



# Evaluation of the complete Sniffin Sticks Test versus its subtests in differentiating Parkinson's disease patients from healthy controls

## *Avaliação do Sniffin Sticks Test completo versus seus subtestes na diferenciação de pacientes com doença de Parkinson de controles sem a doença*

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### Abstract

**Background** Hyposmia is one of the most common, as well as the first nonmotor condition in Parkinson disease (PD). The sniffin sticks test (SST) evaluates three different aspects of olfactory function: threshold (T), discrimination (D), and identification (I). The sum of the scores of these three subtests produce a global score of olfaction, the Threshold-Discrimination-Identification (TDI) score.

**Objective** The aim of this study was to investigate if the TDI score or one of its subtests is better to discriminate PD patients from controls.

**Methods** We recruited 27 PD patients and 17 healthy age-matched controls (HC) who were evaluated through a clinical interview, the Montreal Cognitive Assessment and Movement Disorders Society – Unified Parkinson Disease Rating Scale. The olfaction was assessed using the complete SST.

**Results** The performance of PD patients on the olfactory test was significantly worse when compared with the HC (T: 3.0 vs. 6.5,  $p < 0.001$ ; D: 8.1 vs. 11.2,  $p < 0.001$ ; I: 7.3 vs. 11.7,  $p < 0.001$ ; TDI: 18.8 vs. 29.9,  $p < 0.001$ ). The prevalence of olfaction impairment in our study (PD: 100%, and HC: 56%) was greater than that found in the literature. Cognition influenced the performance on TDI. The olfactory subtests were impaired proportionally between patients and controls. Furthermore, D and I were correlated, but only in PD patients. The TDI showed a tendency to better discriminate PD patients from HC, when compared with its subtests.

### Keywords

- ▶ Parkinson Disease
- ▶ Anosmia
- ▶ Smell

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**Conclusions** Although the complete olfactory evaluation is time consuming, it seems to be a superior tool to identify olfaction impairment in PD patients, when compared with the isolated subtests.

## Resumo

**Antecedentes** Hiposmia é um dos sintomas mais comuns da doença de Parkinson (DP), além de ser um de seus primeiros sintomas não-motores. O Sniffin Sticks Test avalia três diferentes aspectos da função olfatória: limiar (L), discriminação (D) e identificação (I). A soma dos escores desses três subtestes produz um escore global do olfato, o Threshold-Discrimination-Identification (TDI).

**Objetivo** O objetivo deste estudo foi investigar se o TDI ou um de seus subtestes seria melhor em discriminar pacientes com DP de controles saudáveis.

**Métodos** Foram recrutados 27 pacientes com DP e 17 controles saudáveis de mesma faixa etária, que foram avaliados através de uma entrevista clínica, a Montreal Cognitive Assessment e Movement Disorders Society – Unified Parkinson Disease Rating Scale. O olfato foi examinado através da bateria completa do Sniffin Sticks Test (SST).

**Resultados** Os pacientes com DP tiveram pior performance no teste olfatório quando comparados com os controles (L: 3,0 vs. 6,5;  $p < 0,001$ ; D: 8,1 vs. 11,2;  $p < 0,001$ ; I: 7,3 vs. 11,7;  $p < 0,001$ ; TDI: 18,8 vs. 29,9;  $p < 0,001$ ). A prevalência de comprometimento olfatório no nosso estudo (DP: 100%, e controles: 56%) foi maior do que a reportada na literatura. A cognição influenciou a performance no TDI. Os subtestes olfatórios foram afetados proporcionalmente entre pacientes e controles. Além disso, D e I se correlacionaram, mas apenas em pacientes de DP. O TDI mostrou uma tendência em melhor discriminar pacientes de DP dos controles, quando comparado com os seus subtestes.

## Palavras-chave

- ▶ Doença de Parkinson
- ▶ Anosmia
- ▶ Olfato

**Conclusões** Embora a avaliação olfatória completa consuma tempo, ela parece ser superior aos subtestes isolados para identificar comprometimento olfatório em pacientes com DP.

