ABSTRACT

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

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

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

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

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

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

INTRODUCTION

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

Olfactory training is the recommended treatment for PVOD[4,5]. The efficacy of olfactory training, which is safe and non-invasive, has been demonstrated

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

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

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

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

METHODS

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

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

Patient eligibility

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

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

Diagnosis

PVOD was diagnosed by a questionnaire and a clinical examination. All the patients were examined by computed tomography (CT) of the sinus, and nasal endoscopy. The diagnostic criteria were as follows: (1) history of upper respiratory infection before the olfactory loss; (2) sudden onset of olfactory loss; and (3) no evidence of conductive olfactory loss, such as rhinosinusitis, nasal polyps, mucosal edema of the olfactory fissure, deformation of the nasal septum, or neoplastic lesions, on examination by nasal endoscopy and sinus CT scan. Patients were excluded if they had a history of head trauma.

Olfactory assessment

Olfactory function was evaluated using a T&T olfactometer (Daiichi Yakuhin Sangyo Inc., Tokyo, Japan), which is the standard test for measuring the

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

Study design

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

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

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

Statistical analysis

Statistical comparisons between pre- and post-treatment periods were conducted by using univariate generalized estimating equations with adjustment for repeated measurements. Values of $P < 0.01$ were considered to indicate statistical significance. All statistical analyses were performed with R version 3.3.1[14]. Data are shown as the mean ± standard deviation.

RESULTS

Patient characteristics

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

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

Olfactory outcomes

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

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

Adverse events

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

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

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

The plasma level of VPA increased to 46.0 ± 10.1 μg/mL after 1 week of VPA treatment. During the treatment period, the plasma level of VPA was stable within the range of 29.2-69.7 μg/mL in each patient.

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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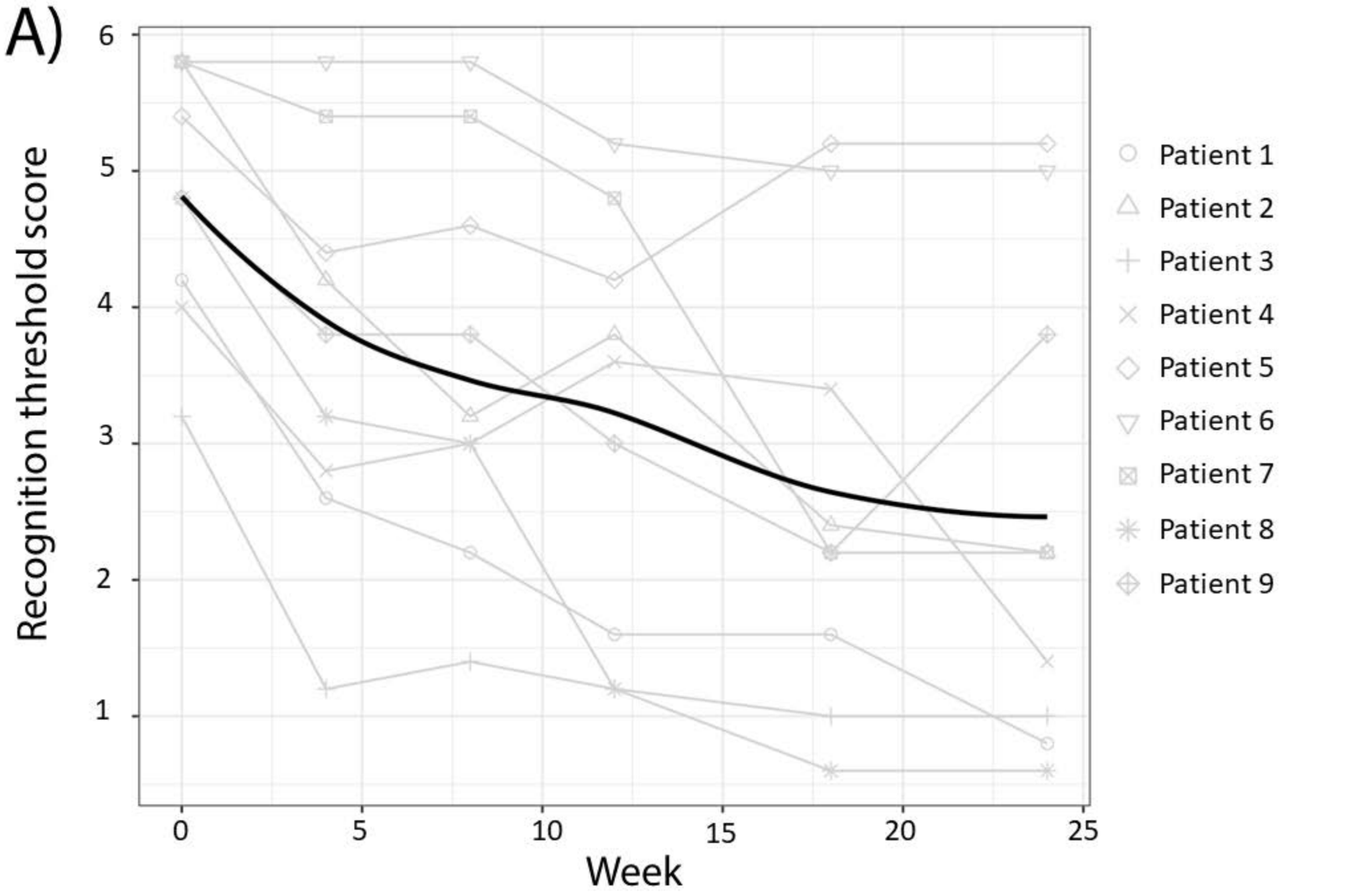
FIGURE LEGEND

