MONASH University

# Evaluating the Progression of Parkinson's Disease with Dynamic 4D Laryngeal CT

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**Abstract**

Parkinson's disease (PD) is a progressive neurodegenerative condition for which there is no treatment to slow or halt its course. Finding effective disease-modifying treatments is made more difficult because of our poor understanding of the disease pathogenesis and lack of reliable biomarkers reflecting the presence and progression of the disease.

Assessment of the vocal changes of PD have been proposed as novel techniques in its diagnosis and assessment, but none appear adequately reliable for use in clinical practice yet. Prior work using laryngoscopy to directly visualise the larynx in participants with Parkinson's disease (pwPD) described abnormalities including abnormal posturing and movement of the arytenoid cartilages, as well as bowing of the vocal folds.

This thesis primarily aims to characterise and analyse the gross anatomical abnormalities which account for the voice changes of PD using dynamic 4D laryngeal CT, as a means of assessing the disease's presence and severity. We performed dynamic 4D laryngeal CT on a cohort of pwPD covering a range of disease durations and severities, as well as healthy control participants. We performed measurements including the 'inter-arytenoid distance' which pertains to the positioning and movement of the arytenoid cartilages, as well as the 'glottic area' which relates to the presence of vocal fold bowing. We found that analysis of laryngeal measures relating to arytenoid cartilage positioning and vocal fold bowing could not only indicate the disease's presence, but that they are also correlated with the disease's duration and severity. However, in contrast to the asymmetric slowing typically seen in the limbs in PD, movements of the arytenoid cartilages were not slowed, nor was there asymmetry in movement between the paired cartilages. Thus, our findings suggest that the voice changes of PD are due to the presence of vocal fold bowing and abnormalities in the posturing of the arytenoid cartilages, and that these laryngeal abnormalities progress as the disease advances.

Secondarily, this thesis aims to improve the feasibility of ongoing research into PD using 4D laryngeal CT. Manual measurement of the inter-arytenoid distance is a laborious process, limiting its utilisation in large-scale research and clinical settings. We set out to validate a machine-learning module which could automatically perform the measurements required. Our results demonstrate

that estimates of the inter-arytenoid distance with our automated machine-learning module are accurate and are a promising tool to be utilised in future work studying the laryngeal changes of Parkinson's disease.

Considered together, the works presented in this thesis further our understanding of the processes responsible for the voice changes seen in the condition. Moreover, they culminate to provide a strong foundation and direction for further study with regards to the use of 4D laryngeal CT in evaluating PD.

**Declaration**

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes two original papers published in peer reviewed journals and one submitted publication. The core theme of the thesis is the use of dynamic laryngeal computed-tomography imaging in evaluating the presence and severity of Parkinson's disease. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Central Clinical School under the supervision of Professors Dominic Thyagarajan and Kenneth Lau.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2, 3 and 5, my contribution to the work involved the following:

| Thesis Chapter | Publication Title and Status | Status | % of student contribution | Co-author name(s) and % of co-author's contribution |
|---|---|---|---|---|
| Two | Voice Changes in Early Parkinson's Disease: What Are They Telling Us? | Published | Andrew Ma (90%) | Dominic Thyagarajan (7.5%)<br>Kenneth K Lau (2.5%) |
| Three | Radiological Correlates of Vocal Fold Bowing as Markers of Parkinson's Disease Progression | Published | Andrew Ma (70%) | Dominic Thyagarajan (20%)<br>Kenneth K Lau (10%) |
| Five | Automated measurement of inter-arytenoid distance on 4D laryngeal CT: A validation study | Submitted | Andrew Ma (65%) | Nandakishor Desai (15%)<br>Dominic Thyagarajan (8.5%)<br>Kenneth K Lau (5%)<br>Marimuthu Palaniswami (2.5%)<br>Paari Palaniswami (2.5%)<br>Terence O'Brien (1.5%) |

The nature of the contribution of my co-authors and I are detailed in the provided manuscripts. None of the co-authors listed for these manuscripts are Monash University students.

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

**Student name:** Andrew Ma

**Student signature:** **Date:** 30/05/2022

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

**Main Supervisor name:** Dominic Thyagarajan

**Main Supervisor signature:** **Date:** 30/05/2022

**Research outputs and awards**

Published peer-reviewed research articles

Ma A, Lau KK, Thyagarajan D. 2021. Radiological Correlates of Vocal Fold Bowing as Markers of Parkinson's Disease Progression. PLOS One. 2021.

Ma A, Lau KK, Thyagarajan D. 2020. Voice Changes in Early Parkinson's Disease: What Are They Telling Us? J Clin Neurosci. 2020;72:1-7.

Submitted research articles

Ma A, Desai N, Lau KK, Palaniswami M, O'Brien TJ, Palaniswami P, Thyagarajan D. 2022. Automated measurement of inter-arytenoid distance on 4D laryngeal CT: A validation study. Manuscript submitted for publication.

Research presentations

Ma A, Lau KK, Thyagarajan D. 2021. Progressive changes in laryngeal posturing and dynamics as biomarkers of progression in Parkinson's disease. E-poster presentation at the Movement Disorders Society Virtual Congress 2021.

Ma A, Thyagarajan D. 2019. Arytenoid cartilage hypokinesis as a marker of Parkinson's disease severity. Poster presentation at the MDSANZ Annual Scientific Meeting, Adelaide.

Research awards

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I would also like to thank the participants who volunteered their time and effort – such work would of course not be possible without your generous contributions. I would also like to thank the clinicians and researchers who contributed to the earlier study on 4D laryngeal CT in Parkinson's disease and laid the foundations for the works to follow in this thesis.

Last but not least, thank you to my family, friends and loved ones for your support. In particular, I thank my mother and father for your love and the opportunities I have been provided, and my brother Tim for your part in shaping who I've become today.

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# Contents

# Chapter 1
# Introduction

## 1.1 Motivation

Parkinson's disease (PD) is a neurodegenerative condition which remains a significant health issue in Australia and worldwide. Unfortunately, the condition is unremittingly progressive and without any significant developments in its diagnosis or management, its impacts are anticipated to continue growing. Despite numerous therapies having undergone clinical trials, there is still no treatment that can slow or halt the course of the disease.

Finding effective disease-modifying treatments is made more difficult because of our poor understanding of the disease pathogenesis and lack of reliable biomarkers reflecting the presence and progression of the disease. By the time patients show early clinical signs of PD, there is already evidence of advanced neurodegeneration[1] with an estimated 30-50% loss of nigrostriatal dopaminergic neurons.[2]

In clinical and research settings, the diagnosis and assessment of severity of PD continues to be based on clinical assessment, despite its limitations.[3, 4] Identifying a biomarker of the disease is of increasing interest in the current research landscape, with many efforts focused on the pursuit of neuroprotective and disease-modifying therapies. Such a biomarker would ideally facilitate earlier diagnosis or more accurate tracking of disease progression than what clinical assessment currently allows.

Assessment of the vocal changes seen in PD has been proposed as novel techniques in its diagnosis and assessment.[5-8] Emerging evidence suggests that voice dysfunction is the earliest sign of motor impairment in PD. However, no modalities used to investigate these voice changes have shown that they are adequately reliable for use research purposes or clinical practice. Accordingly, voice changes are not considered in the current diagnostic criteria for clinical and prodromal PD.[3, 9]

These voice changes have been characterised through multiple different methods. The auditory phenomenology is studied through perceptual analyses by trained assessors, as well as acoustic analyses which provide an objective measure. The physiological and anatomical correlates of these

auditory changes have been investigated using laryngoscopy, videostroboscopy, photoglottography (PGG), laryngeal electromyography (LEMG), lung function studies and aerodynamic studies. However, many of these techniques are invasive and not ideal for transitioning to widespread clinical use.

As a relatively non-invasive investigation, four-dimensional (4D) laryngeal computed tomography (CT) is a promising technique to study the changes to vocal fold and arytenoid movement in PD. It permits the study of the laryngeal apparatus by providing dynamic cross-sectional imaging. It allows the visualisation of the glottis and arytenoid cartilages with high spatial resolution, while also providing high temporal resolution so that the phonatory apparatus can be imaged whilst in motion during vocalisation. Only one previous study by Perju-Dumbrava and colleagues[5] has studied the voice changes of PD using laryngeal CT. Their study measured the 'inter-arytenoid distance' (*IAD*) and 'glottic area' (*GA*) in participants with early PD and healthy controls and found statistically significant reductions in the *IAD* in those with PD when compared to control participants.[5] However, their study does not provide insight into how these measures change with increasing duration and severity of disease. Understanding how these anatomical measures change over the course of the disease would provide a foundation for its use as a marker of disease progression.

This thesis intends to expand upon the existing body of work, with the primary aim of characterising how these anatomical laryngeal measures change as PD progresses using dynamic laryngeal CT. The remainder of 'Chapter 1: Introduction' outlines the research aims and hypotheses. 'Chapter 2: Literature Review' begins by providing relevant background information to understand the anatomy of the larynx and the physiology of vocalisation. It then outlines the changes to the voice which occur in PD, highlights the various modalities which have been used to characterise these changes, and explores how these insights can further inform our understanding and evaluation of PD. It also explores prior works studying automated methods of obtaining the laryngeal measurements in 4D laryngeal CT.

The first experimental study is presented in 'Chapter 3: Dynamic laryngeal CT in the evaluation of Parkinson's disease progression' which aims to determine how measures of laryngeal dynamics and posturing change as PD progresses in duration and motor severity.

Next, 4D laryngeal CT is a relatively novel modality in the evaluation of PD and it is currently unclear which measurements are most useful in determining its presence or severity. Previous laryngoscopic studies have demonstrated asymmetry of the arytenoid cartilages in those with PD[10] and 'Chapter 4: Symmetry of arytenoid cartilage movements in Parkinson's disease' explores whether the presence of left-right asymmetry in the movements of the arytenoid cartilages could be used as a biomarker for the disease's presence or severity.

Additionally, prior work in dynamic laryngeal CT raised the laborious nature of manually marking the CT images as a barrier to its use.[5] Automated methods of measurement provide a solution. If sufficiently precise, automated methods would increase the feasibility of using dynamic laryngeal CT in larger-scale research and clinical settings. 'Chapter 5: Validating automated measurement of inter-arytenoid distance on 4D laryngeal CT' presents a validation study of a machine-learning module developed by our collaborators which obtains these measurements in an automated manner.

Lastly, 'Chapter 6: Integrative Discussion' concludes the thesis by synthesising the findings of this body of work and discusses what it contributes to the current research landscape. The outcomes of this research are appraised and future directions for further work in this area of study are suggested.

### 1.2 Research aims and hypotheses

1. To characterise how arytenoid cartilage position and hypokinesis changes as PD progresses using laryngeal CT, to provide a foundation for its use as a biomarker of disease progression. We hypothesise that with advancing duration and clinical severity of PD, the inter-arytenoid distance, and speed and amplitude of arytenoid cartilage movements reduces.
2. To confirm the results of Perju-Dumbrava and colleagues[5] in a larger data set which includes subjects with advanced PD who have a greater duration and severity of disease.
3. To determine whether there is asymmetry arytenoid cartilage motion in PD, and whether this asymmetry corresponds to the sidedness of parkinsonism in the limbs. We hypothesise that the arytenoid cartilage on the dominant side of the disease will have a greater reduction in the amplitude and speed of movements than the less affected side.
4. Assess the validity of automated detection methods to calculate the inter-arytenoid distance on dynamic laryngeal CT.

# Chapter 2
# Literature review

## 2.1 Overview

This chapter presents my review of the literature across multiple sections. Section 2.2 consists of my published literature review entitled 'Voice changes in Parkinson's disease: What are they telling us?'. It provides background on the anatomy and physiology of vocalisation, outlines the characteristics of the voice dysfunction in PD and explores the possible pathological mechanisms underpinning these voice changes.

As a novel modality, the breadth of research pertaining to dynamic 4D laryngeal CT in the evaluation of PD is limited, and its relatively minor representation in the published literature review is reflective of this. However, as this novel modality forms the focus of this thesis, Section 2.3 delves into greater detail on dynamic 4D laryngeal CT, with close examination of the prior study by Perju-Dumbrava and colleagues.[5] Next, Section 2.4 appraises prior studies which explore the use of voice assessment for the diagnosis and evaluation of PD, while Section 2.5 looks at previous works which investigate automated methods of measurement in laryngeal CT.

This chapter concludes with Section 2.6 which summarises the key concepts raised through this review of the literature.

## 2.2 Published literature review

This section is represented by the following published literature review, 'Voice changes in Parkinson's disease: What are they telling us?' (*Ma A, Lau KK, Thyagarajan D. 2020. Voice Changes in Early Parkinson's Disease: What Are They Telling Us? J Clin Neurosci. 2020;72:1-7*).

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Review article

# Voice changes in Parkinson's disease: What are they telling us?

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ABSTRACT

Emerging evidence suggests voice dysfunction is the earliest sign of motor impairment in Parkinson's disease (PD). The complexity and fine motor control involved in vocalization may result in dysfunction here before the limbs. The voice in PD demonstrates characteristic changes on perceptual and acoustic analyses. The physiological and anatomical correlates of these have been investigated through laryngoscopy, stroboscopy, photoglottography, laryngeal electromyography, computed-tomography, pulmonary function testing and aerodynamic assessments. These have revealed numerous abnormalities including incomplete glottic closure and vocal fold hypoadduction/bowing to account for these voice changes. Many of these phenomena are likely related to rigidity or bradykinesia of the laryngeal muscles. The early onset of voice changes is resonant with the pathophysiological insights offered by Braak's hypothesis and murine models of the disease. These physiological abnormalities and pathological models largely stand to support dopaminergic and non-dopaminergic mechanisms being implicated in the pathogenesis of voice dysfunction. This review focuses on characterizing the voice changes in PD. These stand as a promising area of enquiry to further our understanding of the pathophysiology of the disease and offer potential to be utilized as an early diagnostic biomarker or marker of disease progression.



## 1. Background

Voice disorder in Parkinson's Disease (PD) is prevalent, affecting approximately 70 to 90% of those with PD [1–3]. Abnormalities of acoustic analysis are found as frequently, even in early PD [4,5]. These voice changes are part of a wider constellation of speech changes, collectively described as 'hypokinetic dysarthria' or 'hypokinetic dysarthrophonia' [6].

Speech abnormalities occur across interconnected domains of phonation, articulation and prosody. With respect to phonation, there is reduced voice volume (hypophonia) and altered voice quality (dysphonia). Articulation is impaired by a reduction in the range of articulatory movements (hypokinetic articulation). Dysprosody of speech is manifested by flattened pitch inflection (monopitch) and loss of stress (monoloudness). Other features include festination and hesitancy of speech [1,3,7].

Emerging evidence suggests that voice dysfunction is the earliest sign of motor impairment in PD. Perhaps the complexity and fine motor control involved in vocalization results in dysfunction here before the limbs [8]. Retrospective acoustic analyses have shown reductions in the variability of fundamental frequency of speech up to 5 years before diagnosis [8]. Further understanding of the onset of these speech abnormalities has been derived by studying the prodrome leading to PD in cohorts of individuals with REM sleep behavior disorder (RBD), who have a high risk of developing synucleinopathies and other neurodegenerative syndromes (33.1% at five years, 75.7% at 10 years and 90.9% at 14 years after a diagnosis of RBD) [9]. Speech impairment was identified in 88% of individuals with RBD on acoustic analysis [10] and back-extrapolating a prospective study of individuals with RBD suggested that speech changes first manifest 6–7 years before a diagnosis of PD; i.e. preceding the onset of the all other motor features [11].

PD remains a clinical diagnosis [12] and identifying a suitable early biomarker has remained elusive. Correlates of the vocal changes seen in PD have been proposed as novel techniques in the diagnosis and assessment of PD and RBD [13–16], but none appear adequately reliable for use in clinical practice yet. Consequently, current diagnostic criteria for clinical and prodromal PD do not include them [12,17]. The importance of identifying such

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a biomarker is of increasing interest in the current era of pursuing neuroprotective and disease modifying therapies.

This review will focus on the phonatory and prosodic changes in the voice in PD. We will describe the normal anatomy and physiology of voice production as necessary background to understanding the various modalities employed to characterize voice in PD. We will then review pathological studies of PD in humans and murine models to provide further insight into the pathogenesis of these voice changes.

## 2. Anatomy and physiology of vocalization

The wide range of sounds that the human voice can create occurs through several processes along the respiratory tract. This begins with the creation of air flow from the lungs. Phonation is the result of sound production from vibrations at the vocal folds secondary to change in air pressure. Articulation involves 'shaping' this sound through structures in the phonatory tract such as the lips, teeth and tongue to produce speech [18].

The main cartilages involved in phonation are the singular thyroid and cricoid cartilages and a pair of arytenoid cartilages. The thyroid cartilage is a shield-like structure which rests on top of the ring-shaped cricoid cartilage. The arytenoid cartilage is a pyramidal structure with its base resting on the cricoid cartilage. The muscular process is directed posterolaterally and is the attachment point for many of the intrinsic laryngeal muscles which rotate the cricoarytenoid joint. The vocal process is directed anteriorly towards the thyroid cartilage and is the attachment of the vocal ligament. The true vocal folds are formed by the thyroarytenoid muscle covered by multiple layers of lamina propria and squamous epithelium. The vocal ligament is formed by the intermediate and deep layers of the lamina propria [19,20].

