



Short communication

Feasibility of repeat and bilateral submandibular gland needle biopsies in Parkinson's disease

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ABSTRACT

Introduction: Submandibular gland (SMG) biopsies detect pathological alpha-synuclein (aSyn) in patients with Parkinson's disease (PD). The objectives of this study were to determine 1) the feasibility of performing a second SMG biopsy in previously biopsied patients, 2) the feasibility of doing bilateral SMG biopsies, 3) laterality of aSyn density, 4) whether aSyn density changes over time.

Methods: Seven PD patients (6 males) previously having positive unilateral SMG biopsies underwent bilateral needle biopsies. Staining with a validated antibody to pathologic p-serine 129 aSyn was performed.

Results: Mean age at time of second biopsy was 76 years and mean time between biopsies was 4.1 years. Five subjects had sufficient SMG tissue bilaterally and two only unilaterally for a total of 12/14 glands biopsied having sufficient tissue, all 7 subjects having sufficient tissue on at least one side, and all 12 glands being aSyn positive. There was a 4x increase in aSyn density on average in the repeat biopsy, with 5 subjects having an increase, one no change, and one a decrease in density. Side effects were similar to previous reports; mainly bruising, swelling, slight bleeding.

Conclusions: This is the first published study of bilateral transcuteaneous needle biopsies of the SMG in living patients with PD which showed better tissue acquisition and a change in aSyn density over time. While further study is needed, there is potential for SMG biopsies to serve as a tissue biomarker for PD disease progression and potentially as a peripheral outcome measure for anti-aSyn treatment.

1. Introduction

Currently there are no biofluid or imaging markers of tissue deposition of pathologic alpha-synuclein (aSyn) and therefore no means to monitor tissue-level target engagement in clinical trials of anti-synucleinopathy agents. The use of submandibular gland (SMG) biopsies as a tissue biomarker of aSyn has been studied in Parkinson's disease (PD) and REM sleep behavior disorder (RBD) [1–4]. All studies have been of a single, unilateral biopsy with no longitudinal studies reported. One limitation of the unilateral SMG biopsies has been the success in obtaining adequate tissue in only about 75% of the subjects. Additionally, for SMG biopsy to serve as a disease progression marker it is critical to establish whether aSyn density increases with time and disease progression. The current study was a feasibility study for performing a second SMG biopsy in previously biopsied patients, to assess

aSyn density changes, and do so bilaterally to improve adequate tissue acquisition and determine laterality of aSyn density.

2. Methods

Seven PD patients (1 female, 6 males; 6 from the early PD and one from the advanced PD study) who previously had positive unilateral SMG biopsies were studied [1,2]. All subjects signed written informed consent approved by the Mayo Clinic Institutional Review Board. A stipend was provided to all subjects. The biopsies were performed as outpatient procedures in a standard examination room as previously reported [1,2].

In brief, a commercially-available 16 gauge needle was used to obtain tissue cores (Max-Core Disposable Core Biopsy Instrument, Bard Medical, Covington, Georgia). Submandibular glands were localized by

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palpation and biopsies were performed bilaterally. Local anesthetic (0.5 cc of lidocaine) was injected into the skin overlying both submandibular glands. This was followed by a 3–5 min waiting period to allow the anesthetic to take effect. Four needle cores were taken from each SMG through a single needle puncture on each side, by changing the angle of each insertion, all done at shallow acute angles from a line perpendicular to the tangent line parallel to the skin surface. After the procedure, pressure was used briefly to stop bleeding. Tissue was immediately placed in single standard plastic cassettes between two sponges and immersed in 70 ml of neutral-buffered 10% formalin (Fisher Scientific, Kalamazoo, MI).

