Paediatric Barcelona Olfactory Test − 6 (pBOT-6): Validation of a Combined Odour Identification and Threshold Screening Test in Healthy Spanish Children and Adolescents

Running title: Paediatric Barcelona Olfactory Test − 6 (pBOT-6)

Mariño-Sánchez F[1,2], Valls-Mateus M[2,3], Fragola C[1], de los Santos G[1,4], Aguirre A[1], Alonso J[1], Valero J[5], Santamaría A[1], Rojas Lechuga MJ[2,3], Cobeta I[1,4], Alobid I[2,3]*, Mullol J[2,3]*

*Equal contribution to senior responsibilities

[1]Unidad de Rinología y Cirugía de Base de Cráneo. Servicio de Otorrinolaringología. Hospital Universitario Ramón y Cajal. Madrid, Spain.
[2]Immunoal·lèrgia Respiratòria Clínica i Experimental (IRCE), Institut d'Investigacions Biomédiques August Pi i Sunyer (IDIBAPS). Research Group of Excellence 2017-SGR-1090 (Generalitat de Catalunya). Barcelona, Catalonia, Spain.
[3]Unitat de Rinologia i Clínica de l'Olfacte, Servei d'Otorinolaringologia, Hospital Clínic, Universitat de Barcelona, CIBERES. Barcelona, Catalonia, Spain.
[4]Universidad de Alcalá. Alcalá de Henares, Madrid, Spain.
[5]Departamento de Fisicoquímica. Facultat de Farmàcia. Universitat de Barcelona, Spain.

Corresponding author:
Joaquim Mullol
Unitat de Rinologia i Clínica de l'Olfacte, Servei d'Otorinolaringologia, Hospital Clínic de Barcelona
Carrer de Villarroel, 170, 08036 Barcelona, Spain.
E-mail: jmullol@clinic.cat

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0451

Abstract

Background: Few odour tests have been created for children.

Objectives: The aim of the present study was to develop and validate a simple and quick olfactory test, suitable for the evaluation of odour identification and threshold in a Spanish paediatric population, the paediatric Barcelona Olfactory Test-6 (pBOT-6).

Methods: The pBOT-6 consisted in a set of 6 odorants for a forced-choice identification test (IT), and a 6 dilutions phenyl ethyl alcohol geometric series for the threshold test (TT). The pBOT-6 was compared with the U-sniff test (a validated international paediatric smell test) in 131 Spanish healthy volunteers aged 6-17 years. A Bland-Altman plot was used to determine the agreement between two tests. Reliability was analyzed in fifteenvolunteers using the intraclass correlation coefficient (ICC). Normative data was obtained and 8 children diagnosed with subjective smell loss were tested for validation.

Results: Bland-Altman analysis demonstrated a minimal bias of -1.71% with upper and lower limit of agreement of -31.1% and 27.6%, respectively. The ICC was 0.83 (95% CI 0.6-0.96) for the IT and 0.73 (95% CI 0.36-0.9) for the TT, showing excellent and good consistency between measurements over time. Mean pBOT-6 scores were significantly higher in healthy volunteers compared with patients with smell loss. Discrimination between normosmia and smell loss was achieved with a sensitivity of 96.9% and a specificity of 100%.

Conclusions: The pBOT-6 offers an effectiveand fast method useful in clinical routine to distinguish, with high sensitivity and specificity, between paediatric patients with normosmia and those with smell dysfunction.

Key words: olfaction, smell test, paediatric, children, smell loss.

doi: 10.18176/jiaci.0451

Introduction

The sense of smell provides humans with information on the surroundingenvironment[1,2]. It has been suggested that olfactory function is linked to learning[3], and smell disorders could be an important handicap in children's development.

Studies assessing smell dysfunction in children are scarce, even though several causes of olfactory dysfunction (e.g. congenital anosmia, allergic rhinitis, head trauma, adenoidal hyperplasia, and turbinate enlargement) are common among paediatric population[4-7].

Several odouridentification tests have been developed in different countries for clinical use, mainly in adults[8-11]. However, the nature of odour identification, usually limits the use of olfactory tests to the country or region where they have been developed and validated.

The Barcelona Smell Test (BAST-24)[12] is commonly used in Spain. However, this test may not be adequate for children.Its application takes approximately 30 to 45 minutes and require high level of concentration during the procedure. Therefore, it might be particularly challenging for children who become tired more easily and have shorter attention span than adults, which may result in higher arbitrariness in their answers. Moreover, odour identification score might depend on children's verbal skills[13].

Some smelltests have been created for children[14-17], however, they are more difficult to obtain and they may not be suitable for very young children. Recently, a new international odour identification test for children, the Universal Sniff Test (U-Sniff),has been validated in 19countries[18]. However, this test does not include a threshold test to complement identification task for the assessment of sensorial dysfunction. A composite analysis of several components of olfaction, especially including assessment of odour thresholds, provides the most meaningful approach to human olfactory function[19,20].

The objectives of the present study were to develop a simple and quickolfactory test,suitable for the evaluation of odouridentification and threshold in a Spanishpopulation aged 6-17 years and to assess the reproducibility and validation of the test.

doi: 10.18176/jiaci.0451

Materials and Methods

Study population

One hundred and thirty-one Spanish healthy volunteersaged 6-17 years with subjective normal sense of smell were included in the study from February to September 2016 at a tertiary-care center.All children and adolescents were healthy, community volunteers of middle socioeconomic class. According to age, volunteers were stratified in four groups: 6-8, 9-11, 12-14 and 15-17 years.

Exclusion criteria were: upper respiratory tract infection in the last two weeks, known psychiatric or neurocognitive impairment, nasal inflammatory disorders, previous nasal surgery, diabetes mellitus, renal failure or any other disease linked to olfactory dysfunction.

Study Design

The Ethics Committee of our institution approved the study and signed informed consent was obtained from volunteer's legal guardians and adolescents (≥12 years old) gave their assent. Additionally, children (<12 years old) gave their oral assent.

Each volunteer was tested individually in a noise isolated, well ventilated room with controlled humidity and temperature (21-23ºC).Individuals were tested simultaneously at both nostrils, first for smell identification and then for smell threshold.

To compare the results of our smell test with an already validated and standardized smell identification method in children, all volunteers were also tested using the U-Sniff Test[18].

Children were randomized to perform first the paediatric Barcelona Olfactory Test-6 (pBOT-6) test or the U-Sniff test. The duration of each test was recorded.