The intrinsic laryngeal muscles (see Fig. 1) abduct and adduct the vocal folds, as well as affect their length and tension. Abduction of the folds occur when the arytenoid cartilages rotate outwards and move apart, while adduction occurs when they rotate inwards and move medially. The only abductor is the posterior cricoarytenoid muscle. The main adductors are the thyroarytenoid, lateral cricoarytenoid and interarytenoid muscles [21]. The medial portion of the thyroarytenoid muscle which attaches to the vocal process of the arytenoid is referred to as the vocalis muscle. The vocalis muscle shortens the length and thickens the vocal folds to decrease pitch. Meanwhile the cricothyroid muscle pivots the thyroid cartilage down onto the cricoid cartilage, elongating, tightening and thinning the vocal fold, thereby raising pitch [22].

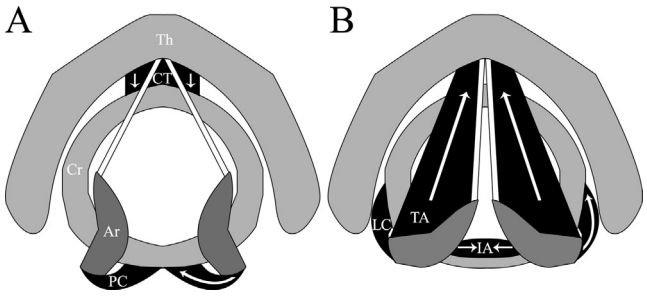


**Fig. 1.** Schematic diagrams of the larynx as viewed from above. The vocal folds are represented in white, the intrinsic laryngeal muscles (PC, posterior cricoarytenoid muscle; CT, cricothyroid muscle; TA, thyroarytenoid muscle; LC, lateral cricoarytenoid; IA, interarytenoid muscle) in black and the cartilages (Th, thyroid cartilage; Cr, cricoid cartilage; Ar, arytenoid cartilage) in grey. A, abduction of the vocal folds by the posterior cricoarytenoid muscle and lengthening of the vocal folds by the cricothyroid muscle pivoting the thyroid cartilage down towards the cricoid cartilage. B, adduction of the vocal folds by the thyroarytenoid, lateral cricoarytenoid and interarytenoid muscles. Adapted from Berke et al. [21].

These muscles are innervated by branches of the vagus nerve. All the intrinsic muscles of the larynx are supplied by the recurrent laryngeal nerve, except the cricothyroid muscle which is supplied by the external laryngeal nerve. The vagus nerve stems from several nuclei of the medulla, with the lower motor neurons of the intrinsic laryngeal muscles arising from the nucleus ambiguus [21].

At its most basic level, the voice is produced by the motor and sensory nuclei of the lower brainstem and spinal cord. These are connected and coordinated by nuclei of the lateral reticular formation. Together, they perform basic control of laryngeal, articulatory and respiratory activity [23].

Higher order neural control of vocalization is complex and not well understood. Recent models propose two functional pathways. One network is thought to coordinate voluntary vocalization. This arises from the upper motor neurons responsible for laryngeal and orofacial control within the primary motor cortex, with additional inputs by the ventral pre-motor cortex and Broca's area. Meanwhile a second network is thought to control innate emotional and non-verbal vocalizations such as crying and laughter. This is mediated by the periaqueductal gray matter, anterior cingulate cortex, parabrachial nucleus and the limbic system [23,24].

## 3. Auditory features of vocal dysfunction in Parkinson's disease

Perceptual analyses involve clinical rating of speech by experienced listeners. While subjective, they are seen to be clinically useful as they are typically more representative of a patient's actual communicative function, deficits and needs [3] than acoustic analyses, which are an objective measure of auditory phenomenology. These involve signal analysis of the sound waveforms in order to extract parameters which reflect voice character [25].

***Changes in quality:*** A rough and weak/'asthenic' voice quality is a common feature [5,26–29]. Meanwhile, acoustic analysis shows reduced harmonics-to-noise ratio (HNR) in those with PD [4,29–34]. HNR measures the proportion of 'harmonic' sound stemming from the vibration of the vocal folds against the 'noise' generated from the glottis [25], with its reduction correlating with a rough and hoarse voice [35].

Jitter is a measure of frequency perturbation. It represents the cycle-to-cycle variability in the fundamental frequency during steady phonation [25]. Increases in jitter have been identified in PD, with further increases as the disease progresses [4,28–31,33,36,37]. Jitter may indicate unstable vocal fold vibration and is associated with a rough vocal quality [4,25,35].

A breathy voice is also evident in PD [5,26–28]. Breathiness is caused by glottic air leakage which adds noise because of turbulent air flow in the larynx [35]. Shimmer is another acoustic parameter which measures the cycle-to-cycle variation in amplitude during steady phonation and increases in this variation are associated with breathiness [25]. Many studies of PD show an increase in shimmer [4,27,29,30,33,36].

***Changes in volume:*** Impairments in volume control producing hypophonia and monoloudness has been noted in PD [26,28,29,31]. This is reflected in acoustic analyses as reduced range and variation of intensity respectively [4,28,29,31,33,38].

***Changes in pitch:*** Perceptual analyses report higher pitch in early PD [28,31,39,40]. This observation correlates with higher mean fundamental frequency, which has been identified on acoustic analysis in many individuals with PD [27,29–31,38]. Fundamental frequency is clinically perceived as pitch [25]. Perceptual analyses show a monopitch character with reduced intonation of voice in PD [5,28,29,31] which correspond to decrease in the variability of fundamental frequency in acoustic analysis [4,28,29,31,33,34,41].

## 4. Physiological observations and measures of vocal dysfunction in Parkinson's disease

Numerous techniques have been employed to identify physiological correlates of the auditory phenomena in PD (Table 1).

***Laryngoscopy:*** Direct visualization with video laryngoscopy has revealed incomplete glottic closure due to impaired vocal fold adduction and bowing in the early and late stages of PD [26,27,31,32,42]. Other non-closure glottic patterns have been identified (see Fig. 2) [26,27,32,42–44]. Incomplete glottic closure results in air leakage across the open glottis during phonation and causes abnormalities of vocal quality such as breathiness, asthenia and roughness [27,45]. It also reduces subglottal pressure, resulting in reduced vocal intensity [46]. The size of visible glottic gap during phonation correlates with increasing breathiness, reducing vocal intensity and difficulty in sustaining prolonged phonation [26].

Asymmetry in vocal fold closure, as well as arytenoid cartilage position and movement has also been described. A laryngoscopic study by Hanson and colleagues [26] identified asymmetry in 26 out of 32 subjects with advanced PD (mean disease duration of 13 years). They found that the side with greater abnormality of vocal fold movement was always the side of the body affected most by motor manifestations of PD. The more affected side typically had a longer vocal fold and its arytenoid cartilage was positioned more posteriorly and laterally than the other. In subjects with shorter disease duration, hypoadduction of the vocal fold on the more affected side was associated with hyperadduction of the other. This resulted in better glottic closure [26,42,44], which was correlated with better voice [26].

***Videostroboscopy/Photoglottography:*** Videostroboscopy is often employed with laryngoscopy to visualize the rapid vibratory movements of the vocal folds during phonation. Modern videostroboscopy systems create a seemingly slow-motion recording of these vibrations by synchronizing parameters of the camera and strobe light to the frequency of vocal fold vibrations [48].

The vibratory pattern of the vocal folds is frequently asymmetric in PD – a finding termed phase asymmetry [32,49]. This may arise from the asymmetry of rigidity typical of PD, creating asymmetric vocal fold tension [49].

During a cycle of normal vocal fold vibration, the time for the vocal folds to move apart (glottal opening time) is longer than the time to come together (glottal closing time). In PD, the glottal opening time becomes abnormally prolonged. Using videostroboscopy, Perez and colleagues [49] identified prolongation of the glottal opening time in 50% of their cohort of individuals with PD (mean disease duration 6.9 years). Analogous findings are obtained on photoglottography, where a light source is placed above or below the glottis, with a light sensor on the other side. The sensor captures the amount of light passing through the glottis, which varies as the vocal folds vibrate [50]. On photoglottography, the speed quotient – a ratio of the glottal opening to closing time – is increased in PD [51,52]. The increase in glottal opening time and speed quotient may reflect rigidity of the vocalis muscle exerting increased resistance to glottal opening [51,52].

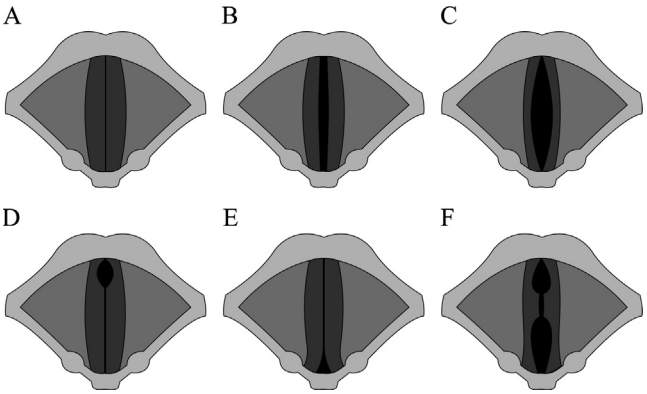


**Fig. 2.** Schematic diagrams of a laryngoscopic view of the larynx demonstrating the vocal fold abnormalities seen in PD. A, normal complete vocal fold closure. B, incomplete closure of the vocal folds. C, vocal fold bowing. D, anterior chink. E, posterior chink. F, hourglass deformity. Adapted from Bless et al. [47].

***Laryngeal EMG:*** In PD, spontaneous intrinsic muscle activity at voice rest is often increased in the thyroarytenoid and cricothyroid [42,53–56] and reduced in the posterior cricoarytenoid muscles [42]. This is occurs in most individuals with PD and voice complaints irrespective of disease severity [56] and it correlates with the severity of clinical speech impairment [42]. These changes may represent loss of normal reciprocal forces between antagonistic muscle groups, analogous to the rigidity typical of parkinsonism [26,42,53].

Rigidity of the intrinsic laryngeal muscles may explain vocal fold bowing. Mismatched co-ordination between the thyroarytenoid, cricothyroid and posterior cricoarytenoid muscles is thought to result in shortening and tensing of the vocal folds, giving rise to the bowed appearance [26,42].

Overactivation of the cricothyroid muscle also accounts for increased mean fundamental frequency. Biomechanical models of phonation demonstrate that increasing vocal fold stiffness increases fundamental frequency and jitter [57,58]. Furthermore, these models show that asymmetrical vocal fold vibration leads to a decreased harmonics-to-noise ratio, increased jitter and decreased voice quality [58]. According to these models, it is not just rigidity in PD which contributes to voice dysfunction, but also its asymmetry.

***Laryngeal CT:*** Perju-Dumbrava and colleagues [13] utilized a 320-slice volume CT scanner with higher spatial and temporal resolutions than older-generation scanners to produce 4-dimensional imaging of the larynx. They obtained measurements of the inter-arytenoid distances and glottic areas over time during repeated phonations. They demonstrated that the inter-arytenoid distances upon vocalization is reduced in PD compared with controls, concordant with previous observations of vocal fold hypokinesis. Meanwhile, glottic area is maintained in PD and did not differ sig-

**Table 1**
Relationships between the clinical, perceptual and acoustic characteristics of the voice changes in PD.

| Speech Domain | Clinical and Perceptual Features | Acoustic Features | Laryngoscopic and Stroboscopic Features | Electromyographic Features |
|---|---|---|---|---|
| Phonation | Dysphonia<br>Rough/asthenic voice<br>Higher pitch<br>Hypophonia<br>Breathy voice | Decreased harmonics-to-noise ratio<br>Increased jitter<br>Increased mean $F_0$<br>Reduced range of intensity<br>Increased shimmer | Asymmetric vocal fold position<br>Phase asymmetry<br>Increased glottal opening time<br>Vocal fold bowing<br>Incomplete glottic closure | Asymmetric intrinsic laryngeal muscle rigidity |
| Prosody | Monoloudness<br>Monopitch | Decreased variation of intensity<br>Decreased variability of $F_0$ | | |

nificantly from controls despite reduction in inter-arytenoid distance, from which they inferred a change in glottic shape corresponding to vocal fold bowing.

***Respiratory assessment:*** The generation of airflow necessary for vocalization can be compromised in PD. The elements of respiratory dysfunction in PD have been reviewed in depth elsewhere [59], but are predominantly characterized by restrictive respiratory dysfunction and reduction in maximal inspiratory and expiratory pressures [60–62]. Aerodynamic studies show reductions in subglottal pressure, laryngeal resistance and peak airflow [63,64]. The combination of glottal incompetence and reduced respiratory driving pressure could account for these findings.

## 5. The roles and effects of dopamine on vocal dysfunction in Parkinson's disease

***Dopamine transporter imaging:*** An analysis of individuals with early untreated PD of less than two years duration demonstrated lower striatal presynaptic dopaminergic uptake on [$^{123}$I] FP-CIT single photon emission computed tomography (SPECT) in those with speech impairment (defined as Unified PD rating Scale Part-III, Item 3.1, Speech $\geq 1$) than those without. Lower FP-CIT uptake was associated with greater speech impairment. Additionally, speech impairment correlated most closely with limb bradykinesia and rigidity, and was more common with an akinetic-rigid phenotype (69.9%) compared to tremor-dominant (18.9%) [65]. It has been shown that akinetic-rigid disease is associated with lower striatal FP-CIT uptake than tremor-dominant disease [66,67]. Taken together, these findings suggest that dopaminergic deficiency causes voice dysfunction in PD, presumably through bradykinesia and/or rigidity of the laryngeal musculature. While this predicts that levodopa would be effective in ameliorating voice dysfunction, studies have yielded mixed results.

***Effects on perceptual and acoustic characteristics:*** A systematic review and *meta*-analysis by Pinho and colleagues [68] pooled the results of studies using acoustic analysis to assess the effects of levodopa on the voice. Four studies were eligible to assess fundamental frequency (n = 67), three for jitter (n = 41) and two for vocal intensity (n = 35); the small number of studies being a limitation of this *meta*-analysis. The pooled data revealed statistically significant reductions in fundamental frequency and jitter; features which likely relate to increased laryngeal muscle rigidity and vocal fold tension [57,58]. On the other hand, vocal intensity did not improve. They did not assess the effects of levodopa on the variability of fundamental frequency, a prosodic feature which has shown improvement with levodopa in several studies [8,34,41], yet not others [38,69,70]. The absence of a robust dopaminergic response suggests that dysprosody is caused partly by non-dopaminergic mechanisms.

***Effects on laryngostroboscopy and LEMG:*** Early laryngoscopic studies did not identify changes after levodopa [26] but later electroglottographic assessments demonstrated reduction of the glottic opening time after levodopa [51]. This suggests that levodopa may normalize vocal fold tension by reducing rigidity, but does not improve gross abnormalities of glottic closure.

Gallena and colleagues [42] assessed LEMG in levodopa-naïve subjects with recently diagnosed PD. They found that those who had vocal fold bowing or clinical voice impairment had increased activation of the thyroarytenoid and cricothyroid muscles, and levodopa reduced thyroarytenoid muscle overactivation.

***Effects on respiration:*** The effects of levodopa on respiratory function are addressed in a systematic review and *meta*-analysis by Monteiro and colleagues [71] which pooled the results of four studies (n = 73). They found that levodopa improves FVC and peak expiratory flow (PEF), but not forced expiratory volume in one second ($FEV_1$) or $FEV_1$/FVC ratio. The authors attributed this to an improvement in chest wall compliance.

Another study by De Letter and colleagues [72] demonstrated dynamic changes in vital capacity across a levodopa cycle, which largely parallel those of motor performance. Additionally, those who developed dyskinesias at peak motor performance often had accompanying reductions in vital capacity. This study demonstrates the value of performing serial measurements following levodopa administration and suggests that dyskinesias may impair both respiration and phonation.

***Effects of neuroleptics:*** The involvement of dopaminergic mechanisms of voice change in PD is also supported by studies in which dopamine neurotransmission is blocked. A cross-sectional study by Sinha and colleagues [73] assessed voice in 140 individuals taking atypical neuroleptic medications for various psychiatric indications. They found that the severity of bradykinesia, tremor, rigidity and postural instability correlated with voice impairment on perceptual measures, reduced harmonics-to-noise ratio, as well as higher mean fundamental frequency in males on acoustic analysis. Thus, similar voice changes are found in idiopathic PD and drug-induced parkinsonism.

## 6. Correlations to the pathological abnormalities of Parkinson's disease

The neuropathological mechanisms underlying the voice abnormalities of PD have not been well characterized. In their cross-sectional neuropathological study of PD, Braak and colleagues [74] inferred that Lewy bodies and neurites (henceforth referred to collectively as Lewy pathology) first occur in the anterior olfactory nucleus and lower brainstem, then the pathology spreads progressively more rostrally in six defined stages. From the brain structures involved in this pathological progression, we can infer the origins of voice dysfunction in PD.