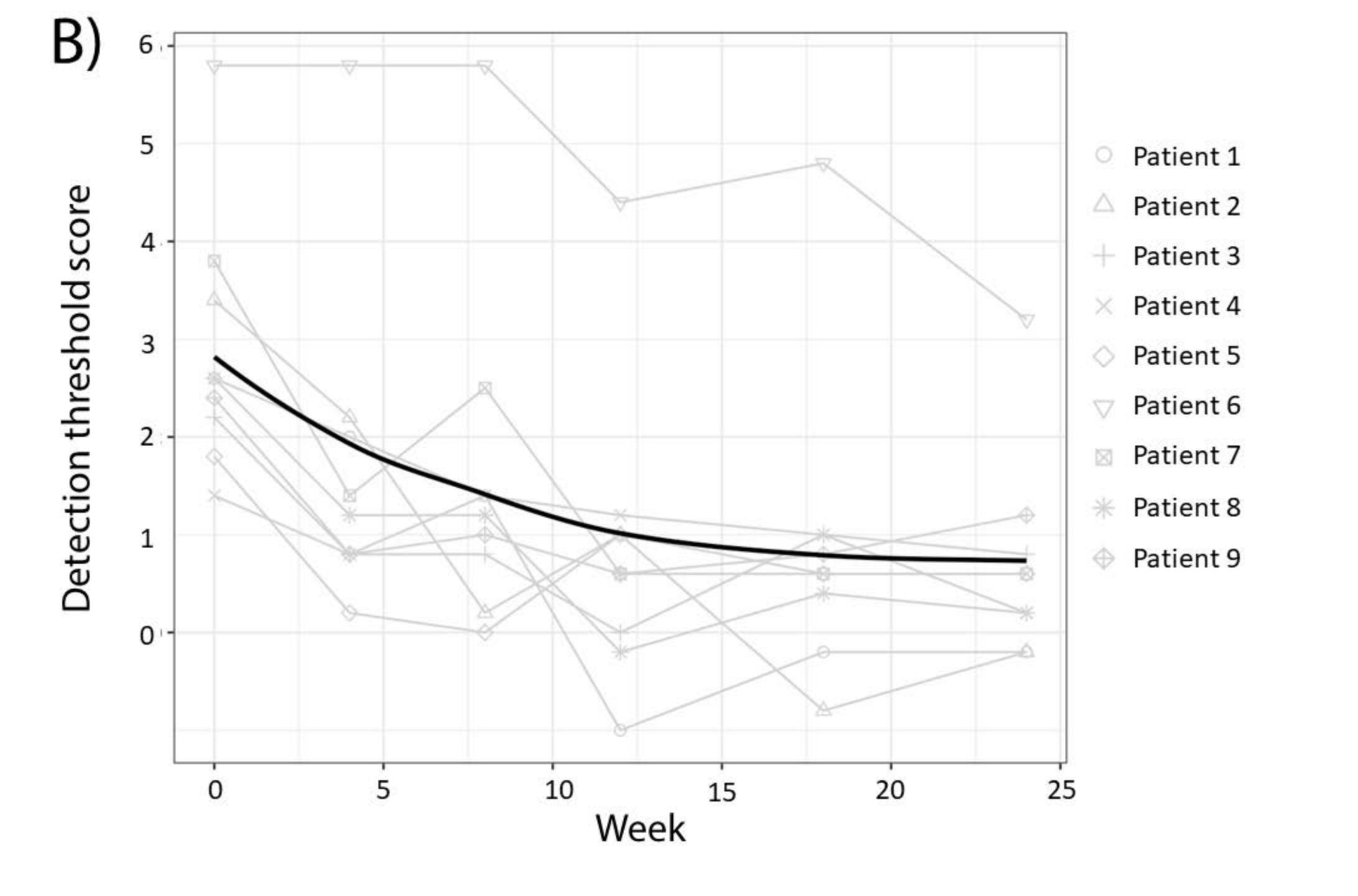
Table 1. Characteristics of the enrolled patients and treatment outcomes.
According to the criteria proposed by the Japan Rhinology Society, the degree of recovery is classified based on the odor recognition threshold score after treatment: ‘cured,’ when the odor score was restored to 2 or less; ‘improved,’ when the score was decreased by ≥1 from the pre-treatment score; ‘no change,’ when the score remained within 1 point of the pre-treatment score; and ‘worsened,’ when the score was increased by ≥1 from the pre-treatment score.