A group of 15children was tested in three separate sessions with a two-weeks interval between examinations to evaluate the test-retest reliability.Additionally, 8children previously assessed with the pediatric Smell Wheel Test [16]: 4diagnosed with isolated congenital anosmia (ICA), and 4 with partial loss of smell due to inflammatory causes (1 nasal polyposis in cystic fibrosis, 2 adenoidal hyperplasia, and 1chronic rhinosinusitis without nasal polyps) were included for test validation.

doi: 10.18176/jiaci.0451

Subjective Olfactometry

1. **Paediatric Barcelona Olfactory Test (pBOT-6)**

1.1.Smell Identification test

Selection of odorants included in the test was based on a comprehensive review of the main olfactory tests reported in the literature. From a list of more than 50 odours, a panel of experienced investigators selected the final odoursto be incorporated in the test. Criteria used to choose odours were: i) easy identifiable and recognizable by young children in Spanish population; and ii) cost-effective and easy to manufacture as chemical compounds (odorants).

Six odorants were selected for inclusion in the identification pBOT-6 (Table 1): i) 5 odours producing little or no trigeminal excitation: banana, chocolate, lemon, mintand flower/rose; and ii)1 odour producing a strong trigeminal stimulation: vinegar. Hermetic glass containers were designed to contain the different odorants (Figure 1A), according to the recommendations of the Meeting of the German Society for Otorhinolaryngology[10].

Volunteers were requested to identify the odour from four given image descriptors (Table 1) labeled with their names, which were shown before odorant presentation in a computer screen using an Excel spreadsheet (Microsoft Office Professional Plus 2013).Each odorant jar was presentedone at a time by holding it 1cm in front of the nose for 2-3 seconds with no contact with explorer's finger orsubject's face. If uncertain, children were allowed to smell the odorant up to 3 times. The test was repeated for each of the 6odours.The explorer clicked on the label selected by the volunteer, and a macro created in Microsoft Excel changed the screen for the image descriptors of the next odour and automatically calculated scores (Figure 1B).

The sum of correct identification answers (0-6/6) was used to obtain theidentification score (IS), which was also expressed as percentage of the total number of presented odorants (0-100%).

1.2.Smell threshold test

doi: 10.18176/jiaci.0451

Phenyl ethyl alcohol (PEA, rose scent) was employed for the threshold test, with 6 dilutions of a geometric series presented in sniff bottlescontaining 20ml of solution (Figure 1C). The solvent for PEA was propylene glycol.

Detection threshold measurement was obtained using a single ascending forcedchoice methodwidely used in Japan [21,22], beginning with the lowest concentration (Bottle 6, 0.0002%), and increasing PEA concentration gradually (Bottle 5, 0.002%; Bottle 4, 0.02%; Bottle 3, 0.2%; Bottle 2, 2%; and Bottle 1, 20%). With each bottle participants were asked to respond "yes" or "no" to the question "do you smell something?" The dilution step at which the odorant stimulus was first detected was used to define detection threshold. Before testing, volunteerswere instructed to say "yes" only when they were certain they had detected the odour, but they were not asked to identify it. If unsure, the subjects were instructed not to guess. PEA threshold measurement was reported in a numeric scale corresponding to the number of the bottle (1 to 6) detected by the subject which defined the subject's threshold score (TS). If the subject was not able to detect the most concentrated dilution (Bottle 1, 2%) a number "0" was assigned.

1.3.Universal-Sniff test for children (U-Sniff)

The pBOT-6 was compared with the U-sniff test, that contains 12 odour items presented as pen-like "sniffin' sticks", administered in a four answer forced choice model using image and name of odours, with an IS of 0 to 12 (0-100%)[18].

Statistical Analysis

Data management and statistical analysis was performed using Epiinfo for Windows (Epiinfo™ 7.1.5; Atlanta, USA) and MedCalc for Windows (MedCalc version 15, Ostend, Belgium; http://www.medcalc.org). A Bartlett's test was performed to evaluate the homogeneity of variances.

Frequencies, means and standard deviations (SD), were calculated for the demographic and clinical characteristics of the participants. ANOVA test was used to analyze gender distribution and smell outcomes differences according to gender and age ($p \leq 0.05$ was considered statistically significant).

A Bland-Altman plot was used to compare pBOT-6withU-Sniff test.For each IS, the average of pBOT-6 and U-Sniff test were calculated and then plotted against the difference of the two measurements. The limits of agreement (LoA) were defined as the

doi: 10.18176/jiaci.0451

mean difference ± 1.96 SD of differences. A 95% confidence interval of the LoA was used to define agreement between the two smelltests[23].

The correlation between smell scores and age was assessed using a linear regression analysis for all patients. Additionally, a Mann-Whitney two-sample test was used to analyze differences of IS and TS between age groups, and between healthy volunteers and smell loss patients.

The reliability over time (test-retest) was analyzed using the intraclass correlation coefficient (ICC). The strength of the ICC values was interpreted according to Shrout and Fleiss[24]as <0.40poor, 0.40-0.75 fair to good, and >0.75 excellent consistency among measurements.Using Walter et al. formula [25], we have calculated that a minimum sample size of 13 healthy children with 3 observations per subject would be required to achieve the statistical significance for an alpha-value set at 0.05 and with the minimum power of at least 80%.

In order to validate the test todifferentiate subjects with a normal smell function from those with partial or total smell loss, the 10^{th} percentile was used as a cut-off point, based on pre-existing tests[8,12,26]. A receiver operator characteristics (ROC) curve analysis was performed in conjunction with the Youden index to define the highest sensitivity and specificity of the "pBOT-6" test. A group of 8 children with smell loss diagnosed by the smell wheel test [16]were also tested for validation.

According to a sample size calculation made by Hugh et al.[17] (p value of 0.05, power of 0.80, clinically significant difference of 1.86 and standard deviation of 1.63), eight participants were required per age group. However, more volunteers aged 6-8 years were recruited in order to validate the test in youngest children, who may presentmore unfamiliarity of the odours.

doi: 10.18176/jiaci.0451

Results

1. Demographic Characteristics

One hundred and thirty-one healthy volunteers (mean age 9±2.6 years; female 58%) were enrolled. The majority of participants were children aged 6-8 years. Age groups were homogeneous in terms of gender (Table 2).

All volunteers understood the task and were able to perform both smell tests. The mean duration of pBOT-6 test (identification + threshold) was 2.33 ± 0.44 minutes. The mean duration of U-Sniff test was 2.55 ± 0.57 minutes. The meanpBOT-6 total IS was 87.5 ± 13.6%. Figure 2 displays the mean IS for each odour. Lemon was the most commonly identified correctly, and banana was the least frequently identified odour. Mean pBOT-6 IS was 88 ± 14.7% for girls and 86.7 ± 11.8% for boys (p=0.5). Mean TS was 3.1 ± 1.2 for girls and 3 ± 1 for boys (p=0.6)

Additionally, 8 children with smell loss (total or partial) were included for test validation (Table 3). Odour identification scores were significantly lower for patients with smell losscompared withhealthy volunteers, but this difference was less pronounced for vinegar odour (Figure 2).