***Stage One:*** In the first stage, Lewy pathology infiltrates the dorsal motor nucleus of the vagus and glossopharyngeal nerves in the medulla [74]. Note that the dorsal motor nucleus of the vagus is a parasympathetic nucleus, and it is actually the nucleus ambiguous – one of few structures spared by Lewy pathology [75] – which innervates the intrinsic laryngeal musculature. Therefore, involvement of the dorsal motor nuclei is unlikely to contribute to voice dysfunction in PD.

***Stage Two:*** In the second stage, Lewy pathology is found within many nuclei of the reticular formation in the pons [74]. Some of these brainstem nuclei are involved in emotional control and the affective modulation of voice and speech. Their dysfunction may contribute to dysprosody. It has been observed that speech impairment in PD is greater in emotional than in neutral contexts [76].

Brain stem nuclei involved in emotional regulation in humans have been identified through a combination of pathological and neuroimaging techniques. It is proposed that these nuclei form emotional control networks that are modulated by a variety of neurotransmitters. These include the raphe nuclei (serotonergic), locus coeruleus (noradrenergic), ventral tegmental area (dopaminergic) and the pedunculopontine and laterodorsal tegmental nuclei (cholinergic) [77]. Lewy pathology has been found extensively in nearly all of these structures [78]. Post-mortem pathological studies of PD demonstrate that neuronal cell loss is greater in the pontine noradrenergic locus coeruleus than the substantia nigra [79]. This may account for the higher prevalence of prosodic over phonatory or articulatory impairment in early PD.

In keeping with the spatio-temporal trajectory of Lewy pathology, a study of early untreated PD (mean duration of disease 30.22 months) found prosodic impairment in 60.87% of the cohort on acoustic analysis of speech. Articulatory and phonatory impairment was less common at 39.13% and 26.09% respectively [4]. On the other hand, another study in a cohort of RBD, some with

parkinsonism, identified mainly articulatory and phonatory speech impairment. The authors hypothesized that the difference in speech profile between these studies arises from a different underlying pattern of neurodegeneration in RBD [10].

***Stage Three:*** In the third stage, there is degradation of dopaminergic neurons of the basal ganglia by Lewy pathology, particularly the substantia nigra [74]. Dopamine deficiency in this phase could cause rigidity and hypokinesia of the laryngeal and respiratory musculature, leading to numerous features of voice dysfunction as previously described.

The basal ganglia may also contribute to dysprosody. A study of individuals with prior ischemic stroke revealed that disruption to the basal ganglia circuits is associated with impairment in the expression and comprehension of emotion [80]. Functional MRI has shown that the basal ganglia are involved in modulating motor control according to affective state [81] and that circuits involving the basal ganglia demonstrate abnormal connectivity in early PD [82].

## 7. Animal models of voice dysfunction in Parkinson's disease

Ultrasonic vocalizations (USV) in rats are an encouraging area of research. As USV are produced by modulation of egressive airflow for the purposes of communication, the study of USV in animal models of PD may cast light on the origins of voice abnormalities in PD [83].

***Dopamine depletion/blockade models:*** There have been several studies of USV in rats with induced defects of dopaminergic neurotransmission. Methods included unilateral infusion of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle as well as the administration of haloperidol or selective $D_1$ and $D_2$ antagonists. These resulted in significant reductions in the intensity and bandwidth of vocalizations as well as an alteration in call profiles when compared to controls [84–86]. Thus, pharmacologically-induced deficiency of nigrostriatal dopaminergic neurotransmission is sufficient to produce changes in USV.

***Neurodegenerative models:*** Alpha-synuclein over-expressing and PTEN-induced putative kinase (PINK1) knock-out mice are models which reflect the broader and gradual neurodegeneration that characterizes PD. Studies of USV in both models showed reduced call intensity and altered call profiles [83,87,88]. Alpha-synuclein overexpressing mice also had reduced duration and rate of calls [83] while the PINK1 knock-out mice had reductions in bandwidth and peak frequency of calls [87].

In both models, USV changes began from 2 to 3 months of age [83,87]. This precedes nigrostriatal dopamine deficiency in both models, which only becomes significant at 14 months of age in the alpha-synuclein overexpression model [83] and 8 months in the PINK1 knock-out model [89,90]. Instead, alpha-synuclein aggregation was found in the periaqueductal gray matter in the alpha-synuclein overexpression model. The periaqueductal gray matter is a structure thought to modulate vocalizations in humans. Stimulating it results in natural-sounding vocalizations and lesioning it results in mutism [91]. Alpha-synuclein was identified within the noradrenergic locus coeruleus in the PINK1 model with corresponding reductions in tyrosine hydroxylase staining [87]; a structure which is affected by Lewy pathology in PD prior to nigrostriatal involvement [74]. Thus, these models suggest the presence of non-dopaminergic mechanisms such as noradrenergic dysfunction being implicated early in vocal dysfunction.

## 8. Conclusion

There have been many approaches to characterizing vocal dysfunction in PD. Objective acoustic analyses confirm what has been apparent through clinical experience and perceptual analyses. Voice disturbance appears to stem from two main pathological processes in the larynx; asymmetric rigidity of the intrinsic laryngeal muscles and incomplete glottic closure due to vocal fold hypokinesis/bowing. These changes may herald the onset of motor dysfunction in PD. The early involvement of the voice is consistent with the proposed progression of Lewy pathology in Braak's hypothesis.

Integrating these analyses with observational clinical research yields numerous insights into the pathophysiology of vocal dysfunction in PD. Phonatory dysfunction in PD correlates with bradykinesia, rigidity and dopamine deficiency. Furthermore, some aspects of phonation improve with levodopa administration. On the other hand, there is an inconsistent effect of dopamine replacement on prosodic dysfunction, suggesting that it may in part relate to non-dopaminergic mechanisms.

The inconsistent findings relating to levodopa responsiveness on voice may also reflect methodological differences, including which parameters of vocal dysfunction have been selected. Voice changes are influenced by sex, disease duration and severity [28,39]. Responses after levodopa administration are also dynamic and may be affected by dyskinesias [72]. Future work requires careful consideration of these factors in the study design.

As we move forward into an era marked by increasing efforts at neuroprotective and disease modifying therapies for PD, seeking relevant therapeutic targets and identifying an early diagnostic marker is of growing importance. The voice changes of PD stand as a promising area of enquiry; particularly by studying people with early PD and RBD, as well as exploring murine models of PD. Listening to what these voice changes are telling us will further our understanding of the pathophysiology of PD and potentially yield novel clinical applications.

## Author contributions

Review of the literature: Andrew Ma, Manuscript preparation – Writing of the first draft: Andrew Ma, Manuscript preparation – Review and Critique: Andrew Ma, Dominic Thyagarajan, Ken Lau, Supervision: Dominic Thyagarajan

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## 2.3 Dynamic 4D Laryngeal CT in the assessment of Parkinson's disease

Dynamic 4D laryngeal CT is a relatively novel modality in the study of PD. The only study which has evaluated its use in PD was performed by Perju-Dumbrava and colleagues.[5] They used 4D laryngeal CT to assess the voice changes in PD and found significant differences in arytenoid cartilage dynamics in those with PD when compared to controls. They recruited 15 patients with PD and 19 controls of a similar age. Subjects with PD had a disease duration less than 6 years and a modified Hoehn and Yahr stage of 2.5 or less (corresponding to mild bilateral disease with recovery on a pull test).[11]

Subjects underwent a perceptual voice analysis, lung function testing and 4D laryngeal CT imaging. These studies were conducted in a practically defined 'off' medication state for subjects with PD by withholding dopaminergic medications overnight. They utilised a 320-slice volume CT scanner to produce 4-dimensional imaging of the larynx during phonation. Subjects were trained to deliver quick and clear phonations of /i/ ('eee') five times during the scan.

From the data obtained, they measured the 'inter-arytenoid distance' (*IAD*) (see ***Figure 2.1***) and 'glottic area' (*GA*) (see ***Figure 2.2***).

The *IAD* was then analysed by taking the area under the curve of the *IAD* over the vocalisation period for each subject. This was then normalised for anatomical factors as well as the vocalisation period. The resulting figure is equivalent to the mean of the *IAD* over the vocalisation period (normalised for anatomical differences) and will henceforth be referred to as the mean *IAD*.

They demonstrated that the mean *IAD* is reduced in PD (Mdn = 0.106, IQR = 0.091-0.116) compared with controls (Mdn = 0.132, IQR 0.116-0.166). The reduction in mean *IAD* was confirmed in a linear mixed-model analysis which accounts for inter and intra-subject variations in sex, anatomy and duration of the vocalisation period. They interpreted the significant difference in *IAD* to imply that there is hypokinesis of arytenoid cartilage movements in PD. Meanwhile, despite the reduction in mean *IAD*, there was no significant difference in mean glottic area between the two conditions, which they inferred to represent the presence of vocal fold bowing.

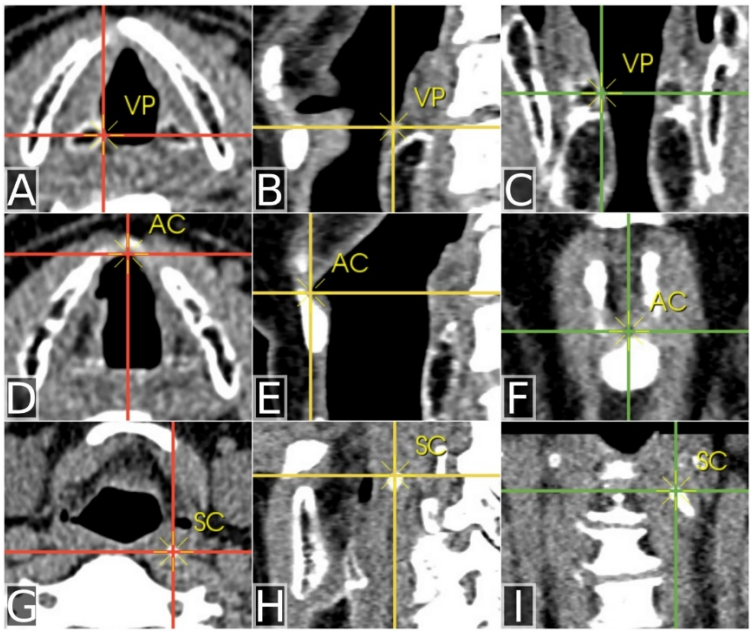


***Figure 2.1. Placement of fiducial markers.*** *Fiducial markers were placed on three anatomical landmarks. Axial, sagittal and coronal images are shown from left to right. A-C: Base of the vocal processes (VP); D-F: Anterior commissure (AC); G-I: Superior cornua (SC). Reproduced from Perju-Dumbrava et al [5], used under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).*

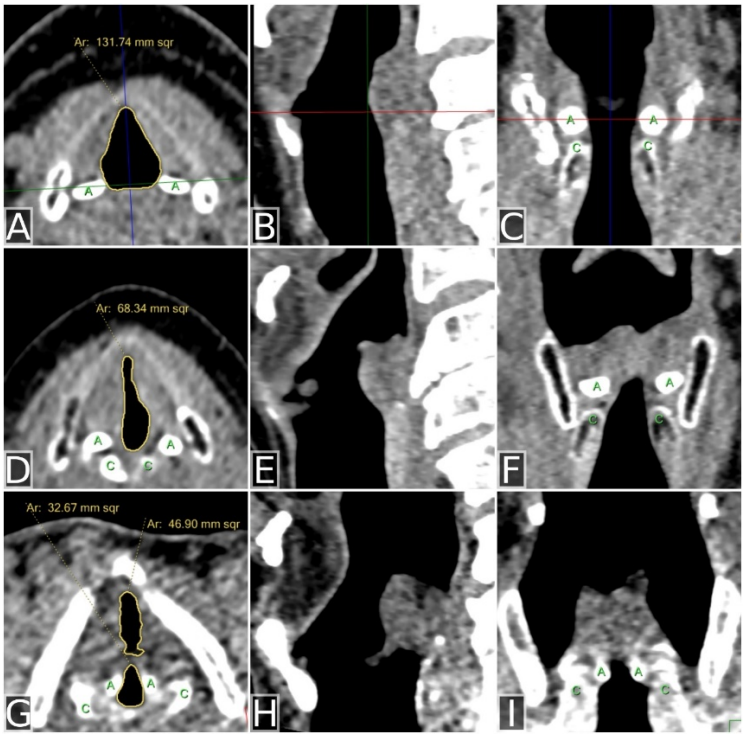


***Figure 2.2. Glottic areas measured by automated segmentation.*** *Axial, sagittal and coronal images are shown left to right. A-C: vocal folds apart with open glottis; D-F: vocal folds adducted with partial glottic closure; G-I: hourglass deformity. Reproduced from Perju-Dumbrava et al [5], used under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).*

## 2.4 Voice assessment in the diagnosis and evaluation of Parkinson's disease

This section will review the studies which have used voice assessment in the diagnosis and assessment of PD. This is with the intention of determining the how effective voice assessment currently is in the diagnosis of PD and inferring how useful assessing CT correlates of these voice changes may be. A secondary objective would be to determine which acoustic parameters have been effective in discriminating between PD and other conditions, which will provide a basis for determining what measurements on CT may be useful. The focus here will be on previous work assessing phonatory features, as articulatory and prosodic features of speech are unlikely to have anatomical correlates on laryngeal imaging.

A systematic review published in 2017 by Brabenec and colleagues[12] assessed studies which used acoustic voice and speech analysis in the diagnosis and assessment of PD. As such, this section will critically appraise the studies identified in their literature search and consider studies published subsequent to their systematic review.

***Voice in the diagnosis of early Parkinson's disease:*** Acoustic assessment of the voice has shown great promise as a means of distinguishing pwPD from healthy controls, even in its early stages. It has also been used to differentiate PD from other parkinsonian conditions,[13-15] but these are outside the scope of this literature review. The systematic review by Brabenec and colleagues[12] identified 34 studies which used acoustic analysis to diagnose PD, with 5 of these studies focusing on early diagnosis.

Rusz and colleagues[16] performed acoustic analysis in subjects with early PD (mean duration of disease 30.22 months) and healthy controls. Parameters of voice which demonstrated statistically significant differences between the PD and control group were used in a classification schema. This classification task managed to correctly identify 21 of the 23 pwPD and all the healthy controls. Acoustic measures of prosody and articulation performed better than phonation in distinguishing between pwPD and controls. It is unclear whether this reflects these domains being more severely affected early in the disease or being more sensitive to acoustic methods of detection.

The acoustic data set used in the previous study was then re-analysed by Bocklet and colleagues[17] using vocal fold, prosodic and acoustic modelling techniques. With this more complex analytical method, assessment of prosodic features again demonstrated superiority in the diagnosis of PD with

an accuracy of 91%. Meanwhile, features extracted based on acoustic and vocal fold modelling had an accuracy of 88% and 79% respectively. They found that having subjects perform more complex tasks such as reading rather than sustained phonation or syllable repetition improved their ability to detect early PD.

The remaining three studies[18-20] demonstrated an accuracy between 80-88% in distinguishing pwPD and controls. However, these studies only assessed articulatory features and are unlikely to be informative regarding the changes occurring at the laryngeal level.

More recently, there has been an emerging trend to extract acoustic features for analysis by using machine-learning algorithms. These studies have reported accuracies between 82.5-100% in distinguishing between pwPD and healthy controls.[21-26] However, many of these do not report baseline data such as disease duration, doses of dopaminergic treatment being taken or the severity of motor or speech impairment. Therefore, it is unclear how these methods perform in an early PD population. There has also been much scepticism raised over the veracity of these results, particularly with concerns of 'data-mining' and the absence of hypothesis-driven selection of speech characteristics.[27]

***Voice in the diagnosis of prodromal Parkinson's disease:*** Voice assessment in the diagnosis of PD would be most beneficial if it could detect its presence earlier than what clinical assessment currently allows. To that end, Rusz and colleagues[28] studied a cohort of 16 participants with REM sleep behaviour disorder, given their high rate of conversion to PD, together with 16 sex-matched healthy controls. They achieved 96% sensitivity and 79% specificity in distinguishing between the two groups. Therefore, voice assessment appears accurate in distinguishing those with RBD from controls, and therefore offers potential in identifying subjects with prodromal PD.

It remains unclear whether individuals with prodromal PD who do not have RBD demonstrate any speech abnormalities, and there is currently no practical method of studying this. It is estimated that in a prospective population-based study, 10,000 subjects would need to be followed in order to find 20 cases of incident parkinsonism.[29]

***Voice as a marker of progression in Parkinson's disease:*** The use of voice and speech analysis as a marker of progression of PD has been assessed across multiple studies by Skodda and colleagues.

They demonstrated that there are progressive changes in phonation,[30] prosody,[31] and articulation[32, 33] which show statistically significant correlations with increasing disease duration. However, these did not correlate with motor function as determined by the Unified Parkinson's Disease Rating Scale (UPDRS) Part-III.

In their study in 2013,[30] they assessed speech and voice in 80 pwPD and 60 controls. A baseline assessment was performed, with a follow-up assessment made after at least 12 months (mean time interval of 32.5 months). From the phonatory features assessed, they found that shimmer and the noise-to-harmonics ratio progressed but mean fundamental frequency and jitter did not. It is important to note that subjects were on medication for both voice assessments. Therefore, it is unclear whether mean fundamental frequency and jitter do not progress as the disease advances, or whether any change was masked by the effects of medication. These results suggest that the phonatory abnormalities of PD progress as the disease advances in duration, but it is unclear if they are correlated with motor impairment due to the confounding effects of medication in that study.

