  
8 can cause hematologic toxicity<sup>22</sup>. During the treatment period in our study, the  
9 plasma level of VPA did not exceed 100 µg/mL, and no serious adverse events  
10 were observed in our study. However, the occurrence of adverse effects often  
11 unrelated to the concentration of VPA<sup>21</sup>, and VPA is associated with several  
12 potentially serious adverse effects, including liver toxicity, blood, or hepatic  
13 disorders, and pancreatitis<sup>23</sup>. Therefore, careful observation of the overall  
14 condition is required when patients with PVOD are being treated with VPA.

15 There are several limitations in our study. Firstly, this study lacked a  
16 control group because of its exploratory nature. In our single-arm study, the  
17 beneficial effects in patients with PVOD remain unclear  
18 due to the spontaneous recovery potential seen in patients with PVOD.

1 Furthermore, practice effects must be considered, which are observed  
2 commonly in psychophysical testing. Due to the lack of a control group, we  
3 cannot rule out the possibility that the present positive results were affected by  
4 positive practice effects caused by the shorter measurement intervals of  
5 olfactory testing. Secondly, the sample size in this study was small. Therefore, a  
6 randomized controlled trial or a comparative trial with a larger sample size is  
7 necessary to assess the efficacy and safety of VPA treatment for patients with  
8 PVOD. Thirdly, diagnosis of PVOD mainly depends on taking the history of  
9 olfactory loss after upper respiratory infection from the patient, and it is difficult to  
10 prove directly by examinations whether the olfactory dysfunction is indeed  
11 caused by viral infection. Therefore, it remains difficult to fully distinguish viral  
12 from non-viral etiologies. In the present study, nasal endoscopy and CT were  
13 performed in all cases to exclude obstructive lesions such as sinusitis and  
14 olfactory cleft disease, thereby increasing the reliability of the diagnosis that  
15 sensorineural dysfunction was the cause of olfactory dysfunction.

16 A comprehensive medical evaluation should be performed to ensure that  
17 the patient can tolerate VPA treatment, and the medication should be  
18 administered with caution to patients at risk for liver disease. However, VPA

1 seems to be a safe treatment option in patients with PVOD. VPA treatment was  
2 well tolerated, and severe adverse events were not observed. Effects of VPA  
3 treatment in PVOD patients observed here are worthy of further investigation  
4 with a controlled study design in the future.  
5

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2

3 **CONFLICT OF INTERESTS:** All authors report no conflict of interest.

4

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7

8

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1

2 **FIGURE LEGEND**

3

4 **Table 1. Characteristics of the enrolled patients and treatment outcomes.**

5 According to the criteria proposed by the Japan Rhinology Society, the degree  
6 of recovery is classified based on the odor recognition threshold score after  
7 treatment: 'cured,' when the odor score was restored to 2 or less; 'improved,'  
8 when the score was decreased by  $\geq 1$  from the pre-treatment score; 'no change,'  
9 when the score remained within 1 point of the pre-treatment score; and  
10 'worsened,' when the score was increased by  $\geq 1$  from the pre-treatment score.

11

12 **Figure 1. Odor scores for each patient at different timepoints during**  
13 **valproic acid (VPA) treatment. A) Odor recognition threshold scores. B)**  
14 **Odor detection threshold scores. C) Visual analog scale (VAS).**