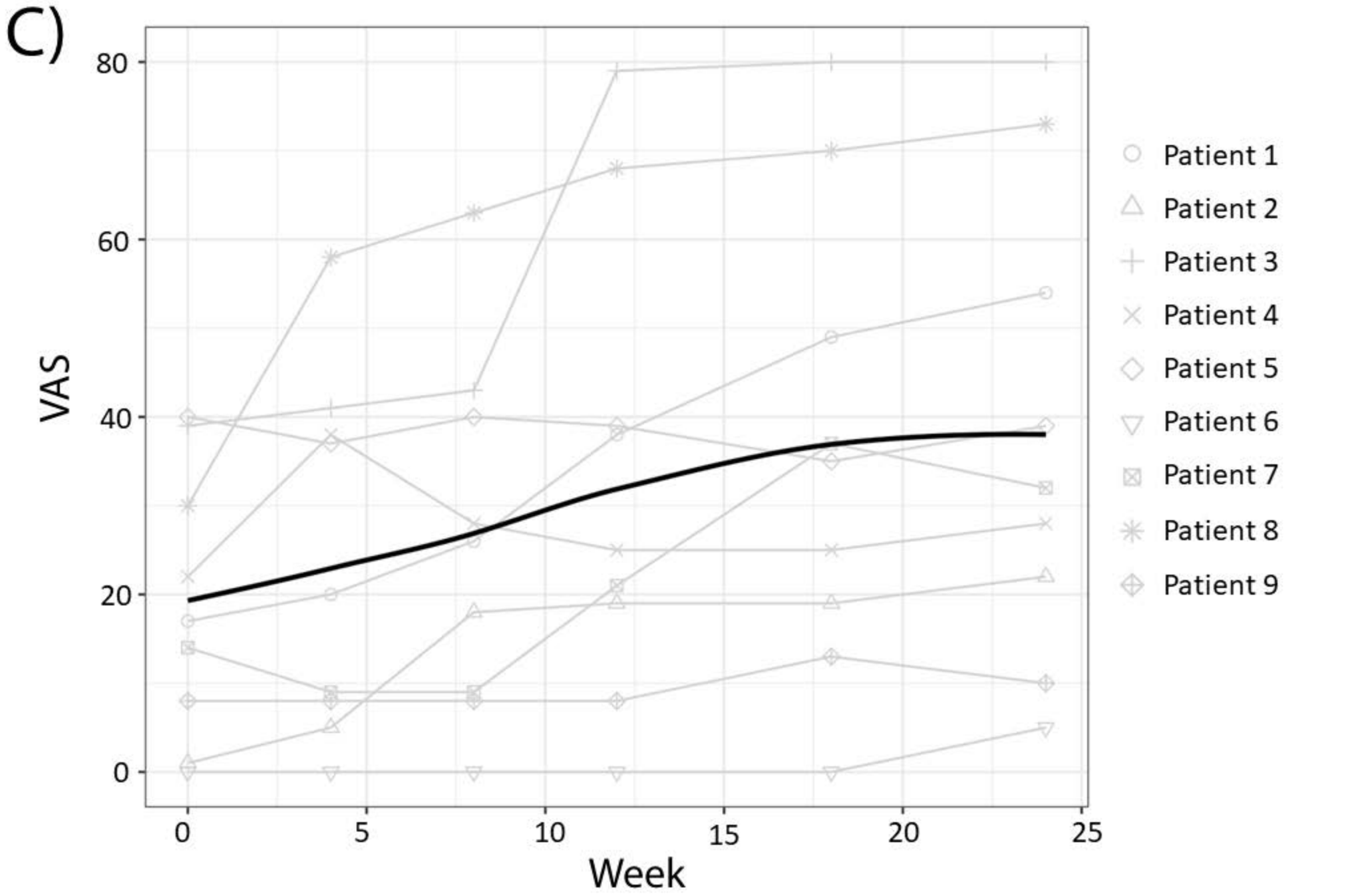
Figure 1. Odor scores for each patient at different timepoints during valproic acid (VPA) treatment. A) Odor recognition threshold scores. B) Odor detection threshold scores. C) Visual analog scale (VAS).
Bold lines indicate the mean values. There was a significant improvement over time in all olfactory assessments. In addition, there was a significant improvement in the odor recognition threshold and odor detection threshold at 4 weeks of treatment with valproic acid compared to the pre-treatment threshold.

Table 1. Characteristics of the enrolled patients and treatment outcomes

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


A)
Recognition threshold score
6
5
4
3
2
1
0
5
10
15
20
25
Week
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9


B)
Detection threshold score
6
5
4
3
2
1
0
0
5
10
15
20
25
Week
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9


C)
VAS
80
60
40
20
0
0
5
10
15
20
25
Week
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9