### 2.5 Barriers to utilisation in current clinical practice

As acknowledged by Perju-Dumbrava and colleagues,[5] measuring the *IAD* and *GA* on 4D laryngeal CT is laborious and time consuming. Therefore, automated tools which can achieve sufficient accuracy will assist in further research using 4D laryngeal CT and facilitate its potential utilisation in clinical practice.

***Automated point detection of the arytenoid cartilages:*** An automated process to detect the arytenoid cartilages has been described by Desai and colleagues.[34] Their method employs classical imaging processing techniques to detect and mark the arytenoid cartilages in a manner similar to the manual annotations performed in the study by Perju-Dumbrava and colleagues.[5] This algorithm depends upon finding the arytenoid cartilages then marking their most anteromedial aspect (see ***Figure 2.3***). Their algorithm demonstrated 83.33% accuracy in determining the location of these points as compared to manual marking from expert assessors. The distance between these two points would be essentially equivalent to the *IAD*. Therefore, automated processes to detect relevant feature points on the arytenoid cartilages demonstrate reasonable accuracy at present, and these could be used to automatically measure the *IAD*.

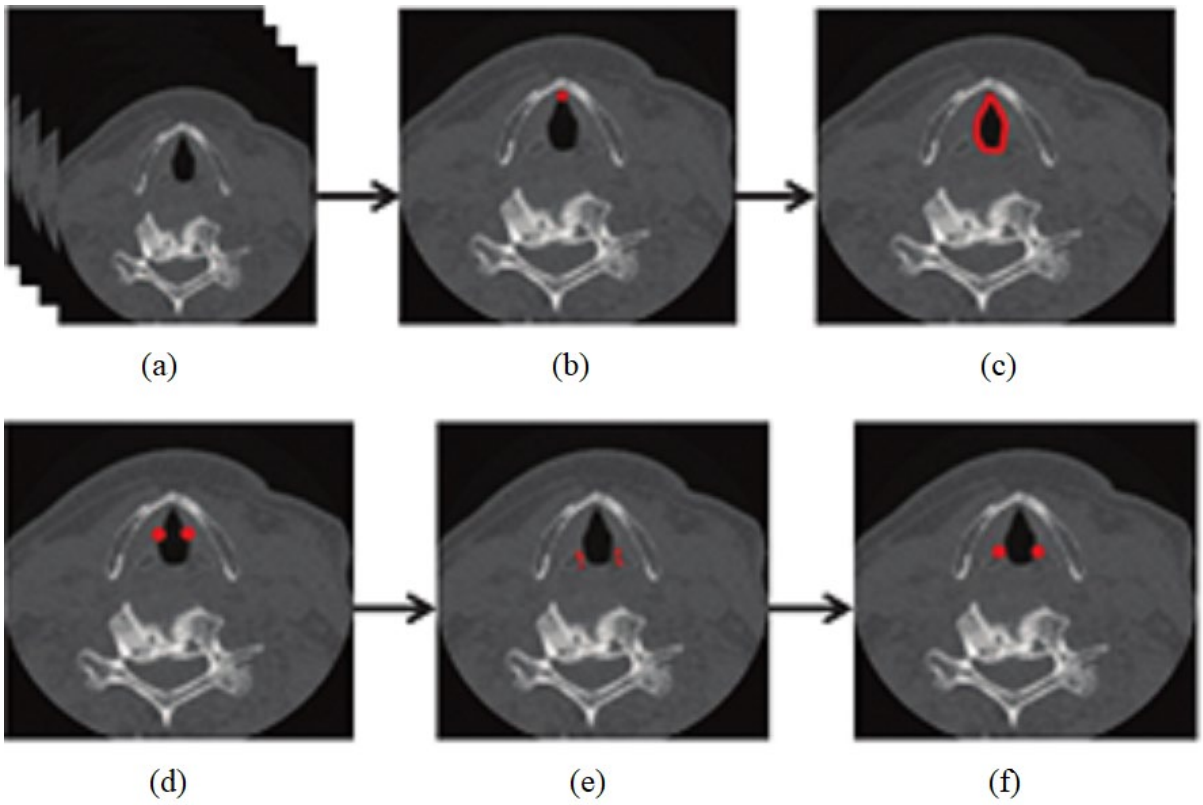


***Figure 2.3: Workflow for the automated detection of the vocal process.*** *A, laryngeal CT viewed in the axial plane; B, localisation of the anterior commissure; C, detection of the glottis; D, pre-processing; E, detection of potential points; F, finalised points after post-processing. Reproduced with permission from Desai et al*[34] *© 2017 IEEE.*

***Automated segmentation of glottic area:*** Hewavitharanage and colleagues[35] developed an automated method to determine the glottic area. Their technique involved segmenting areas of air density on the CT scan for each slice to create a volume (see ***Figure 2.4***). As the glottis is anatomically defined as the narrowest part of the laryngeal tract, the rima glottidis corresponds to the cross-sectional area of the narrowest point within this segmented volume. The results obtained by their algorithm correlated well to measurements taken manually by expert assessors in 65% of subjects. Issues which the authors felt degraded the accuracy of the algorithm were in part due to technical factors with the CT images (noise and blurring) and anatomical factors (the presence of laryngeal ventricles). Therefore, improvements in the algorithm to recognise the laryngeal ventricles, as well as improvements in the technical quality of the CT images would presumably increase the accuracy to facilitate its use in clinical practice.

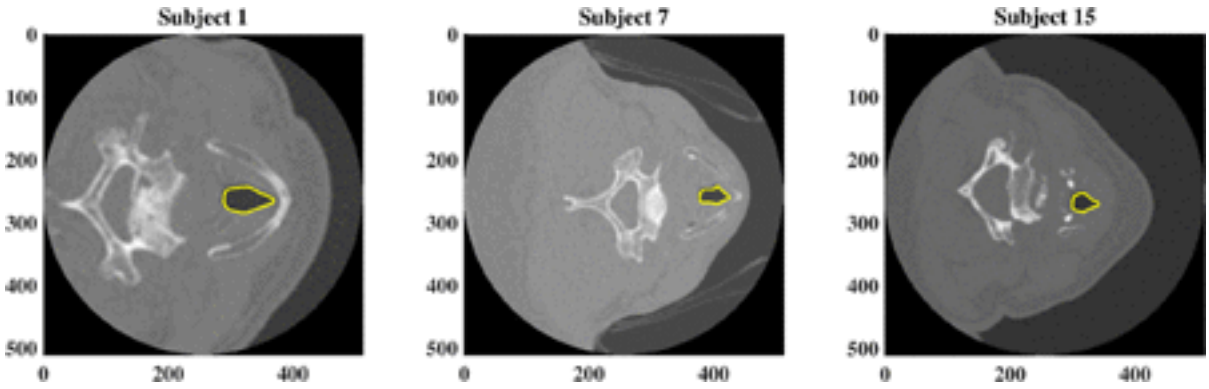


***Figure 2.4: Outputs from the automated detection of glottic area.*** *Reproduced with permission from Hewavitharanage et al[35] © 2017 IEEE.*

## 2.6 Conclusion

Voice dysfunction has been recognised as a feature of PD through clinical experience for some time. The voice changes of PD occur early in the course of the disease and their development may herald the beginnings of motor impairment. Its features have now been characterised using a variety of modalities. These show anatomical changes which contribute to the phonatory disturbances of PD including incomplete glottic closure due to asymmetric posturing and movement of the arytenoid cartilages, as well as bowing of the vocal folds. There is also asymmetric rigidity of the intrinsic laryngeal muscles and the vocal folds.

Nevertheless, there continue to be significant knowledge gaps in our understanding of these voice changes. The neuropathological changes which underpin these voice changes remain poorly understood, but indications that these voice changes manifest prior to the onset of motor dysfunction suggest the involvement of non-dopaminergic mechanisms. However, there are also indications of dopaminergic mechanisms being involved and the literature is conflicting regarding the effects of dopaminergic therapies on the voice in PD. This complicates the interpretation of prior studies, which have been performed in a variety of medication states.

Voice analysis has already shown promise in the diagnosis of PD. Its use as a marker of progression has so far been limited, but the available studies suggest that some correlates of these voice changes do progress over the course of the disease.

It has already been demonstrated that subjects with early PD demonstrate significant differences on dynamic laryngeal CT when compared to healthy controls. Subjects with more advanced disease have not previously been examined. Moving forward, assessing these measures in a cohort of subjects with PD of varying duration and severity may reveal trends that could be used in the diagnosis of PD or assessment of its progression. Identifying other correlates of arytenoid cartilage hypokinesis, vocal fold bowing or their asymmetry could also uncover new insights into the disorder.

Automated methods for analysing dynamic laryngeal CT have been shown to be reasonably accurate. However, their ability to be used in obtaining clinically relevant measures such as the *IAD* need to be validated.

In essence, there are many indications that the voice changes of PD have much to tell us about the disease. Despite investigating these voice changes through many modalities, there are many questions left unanswered. Proceeding to further characterise these voice changes using dynamic, cross-sectional imaging with laryngeal CT offers much potential in elucidating these knowledge gaps.

# Chapter 3
# Dynamic laryngeal CT in the evaluation of Parkinson's disease progression

### 3.1 Overview

As detailed in 'Chapter 2: Literature Review', while there are many indications of changes to the voice occurring early in PD, and that these progress over the course of the disease, objective measures of these alterations are lacking.

A key aim of this thesis was to characterise how these alterations to laryngeal structure and function change as the disease advances using dynamic laryngeal CT. As the only previous work[5] using dynamic laryngeal CT assessed patients with relatively early disease, we supplemented that cohort with pwPD with more advanced disease to cover a wider spectrum of disease severity and duration. We also performed new measures to characterise arytenoid cartilage movement, rather than just arytenoid cartilage position which was the focus of the prior work.

We found that analysis of laryngeal measures relating to arytenoid cartilage position and vocal fold bowing did not only indicate the disease's presence, but that they are also correlated with the disease's duration and severity. However, in contrast to the slowing typically seen in the limbs in PD, movements of the arytenoid cartilages were not slowed. Thus, our findings suggest that the voice changes of PD are in part due to vocal fold bowing and abnormal posturing of the arytenoid cartilages, and that these laryngeal abnormalities progress as the disease advances. These findings provide a foundation for further research into 4D laryngeal CT as well as other modalities evaluating the voice changes of PD.

### 3.2 Published Manuscript

Our findings are detailed in the following published manuscript entitled 'Radiological correlates of vocal fold bowing as markers of Parkinson's disease progression: A cross-sectional study utilizing dynamic laryngeal CT' (*Ma A, Lau KK, Thyagarajan D. 2021. Radiological Correlates of Vocal Fold Bowing as Markers of Parkinson's Disease Progression. PLOS One. 2021*).

PLOS ONE

RESEARCH ARTICLE

# Radiological correlates of vocal fold bowing as markers of Parkinson's disease progression: A cross-sectional study utilizing dynamic laryngeal CT

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**Data Availability Statement:** Data will be publically available in the figshare public repository at http://

## Abstract

### Objective

To determine whether arytenoid cartilage position and dynamics change with advancing duration and severity (as graded by MDS-UPDRS part III scores) in Parkinson's disease, in a cross-sectional study design, we performed laryngeal four-dimensional computed tomography (4D-CT) in people with Parkinson's disease and controls.

### Methods

31 people with Parkinson's disease covering a range of disease duration and severity and 19 controls underwent laryngeal 4D-CT whilst repeatedly vocalizing. We measured on each CT volume the glottic area (*GA*), inter-arytenoid distance (*IAD*), IAD-Area index (*IAI*) and arytenoid cartilage velocity ($\overrightarrow{av}$).

### Results

People with Parkinson's disease had reductions in the mean/effective minimum *IAD* when compared to controls, while mean/effective minimum *GA* and mean/effective maximum *IAI* were increased. Arytenoid cartilage velocities showed no difference. On Spearman correlation analyses, advancing disease duration and severity of PD showed moderately strong and significant correlations with increasing mean/effective minimum *GA*, increasing mean/effective maximum *IAI* and decreasing effective minimum *IAD*. Linear mixed models which considered the effects of intra and inter-individual variation showed that both disease duration (b = -0.011, SEb = 0.053, 95% CI [-0.022, 0], t(27) = -2.10, p = 0.045) and severity (b = -0.069, SEb = 0.032, 95% CI [-0.14,-0.0039], t(27) = -2.17, p = 0.039) were significant predictors for *IAD*, and also for transformed values of the *GA* and *IAI*.

doi.org/10.26180/14214056 (available after acceptance of manuscript).

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**Competing interests:** A. Ma received salary from Ipsen Biopharmaceuticals as support for a Movement Disorders Fellowship and received support from an Australian Government Research Training Program (RTP) Scholarship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. D. Thyagarajan and K.K. Lau report no disclosures relevant to the manuscript. This does not alter our adherence to PLOS ONE policies on sharing data and materials."

## Conclusions

There are progressive alterations in phonatory posturing as Parkinson's disease advances. The increases in *GA* despite reductions in *IAD* are concordant with prior observations of vocal fold bowing. Our study provides a basis for using laryngeal 4D-CT to assess disease progression in Parkinson's disease.

## Introduction

Parkinson's disease (PD) is a neurodegenerative condition which remains a significant health issue worldwide. No current intervention alters the course of the disease. Lack of biomarkers of disease progression hampers the search for disease-modifying treatments. For lack of alternatives, assessing progression in PD is still primarily focused on clinical assessment of motor function, often with the aid of structured rating scales such as the Unified Parkinson's Disease Rating Scale (UPDRS). Yet, these assessments are subjective with high inter- and intra-rater variability [1, 2]. More reliable assessment tools would allow for shorter follow-up periods and smaller sample sizes when studying the effects of novel disease-modifying therapies [3].

Voice disorder affects 70–90% of people with Parkinson's disease (pwPD) [4–6]. There is evidence that voice dysfunction is the earliest sign of motor impairment in PD [7–9]. Despite this, perceptual, acoustic or other measures of hypokinetic dysarthrophonia in PD (e.g. vocal cord dysfunction) have not yet found a place in the assessment of disease progression.

Laryngeal computed tomography (CT) is a potential technique to study these voice changes. Our previous study [10] used laryngeal CT during vocalization to measure glottic area (*GA*) and arytenoid cartilage position. It found differences in the inter-arytenoid distance (*IAD*) between patients with early PD and healthy controls. In that study, participants also underwent perceptual analysis and we found significant increases in breathiness and articulatory dysdiadochokinesis, and reductions in loudness variability and mean phonation time in Parkinson's disease. We found no effect of age or sex on *IAD* or *GA*. However, that study provided no insight into how these measures change with disease duration and severity. Yet, such objective measures of vocal cord dysfunction would be valuable in measuring the effects of potential disease-modifying therapies.

Based on our prior findings, our hypotheses are that disease duration or motor severity is: 1) positively correlated with mean and effective minimum *GA*, 2) negatively correlated with mean and effective minimum *IAD*, and effective maximum arytenoid cartilage velocities. The aim of this study is to characterise how these laryngeal measures (*GA* and *IAD*) change as PD progresses.

## Materials and methods

### Patients and recruitment

We previously studied 19 healthy controls and 15 pwPD. Those patients had a disease duration less than 6 years and modified Hoehn and Yahr (H&Y) stage of 2.5 or lower [10]. We expanded this cohort by recruiting patients with more advanced PD from the Movement Disorders Clinic at Monash Medical Centre. For the purposes of obtaining adequate representation across the spectrum of the disease, we recruited patients who were H&Y stage 3 or more and a disease duration of 5 years or greater. Diagnoses of idiopathic PD were made in accordance with the UK Brain Bank Criteria. Control participants either responded to

advertisements or were spouses of the pwPD who were unaffected by Parkinson's disease or other neurological disorders apparent on clinical assessment by a neurologist.

## Standard protocol approvals, registrations and patient consents

In a cross-sectional design, all participants were imaged only once. Written informed consent was obtained from all participants according to the Declaration of Helsinki. Ethics approval was granted by the Research Ethics Committee of Monash Health (HREC reference number 11230B).

## Image acquisition and analysis

Four-dimensional imaging data with an anatomical z-axis of 16 cm over the larynx was acquired using a 320 multi-detector row CT (Aquilion One, Tokyo, Canon Medical Systems). Participants were imaged in the supine position without CT table movement. During a continuous CT acquisition scanning period of 5 seconds, participants were instructed to produce five short phonations of /i/ quickly and clearly at a comfortable speaking volume and pitch. Patients practiced the vocalization task prior to undergoing the scan. Multiple phonations were performed to allow study of the arytenoid cartilages in motion in as repeatable a way as possible. Imaging acquisition was terminated before five seconds in those who had completed the vocalization task early. Images were re-constructed to produce continuous multiplanar images of the larynx at 100ms per frame. pwPD were assessed in the practically-defined 'off' state by withholding their regular PD medications overnight. In the newly recruited participants, fiducial coordinates of the arytenoid cartilages were obtained using the open-source ImageJ software [11].