2. Agreement between BOT-6 and U-Sniff test

Bland-Altman analysis demonstrated a minimalbias of -1.71% with upper and lowerlimit of agreement of -31.1% and 27.6%, respectively. After calculating the mean difference and the standard deviation of the difference, we would expect most of the differences to lie between the limit of agreement. Hence, according to the Bland-Altman method, there was agood degree of correlation and agreement between pBOT-6 and U-Sniff test (Figure 3).

Figures4A and 4B show a moderate correlation between pBOT-6 IS and age (r=0.26; 95% CI 0.09-0.41; p<0.05), and between U-Sniff IS and age (r=0.31; 95% CI 0.14-0.45; p<0.001), respectively. Figure 4C shows no significant correlation between PEA threshold score and age (r=0.14; 95% CI -0.04-0.29; p>0.05).

Figure 5A shows a significant increaseof IS in older age groups (p<0.001) without significant differences between U-Sniff and pBOT-6 tests. Figure 5B shows no differences (p>0.05) in TS between age groups.

doi: 10.18176/jiaci.0451

3. Reliability (test-retest)

When analyzing olfactory scores at weeks 0, 2 and 4 in fifteenvolunteers (table 4), the ICC was 0.83 (95% CI 0.6-0.96) for the pBOT-6 IS and 0.73 (95% CI 0.36-0.9) for the TS, showing excellent and good consistency between measurements over time respectively.

4. Normative values

To separate normosmia from olfactory dysfunction we applied the 10^{th} percentile cutoff to our data sample for the IS at every age. According to the 10^{th} percentile an IS of 4/6 in children aged6-8 and 9-11 years; and an IS of 5/6 in subjects aged 12-14 and 15-17 yearsis considered normosmic. Therefore, scores below these values can be considered as smell loss. Regarding PEA threshold test, the 10^{th} percentile cutoff defined normosmiaas a TS of 2/6 for all age groups.

5.Validation

A group of eight children diagnosed with subjective smell loss (4 children with ICA and 4 children with hyposmia caused by inflammatory conditions) were analyzed (Table 3).

The 4 patients included with partial loss of smell had very low Smell Wheel test identification scores (<4/11). None of the 4 patients with total smell loss (ICA) were able to detect or identify any of the Smell Wheel scratch and sniff odorants.

Mean pBOT-6 IS(Figure 6A) and TS (Figure 6B) were significantly higher in healthy volunteers compared with patients with smell loss.By using the highest Youden index, a sensitivityof 96.9% and a specificity of 100% to confirm a normal senseof smell were reached when a cut-off of ≥4/6 points in IS was used. For PEA threshold test, a sensitivity of 66.4% and a specificity of 87.5% to confirm a normal sense of smell were reached when a cut-off of ≥2/6 points in TS was used.

doi: 10.18176/jiaci.0451

Discussion

In the current study, we developed and validated the "pBOT-6" smell identification and threshold test for childrenaged 6-17 years. This is the first smell test designed specifically for children that includes a threshold test. Normative values for healthy Spanish population were determined and reliability of the test was corroborated. All participants, including children as young as 6 years old, were able to understand and complete the test.

Bland-Altman analysis showed a significant correlation and agreement between pBOT-6 and U-Sniff tests. Additionally, ICC values showed consistency between measurements of pBOT-6 identification and threshold tests over time. Moreover, normative values showed high sensitivity and specificity to diagnose smell loss in children with ICA or inflammatory conditions associated with hyposmia.

Performance of both U-Sniff and pBOT-6 identification scorescorrelated and increased with age. This is in the same line with previous studies demonstrating age-related increases in children's IS[14,17,18,27]. However, in the current study,although we observed a tendency toward a better threshold score with age, differences did not reach statistical significance.A previous study evaluated olfactory threshold in children using a modified "Sniffin' Sticks" threshold test[20]. They reported an increase in threshold scores with age. However, they used a three-alternative-forced-choice test which might take a longer time and requiresa higher level of concentration, making difficult for young children to perform adequately. In the pBOT-6 threshold test, we used a single ascending non-forced choice method. This is a fast and very easy method for young children in which they are asked to detect, and not to identify, an odorant. These results are in line with other studies that have found noodour threshold differences between children and young adults[1,28], suggesting that the ability to identify odours is related to perceptual learning with age, but this cognitive ability does not extend to sensorial smell threshold detection, as detection is purely sensorial and therefore not affected by experience[19,29].The importance of using a threshold test lies in the fact that a composite analysis of several components of olfaction provides a more comprehensive approach to olfactory function than smell identification alone, facilitating diagnosis of early stages of hyposmia[19].

doi: 10.18176/jiaci.0451

It's well known that adult woman outperform men in olfactory tasks[30,31]. However, gender difference in smell function in children is controversial. Some studies have reported that girls outperform boys[13,15,18]. Nevertheless, in accordance with other studies[14,16,32,33], wefound no differences in pBOT-6 identification and threshold scores between girls and boys. As pBOT-6 was designed to be a simple, quick and easy to perform screening test, it might be not sufficient to detect subtle gender differences. Furthermore, some studies show that female smell function superiority decreases when men are provided with some help in the retrieval of odour names[32] (pictures and labels in pBOT-6).

Healthy volunteers showed higher pBOT-6 identification scores than smell loss patients for all odorants, but this difference was less noticeable for acetic acid odorant (vinegar). Probably, some children with olfactory loss are able to detect vinegar due to its strong stimulation of trigeminal receptors[34]. Acetic acid (AcOH) has been described as a trigeminally potent chemical stimuli [35]. It produces a stimulation of a specific trigeminal receptor (TRPV1) even in very low concentrations leading to a tingling perception, which in higher concentrations becomes sharp, burning, and even painful[36].

Someodour identification tests have been designedfor children to distinguish between normosmia and smell dysfunction[14-16,18]. However, odour identification differs significantly across countries[18]. Furthermore, performance relies on prior exposure to and familiarity with the presented odours, which may differ acrosscultures[37,38]. This limitation is particularlyrelevant for paediatric population where experience, semantic memory, and verbal skills affectodour tasks proficiency.pBOT-6 was developed specifically for Spanish children, as a short olfactory screening test, easy to perform, and designed to be used in daily clinical practice. Total IS was near 88%, comparable with other smell tests developed for children such as NIH-Toolbox[39] (72%), Smell Wheel[16] (70-90%) and U-Sniff[18] (69-93%).

When compared with the U-Sniff test, which has been recently validated across different countries[18], the pBOT-6 showed a good correlation and agreement according with Bland-Altman plot. The time required to perform the combinedidentification and threshold test was less than 3 minutes, a duration similar to U-Sniff identification test alone. The main advantage of the rapidity of pBOT-6 is that young children are able to maintain attention, decreasing the probability of randomness

doi: 10.18176/jiaci.0451

in their responses. Additionally, the test can be used as part of the standard in-office clinical assessment.