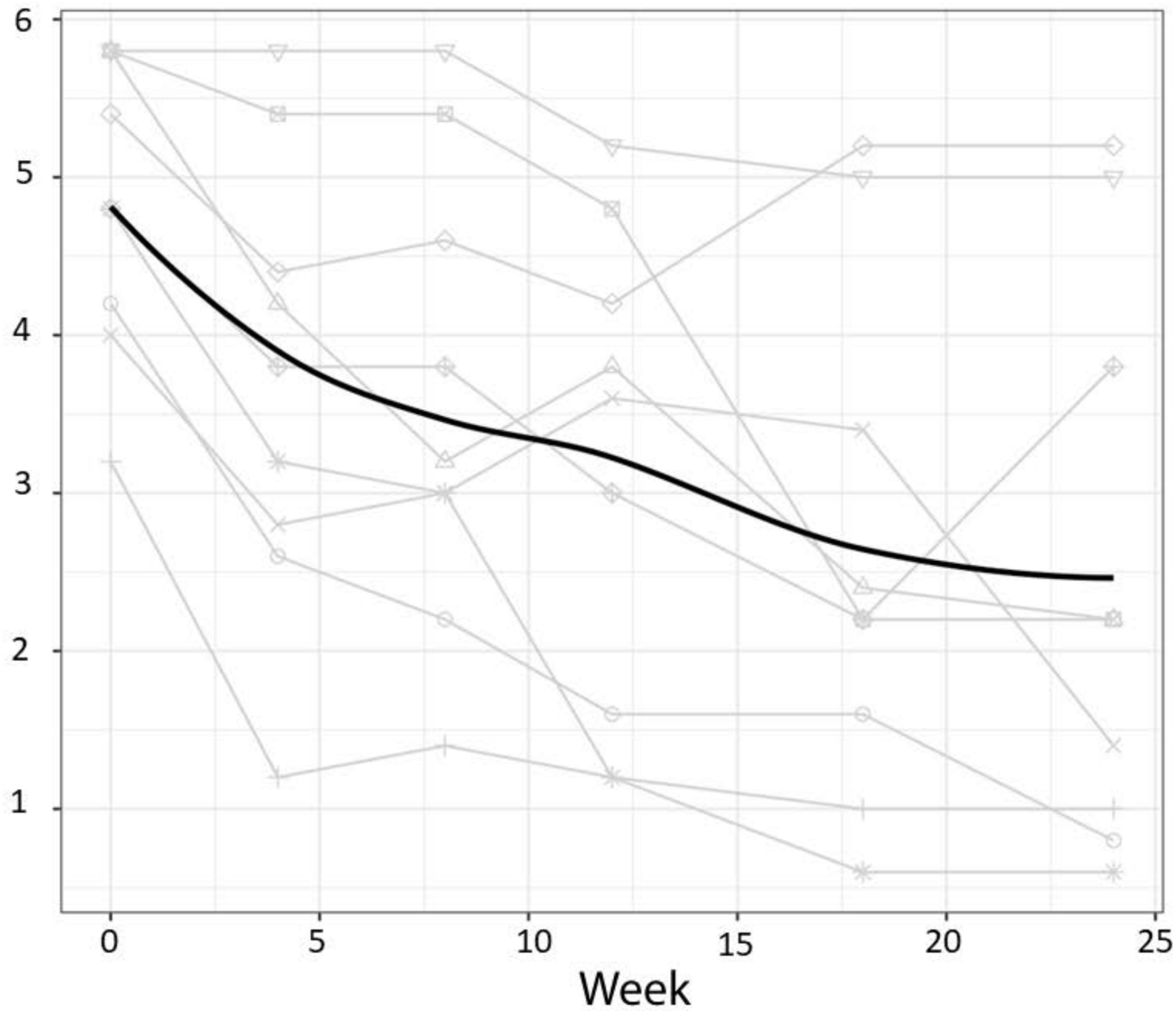
15 Bold lines indicate the mean values. There was a significant improvement over  
16 time in all olfactory assessments. In addition, there was a significant  
17 improvement in the odor recognition threshold and odor detection threshold at 4  
18 weeks of treatment with valproic acid compared to the pre-treatment threshold.

**Table 1. Characteristics of the enrolled patients and treatment outcomes**

	Patient number										
<b>Characteristics</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>
<b>Age, years</b>	51	59	49	60	62	57	52	57	63	35	59
<b>Sex</b>	F	F	F	F	F	F	F	F	F	M	F
<b>Duration of disease until the first visit, months</b>	9	2	6	15	33	3	13	2	2	2	40
<b>Olfactory score</b>											
<b>Detection threshold</b>											
<b>Before treatment</b>	2.6	3.4	2.2	1.4	1.8	5.8	3.8	2.6	2.4	0.4	1.8
<b>After treatment</b>	-0.2	-0.2	1	0.2	0.6	3.2	0.6	0.2	1.2	-0.2	0.6
<b>Recognition threshold</b>											
<b>Before treatment</b>	4.2	5.8	3.2	4	5.4	5.8	5.8	4.8	4.8	4.4	2.2
<b>After treatment</b>	0.8	2.2	1	1.4	5.2	5.0	2.2	0.6	3.8	2.0	2.2
<b>VAS, mm</b>											
<b>Before treatment</b>	17	1	39	22	40	0	14	30	8	15	25
<b>After treatment</b>	54	22	80	28	39	5	32	70	10	42	42
<b>Week when treatment was stopped</b>	24	24	18	24	24	24	24	24	24	8	4
<b>Patient outcome</b>	Cured	Improved	Cured	Cured	No change	No change	Improved	Cured	Improved	Cured	No change