## Statistical analysis

Data transformation, statistical analysis and graphics were performed using the R statistical and graphing package [12] and nlme [13].

In pre-processing, we manually removed *IAD* and *GA* values from the pre and post-vocalization period. Whilst not vocalizing, values of both the *GA* and *IAD* are strikingly higher as the vocal folds are apart. Trimming was performed by visually inspecting the raw *GA* and *IAD* data sets and excluding periods containing these larger values. This was done as the study was focused on assessing the movement and posturing of the vocal folds during vocalization.

The raw data set consisted of *GA* and fiducial markers on each arytenoid cartilage for each timepoint, with successive timepoints at 100 msec intervals. Workstation software (IntelliSpace Portal, Philips Healthcare, Cleveland, USA) was utilised to adjust the continuous dynamic CT images to the plane of the vocal folds. The glottic area (GA) was then segmented at each 100ms frame. *IAD* was the calculated Euclidean distance between the fiducial markers on the left and right arytenoid cartilages. These measures are illustrated in Fig 1. We also calculated a dimensionless index, the IAD-Area index (*IAI*), which we defined as $\frac{\sqrt{GA}}{IAD}$. The instantaneous velocity of arytenoid cartilage movements ($\overrightarrow{av}$) was calculated as the sum of the distance in mm that the left and right fiducial markers moved between successive 100ms timepoints. We also separated these values as either representing abduction velocity ($\overrightarrow{abv}$) or adduction velocity ($\overrightarrow{adv}$), based on the direction of arytenoid cartilage movement over that time interval.

We defined an 'effective minimum' for *IAD* and *GA* as the median of the lowest five values, as the vocalization task involved phonating /i/ five times. Similarly, the 'effective maximum' *IAI, AS*, $\overrightarrow{abv}$ and $\overrightarrow{adv}$ was defined as the median of the highest five values. This was done to minimise the potential distorting effects of outliers.

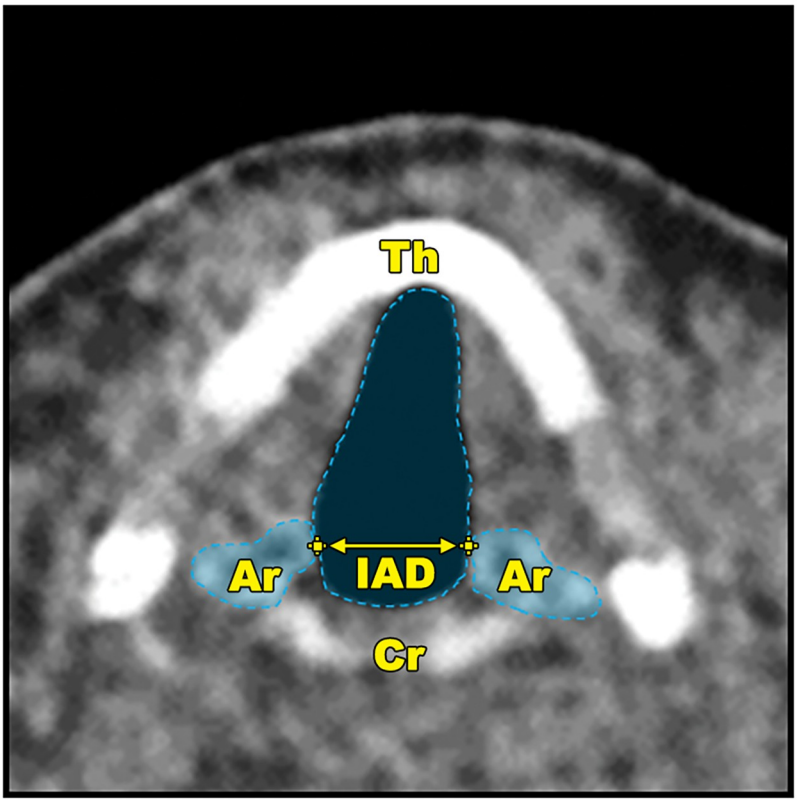


**Fig 1. The laryngeal measures.** Annotated CT image at the level of the glottis depicting the laryngeal measures used in this study. Fiducial markers (shown as yellow crosshairs) are placed at the most medial aspect of the arytenoid cartilages (Ar). The inter-arytenoid distance (*IAD*) is defined as the Euclidean distance between them, as depicted by the arrow. The arytenoid velocity ($\overrightarrow{av}$) is equivalent to the change in *IAD* between successive timepoints (not illustrated). The glottic area (*GA*) is the segmented area of air density at the level of the vocal folds. Also shown are the thyroid cartilage (Th) and cricoid cartilage (Cr).

https://doi.org/10.1371/journal.pone.0258786.g001

Statistical tests utilised the mean *GA* and *IAD* (analogous to our previously reported key measures $AUC_{ga}$ and $AUC_{iai}$ respectively) [10], effective minimum *GA* and *IAD*, mean IAI and $\overrightarrow{av}$, and the effective maximum *IAI*, $\overrightarrow{av}$, $\overrightarrow{abv}$ and $\overrightarrow{adv}$. We set an alpha level of 0.05 for all statistical tests. Wilcoxon's rank sum test was then used to compare the above against condition (PD/control).

**Table 1. Demographic details.**

| | Controls | PD |
|---|---|---|
| n | 19 | 30 |
| F:M | 8:11 | 9:20 |
| Mean age in years | 70.8 | 69.7 |
| SD age in years | 7.38 | 7.32 |
| Median disease duration (months) | NA | 83.5 |
| Lower bound IQR of disease duration (months) | NA | 48.8 |
| Upper bound IQR of disease duration (months) | NA | 138 |
| Median UPDRS Part III | 0.0 | 19.5 |
| Lower bound IQR of UPDRS Part III | NA | 15 |
| Upper bound IQR of UPDRS Part III | NA | 30.8 |

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Spearman's rank correlation analysis as well as linear mixed effects modelling were performed to determine how these measures relate to PD duration (in months) and severity (as graded by UPDRS part-III scores). Models considered the laryngeal measures as the dependent variable, with non-linear transformations applied to the *GA* and *IAI* to achieve linearity of the data. Disease duration, UPDRS part-III scores, age and sex were considered as fixed effects. The intercepts were considered as a random effect to account for inter-participant variability.

## Results

### Study population and baseline characteristics

Since the prior study, we recruited 14 further pwPD, with the overall cohort consisting of 31 pwPD and 19 controls. Their baseline characteristics are presented in Table 1. No pwPD recruited had previously undergone voice training. One patient was excluded because gross imaging artefact precluded accurate image analysis. Another patient was excluded from analysis of arytenoid motion as the images were inadvertently captured at 300 ms/frame, rather than our standard of 100 ms/frame. After exclusion of these patients and data trimming, the total number of timepoints analysed was 1671 for the *IAD*, 1705 for the *GA* and 1607 for the *IAI*.

### Arytenoid cartilage position and glottic area is altered in Parkinson's disease

In comparing participants with PD and controls, statistically significant differences were found in the median values across the vocalization period of the mean *IAD* (*Mdn* = 4.24 vs 5.21, $p = 0.006$), mean *GA* (*Mdn* = 53.0 vs 21.2, $p = 0.024$) and mean *IAI* (*Mdn* = 1.54 vs 0.852, $p = 0.001$). Significant differences were also seen for the effective minimum *IAD* (*Mdn* = 2.66 vs 3.69, $p = 0.009$), effective minimum *GA* (*Mdn* = 23.2 vs 3.60, $p = 0.002$) and effective maximum *IAI* (*Mdn* = 2.68 vs 1.35, $p < .001$). Mean and effective maximum $\overrightarrow{av}$, as well as effective maximum $\overrightarrow{abv}$ and $\overrightarrow{adv}$, all were not significantly different in pwPD when compared to controls (see Fig 2 and S1 Table).

### The IAD, GA and IAI is correlated with the duration and severity of Parkinson's disease

Plots of the mean/effective minimum *GA* and mean/effective maximum *IAI* show that these measures increase with disease duration and UPDRS part-III scores, while mean/effective minimum *IAD* decrease. As there was no significant difference seen in the $\overrightarrow{av}$, $\overrightarrow{abv}$ and $\overrightarrow{adv}$ in those with pwPD when compared to controls, these measures were not included in the correlation analyses.

Spearman correlation analysis demonstrates moderately strong and significant correlations between all laryngeal measures, except for the mean *IAD*, with both disease duration and UPDRS. The strongest correlation was between the effective minimum *GA* and disease duration (Spearman Rho = 0.692) (see Fig 3).

Linear mixed regression models showed statistically significant effects of both the duration and severity of PD on the *IAD*, as well as transformed values of the *GA* and *IAI*. Non-linear transformations were applied to the *GA* and *IAI* to achieve linearity of the data set. This allowed all models to meet all the standard statistical assumptions. Duration as a fixed effect significantly predicted the *IAD* with b = -0.011, SEb = 0.053, 95% CI [-0.022, 0], t(27) = -2.10, p = 0.045. Similarly, the UPDRS as a fixed effect predicted the *IAD* with b = -0.069, SEb = 0.032, 95% CI [-0.14, -0.0039], t(27) = -2.17, p = 0.039. The transformed values of the

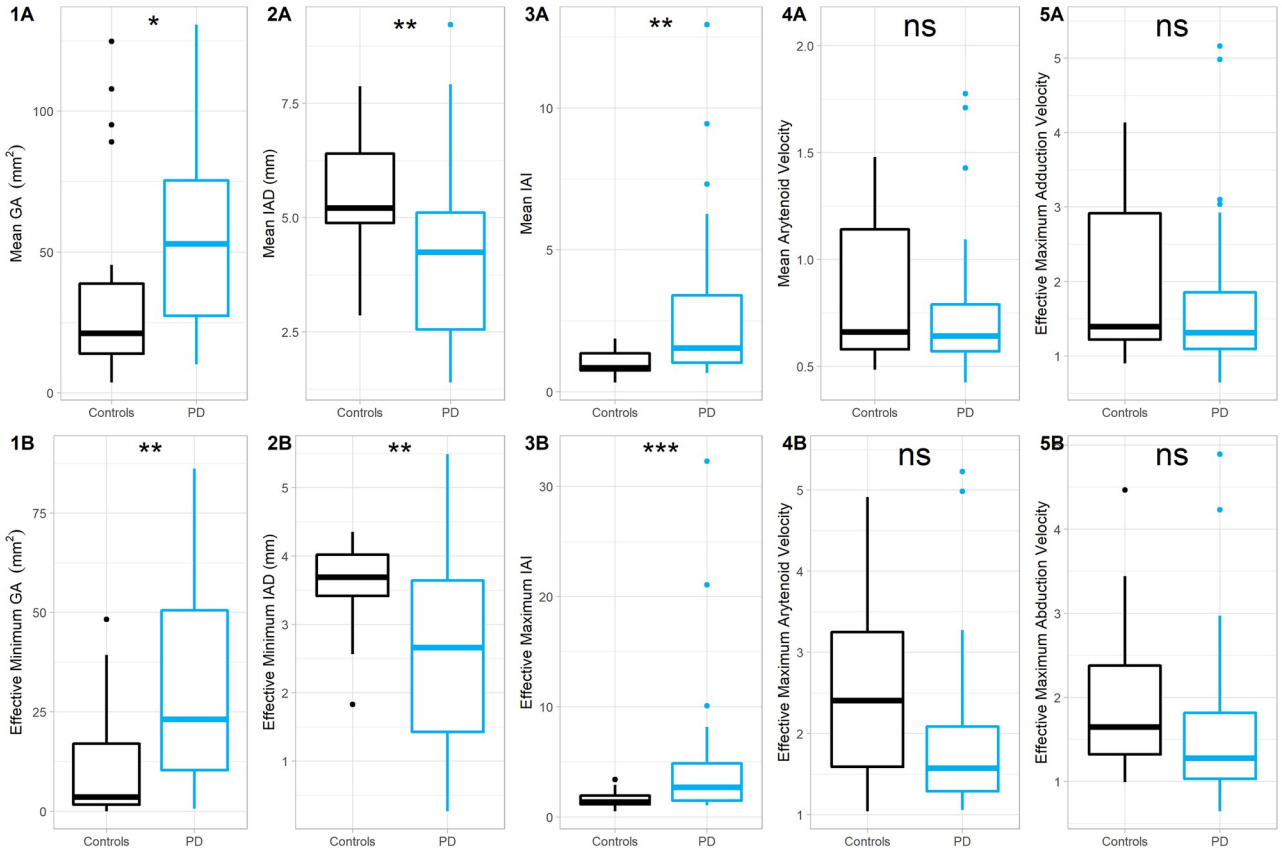


**Fig 2. Comparison of the laryngeal measures during vocalization between pwPD and controls.** Boxplots comparing measures of laryngeal dynamics in participants with Parkinson's disease (blue boxes) against controls (black boxes). (1-3A) Comparison of the means of the *GA*, *IAD* and *IAI* during the period of vocalization. (1-3B) Comparison of the values of the laryngeal measures at maximal vocal fold adduction, corresponding with the effective minimum *GA*, effective minimum *IAD* and effective maximum *IAI*. The mean and effective maximum velocities of the arytenoids is assessed in (4A) and (4B) respectively, calculated by assessing the extent of movement of the arytenoids between successive 100 ms volumes. Sub-analysis of the maximal velocities by the direction of vocal fold movements is shown in (5A) (adduction; $\overrightarrow{adv}$) and (5B) (abduction; $\overrightarrow{abv}$). ns $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

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*IAI* in model 3, predicting the duration (b = 0.0054, SEb = 0.0014, 95% CI [0.0024, 0.0083], t(27) = 3.69, p < .001) and model 6, predicting severity (b = 0.031, SEb = 0.0089, 95% CI [0.012, 0.049], t(27) = 3.41, p = 0.002) were the preferred models for interpretation based on having the highest marginal R-squared values (see S2 Table for model details and comparisons). Models were all adjusted to account for the age and sex of participants, although neither demonstrated a significant interaction with the laryngeal measures. Additionally, no significant interaction was found between duration of disease or severity and participant age or sex.

## Discussion

The main findings of this study are that in PD, disease duration and severity are correlated positively with mean/effective minimum *GA* and negatively with the mean/effective minimum *IAD*. This relationship was confirmed after controlling for age and sex with a linear mixed random effects model. Thus, these laryngeal measures have potential utility as a marker of disease progression.

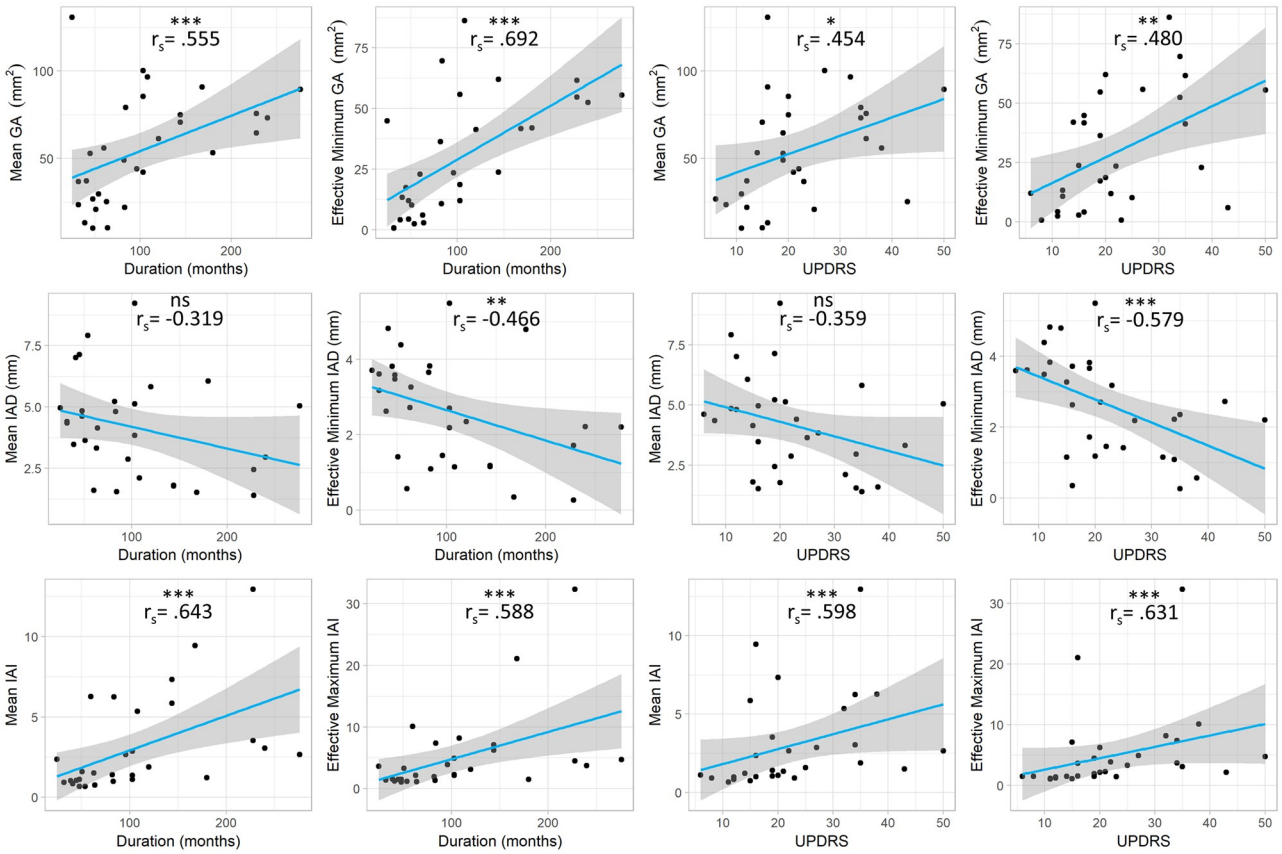


**Fig 3. Change in laryngeal measures with disease duration and UPDRS part III scores.** Plots of the raw data of the laryngeal measures against duration in months and UPDRS part-III scores. The linear regression 'line of best fit' is marked in blue, with the 95% confidence interval shaded in grey. Spearman correlation coefficients ($r_s$) between the laryngeal measures and disease duration or UPDRS are annotated for each. ns $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

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How do these laryngeal measures relate to previous knowledge of phonatory dysfunction in PD? Prior laryngoscopic studies have identified abnormalities of phonatory posture in pwPD characterized by glottic incompetence due to bowing of the vocal folds, as well as alterations in the position of the arytenoid cartilages, vocal folds and ventricular folds (false cords) [14–16]. One of these studies followed-up patients for up to 4 years but did not identify any change in these laryngeal characteristics on repeat laryngoscopic assessment for any given patient [14]. Our study, possibly owing to the capacity to perform precise anatomical measurements rather than observation alone, has identified change with disease progression.