The rest of the tissue preparation was as previously described [1,5,6]. In brief, after 24 h fixation at 4 °C, the tissue cores were dehydrated in alcohols, infiltrated with paraffin and serial sections cut at 5–7 μm with a rotary microtome. Every section was collected and mounted on a separate slide, beginning with the first appearance of tissue fragments and ending when tissue fragments were no longer visible. Slides were stained with an extensively characterized antibody raised against aSyn phosphorylated at serine 129 (gift of Dr. Haruhiko Akiyama, Yokohama Brain and Spinal Center, Yokohama, Japan) [7]. As previously published, non-specific staining was defined as epithelial cell cytoplasmic staining [8]. The sensitivity and specificity of this method, when used on SMG tissue from both autopsies and biopsies, has previously been reported [1,2,9,10]. The neuropathologist was blinded to the previous biopsy results including the side biopsied and whether the subject had been in the early or advanced PD study. Quantification of aSyn density was assessed by a single neuropathologist (TGB) as the mean of semi-quantitative densities (0–4) in all stained slides from all of a subject's biopsies, with reference to scoring templates published by McKeith et al. [11] The density of each slide was scored at the highest density on the slide, or scored as zero if there were no positive, specifically-stained tissue elements. An example of slides from a single subject at first and then bilateral second biopsies is shown in Fig. 1.

2.1. Statistical methods

The Wilcoxon signed-rank test was used to test if there were differences in the aSyn density from left and right second biopsies, between first biopsy and ipsilateral second biopsy, or between first biopsy and the density mean of the second bilateral biopsies.

Table 1
Demographics and clinical characteristics of subjects.

	Subject						
	1	2	3	4	5	6	7
1st biopsy							
Age (yrs)	80	65	67	71	71	74	75
Sex	Male	Male	Female	Male	Male	Male	Male
Disease Duration (yrs)	3	15	1	2	2.5	3	3
UPDRS Part II	8	4.5	11	NA	13	7	7
UPDRS Part III	22	5	32	25	25	18	18
2nd biopsy							
Age	83	70	72	75	74	78	80
Years after 1st biopsy	3.4	5.4	4.25	3.5	3.6	4.25	4.5
UPDRS Part II	15	26	12	7	12	13	2
UPDRS Part III	41	21	29	26	26	17	19

3. Results

3.1. Demographics

The mean age at the time of the second biopsy was 76 yrs, with a range of 70–83 years (Table 1). The mean interval from the first to the second biopsy was 4.1 yrs, with a range of 3.5–4.5 years (Table 1).

3.2. Submandibular gland biopsy findings

All 7 subjects had sufficient tissue obtained from at least one gland. Two subjects had sufficient tissue from only one of the biopsied gland so overall, 12/14 (85.7%) glands biopsied had sufficient tissue. There was a trend for increased aSyn mean and median density from first to ipsilateral second biopsy and for the mean of the second bilateral biopsies (Table 2). There was an approximately 4-fold increase in aSyn density seen in the second biopsy compared to the first, when comparing either the ipsilateral sides or the mean for the bilateral biopsies. This increase approached, but failed to meet, the significance level ($p = 0.078$). The effect size however was 0.82 and 0.88 respectively (Table 2) for the ipsilateral or bilateral mean comparisons. Individually, 5 of the subjects had an increase in aSyn density ipsilaterally, one had no significant change, and one had a decrease (Fig. 2).

When comparing the right vs. the left aSyn density for the 5 subjects that had sufficient SMG tissue from both sides there was no difference in the mean densities when combining the subjects but each individual subject aSyn density did differ to some degree (Table 3).