We believe U-Sniff test is an excellent tool to evaluate olfaction in Spanish children. However, we think of pBOT-6 smell identification test as a fast screening tool feasible to use in daily clinical practice. Children with smell loss screened by pBOT-6 can be further studied and diagnosed with U-Sniff test and PEA threshold test to complement olfactory function assessment.

Test-retest pBOT-6 ICC values showed excellent (0.8) and good (0.7) consistency among identification and threshold measurements over time, respectively. Similar levels of reliability using Pearson correlation have been noted in Smell Wheel[16] (r=0.7) and U-Sniff[18] (r=0.83) identification tests. However, pBOT-6 was more reliable than other paediatric tests such as the Sniffin' kids[14] (r=0.44) or the NIH-Toolbox[39] (r=0.45).

Only three paediatric smell identification tests have included patients with olfactory dysfunction for validation[14,15,18] during test development. Although we included only 8 children diagnosed with smell loss in the present study, children with ICA and sinonasal inflammatory disorders scored significantly lower p-BOT-6 identification and threshold scores than healthy volunteers. Additionally, Youden index cutoff points (4/6 for IS and 2/6 for TS) were able to differentiate normosmia from smell loss with high levels of sensitivity and specificity.This cutoff points coincided with the 10^{th} percentile values, which are frequently used to separate normal from reduced sense of smell in olfactory testing[8,14,15,18,39].

Limitations

First, the main limitation of the present study was the number of odorants used for the identification task, which might be insufficient to characterize accurately smell function and to define the severity of hyposmia. However, we decided to include only 6 odorants, in order to maintain as much as possible the attention span of children, and to be able to use the test in daily clinical practice as a screening tool. Some brief smell identification tests with less than 6 items have been developed to identify anosmia in adults with a high degree of specificity[40-42]. Richman et al.[43] validated a rapid 5 microencapsulated odorant test based on "Scratch and Sniff'' technique in a large population of healthy children and adolescents. However, they did not study the

doi: 10.18176/jiaci.0451

efficacy of the test in children with olfactory dysfunction. In the present study, pBOT-6 showed a high degree of sensitivity and specificity to diagnose smell dysfunction in 8 children with well-known causes of olfactory loss. However, smell loss patients were initially evaluated with the Smell Wheel test[16], which has no normative/reference values published to date. Therefore the difference between partial or total loss of smell was initially based on patient's subjectivity and parent's opinion. A much larger sample of such children should be evaluated with the test to characterize its efficacy for evaluating olfactory ability.Second, we did not conduct any cognitive test. Hence, the influence of cognition on odour identification ability could not been observed in the current study. Third, selection of odorants was madebased on the experience of participating researchers and consequently, it is possible that other odour items also would have been appropriate for inclusion.And forth, the lack of objective smell measurements. Although objective smell tests, such as odour-evoked response potentials and functional magnetic resonance imaging have been used in olfaction research, they are expensive and its clinical use in humans has been limited to specialized smell and taste clinics. Forth, this is the first study to use this olfactory threshold test in chidren. Therefore, validation of the test correlating it with an already validated pediatric threshold test would be necessary. However, to the best of our knowledge, there are no odor detection threshold tests specifically designed for children in the literature.Few adult odor threshold test have been previously used in pediatric population. The Lyon Clinical Olfactory Test [13] and the "Sniffin' Sticks" olfactory threshold test [20] seem suitable and reliable for children and adolescents. However, these tests were developed in a specific country with country specific odors that may not be suitable for Spanish children.

Conclusions

With the 6-item odour identification test and the 6-dilution odour threshold tests, we propose a valid and reliable tool, the "paediatric Barcelona Olfactory Test – 6" (pBOT-6), to rapidly assess olfactory function in Spanish children and adolescents. This test offers anefficient and fast method useful in clinical routine to distinguish,with high sensitivity andspecificity,between paediatric patients with normosmia and those with a partial (hyposmia) or total (anosmia) loss of smell.

doi: 10.18176/jiaci.0451

Funding
The authors declare that no funding was received for the present study

Conflicts of interests:
The authors declare they have nothing to disclose.

References

[1] Hummel T, Bensafi M, Nikolaus J, Knecht M, Laing DG, Schaal B. Olfactory function in children assessed with psychophysical and electrophysiological techniques. Behav Brain Res. 2007;180(2):133-8.
[2] Hummel T, Whitcroft KL, Andrews P, Altundag A, Cinghi C, Costanzo RM, et al. Position paper on olfactory dysfunction. Rhinol Suppl. 2017;54(26):1-30.
[3] Li W, Luxenberg E, Parrish T, Gottfried JA. Learning to smell the roses: experience-dependent neural plasticity in human piriform and orbitofrontal cortices. Neuron. 2006;52(6):1097-108.
[4] Langdon C, Guilemany JM, Valls M, Alobid I, Bartra J, Davila I, et al. Allergic rhinitis causes loss of smell in children: The OLFAPEDRIAL study. Pediatr Allergy Immunol. 2016;27(8):867-70.
[5] Marino-Sanchez F, Valls-Mateus M, Haag O, Alobid I, Bousquet J, Mullol J. Smell loss is associated with severe and uncontrolled disease in children and adolescents with persistent allergic rhinitis. J Allergy Clin Immunol Pract. 2018;6(5):1752-5.e3.
[6] Bousquet J, VandenPlas O, Bewick M, Arnavielhe S, Bedbrook A, Murray R, et al. The Work Productivity and Activity Impairment Allergic Specific (WPAI-AS) Questionnaire Using Mobile Technology: The MASK Study. J Investig Allergol Clin Immunol. 2018;28(1):42-4.
[7] Del Cuvillo A, Sastre J, Colas C, Navarro AM, Mullol J, Valero A. Adaptation to Spanish and validation of the Rhinitis Control Assessment Test (RCAT) questionnaire. J Investig Allergol Clin Immunol. 2019:0. doi: 10.18176/jiaci.0420 (in press).
[8] Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol Behav. 1984;32(3):489-502.
[9] Cain WS. Bilateral interaction in olfaction. Nature. 1977;268(5615):50-2.
[10] Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S. "Sniffin' sticks": screening of olfactory performance. Rhinology. 1996;34(4):222-6.
[11] Briner HR, Simmen D. Smell diskettes as screening test of olfaction. Rhinology. 1999;37(4):145-8.
[12] Cardesin A, Alobid I, Benitez P, Sierra E, de Haro J, Bernal-Sprekelsen M, et al. Barcelona Smell Test - 24 (BAST-24): validation and smell characteristics in the healthy Spanish population. Rhinology. 2006;44(1):83-9.
[13] Monnery-Patris S, Rouby C, Nicklaus S, Issanchou S. Development of olfactory ability in children: sensitivity and identification. Dev Psychobiol. 2009;51(3):268-76.
[14] Schriever VA, Mori E, Petters W, Boerner C, Smitka M, Hummel T. The "Sniffin' Kids" test--a 14-item odor identification test for children. PLoS One. 2014;9(6):e101086.