## INTRODUCTION

Olfaction impairment is present in approximately 90% of Parkinson disease (PD) patients.<sup>1</sup>

According to the Movement Disorder Society's (MDS) clinical diagnostic criteria, the diagnosis of PD can only be made when motor symptoms appear. Bradykinesia plus resting tremor and/or rigidity are required for the diagnosis of parkinsonism.<sup>2</sup> However, olfactory reduction may occur years before the onset of motor symptoms, during the prodromal phase.<sup>1</sup> There are some possible mechanisms that can contribute to olfactory loss, like early deposition of Lewy body on the olfactory bulb, and, at a later stage, on the olfactory cortex and limbic structures, which are important for the interpretation of the olfactory stimulus.<sup>3</sup> It is important to mention that although PD is an important cause of olfaction impairment, there are several other conditions that can also alter smell, such as sinusitis, traumatic brain injury, and aging. One study estimated a prevalence of over 20% of olfactory impairment in the general population, even after excluding people with chronic sinonasal problems.<sup>4</sup> The way to objectively test the olfaction was standardized through psychophysical tests of olfaction, which has been widely used around the world.

However, these tests can be influenced by social, cultural, and cognitive factors.

The sniffin sticks test (SST, Burghart Medizintchnik, Germany) is an olfactory test divided into three subtests that assess olfactory threshold (T), discrimination (D) and identification (I). The results of the subtests are summed to compose a total score, which allows categorizing the patient as having normal olfaction, hyposmia, or anosmia. First, T is measured by the lowest concentration of a particular aroma that the subject tested can feel; then, D is the ability to know which of options is the different one between three alternatives; and, finally, I is a forced response test in which the subject must choose from four possible alternatives the smell descriptor that better matches the odor presented.

The execution of the whole SST is time consuming. By using a single subtest evaluation, one could decrease the time spent. Many studies have shown a predilection for the I subtest. However, olfactory testing based in I alone may suffer from cultural differences, because it is strongly dependent on familiarity with the odors used in the test. Furthermore, there is a risk of losing diagnostic accuracy when applying a single subtest.

The aim of this study was to investigate these three olfactory evaluations (TDI) in PD patients to know if there

are correlations between them and to identify if one of them is more accurate in identifying olfactory dysfunction in this population.

## METHODS

### Study participants

This study was designed as a cross-sectional, observational study. We recruited 27 PD patients and 17 healthy age-matched controls (HC) from southern Brazil, from the Movement Disorders Outpatient Clinic at the Hospital São Lucas from PUC-RS. All patients had long-term follow-up at the hospital and were diagnosed with PD by a neurologist, according to the MDS clinical diagnostic criteria for Parkinson disease.<sup>2</sup> The HC were spouses of the patients. Individuals with psychosis, an established diagnosis of dementia, or any condition which could cause a change in olfaction such as a history of severe head injury, chronic nasal diseases, chronic use of nasal solutions, and use of certain medications or drugs were excluded.

All participants were submitted to a clinical interview where data were recorded regarding previous health history, medication use, smoking history, and education level. At this time, cognitive assessment was also tested through Montreal Cognitive Assessment (MoCA). In another consultation, the patients were evaluated using Movement Disorders Society – Unified Parkinson Disease Rating Scale (MDS-UPDRS). During the third visit, to avoid tiring the patients, the olfactory evaluation was applied. The maximum time interval between the first and last evaluation was 1 month.

The study was approved by the local ethics committee, and informed consent was obtained from all participants.

### Olfactory test

Participants underwent a standardized psychophysical olfactory test, the SST which, in its most complete version, comprises 3 subtests of olfactory function: T, D, and I.