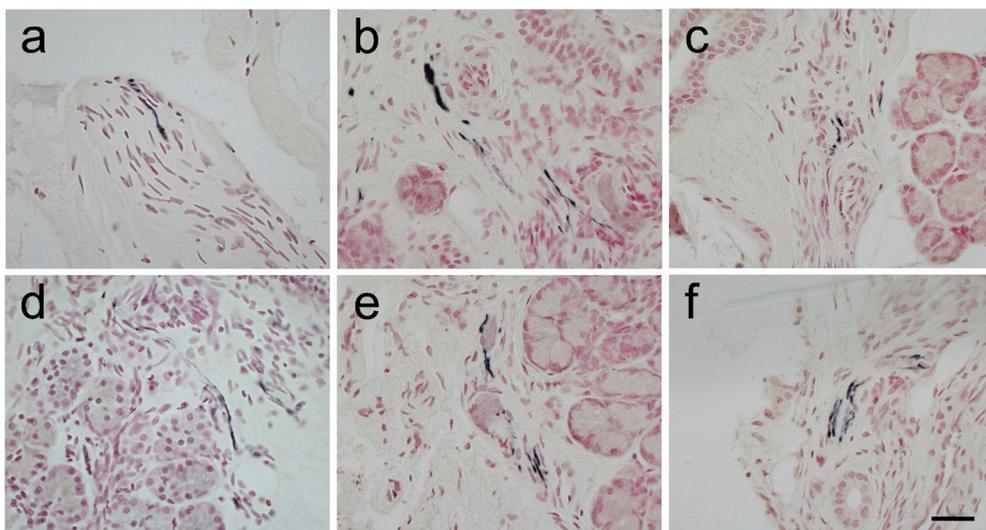


Fig. 1. Photomicrographs of biopsies from a single subject immunohistochemically stained for aSyn pathology. Portrayed are representative images from the first biopsy (a,d), images from the ipsilateral second biopsy (b, e) and from the contralateral second biopsy (c, f). Nerve fibers positive for aSyn pathology are black while the counterstain is Neutral Red. The calibration bar (f) represents 40 μm and serves for all panels. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Comparison of aSyn density in the first biopsy with the mean and median aSyn density in the second biopsy. Data are shown for comparing the ipsilateral glands in the first and second biopsy as well as for the first biopsy versus the mean of the bilateral second biopsies.

Variable	Statistics	First biopsy	Second biopsy ipsilateral comparison	Second biopsy using mean of the right and left sides	pvalue ^a	pvalue ^b	Effect Size ^c	Effect Size ^d
Mean aSyn density	Mean (SD)	0.25 (0.26)	1.14 (0.95)	1.03 (0.77)	0.078	0.078	0.82	0.88
	Median (Range)	0.21 (0.03–0.77)	1.34 (0.08–2.30)	1.22 (0.08–2.04)				

^a Wilcoxon signed-rank test for comparing the density of first and second biopsies on matched sides.

^b Wilcoxon signed-rank test for comparing the density of first and second biopsies using mean of bilateral second biopsies.

^c Effect size for comparison of means between first biopsy and repeat biopsy using mean of matched sides for the repeat biopsy.

^d Effect size for comparison of means between first biopsy and repeat biopsy using mean of bilateral repeat biopsies.

3.3. Side effects from biopsy

No serious adverse events occurred. Five of the subjects reported swelling and/or bruising following the biopsies, all transient. Two reported bleeding inside the mouth immediately following the biopsy. One subject reported no side effects.

4. Discussion

This study establishes that repeat transcutaneous needle biopsies of the submandibular gland in patients with PD is feasible, can be performed bilaterally to increase adequate tissue acquisition, and might be able to serve as a disease progression marker. The SMG is readily accessible to percutaneous biopsy but previous studies of unilateral biopsies found adequate tissue for staining and analysis in only ~75% or less of cases [1–3], although the most recent study of 16 patients had adequate tissue in 100% of cases [4]. This has been considered a significant limitation by some in the field and therefore the feasibility of doing bilateral biopsies was studied here. Adequate tissue was obtained in 85.7% of the glands biopsied and in 100% of subjects. This suggests future studies should consider bilateral biopsies. While there has been criticism of doing SMG biopsies we would note that the adequacy of obtaining tissue in SMG biopsies is markedly better than obtaining adequate cerebrospinal fluid samples from patients with PD and controls, due to deficiencies in lumbar puncture success and the need to exclude samples with hemoglobin concentrations > 200 ng/ml; in some studies these exclusions resulted in a sample adequacy rate of only 47.4% [12]. Additionally, the density of aSyn in the SMG is much greater than in skin or colon. Bilateral biopsies did not result in a

Table 3

Comparison of aSyn density in the right and left submandibular gland at the time of the bilateral biopsy. Data is shown for the mean + standard deviation.

Subject	Left	Right
1	1.08 ± 1.20	1.75 ± 0.98
2	0.22 ± 0.52	0.73 ± 0.76
3	1.34 ± 0.91	2.17 ± 1.03
4	2.09 ± 0.64	0.35 ± 0.59
5	2.30 ± 0.99	1.77 ± 1.07

greater number of side effects nor did any serious side effects occur. Additionally, there have been no serious side effects in any of the 102 subjects previously biopsied by two independent centers, or the 7 subjects in this study [1–3]. Data from a multi-center study of tissue biopsies, the S4 study, reported 1 serious adverse event in the 76 subjects having SMG biopsies [13], so a total of only 1 serious adverse event in 178 (0.0056%) biopsied subjects. Yet while many have stated that the risks of SMG biopsies are too high, this may be a safer procedure than a lumbar puncture. In the PPMI study it has been reported that 153/679 (22.5%) of subjects had a total of 180 adverse events with 10 (5.6%) being serious adverse events [14].