[15] Richman RA, Post EM, Sheehe PR, Wright HN. Olfactory performance during childhood. I. Development of an odorant identification test for children. J Pediatr. 1992;121(6):908-11.
[16] Cameron EL, Doty RL. Odor identification testing in children and young adults using the smell wheel. Int J Pediatr Otorhinolaryngol. 2013;77(3):346-50.
[17] Hugh SC, Siu J, Hummel T, Forte V, Campisi P, Papsin BC, et al. Olfactory testing in children using objective tools: comparison of Sniffin' Sticks and University of Pennsylvania Smell Identification Test (UPSIT). J Otolaryngol Head Neck Surg. 2015;44:10.
[18] Schriever VA, Agosin E, Altundag A, Avni H, Cao Van H, Cornejo C, et al. Development of an International Odor Identification Test for Children: The Universal Sniff Test. J Pediatr. 2018;198:265-72.e3.
[19] Lotsch J, Reichmann H, Hummel T. Different odor tests contribute differently to the evaluation of olfactory loss. Chem Senses. 2008;33(1):17-21.
[20] Gellrich J, Stetzler C, Oleszkiewicz A, Hummel T, Schriever VA. Olfactory threshold and odor discrimination ability in children – evaluation of a modified “Sniffin’ Sticks” test. Sci Rep. 2017;7:1928.
[21] Takagi S. Olfactory Tests. In: Takagi S, editor. Human olfaction. Tokyo: University of Tokyo Press, 1989; 35-71.
[22] Tsukatani T, Miwa T, Furukawa M, Costanzo RM. Detection Thresholds for Phenyl Ethyl Alcohol Using Serial Dilutions inDifferent Solvents. Chemical Senses. 2018;28(1):25-32.
[23] Stockl D, Rodriguez Cabaleiro D, Van Uytfanghe K, Thienpont LM. Interpreting method comparison studies by use of the bland-altman plot: reflecting the importance of sample size by incorporating confidence limits and predefined error limits in the graphic. Clin Chem. 2004;50(11):2216-8.
[24] Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979;86(2):420-8.
[25] Walter SD, Eliasziw M, Donner A. Sample size and optimal designs for reliability studies. Stat Med. 1998;17(1):101-10.
[26] Cain WS, Gent JF, Goodspeed RB, Leonard G. Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center. Laryngoscope. 1988;98(1):83-8.
[27] van Spronsen E, Ebbens FA, Fokkens WJ. Olfactory function in healthy children: normative data for odor identification. Am J Rhinol Allergy. 2013;27(3):197-201.
[28] Cain WS, Stevens JC, Nickou CM, Giles A, Johnston I, Garcia-Medina MR. Life-span development of odor identification, learning, and olfactory sensitivity. Perception. 1995;24(12):1457-72.
[29] Marino-Sanchez FS, Alobid I, Cantellas S, Alberca C, Guilemany JM, Canals JM, et al. Smell training increases cognitive smell skills of wine tasters compared to the general healthy population. The WINECAT Study. Rhinology. 2010;48(3):273-6.
[30] Mullol J, Alobid I, Marino-Sanchez F, Quinto L, de Haro J, Bernal-Sprekelsen M, et al. Furthering the understanding of olfaction, prevalence of loss of smell and risk factors: a population-based survey (OLFACAT study). BMJ Open. 2012;2(6):e001256.
[31] Liu G, Zong G, Doty RL, Sun Q. Prevalence and risk factors of taste and smell impairment in a nationwide representative sample of the US population: a cross-sectional study. BMJ Open. 2016;6(11):e013246.
[32] Sorokowska A, Schriever VA, Gudziol V, Hummel C, Hahner A, Iannilli E, et al. Changes of olfactory abilities in relation to age: odor identification in more than 1400 people aged 4 to 80 years. Eur Arch Otorhinolaryngol. 2015;272(8):1937-44.
[33] Dzaman K, Zielnik-Jurkiewicz B, Jurkiewicz D, Molinska-Glura M. Test for screening olfactory function in children. Int J Pediatr Otorhinolaryngol. 2013;77(3):418-23.
[34] Wang YY, Chang RB, Allgood SD, Silver WL, Liman ER. A TRPA1-dependent mechanism for the pungent sensation of weak acids. J Gen Physiol. 2011;137(6):493-505.

doi: 10.18176/jiaci.0451

[35] Hucke CI, Pacharra M, Reinders J, van Thriel C. Somatosensory Response to Trigeminal Stimulation: A Functional Near-Infrared Spectroscopy (fNIRS) Study. Sci Rep. 2018;8(1):13771.
[36] Cometto-Muniz JE, Cain WS, Abraham MH. Nasal pungency and odor of homologous aldehydes and carboxylic acids. Exp Brain Res. 1998;118(2):180-8.
[37] Goldman WP, Seamon JG. Very long-term memory for odors: retention of odor-name associations. Am J Psychol. 1992;105(4):549-63.
[38] Sorokowska A, Sorokowski P, Hummel T. Cross-Cultural Administration of an Odor Discrimination Test. Chemosens Percept. 2014;7(2):85-90.
[39] Dalton P, Mennella JA, Maute C, Castor SM, Silva-Garcia A, Slotkin J, et al. Development of a test to evaluate olfactory function in a pediatric population. Laryngoscope. 2011;121(9):1843-50.
[40] Hummel T, Pfetzing U, Lotsch J. A short olfactory test based on the identification of three odors. J Neurol. 2010;257(8):1316-21.
[41] Jackman AH, Doty RL. Utility of a three-item smell identification test in detecting olfactory dysfunction. Laryngoscope. 2005;115(12):2209-12.
[42] Mueller C, Renner B. A new procedure for the short screening of olfactory function using five items from the "Sniffin' Sticks" identification test kit. Am J Rhinol. 2006;20(1):113-6.
[43] Richman RA, Wallace K, Sheehe PR. Assessment of an abbreviated odorant identification task for children: a rapid screening device for schools and clinics. Acta Paediatr. 1995;84(4):434-7.

Figure legends

Figure 1. Paediatric Barcelona Olfactory Test with the 6 odorant glass jars (A) for odour identification, a computer screen capture of image descriptors and labels used for the forced choice identification task (B) for banana odour, and the 6 plastic sniff bottles (C) with phenyl ethyl alcohol dilutions for the threshold detection task. Original descriptors labels in Spanish where, plátano, banana; césped, grass; cebolla, onion; café, coffee; umbral rosa, rose threshold.