According to manufacturer's recommendations, the time interval between each of these subtests must be 3 minutes. Odorants were presented in pen-like odor dispensing devices, in a quiet and well-ventilated room, always by the same investigator. The subject tested could not have ingested anything within 15 minutes prior to testing, only water. The examiner wore odorless gloves, changed for each patient. For odor presentation, the pen's cap was removed by the experimenter for around 3 seconds, and the pen's tip was placed approximately 2 cm in front of both nostrils, without touching the skin. The interval between odor presentations was approximately 20 seconds. For T and D subtests, triplets of SST pens were presented to the patients, who were blindfolded to prevent them from associating specific odors with the colors of the pens.

The T subset consisted of the presentation of three sticks in randomized order, two containing only a solvent and the third the odorant at a particular dilution of *n-butanol*. The subjects had to identify the stick with the odorant. For the D test, triplets of odorants (two with the same odorant and one with a different one) were presented, and subjects were

asked to identify the different one. The I test was performed on a multiple-forced-choice task, from a list of 4 descriptors each. The T subtest score ranges from 1 to 16, and the other two subtests (D and I) range from 0 to 16. The sum of the three subtests obtained a global score of olfaction, the Threshold-Discrimination-Identification (TDI) score. For this score, normative values are available allowing the diagnosis of anosmia (TDI score <16), hyposmia (TDI score 16–31) and normosmia (TDI score >31).<sup>5</sup>

### Statistical analysis

The statistical analysis was performed through the Statistical Package Social Sciences (SPSS, Inc. Chicago, IL, USA) software, version 15.0, MedCalc Statistical Software, version 19.3.1 (MedCalc Software Ltd., Ostend, Belgium) and RStudio (RStudio, Inc., PBC, Boston, MA, USA), version 1.2.5033.

Gaussian distribution was confirmed by visual analysis of Q-Q plots and the Kolmogorov-Smirnov test. To compare SST scores among groups, the Student *t* tests were used for D and I subtests and TDI total score, and for the analysis of non-normal data (T subtest) we performed Mann-Whitney U-tests for independent sample comparisons. To study the influence of different variables on olfaction performance, we used the Student *t* test or analysis of variance (ANOVA) for factors with two or more categories respectively. For continuous numerical variables, the Pearson correlation coefficient was calculated.

To assess the olfactory evaluation which best discriminates PD patients from HC, we performed the receiver operator curves (ROC) and calculated the area under the curve (AUC) for the TDI and each subtest. The ROC curves were compared with each other to verify if they were statistically significantly different using the DeLong test.

To investigate potential differences in the pattern of olfactory loss between patients and HC, we calculated the proportion that each subtest contributes to the composition of the TDI in both groups, considering only subjects with olfactory impairment.

To assess correlation between the different subtests, the Spearman rank correlation test was used.

Multiple comparisons were Bonferroni corrected and the  $\alpha$  value considered was 0.05.

## RESULTS

### Demographics and clinical assessment

The comparison of clinical and demographic profiles between the two groups, including age, sex, schooling, cognition, and smoking history, showed no significant difference. The demographic and clinical variables of the subjects are shown in ▶Table 1.

### Olfaction assessment

A total of 27 PD patients and 17 HC underwent formal olfactory testing. According to the SST TDI scores, 8 controls (47%) were classified as having normal olfaction, and 9 (53%) had hyposmia. None of the participants from the control group had anosmia. Among the PD patients, 7 (26%) had

**Table 1** Demographic and clinical variables in Parkinson disease and healthy controls

	Control (mean ± SD) N = 17	PD (mean ± SD) N = 27	p-value
Age, years	61.4 (7.4)	65.6 (9.7)	0.13
Male sex, No (%)	3 (17.6)	11 (40.7)	0.18
Schooling, years	7.0 (3.5)	6.7 (4.5)	0.59
MoCA	22.4 (4.1)	20.5 (3.5)	0.09
Smoking history	10 (58.8)	13 (48.1)	0.75
Disease duration	–	8.4 (0.7)	–
MDS-UPDRS	–	53.04 (4.8)	–
Hoehn & Yahr	–	2.3 (0.1)	–

**Abbreviations:** MDS-UPDRS, Movement Disorders Society – Unified Parkinson Disease Rating Scale; MoCA, Montreal cognitive assessment; PD, Parkinson disease; SD, standard deviation.