This study is limited by the small number of subjects as it was a feasibility study and needs to be replicated. It is unclear whether the lack of symmetry in aSyn density is due to random sampling of the SMG by needle biopsy, heterogeneity of aSyn density from one gland to another, or other factors. The sample size is too small to address the individual differences in aSyn density between sides, but did demonstrate that both sides in a single subject were positive. The sample size is insufficient to assess any correlation to clinical asymmetry and as

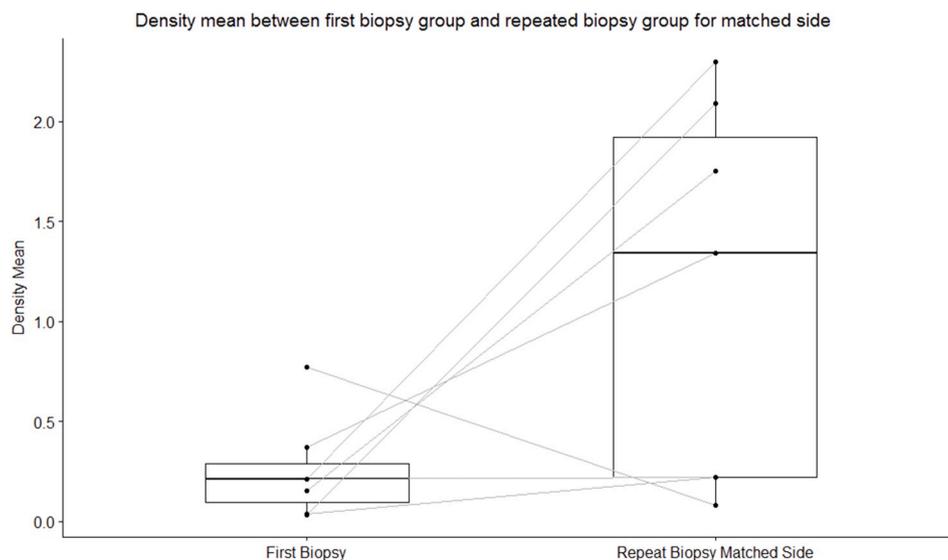


Fig. 2. The aSyn density mean between the first biopsy and the second biopsy on the ipsilateral side. aSyn density is on a 0–4 scale.

these are living subjects it is not possible to correlate to brain aSyn density. A strength, as well as a limitation of the study, was that the biopsies were performed by an experienced head and neck surgeon which might limit generalization of the adequate tissue results to centers with surgeons experienced in this procedure. However, as with any procedure this is one that can be learned and other centers have shown good tissue acquisition [4].

Overall conclusions from this study are that bilateral SMG biopsies are feasible and safe, longitudinal SMG biopsies are feasible, and aSyn density in the SMG appears to increase over time. As aSyn density is not completely symmetric, bilateral or ipsilateral biopsies should likely be considered for longitudinal studies. Patients with PD are willing to undergo longitudinal and bilateral biopsies and this should help reduce criticism regarding this procedure being too invasive. At this time there is no definitive diagnostic test for PD nor is there a tissue biomarker for disease progression and thus further study of SMG biopsy is needed. This study did not assess skin biopsies and whether SMG biopsies, or other tissue biopsies, could eventually serve as an outcome measure for anti-synuclein therapy is a critical question that requires further study.

Authors' roles

Adler: Led all aspects of the study, drafted manuscript.
 Serrano: Performed pathology work, revised the manuscript.
 Zhang: Performed statistics, revised the manuscript.
 Hinni: Performed biopsies, revised the manuscript.
 Lott: Performed biopsies, revised the manuscript.
 Mehta: Performed clinical assessments and patient recruitment, revised the manuscript.
 Sue: Coordinated the pathological assessments, revised the manuscript.
 Intorcica: Performed pathology work, revised the manuscript.
 Beach: Performed and oversaw all pathology work, revised the manuscript.

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Declaration of competing interest

The authors have no conflicts of interest for this study.

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