Figure 1

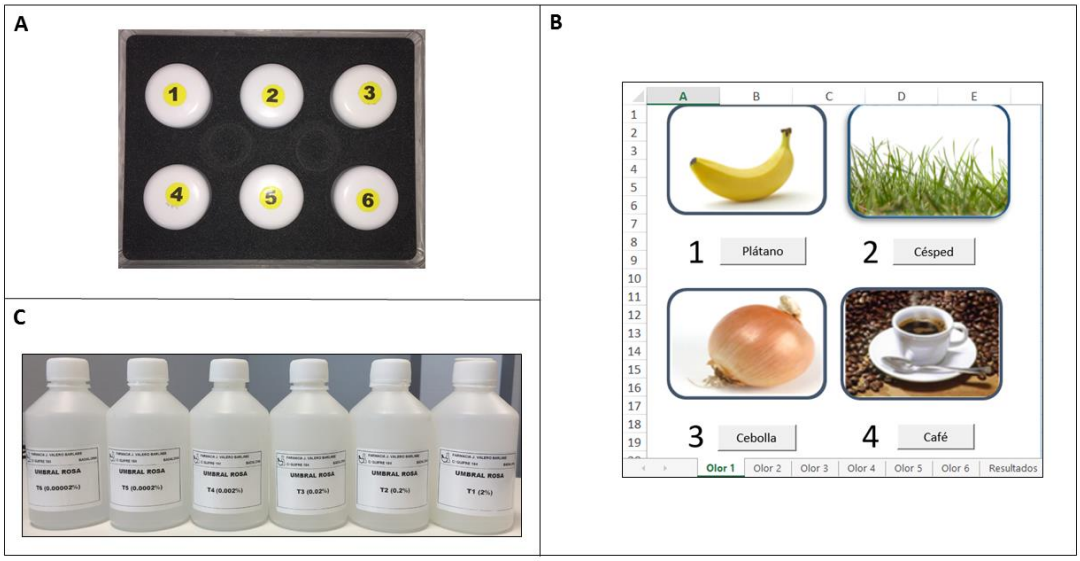


Figure 2. Correct odour identification frequency (%) in healthy volunteers (gray columns) and patients with olfactory dysfunction (black columns). ANOVA test was performed and difference between healthy volunteers and smell loss patients was evaluated where, **, p<0.001; *, p<0.01.

Figure 2

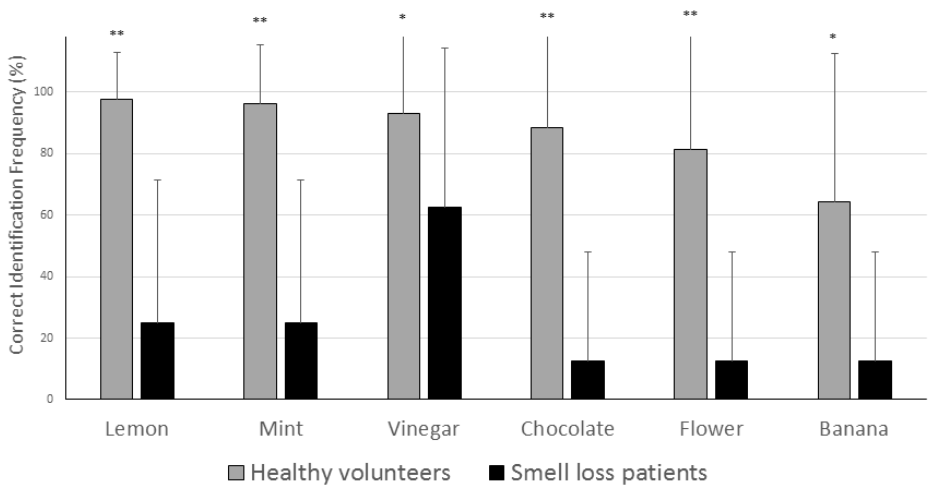


Figure 3. Bland-Altman plot comparison between pBOT-6 and U-Sniff tests within 95% limits of agreement. The X axis represents the average of the identification score values (pBOT-6 + U-Sniff) and the Y axis represents the difference of the values (pBOT-6 – U-Sniff), were, SD, standard deviation.

Figure 3

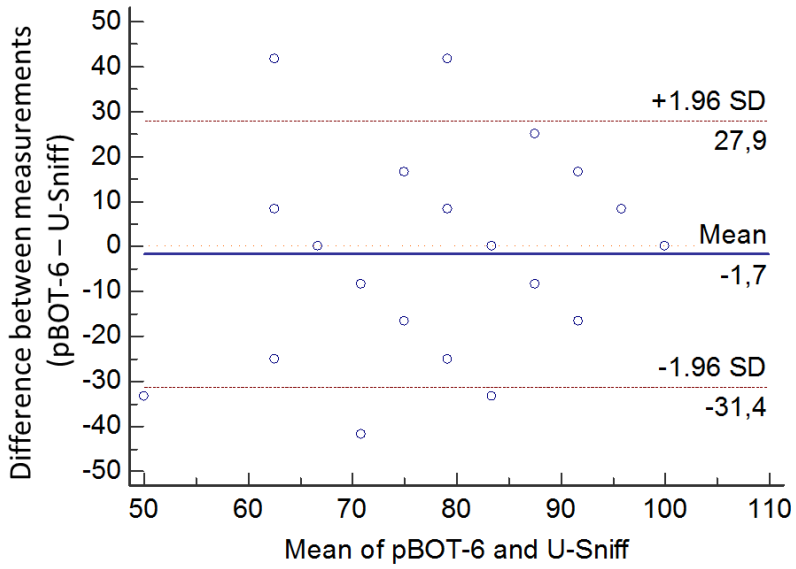
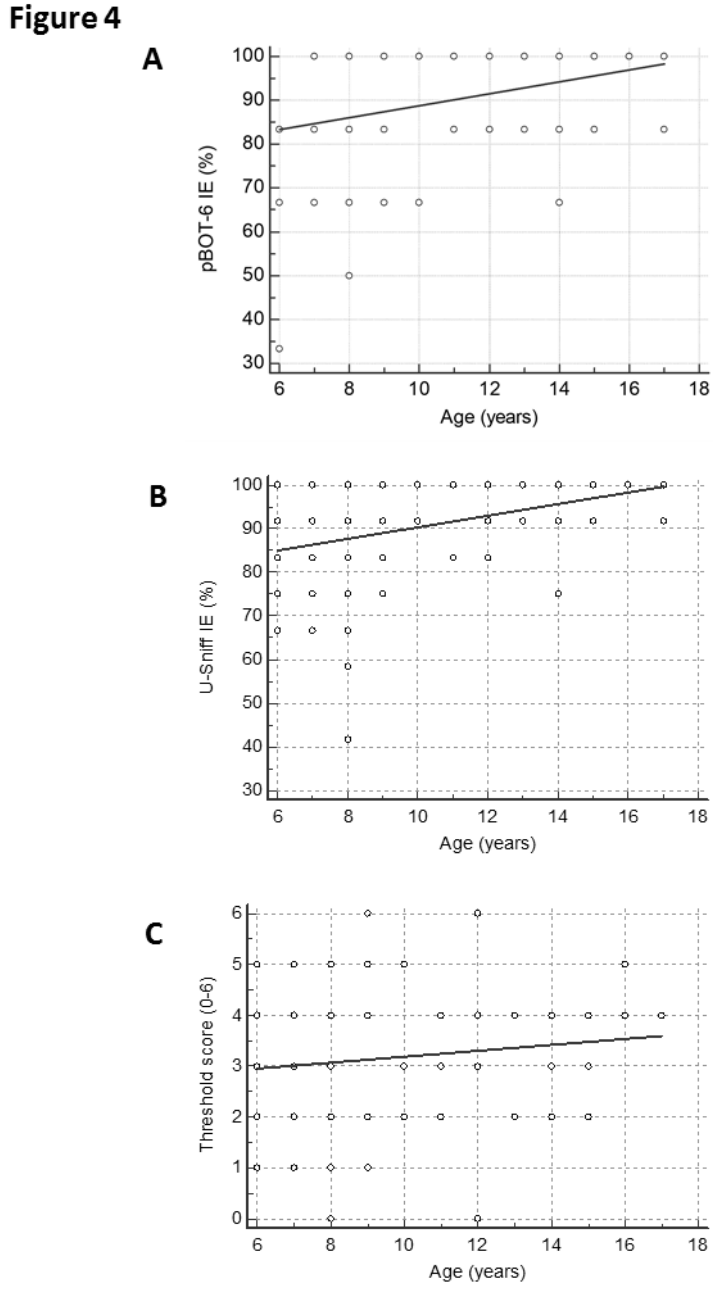