**Table 2** Subtests of olfactory performance in Parkinson disease and healthy controls

Olfactory Test – SST	Control (mean ± SD) N = 17	PD (mean ± SD) N = 27	p-value
Threshold	6.5 (2.8)	3.0 (2.2)	<0.01
Discrimination	11.2 (2.5)	8.1 (1.8)	<0.01
Identification	11.7 (2.1)	7.3 (2.9)	<0.01
TDI	29.9 (4.9)	18.8 (5.0)	<0.01

**Abbreviations:** SD, standard deviation; PD, Parkinson disease; SST, sniffin sticks test; TDI, Threshold-Discrimination-Identification.

anosmia, and 20 (74%) had hyposmia. No PD patient was classified as having normal olfaction.

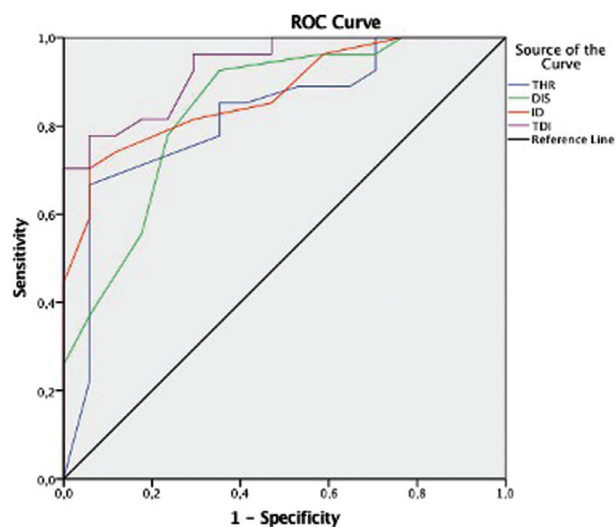
The performance of PD patients on the three olfactory subtests, as well as on the final score (TDI), was significantly worse when compared with the HC group, as shown on **Table 2**.

The analysis of clinical variables that could influence the performance in the olfactory test showed that only cognition, measured by MoCA, correlated in a statistically significant way with TDI ( $r=0.42$ ;  $p=0.03$ ). All olfactory subtests correlated with MoCA in a similar degree (T:  $r=0.33$ ,  $p=0.02$ ; D:  $r=0.39$ ,  $p<0.01$ ; and I:  $r=0.32$ ,  $p=0.02$ ). When patients and HC were analyzed separately, this correlation was not statistically significant, probably due to the small sample size. However, for HC, the statistical significance was very close to the limit ( $p=0.06$ ). Characteristics such as age, sex, smoking history, family history of PD, or olfactory self-perception did not influence the value of TDI in either group. In PD patients, disease duration, levodopa equivalent daily dose (LEDD), or disease clinical subtype also did not influence test performance.

Regarding the ROC analysis, the highest AUC was observed for TDI (AUC: 0.93; 95% confidence interval [CI]: 0.86–1.00), followed by I subtest (AUC: 0.87; 95% CI: 0.77–0.97), then D (AUC: 0.84; 95% CI: 0.71–0.96), and, finally, T (AUC: 0.82; 95% CI: 0.69–0.95) (**Figure 1**). When comparing the AUC between the TDI and its subtests, no statistically significant differences were found. However, there was a tendency favoring TDI over T ( $p=0.06$ ), D ( $p=0.06$ ), and I ( $p=0.08$ ). The sensitivity and specificity for the optimal cut-offs were calculated for all olfactory tests, as follows: T (cut-off  $\leq 4$ ; sensitivity 67%; and specificity 94%); D (cut-off  $\leq 10$ ; sensi-

tivity 93%; and specificity 65%); I (cut-off  $\leq 8$ ; sensitivity 70%; and specificity 94%); and TDI (cut-off  $\leq 24$ ; sensitivity 78%; and specificity 94%) (**Table 3**).