The increase in mean and effective minimum *GA* in pwPD compared with controls is in keeping with the presence of glottic incompetence in pwPD. This is thought to contribute to the breathiness of the voice that is often clinically appreciable in pwPD [17, 18]. It should be noted that our previous study did not find significant differences in the mean *GA* between PD and healthy controls. However, this study only assessed patients earlier in the course of the disease. As *GA* is correlated with duration of disease, in this cohort including patients with more advanced PD, we found that the mean *GA* is also significantly increased.

With regards to the *IAD*, the mean and effective minimum values are reduced during vocalization in pwPD when compared to controls i.e. the arytenoid cartilages are closer together during vocalization in PD. This is in keeping with laryngoscopic observations that in pwPD

whose motor symptoms are asymmetric, on the side of the body with more severe parkinsonism, the arytenoid cartilage is hyperadducted, its vocal fold closes underneath the contralateral vocal fold and there is increased contraction of the adductor muscles of the vocal folds. This results in the arytenoid cartilage on the more affected side being positioned with its vocal process and apex more posterior, and its apex more lateral, when compared with the other side [14]. Their observations are supported by laryngeal EMG studies of pwPD. These studies have shown increased spontaneous muscle activity in the laryngeal adductors (thyroarytenoid, cricothyroid and lateral cricoarytenoid muscles) at rest [15, 19–21]. Also, EMG activity of the only abductor of the vocal folds (the posterior cricoarytenoid muscle) is decreased [15]. These laryngoscopic and EMG findings (see Fig 4) explain the hyperadduction of the arytenoid cartilages seen on laryngoscopy, and which we have now also demonstrated on the 4-dimensional CT.

The change in glottic shape caused by vocal fold bowing was indirectly measured by defining the *IAI*, a ratio which relates *GA* to *IAD*. A disproportionately elevated *GA* for a given *IAD* implies bowing of the vocal folds. Although vocal fold bowing can also occur with ageing due to vocal fold atrophy [22], we showed that the mean/effective maximum *IAI* is increased in pwPD when compared to age-matched controls. The mean/effective maximum *IAI* is more

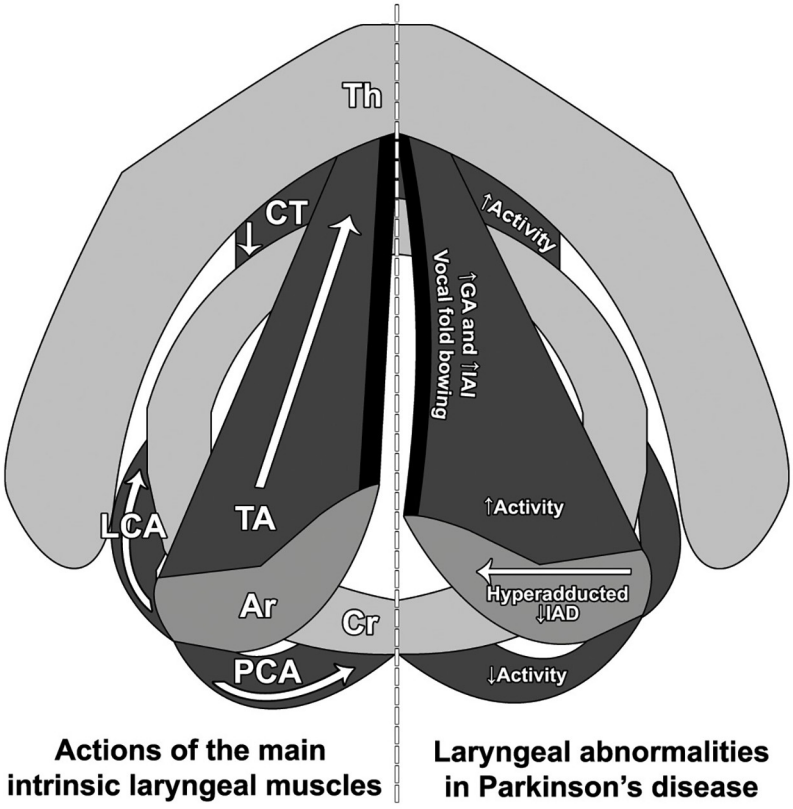


**Fig 4. Actions of the main intrinsic laryngeal muscles and the laryngeal abnormalities in Parkinson's disease.** Schematic diagrams of the larynx viewed from above. The left panel demonstrates the normal anatomy of the main laryngeal cartilages (Th, thyroid cartilage; Cr, cricoid cartilage; Ar, arytenoid cartilages), as well as the main abductors (LCA, lateral cricoarytenoid muscle; TA, thyroarytenoid muscle) and adductor (PCA, posterior cricoarytenoid muscle), with their primary movements depicted by the accompanying arrows. The right panel highlights the prior laryngoscopic findings, as well as the EMG abnormalities described which account for these. The vocal fold bowing explains the increase in glottic area (*GA*) and IAD-Area Index (*IAI*) seen on laryngeal CT, while hyperadduction of the arytenoids causes the reduction in inter-arytenoid distance (*IAD*).

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strongly correlated with UPDRS part-III scores than either the *GA* or *IAD* alone. The preferred linear mixed models based on effect size were those which treated transformed values of the *IAI* to explain disease duration or severity. As the *IAI* considers both the changes to *GA* and *IAD* which occur in the disease, increased *IAI* may also be more specific for PD, although this would need to be confirmed in future studies.

We did not identify the presence of bradykinesia in the arytenoid cartilage movements during vocalization, with the mean and effective maximum $\overrightarrow{av}$ both not significantly reduced in pwPD when compared to control subjects. We also demonstrated hyperadduction of the arytenoid cartilages, with a reduction in *IAD*, suggesting against the presence of arytenoid cartilage hypokinesis. However, our method of image analysis only measures the sliding adduction movements, but we could not easily measure rotational movements of the arytenoid cartilages. To do so, suitable landmarks (e.g. the vocal and muscular processes) would need to be marked with fiducials but these are not always reliably identifiable on CT. Therefore, we cannot exclude the presence of bradykinesia or hypokinesia in arytenoid cartilage movements in other planes.

Effective minimum *GA* and *IAD* during vocalization, which occur at maximal vocal fold adduction, showed significant differences between PD and controls. They also change as the disease progresses, with the *GA* increasing and the *IAD* decreasing, with advancing disease duration and severity. Measuring the effective minimum *GA* and *IAD* reduces variability introduced by differences in how participants performed the vocalization task. Future studies assessing the laryngeal dynamics in PD could consider measuring the *IAD* and *GA* just at the point of maximal vocal fold adduction during vocalization.

Limitations of our study include the possibility of variance being introduced by differences amongst participants in volume, pitch and cadence. Vocalization during image acquisition could not be recorded due to the loudness of the scanning apparatus. As such, we could not account for these acoustic parameters which may influence arytenoid cartilage dynamics.

The presence of vocal tremor was also not assessed. Vocal tremor occurs in some pwPD, with a frequency depending on technique and definition– 13–68% on perceptual studies and about 15–55% on endoscopic studies—but at a similar prevalence to controls on acoustic analysis [23]. The contribution of vocal tremor is therefore uncertain. The addition of a sustained phonation task may have helped us to understand the contribution of vocal tremor, but this would have exposed the subjects to more radiation.

Another limitation of our study is its cross-sectional design. To establish these radiographic laryngeal measures as markers of disease duration or severity, a prospective study would be ideal. Imaging modalities which do not involve the use of ionising radiation such as ultrasound would be preferable in a prospective study. Ultrasound can reliably detect the arytenoid cartilages and the movements of the vocal folds [24, 25] and we are exploring the use of this modality.

In summary, this study has identified radiographic measures which correlate with the anatomic changes previously observed in laryngoscopic studies of pwPD. We conclude that certain measures of laryngeal dynamics change with increasing duration and severity of PD. Therefore, our results demonstrate the utility of dynamic laryngeal CT as a means of objectively tracking the duration and severity of PD. Validating these results in a prospective cohort with other imaging modalities (e.g. ultrasound) is a useful direction in a future study. Further study in atypical parkinsonian conditions, as well as determining the effect of levodopa administration would also prove to be of great interest. The use of imaging modalities may elucidate the disease trajectory and offer opportunities for objective monitoring of PD.

## Supporting information

**S1 Table. Laryngeal measures during vocalization per participant.** Table of summary statistics for each laryngeal measure by patient. Mean *GA*, *IAD* and *IAI* and their standard deviations (SD) during the vocalization period are listed. The effective minimum *GA* and *IAD* and effective maximum *IAI* listed refers to the median of the five lowest or highest values, whilst the inter-quartile range (IQR) given corresponds to the IQR for these five values.
(DOCX)

**S2 Table. Linear mixed models.** Table listing the details of the linear mixed models used to analyse the effect of duration or severity (as graded by UPDRS part-III scores) of PD on the *IAD* and transformed values of the *GA* and *IAI*.
(PDF)

## Author Contributions

**Conceptualization:** Kenneth K. Lau, Dominic Thyagarajan.

**Data curation:** Andrew Ma, Dominic Thyagarajan.

**Formal analysis:** Andrew Ma, Dominic Thyagarajan.

**Funding acquisition:** Kenneth K. Lau, Dominic Thyagarajan.

**Investigation:** Andrew Ma, Dominic Thyagarajan.

**Methodology:** Andrew Ma, Dominic Thyagarajan.

**Project administration:** Andrew Ma, Dominic Thyagarajan.

**Resources:** Andrew Ma, Kenneth K. Lau, Dominic Thyagarajan.

**Software:** Andrew Ma, Dominic Thyagarajan.

**Supervision:** Kenneth K. Lau, Dominic Thyagarajan.

**Visualization:** Andrew Ma.

**Writing – original draft:** Andrew Ma.

**Writing – review & editing:** Andrew Ma, Kenneth K. Lau, Dominic Thyagarajan.

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# Chapter 4
# Symmetry of arytenoid cartilage motion in Parkinson's disease

**4.1 Overview**

This thesis set out to further characterise the changes which occur to the larynx in PD using dynamic laryngeal CT. Previous laryngoscopic studies showed asymmetry in the positioning and movements of the arytenoid cartilages and vocal folds.[10] These descriptions led us to hypothesise that laryngeal CT could identify asymmetry in arytenoid cartilage movement, and that the arytenoid cartilage on the side of the body exhibiting more motor impairment would have greater reductions in the amplitude and speed of movements than the less affected side.

To determine whether there is asymmetry in the movement of the arytenoid cartilages in PD, I performed a post-hoc analysis of the data set utilised in Chapter 3. Results of these analyses are presented in Chapter 4 in a manuscript format. These results were not included in the manuscript presented in Chapter 3 as it was outside the scope of that study's aims.

Essentially, this analysis did not demonstrate evidence of asymmetry in the *velocity* of the arytenoid cartilages in pwPD during vocalisation. In view of the findings presented in Chapter 3, which did not find any significant difference in the velocity of the arytenoid cartilages in pwPD compared to healthy controls, the results of this analysis are not unexpected. Thus, the changes to the arytenoid cartilages in PD appear to relate to their positioning, rather than their movement.

When considered together, the findings of Chapter 3 and Chapter 4 pose an interesting question for future study as to whether asymmetry exists in the *posturing* of the arytenoid cartilages. Answering this question was not possible with the existing data set of *IAD* and *GA* values and fiducial coordinates. A reference point in the mid-line would need to be marked for each CT timepoint. Taking measurements between a mid-line reference point to each arytenoid cartilage could identify asymmetry in the position of the arytenoid cartilages. I plan to pursue this research question in future and intend to publish those findings together with those presented in this chapter.

## 4.2 Symmetry of arytenoid cartilage motion in Parkinson's disease

### Introduction

Laryngoscopic studies during vocalisation described asymmetry in the positioning and movement of the arytenoid cartilages and vocal folds in pwPD. Hanson and colleagues[10] observed that in PD, the arytenoid cartilage on the side of the body with greater motor impairment was positioned more posterolaterally, while its vocal fold would close incompletely, be further away from the midline and move less than the contralateral side. PD is recognised to be asymmetrical in its motor symptoms, particularly in its early stages, and bilateral symmetric parkinsonism is a 'red-flag' which argues against the presence of the condition.[3] Asymmetry in its motor manifestations are used as a means of clinically diagnosing the disorder. It is unclear whether asymmetry exists in arytenoid cartilage movements of pwPD, and if this could be used as a marker of the condition's presence or severity.

Based on the prior literature, we hypothesise that laryngeal CT will detect asymmetry in arytenoid cartilage movements in pwPD, and that the arytenoid cartilage on the side of the disease exhibiting more motor impairment will have greater reductions in the amplitude and speed of movements than the less affected side. If there is asymmetry in the movements of the arytenoid cartilages in pwPD but not control participants, this work could provide a basis for further research into its utility as a biomarker for determining the presence or severity of PD.

### Materials and methods

The raw data set of laryngeal measurements (IAD, GA, $\overrightarrow{av}$, $\overrightarrow{abv}$ and $\overrightarrow{adv}$) was generated from the 4D laryngeal CT imaging study detailed in the manuscript in 'Chapter 3'.

From the fiducial coordinates placed on each arytenoid cartilage, we derived the interval movement in mm for the left and right arytenoid cartilages between each 100 ms frame. These measurements were denoted as the left arytenoid velocity ($\overrightarrow{av_L}$) and right arytenoid velocity ($\overrightarrow{av_R}$), as they represent the instantaneous velocity of each arytenoid cartilage over the 100 ms interval.

The difference in arytenoid velocity ($\Delta\overrightarrow{av}$) was then defined as $\Delta\overrightarrow{av} = \overrightarrow{av_R} - \overrightarrow{av_L}$. That is, the $\Delta\overrightarrow{av}$ represents the difference in distance moved by the right arytenoid cartilage compared to the left arytenoid cartilage over each 100 ms interval. $\Delta\overrightarrow{av}$ values of 0 imply symmetrical movement of the

arytenoids, positive values represent less movement of the left arytenoid cartilage relative to the right, and vice versa for negative values.

Using the R statistical and graphing package,[36] $\Delta\overrightarrow{av}$ values were calculated for each participant at every timepoint during the vocalisation period. We used an independent samples t-test to compare the distribution of $\Delta\overrightarrow{av}$ measurements with respect to condition (PD against control group), with a pre-specified alpha level of 0.05. We also analysed a sub-group of those with relatively early PD – which we defined here as a Hoehn & Yahr grade of less than 2.5 – as pwPD with relatively early disease are expected to have more considerable left-right asymmetry in motor impairment.

**Results**

We did not identify any asymmetry in instantaneous arytenoid cartilage velocities. The spread of $\Delta\overrightarrow{av}$ approximates the normal distribution in participants with PD, those with relatively early disease, as well as control subjects (see ***Figure 4.1***).