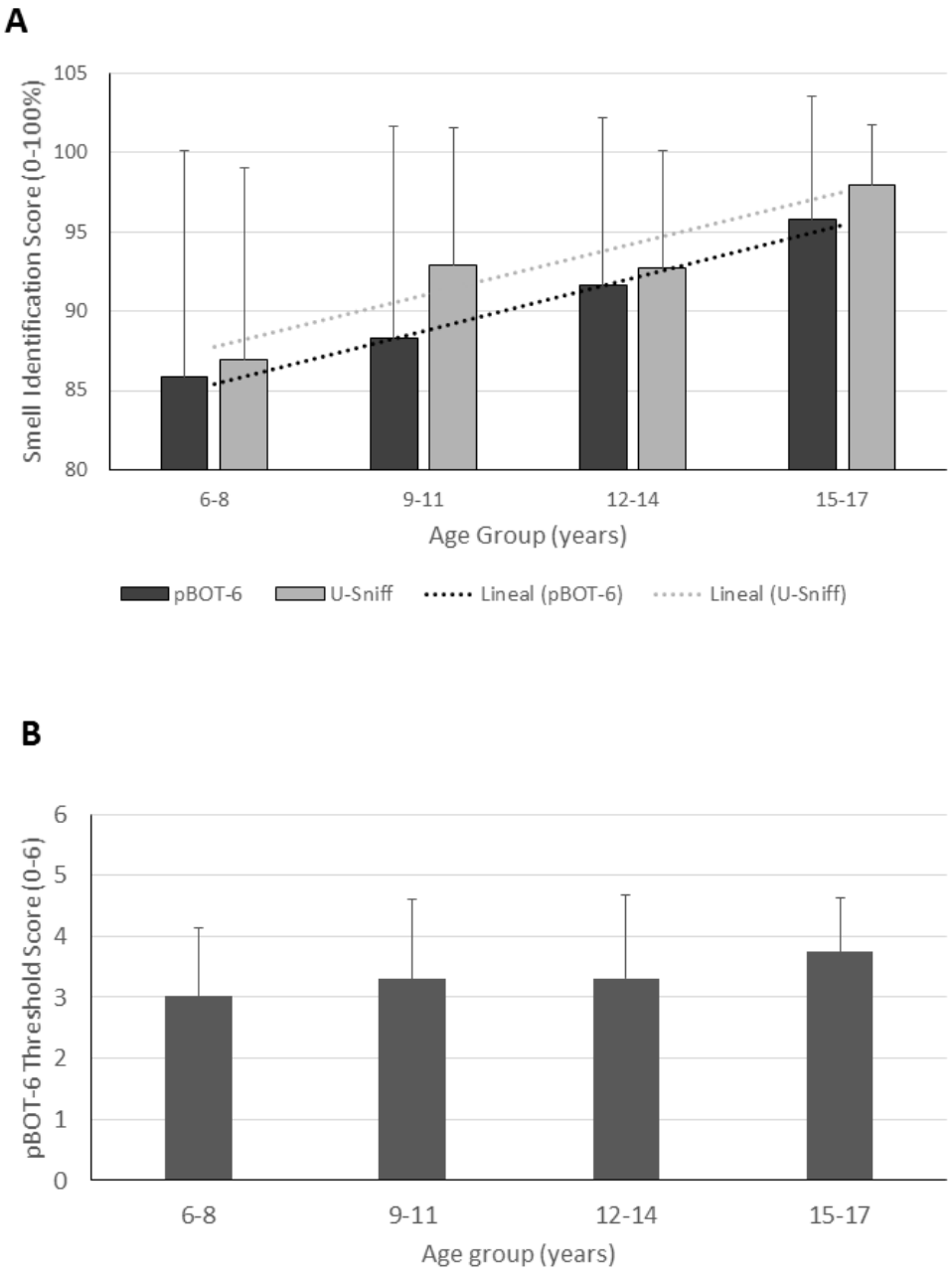
Figure 4. Linear regression analysis of correlation between pBOT-6 identification score (A), U-Sniff identification score (B) and pBOT-6 threshold score (C), and age.