We also investigated whether there was a difference in the pattern of olfactory loss between PD patients and HC. For this, we calculated the proportion that each subtest contributed to the composition of the TDI in both groups. In this calculation, all patients and only 9 HC were included, as the others had no olfactory impairment. There was no statistically significant difference between the proportions of each



**Figure 1** Receiver operator curve (ROC) analysis for the TDI and subtests. Abbreviations: T, threshold; D, discrimination; I, identification; TDI, Threshold-Discrimination-Identification.

**Table 3** The sensitivity and specificity for the optimal cut-offs for all olfactory subtests

Olfactory Test – SST	Cut-off	Sensitivity	Specificity
Threshold	≤ 4	67%	94%
Discrimination	≤ 10	93%	65%
Identification	≤ 8	70%	94%
TDI	≤ 24	78%	94%

**Abbreviations:** SST, sniffin sticks test; TDI, Threshold-Discrimination-Identification.

subtest of the TDI between PD patients and HC with olfactory loss (►Figure 2).

We observed a moderate correlation between D and I subtests scores in PD patients ( $r_s = 0.41$ ;  $p = 0.03$ ). For each reduction of one point in the I score, a reduction of 0.2 in D is expected. A correlation coefficient of similar degree was observed for HC ( $r_s = 0.44$ ;  $p = 0.079$ ), which was statistically insignificant, probably due to sample size ( $n = 17$ ). No correlation was found between other subtests, both in PD patients and HC.

## DISCUSSION

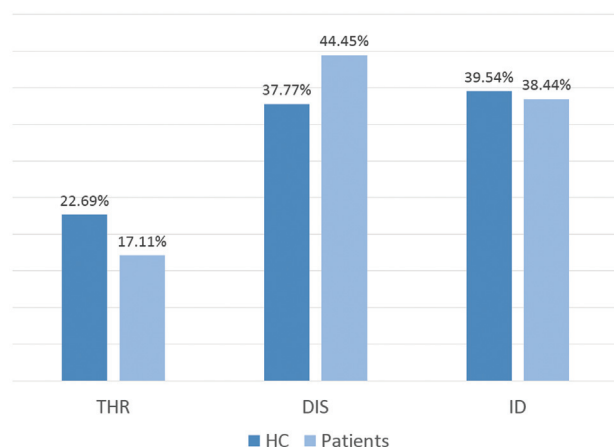
As was expected, PD patients performed significantly worse than HC on SST. All PD patients analyzed in this study showed olfaction impairment. Furthermore, around half of the HC group had hyposmia. These rates are greater than what is seen in the literature.<sup>1,4</sup> This higher prevalence of olfaction impairment, even in HC, could be a reflex of sociocultural differences rather than a worse sense of smell in the Brazilian population. We have not found another study that applied the complete SST in PD patients in the Brazilian population.

Although it is described in the literature that age is a factor that is associated with a worsening of olfaction,<sup>6</sup> in this

study, it was not found a statistically significant influence of age on olfactory test performance, although the older subjects showed a tendency to have a worse sense of smell. This could be explained by the small number of subjects studied and due to a small difference in age between the subjects. A similar study in the Honduran population, with 46 PD patients and 46 HC also failed to show influence of age as well as education level and gender in the olfactory performance.<sup>7</sup>