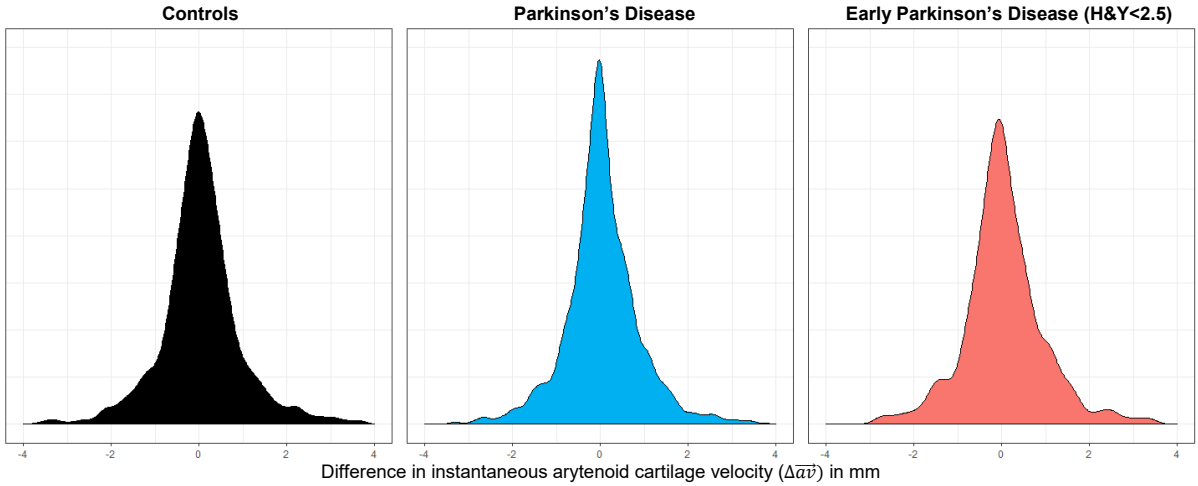


***Figure 4.1: Symmetry in arytenoid cartilage movements during vocalisation in Parkinson's disease.*** *Density plots of* $\Delta\overrightarrow{av}$ *comparing pwPD (blue plot) against healthy controls (black plot). A sub-group analysis was done for those with relatively early PD (red plot).*

An independent-samples t-test showed no significant difference in the $\Delta\overrightarrow{av}$ between pwPD (M = 0.031 mm, SD = 0.93) and controls (M = 0.046 mm, SD = 1.09), t(1749) = 0.34, p=0.74. Comparing those with relatively early PD against controls, there is still no significant difference seen (M = 0.022mm, SD = 1), t(1649) = 0.47, p=0.64. Therefore, there is no evidence of asymmetry in the velocities of the arytenoid cartilages in pwPD.

**Discussion**

We did not find evidence of asymmetry in the movement of the arytenoid cartilages in pwPD with 4D laryngeal CT. Had there been asymmetry, we would expect to see a bimodal distribution in the density plots, with one peak representing a mean tendency for the right arytenoid cartilage to move relatively less than the left, and vice versa for the second peak. Instead, density plots for all groups approximate the normal distribution, implying symmetry in the movements of the arytenoid cartilages, with variance around this introduced by the effect of random error.

Our results may reflect the true absence of asymmetry in vocal fold or arytenoid cartilage movements in PD. In the laryngoscopic study by Hanson and colleagues,[10] they observed that the arytenoid cartilage on the side more affected by the disease was positioned more posterolaterally, and its vocal fold would be further from the midline than the other side. Meanwhile, the vocal fold on the side less affected by the disease would come closer to the midline, and sometimes even cross the midline to move underneath the contralateral vocal fold – a phenomenon which they interpreted as a compensatory mechanism to achieve vocal fold closure. However, they did not measure the distance over which the vocal folds moved, so it is not clear whether there is any true difference in the distance moved by each of the vocal folds. Instead, the asymmetry in movement that they reported may only reflect differences in arytenoid cartilage positioning – that is, the vocal fold and arytenoid cartilage on the side of the body more affected by the disease would not come as close to the midline because they are positioned further away from the midline to begin with.

It needs to be acknowledged that this is a post-hoc analysis and the initial data set was not collected for the purposes of answering this research question. Furthermore, guided by the slowing of movement seen elsewhere in the body in PD, our hypothesis pertained only to abnormalities of movement of the arytenoid cartilages. The laryngeal measures performed in this analysis do not provide an indication of the position of the arytenoid cartilages. Therefore, we were unable to determine whether there was any asymmetry in the positioning of the arytenoid cartilages, as had been previously observed on laryngoscopy.[10] This poses an interesting question which would need to be answered in future work.

In summary, this analysis did not demonstrate any asymmetry in the movement of the arytenoid cartilages during vocalisation. However, as the data set utilised was concerned only with arytenoid cartilage movement, we could not assess for asymmetry in the position of the arytenoid cartilages. Further studies should address whether there is asymmetry in both the positioning and movements of the arytenoid cartilages in PD. Such work could confirm the results of this analysis, whilst also determining whether asymmetry of arytenoid cartilage positioning could be used as a potential biomarker for PD.

# Chapter 5
# Validating automated measurement of inter-arytenoid distance on 4D laryngeal CT

## 5.1 Overview

The prior chapters demonstrate that dynamic laryngeal CT has potential as a means of assessing for the presence and severity of Parkinson's disease. While the *GA* was derived in a semi-automated manner using CT workstation software, the *IAD* had to be manually obtained by annotating the locations of the arytenoid cartilages. After having personally laboured to perform these measures manually, it became apparent that obtaining these measurements may be impractical when conducted at greater numbers in larger scale studies or in a clinical setting. The CT imaging data for each participant was formatted so that there were 160 axial imaging slices produced for each 100 ms interval during the vocalisation period. Each timepoint required inspection of the 160 axial imaging slices to determine the anatomical level where the paired arytenoid cartilages lie closest together, after which fiducial markers were placed on their most medial aspect. With a vocalisation period of five seconds, this process would then be repeated 49 times for each participant.

The application of machine-learning techniques to the field of radiology have demonstrated numerous benefits. In particular, it can reduce time consuming practices while producing results which are comparable, or even superior to those, obtained by well-trained operators.[37] Developing automated methods of performing these laryngeal measurements will enable the study of larger data sets of laryngeal CT.

In the following validation study, we compared the results of manual markings which I performed, against those derived by a machine-learning module developed by our collaborators. We found that estimates of the inter-arytenoid distance measured by an automated machine-learning module are accurate. Future work using 4D laryngeal CT to evaluate the laryngeal changes in PD should consider the use of automated methods of measuring the *IAD*.

### 5.2 Submitted Manuscript

Our results are detailed in the following submitted manuscript 'Automated measurement of inter-arytenoid distance on 4D laryngeal CT: A validation study' *(Ma A, Desai N, Lau KK, Palaniswami M, O'Brien TJ, Palaniswami P, Thyagarajan D. 2022. Automated measurement of inter-arytenoid distance on 4D laryngeal CT: A validation study. Manuscript submitted for publication).*

# Automated measurement of inter-arytenoid distance on 4D laryngeal CT: A validation study

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## Abstract

Changes to the voice are prevalent and occur early in Parkinson's disease. Correlates of these voice changes on four-dimensional laryngeal computed-tomography imaging, such as the inter-arytenoid distance, are promising biomarkers of the disease's presence and severity. However, manual measurement of the inter-arytenoid distance is a laborious process, limiting its feasibility in large-scale research and clinical settings. Automated methods of measurement provide a solution. Here, we present a machine-learning module which determines the inter-arytenoid distance in an automated manner. We obtained automated inter-arytenoid distance readings on imaging from participants with Parkinson's disease as well as healthy controls, and then validated these against manually derived estimates. On a modified Bland-Altman analysis, we found a mean bias of 1.52 mm (95% limits of agreement -1.7 to 4.7 mm) between the automated and manual techniques, which improves to a mean bias of 0.52 mm (95% limits of agreement -1.9 to 2.9 mm) when variability due to differences in slice selection between the automated and manual methods are removed. Our results demonstrate that estimates of the inter-arytenoid distance with our automated machine-learning module are accurate, and represents a promising tool to be utilized in future work studying the laryngeal changes in Parkinson's disease.

## Introduction

Parkinson's disease (PD) is a progressive neurodegenerative condition which leads to increasing disability from motor dysfunction as well as a variety of non-motor symptoms. The diagnosis of PD as well as assessment of its progression both remain primarily dependent on clinical assessment of motor function [1]. However, by the time patients with Parkinson's disease (pwPD) can be diagnosed clinically, there is already a 50% loss of nigrostriatal dopaminergic neurons [2, 3]. Developing biomarkers which could diagnose PD earlier would make it possible to initiate disease-modifying therapies prior to the onset of advanced neurodegeneration. Furthermore, in trials of disease-modifying therapies, utilizing biomarkers which could track disease progression more accurately than current clinical assessment tools may allow for smaller sample sizes and shorter follow-up periods [4].

The voice changes seen in PD are a promising candidate for such a biomarker. Voice disorder is prevalent in the disease [5-7] and changes to the voice may herald the onset of the disease, manifesting several years prior to the limb dysfunction [8, 9]. While the voice changes of PD have been studied by numerous modalities including acoustic analysis and direct visualization [10], we utilized dynamic 4-dimensional computed-tomography (4D-CT) scans of the larynx to study the structural movement during phonation [11, 12]. 4D-CT is a CT technique which involves continuous image acquisition of anatomical structures over time (the 'fourth dimension'), thereby creating a dynamic volume data set. This allows quantitative analysis of the motion of anatomical structures.

Parkinson's disease is characterized by slowed movements, decrementing in amplitude and/or speed upon repetition (i.e. bradykinesia). We hypothesized that a repetitive vocalization task with laryngeal 4D-CT could reveal differences in arytenoid cartilage movements between pwPD and healthy control participants. In our prior work [11, 12], two key measurements were taken which were 1) the minimum distance between the arytenoid cartilages (denoted the inter-arytenoid distance or IAD), and 2) the glottic area. We previously demonstrated that changes to arytenoid cartilage posturing distinguish pwPD from healthy controls. pwPD have a reduction in the IAD and an increase in the glottic area during vocalization [11, 12]. We also found that as the condition advances over both time and clinical severity, the IAD progressively decreases while the glottic area increases [12]. Therefore,

4D laryngeal CT shows promise in both the diagnosis of PD, as well as in monitoring its progression. However, undertaking further research of a prospective nature and utilizing larger data sets would be necessary before 4D laryngeal CT could be more widely adopted in a clinical or research setting.

Our study protocol required manually placing a fiducial marker on the most anteromedial aspect of each of the arytenoid cartilages, which would correspond with the base of the vocal process. The Euclidean distance between these markings was then derived to determine the IAD. On the other hand, the glottic area was automatically segmented using workstation software (IntelliSpace Portal, Philips Healthcare, Cleveland USA). Manual determination of the IAD at 100 ms intervals on the large volume 4D dynamic CT dataset during phonation is a laborious and time-consuming process, so automated means of measuring the IAD could facilitate the study of larger data sets. Placement of fiducial markers is also subjective and expected to have a degree of inter- and intra-observer variability – an issue which could be largely overcome through automated measurement techniques.

We previously described an automated method which used classical imaging processing techniques to detect the most anteromedial aspect of the arytenoid cartilages. Comparing the feature points identified by this automated rule-based approach to fiducial markers manually placed by investigators, an accuracy of 83.33% was achieved within an error tolerance of 15 pixels (equivalent to an anatomical length of 3.33 mm) [13]. This represents the error in the placement of the individual fiducial markers; the IAD between the fiducial markers was not calculated in this study. The mean IAD during a vocalization task in our cohort of pwPD of varying duration and severities was 4.24 mm, while it was 5.21 mm in control participants (comparing the unadjusted median) – a difference of only 0.97 mm [12]. In our study comparing pwPD with relatively early-stage disease against controls, adjusted for sex, the mean difference was 0.87 mm [11]. The error tolerance of 3.33 mm is therefore rather high given our findings of such small differences in the IAD between pwPD and healthy controls.

In this study, we employ a novel automated technique for deriving IAD estimates which differs in two key aspects from the method previously described. First, it employs a computer algorithm which is trained with machine-learning techniques to automatically identify the regions of interest, as compared to the fixed rule-based approach adopted previously. Next, it marks bounding boxes

around the entirety of each arytenoid cartilage, in as many axial slices of the larynx as possible, for each imaging volume.

By processing a wider range of inputs, we expect that the machine-learning module will provide IAD estimates closer to those which are manually derived than the previous rule-based method. The machine-learning module considers factors both within and across image slices to accurately identify the position of the arytenoid cartilages. In contrast, the rule-based approach operates by only considering the individual axial slices around the plane of the glottis. This may lead to inaccuracies if there is misalignment of the imaging axis due to technical or anatomical reasons. Additionally, marking bounding boxes around the entirety of the arytenoid cartilages provides a measurement of their position. This offers the potential to conduct novel measurements of laryngeal function which have not been previously considered, such as measurement of movement in the vertical plane as can be seen in laryngeal tremor [14].

The aim of this study is to validate the IAD measurements derived from the automated detection of bounding boxes around the arytenoid cartilages by the machine learning module, against the current gold-standard of manual markings by investigators which we utilized in our prior work. In doing so, we intend to provide a basis for its use in future research using 4D dynamic laryngeal CT to study PD and other similar disorders.

## Materials and methods

### Patients and recruitment

We used the existing four-dimensional laryngeal CT image data set from our prior work. We recruited participants from the Movement Disorders clinic at Monash Medical Centre. Our initial study [11] recruited healthy controls as well as pwPD with early-stage disease, which we defined as a disease duration less than 6 years and a modified Hoehn and Yahr stage of 2.5 or lower. Our subsequent study [12] recruited pwPD with more advanced disease, which we defined as a disease duration of 5 years or greater and a Hoehn & Yahr stage of 3 or more. Control participants were not affected by Parkinson's disease or other neurological disorders on clinical assessment by a neurologist. We excluded participants with respiratory or laryngeal disorders, as well as those with brain, head and neck cancers. From this data set, we randomly selected 4 pwPD with early-stage

disease, 4 pwPD with more advanced disease and 4 healthy controls to validate the technique across the populations we have studied thus far.

**Protocol approvals, registrations and patient consents**

All participants provided written informed consent in accordance with the Declaration of Helsinki. The Research Ethics Committee of Monash Health granted ethics approval (HREC approval number 11230B).

**Image acquisition and processing**

Using a 320 multi-detector row CT (Aquilion One, Tokyo, Canon Medical Systems), non-contrast four-dimensional dynamic volume CT imaging of the larynx was acquired over an anatomical z-axis length of 16 cm without CT table movement. During a continuous imaging period of 5 seconds, participants were positioned supine and produced five quick and clear phonations of /i/ ('eee') at a comfortable speaking volume and pitch. Image acquisition was terminated before five seconds in those who had completed the vocalization task early. pwPD underwent imaging in a practically-defined 'off' state by withholding their usual PD medications overnight.

Continuous multiplanar images were produced at 100 ms intervals and were analyzed in the Neuroimaging Informatics Technology Initiative (NIfTI-1) image format at a resolution of 512 x 512 pixels and a voxel size of 0.222 x 0.222 x 0.5 $mm^3$. Although our prior work [11, 12] only considered measurements taken during the vocalization phase, we performed manual and automated measurements of the IAD across the entire image acquisition period (including incidental periods of voice rest and respiration) in this validation to increase the generalizability of our results to different states of the arytenoid cartilages.

**Manual image analysis**

Images were manually annotated using the open-source ImageJ graphics software [15]. For each subject, at every timepoint within the vocalization period, the most medial aspect of each arytenoid cartilage was identified by scrolling through the image stack. A fiducial marker was then manually placed on the most medial aspect of each arytenoid cartilage. The manually estimated IAD ($IAD_M$) was then calculated by finding the Euclidean distance between the two paired fiducial markers for every timepoint in each subject (see **Fig 1B**).

Rather than using the previously measured IAD data set, all images were re-annotated by investigator A.M for the purposes of this study to eliminate error due to inter-observer variability. Images were manually annotated in a randomized order across both subjects and timepoints to reduce auto-correlation. There was a risk of introducing bias by sequentially analyzing timepoints for a single subject, as the preceding annotations could influence the location of the markings for the following timepoint and thereby increasing dependence between data points.

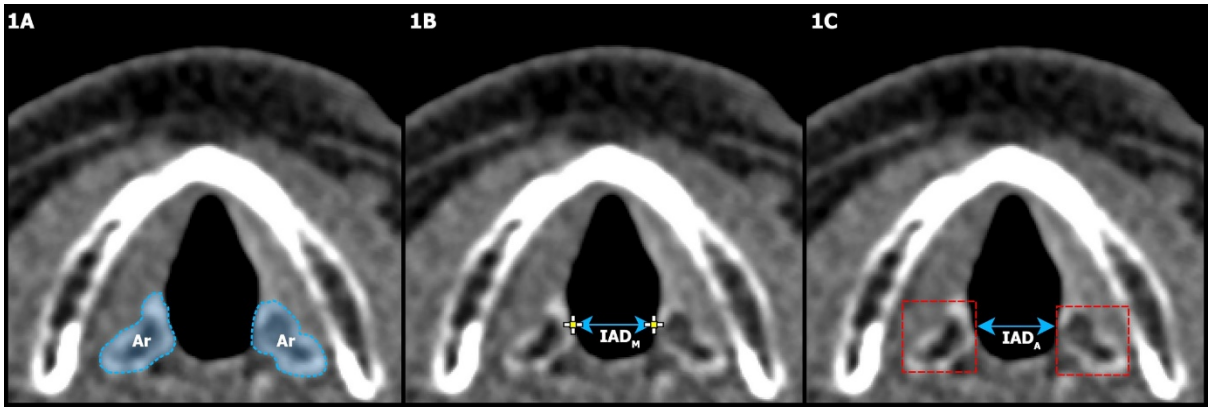


***Fig 1. Illustrative diagrams comparing the image annotation techniques.*** *1A, axial slice through the larynx during voice rest, with the left and right arytenoid cartilages (Ar) marked in blue. 1B, fiducial markers manually placed at the anteromedial aspect of each arytenoid cartilage, with the corresponding* $IAD_M$ *marked. 1C, bounding boxes marked in red around each arytenoid cartilage by the automated machine learning algorithm, with the corresponding* $IAD_A$ *marked.*

**Automated image analysis**

Images were analyzed using the automated image analysis technique developed by investigators N.D and colleagues [16]. The algorithm operated by detecting slices which contained the arytenoid cartilages and subsequently localized them before computing the IAD.