doi: 10.18176/jiaci.0451

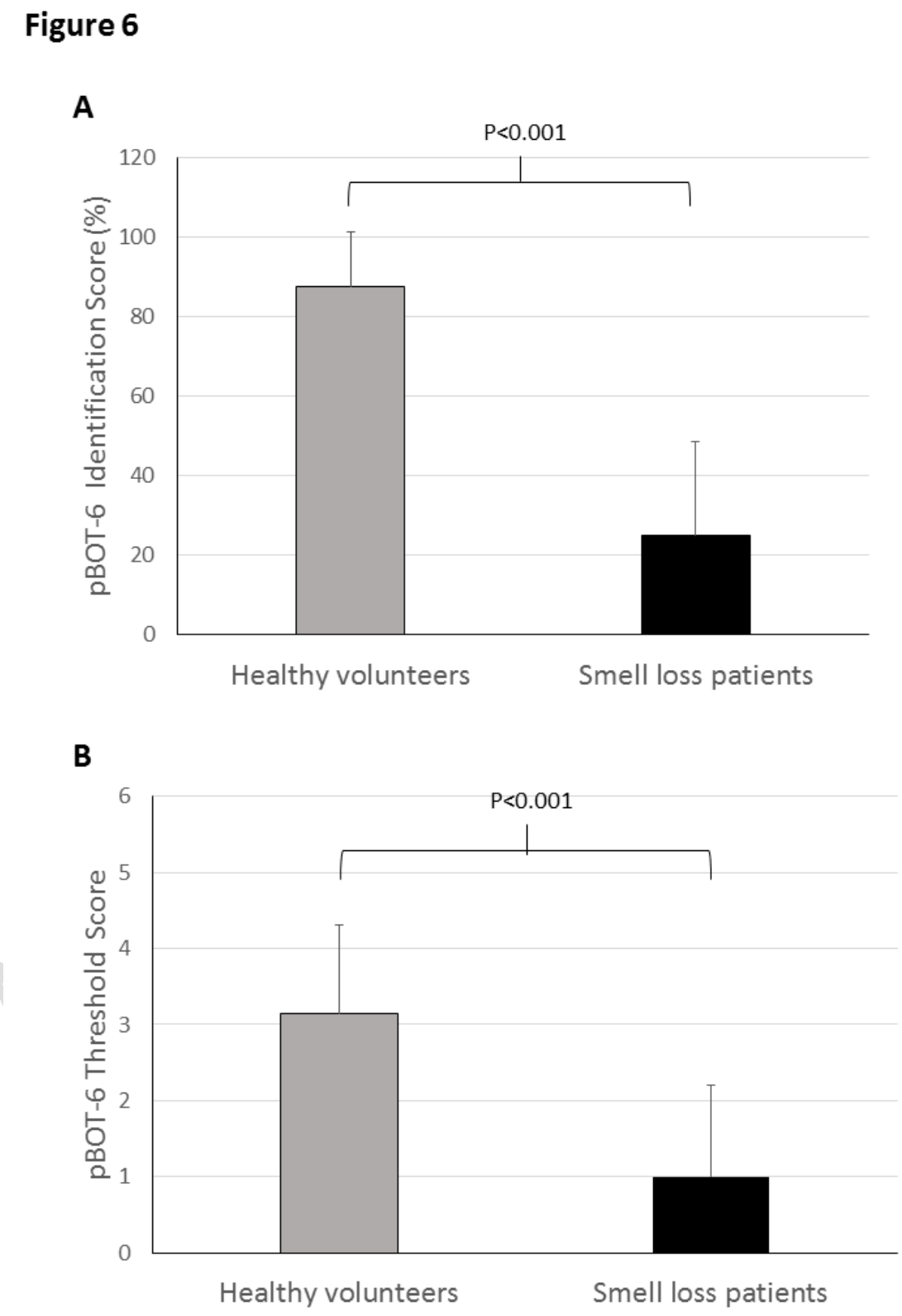
Figure 5. Mean pBOT-6 (dark gray column) and U-Sniff test (light grey column) smell identification scores (A), and mean threshold scores (B) according to age group where, black dotted line, pBOT-6 tendency line; grey dotted line, U-Sniff tendency line.

Figure 5



doi: 10.18176/jiaci.0451

Figure 6. Mann-Whitney two-sample test comparison of mean pBOT-6 identification (A) and threshold (B) scores between healthy volunteers (grey columns) and smell loss patients (black columns).



doi: 10.18176/jiaci.0451

Table 1. Odorants selected for pBOT-6 identification test with their chemical compounds and descriptors used for the forced choice task.

Odorant	Chemical compound	Descriptors
Banana	Isoamyl acetate	Banana, grass, onion, coffee
Chocolate	Pyrazines	Pineapple, tangerine, soap, chocolate
Vinegar	Acetic acid	Strawberry, vinegar, fish, poop
Lemon	Citral	Lemon, smoke, popcorn, cheese
Mint	Menthol	Gasoline, peach, mint, tomato
Flower	Phenethyl alcohol	Honey, flower, apple, cookies

Table 2. Demographic data of volunteers

AgeGroup	Females N (%)	Males N (%)	P
6-8 years	53 (61)	34 (39)	>0.05
9-11 years	12 (60)	8 (40)	
12-14 years	8 (50)	8 (50)	
15-17 years	4 (50)	4 (50)	
Total	77 (59)	54 (41)	

P, ANOVA comparison between males and females

Table 3. Demographic and Clinical Data of Smell Loss Patients

Patient	Age (years)	Gender	Cause of Loss of Smell	pBOT-6 IS	pBOT-6 TS
1	8	Male	AdenoidHyperplasia	3	1
2	6	Male	AdenoidHyperplasia	3	2
3	14	Male	Cystic Fibrosis CRSwNP	3	3
4	12	Female	CRS	2	2
5	6	Male	ICA	0	0
6	8	Female	ICA	1	0
7	6	Female	ICA	0	0
8	10	Male	ICA	0	0

CRS, Chronic rhinosinusitis; CRSwNP, Chronic rhinosinusitis with nasal polyps; ICA, Isolated congenital anosmia; pBOT-6 IS, pediatric Barcelona Olfactory Test Identification Score; pBOT-6 TS, pediatric Barcelona Olfactory Test Threshold Score

Table 4. Data and smell scores of volunteers used for test-retest reliability analysis

Subject	Age	Gender	Week 0		Week 2		Week 4	
			IS (0-6)	TS (0-6)	IS (0-6)	TS (0-6)	IS (0-6)	TS (0-6)
1	14	Female	4	4	4	4	5	4
2	12	Female	6	3	6	3	6	4
3	14	Male	5	3	5	4	6	4
4	8	Male	6	4	6	4	6	4
5	12	Female	5	3	5	4	6	4
6	14	Female	6	4	6	4	6	4
7	7	Male	6	3	6	2	6	3
8	7	Male	5	3	6	3	6	2
9	7	Male	5	3	6	4	6	3
10	7	Male	6	4	6	4	6	3
11	7	Male	6	3	6	3	6	4
12	7	Male	6	3	6	3	6	3
13	7	Male	5	4	5	4	6	4
14	7	Female	6	3	6	3	6	3
15	7	Female	5	3	6	3	6	3

IS, Identification Score; TS, Threshold Score

doi: 10.18176/jiaci.0451