A)

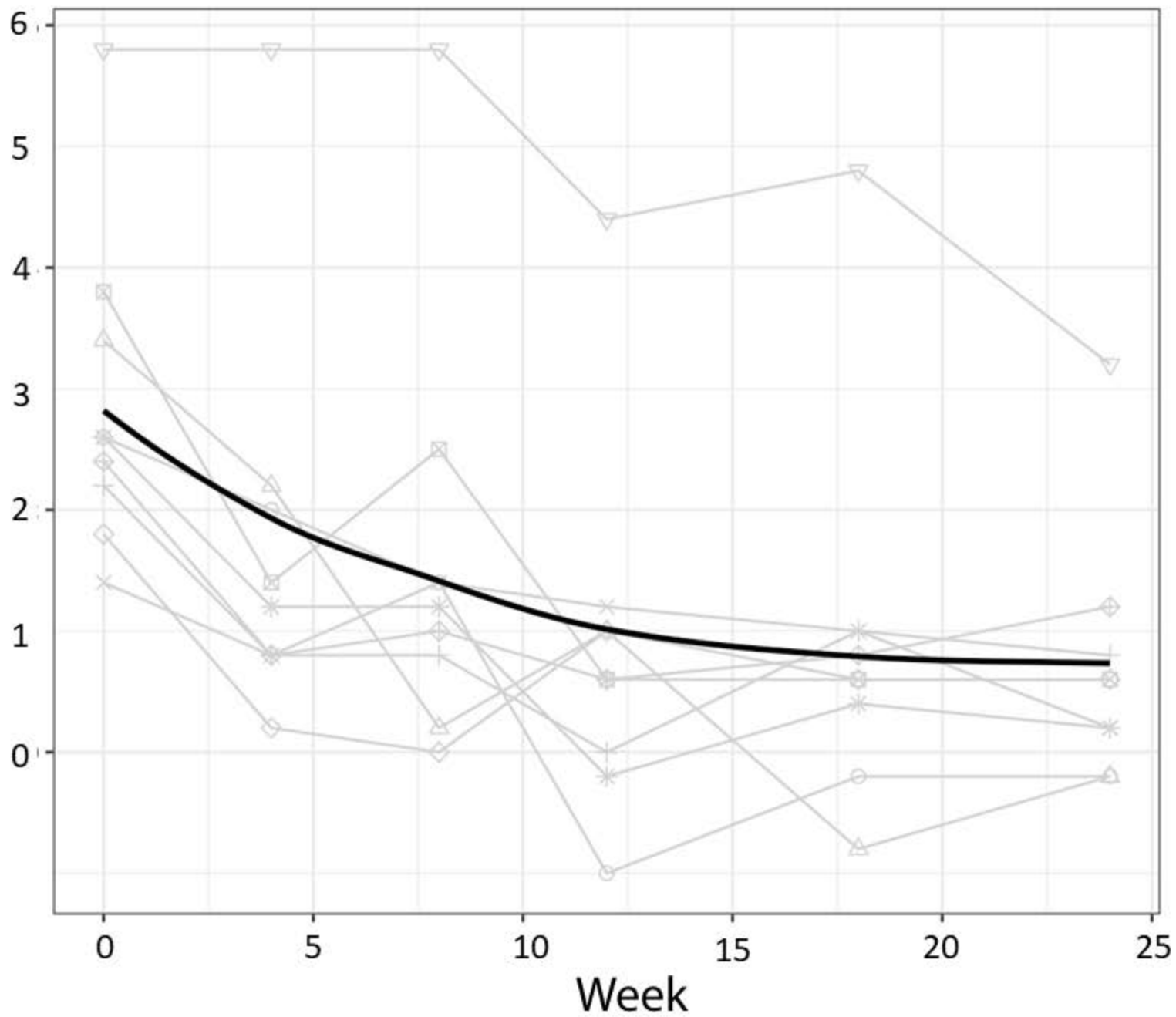
Recognition threshold score



- Patient 1
- △ Patient 2
- + Patient 3
- × Patient 4
- ◇ Patient 5
- ▽ Patient 6
- ⊠ Patient 7
- \* Patient 8
- ⊠ Patient 9

B)

Detection threshold score



- Patient 1
- △ Patient 2
- + Patient 3
- × Patient 4
- ◇ Patient 5
- ▽ Patient 6
- ⊠ Patient 7
- \* Patient 8
- ◊ Patient 9

C)