The influence of cognition has been described in previous studies<sup>8–10</sup> and was confirmed in this one when analyzing all subjects together, even when using just a limited screening tool like MoCA. Furthermore, the cognition seems to have a similar influence across all olfactory domains. In contrast, a previous study found a correlation between MoCA and T ( $r = 0.203$ ,  $p < 0.05$ ), I ( $r = 0.206$ ,  $p < 0.05$ ), and TDI ( $r = 0.234$ ,  $p < 0.05$ ), but not with D.<sup>11</sup> In another study that assessed the olfaction in PD patients with and without mild cognitive impairment (MCI), the I subtest was the only olfactory domain that differed between groups.<sup>12</sup> In our study, when PD patients and HC were analyzed separately, the HC group showed a tendency of correlation between MoCA and TDI, which was not shown in PD patients. One hypothesis could be that olfaction is more influenced by cognition in healthy subjects than in PD patients, in which other factors could play a major role. One could speculate that from a certain degree of olfactory loss, perhaps cognition is no longer so determinant in the interpretation of the stimulus.

The complete battery of SST showed a tendency in being the most efficient tool for differentiating PD patients from HC than its subtests. The comparisons between the AUC of the TDI and its subtests, though, was not statistically significant by a narrow margin, probably due the small sample size. However, the complete test is very time consuming to perform and, perhaps, that is the reason why is not universally used throughout the studies. One prospective, cross-sectional study whose objective was to define the optimum SST cut-offs that best discriminate PD patients from HC had similar findings, since ROC analysis showed the largest areas under the curve for the sum score (TDI AUC: 0.96; 95% CI: 0.91–1.00) and the I subscore (AUC: 0.94; 95% CI: 0.88–1.00), while the performance of the D and T subscores did not surpass the pre-defined threshold.<sup>13</sup> The TDI and I tests were also superior to the other subtests in discriminating PD patients from other tremor syndromes (TDI AUC: 0.85, 95% CI: 0.80–0.89; I AUC: 0.86, 95% CI: 0.82–0.90; D AUC: 0.77, 95% CI: 0.71–0.81; and T AUC: 0.71, 95% CI: 0.65–0.77).<sup>14</sup> Another study, which applied only the I subtest of the SST (SST-16), also found a good diagnostic accuracy in discriminating PD from HC (AUC: 0.90; sensitivity 83.3%; specificity 82.0%).<sup>15</sup> Regarding the aim to enhance the accuracy in discriminating PD patients from HC, studies confirmed that the I evaluation is the best single subtest of SST to accomplish this objective. Furthermore, it has been shown that the extended version of the olfactory subtests – for example, the 32-item odor I and D – is not superior to their short versions (16-item).<sup>16</sup> However, the combination of I plus T subtests (but not the I plus D combination) is superior



**Figure 2** Percentage contribution of each subtest to the TDI. Considering only subjects with olfaction impairment: 9 HC with hyposmia, and all patients evaluated (7 with anosmia and 20 with hyposmia). Threshold:  $p = 0.20$ ; Discrimination:  $p = 0.08$ ; Identification:  $p = 0.97$ .

to one test alone.<sup>16,17</sup> Considering all these data, it is questionable if the gain in accuracy in discriminating PD patients from HC by the use of the complete battery is justified by the considerable additional time spent in executing all the three olfactory subtests.

The different olfactory capacities (T, D, and I) seem to be decreased in a similar proportion between groups; D and I only showed a correlation in PD patients.

The limitations of the present study are the small sample size and the restriction to a single center in a country with big dimensions, which could compromise the extrapolation of the results to the whole Brazilian population. Besides, the high prevalence of olfactory loss in the HC group could have interfered with the statistical analysis.

In conclusion, the complete olfactory evaluation using the SST tends to be superior to isolated subtests (T, D, and I) in identifying olfaction impairment in Brazilian PD patients. Cognitive aspects seem to have some interference in olfaction performance even in otherwise healthy people. Cultural and cognitive aspects should be considered during olfactory assessment.

#### Authors' Contributions

CRM: conceptualization; BSFO, ST: data collection and writing; CRM, YFFB: review and editing.

#### Conflict of Interest

The authors have no conflict of interests to declare.

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