The image analysis algorithm consisted of a machine learning module which was designed using feedforward artificial neural nets that operated on orthogonal 2D views of the 4D CT data. The CT images acquired during the phonation task described previously, and those acquired during a standard breathing examination were combined to construct a dataset of 100 subjects. Only slices containing arytenoid cartilages were used for training the algorithm, with three-quarters of the included imaging data used in the training subset and one-quarter in the validation subset. An ubuntu 18.04 computing machine with Nvidia Quadro 6000 graphics card (24 gigabyte global

memory) was used to train and validate the machine learning module. The implementation was done using the Pytorch library [17] and detectron2 [18] in Python.

Firstly, the algorithm marked bounding boxes around each arytenoid cartilage for each slice in which they were identified. Further, the arytenoid bounding boxes are localized in all the possible slices in the 3D volume for each timepoint. This process was repeated for each subject. The estimated IAD derived by the automated technique ($IAD_A$) for a given timepoint was then calculated by finding the minimum distance between the paired structures in the x-axis/coronal plane (see **Fig 1C**).

The algorithm was also tuned to compute a 'same slice' automated IAD estimate ($IAD_S$) on the same axial slice from which the $IAD_M$ was derived. If the automated algorithm was unable to localize a pair of arytenoid cartilages on the slice marked by the manual observer, then that data point was excluded from analysis. Comparing the $IAD_S$ to $IAD_M$ removes the proportion of bias attributable to differences in slice selection between the manual and automated techniques. Therefore, the remaining bias is largely attributable to differences between the techniques in where the markings are placed within a slice.

**Statistical analysis**

We performed all data processing, statistical analysis and creation of graphics with the R statistics and graphing software [19] and nlme package. The estimates obtained by the automated ($IAD_A$) and same-slice automated ($IAD_S$) techniques were compared to the estimates obtained by the manual technique ($IAD_M$). We adjusted for repeated measures in our data by using linear mixed models to form regression lines. Similarly, considering the repeated measures, we utilized a modified Bland-Altman method to calculate the mean of the paired differences between the techniques, as well as their corresponding 95% limits of agreement.

The regression lines considered the $IAD_M$ as explained by the fixed effect of either the $IAD_A$ or $IAD_S$, with random slopes and intercepts considered per participant to account for the repeated measures.

We performed a modified Bland-Altman analysis utilizing linear mixed models based on techniques previously described in the literature [20-22]. These modifications were necessary as our data consisted of repeated measures within individual subjects, thereby violating the assumption of independence inherent to the standard Bland-Altman method. Our models considered the paired

differences between the $IAD_M$ and $IAD_A$, and $IAD_M$ compared to $IAD_S$, after considering inter-participant variation as a random effect. The R code for these analyses is provided in the **S1 Appendix**.

**Data availability**

The data that support the findings of this study are openly available in the figshare repository at http://doi.org/10.26180/19306916.

## Results

Imaging data was analyzed for 12 participants. 4 participants (1 female, 3 male) were healthy controls and had a mean age of 67 years (SD 7.4). 8 participants (2 female, 6 male) had a diagnosis of Parkinson's disease and had a mean age of 71 years (SD 8.1). pwPD had a median disease duration of 94 months (IQR 51-126) and median UPDRS part-III score of 22 points (IQR 14-34).

Paired $IAD_A$ and $IAD_M$ measurements were taken on 560 CT volumes while only 515 paired measurements of the $IAD_S$ and $IAD_M$ were acquired. There were fewer $IAD_S$ measurements as the automated technique did not detect the arytenoid cartilages on some slices marked by the manual observer.

**Fig 2** plots the $IAD_M$ estimates paired against the $IAD_A$ and $IAD_S$. Regression lines considering repeated measures within subjects are overlayed.

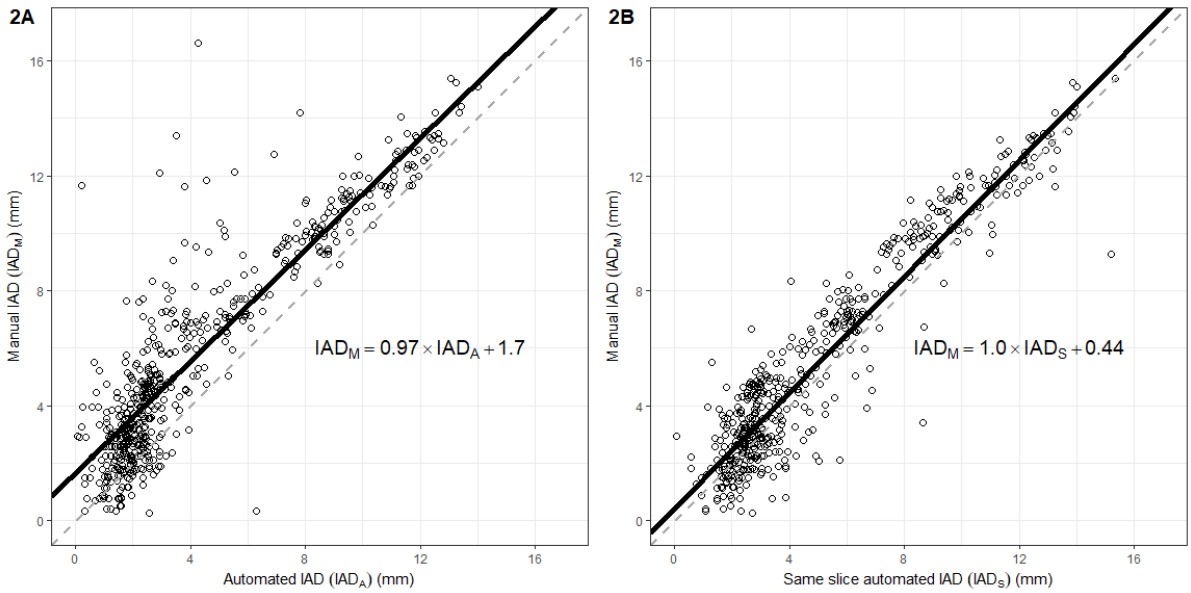


***Fig 2. Scatter plots of the paired estimates.*** *2A compares the paired estimates of the $IAD_M$ with $IAD_A$, while 2B compares the paired estimates of $IAD_M$ with $IAD_S$. Regression lines are shown as solid black lines with their respective equations provided. The grey dashed lines represent the lines of equivalence.*

The modified Bland-Altman analysis accounting for repeated measures demonstrated that compared to the $IAD_M$, the $IAD_A$ had a mean difference of 1.52 mm with 95% limits of agreement from -1.7 to 4.7 mm. On the other hand, the $IAD_S$ compared with the $IAD_M$ showed a mean difference of 0.52 mm with 95% limits of agreement from -1.9 to 2.9 mm. The corresponding Bland-Altman plots are given in **Fig 3**. Further statistics pertaining to the Bland-Altman analysis are given in **Table 1**.

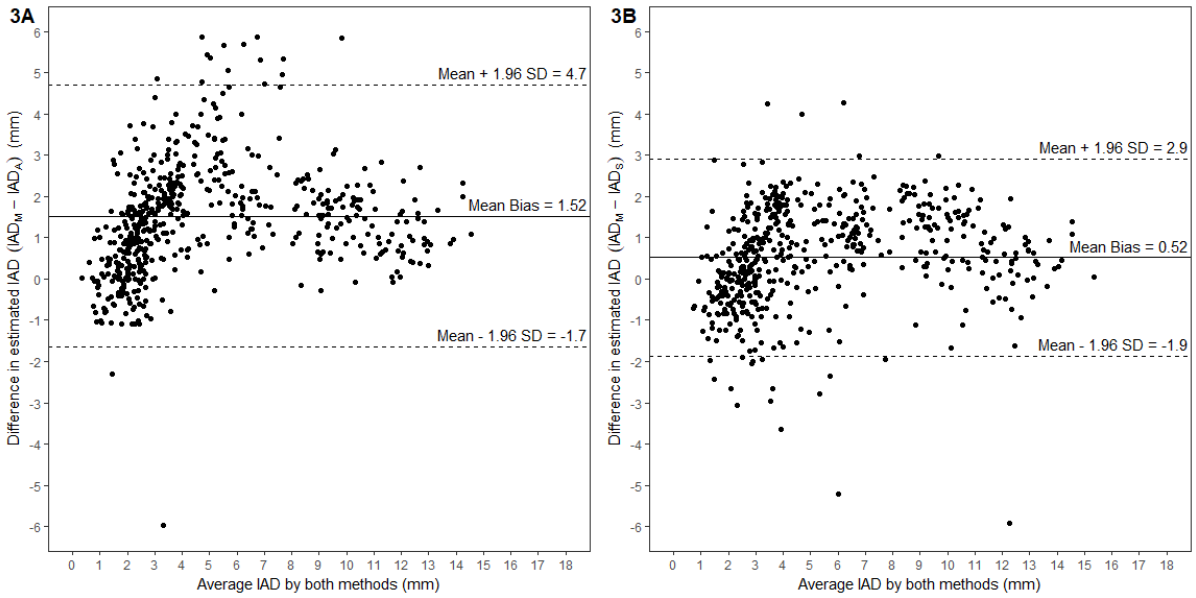


***Fig 3*: *Bland-Altman plots.*** *3A compares the $IAD_M$ against the $IAD_A$, while 3B compares $IAD_M$ to the $IAD_S$. The mean bias (solid line), 95% upper and lower limits of agreement (dotted lines) are shown.*

**Table 1: Bland-Altman comparisons of the $IAD_A$ and $IAD_S$ against $IAD_M$**

| | $IAD_A$ | $IAD_S$ |
|---|---|---|
| Number of paired measurements | 560 | 515 |
| Mean bias (mm) | 1.52 | 0.52 |
| 95% limit of agreements (mm) | -1.7 to 4.7 | -1.9 to 2.9 |
| Within participant standard deviation (mm) | 1.45 | 0.94 |
| Between participant standard deviation (mm) | 0.73 | 0.77 |
| Total standard deviation (mm) | 1.6 | 1.2 |

## Discussion

This study evaluates the concordance of automated estimates of the IAD compared to those obtained through manual markings. We expect manual measurement of the IAD, as performed in our prior studies, to have a degree of inter- and intra-rater variability (although we have not previously formally determined this) and be subject to the influence of measurement error. This is a key motivation in exploring automated techniques of measurement, which would be more reproducible, less laborious and eliminate inter- and intra-rater variability. Our prior work utilized manual measurement of the IAD and by default is the current 'gold standard', but these measurements do not necessarily closely approximate the ground truth value.

In comparing the $IAD_M$ with the $IAD_A$, Bland-Altman analysis demonstrates a mean bias of 1.52 mm (i.e. the estimate of $IAD_A$ is 1.52 mm less than the estimate of $IAD_M$). Much of this discrepancy arises from differences introduced by differing slice selection between the automated and manual techniques. To remove this source of variability, we obtained the $IAD_S$ – automated measurements taken on the same CT slice as the $IAD_M$. When comparing the $IAD_M$ with the $IAD_S$, there was an average discrepancy of 0.52 mm in the estimates given, which is less than 3 pixels difference. Thus, differences in slice selection by the manual and automated techniques accounts for a significant portion of the variability between the $IAD_M$ and $IAD_A$; an increase in the mean bias of 1.0 mm and a widening of the Bland-Altman limits of agreement by 1.6 mm.

The slice on which the $IAD_M$ is measured is determined by gross visual inspection of where the arytenoid cartilages appear to be closest together. It is expected that the manual observer will not always select the slice where the ground truth IAD lies (i.e. where the pair of arytenoid cartilages are closest together). The automated technique calculates a distance between the arytenoid cartilages on every slice, with the minimum of this set of measurements taken as the $IAD_A$. This approach would be expected to be more reliable in identifying the slice where the ground truth IAD lies than the manual technique. Having a manual observer perform measurements on every slice may achieve similar reductions in variability, but this would be unfeasible and markedly increase the work involved in what is already a laborious task. Ultimately, by removing variability due to imprecise slice selection by a manual observer, automated methods of IAD measurement may provide closer approximations of the ground-truth IAD.

The bounding box outputs of the machine-learning algorithm may also provide potential for more clinical applications. Capturing the positions of each arytenoid cartilage within three-dimensional space allows for novel measures to be considered, such as assessment of movements of the arytenoid cartilages in the vertical plane or z-axis over the imaging period. This could be applied to the study of vocal tremor, a phenomenon seen in PD which on laryngoscopic studies occurs predominantly in the vertical plane.[38] Future work on laryngeal CT could assess these novel measures, which may offer additional benefits in the diagnosis or assessment of progression of PD beyond those which have been previously studied.

The 95% limits of agreement in comparing $IAD_M$ to the $IAD_A$ or $IAD_S$ are still quite wide (-1.7 to 4.7 mm and -1.9 to 2.9 mm respectively). Random error is a major contributor to the variance in the agreement. A source of this random error is the subjective nature of fiducial placement which depends on where the observer judges the edge of the arytenoid cartilages to lie. On the other hand, the automated algorithm is consistent in its thresholds for edge detection. Whilst this normally distributed random error will be averaged out in a study such as ours using repeated measures, the automated algorithm will be preferred because of its consistency.

Limitations of fully automated techniques of measurement include their susceptibility to artifactual measurements. Inspection of **Fig 2A** shows numerous outliers in the upper left quadrant of the scatter plot, with the $IAD_A$ estimate being substantially lower than the $IAD_M$. Differences this large suggest that the estimates taken by the automated technique are artifactual, as it is unlikely the manual observer would have made such a substantial deviation from the ground truth. The automated algorithm selects the minimum of the IAD estimates across all measured slices for a given timepoint. Therefore, a single outlier which underestimates the ground truth IAD will be chosen, rendering the automated technique susceptible to artifacts of this nature. However, these outliers all occurred at high $IAD_M$ readings, which correspond to periods of vocal rest where the vocal folds lie abducted. Therefore, this shortcoming of the automated algorithm is not relevant to studies (such as ours) [12] of vocal fold motion during phonation.

Further analysis to assess the performance of the automated method in specific populations would have been ideal, such as comparisons between sexes, or between participants with earlier and more

advanced disease. However, this study was limited to only 12 participants, preventing meaningful sub-group analyses. Although the algorithm performs quite consistently across a wide range of IADs (aside from very high values as discussed above), it is possible that other factors such as anatomical differences between groups could lead to reduced performance. Performing a further validation study with greater patient numbers could permit such sub-group analyses.

We performed a modified Bland-Altman analysis utilizing linear mixed models given the repeated measures nature of our data. Regarding the construction of our linear mixed models, we considered participants as a random effect as suggested by Parker and colleagues [21] given the nature of our study design. As timepoints were only 100 ms apart, there would be a high degree of auto-correlation of the IAD over successive measures. Additionally, our participants included both healthy controls, as well as people with Parkinson's disease covering a range of disease durations. Parkinson's disease could affect phonation, but our sample was not large enough to consider it as a fixed effect. Therefore, we accounted for these differences by considering participant as a random effect.

Ultimately, the question remains whether automated methods of estimating the IAD are suitable to replace manual techniques in future research, and as a clinical biomarker. Based on our previous work, we found a 'threshold of detection' of early PD in a controlled study was a 0.87 mm sex-adjusted mean difference in IAD when compared to controls [11]. In this validation study, we found close agreement between a novel automated measurement technique and the manual approach used in that study. The $IAD_A$ was consistently 1.52 mm less than the $IAD_M$ across the entire range of measurements. Therefore, provided a single technique is applied in such repeated measures group studies, PD may be reliably detected. The automated method should also have greater consistency in edge detection and slice selection than manual fiducial placement. Thus, the $IAD_A$ may more closely represent the ground truth IAD than $IAD_M$. Automation would also facilitate analysis of much larger data sets, through which measures such as the IAD could be further validated, thereby fostering their broader adoption into research and clinical settings. Automation could also facilitate novel measures such as vertical motion of the arytenoid cartilages. After consideration of the above, we propose that automated techniques for estimating the IAD are a valid and superior tool for use in future work assessing the position and movement of the arytenoid cartilages during 4D laryngeal CT phonation imaging.

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## S1 Appendix. Modified Bland-Altman analysis using linear mixed models in R.

*Provided is the code used in the R software to perform the modified Bland-Altman analysis, which accounts for repeated measures within our data through the use of linear mixed models.*

```
# Data structure: All data is stored in the data frame 'Data',
# in which the paired differences between the manual IAD and
# automated IAD are stored as 'a.diff', and those between the
# manual IAD and 'same-slice' automated IAD are stored as
# 's.diff'.


# Dependencies
library(nlme)


# Manual IAD vs automated IAD
a.lme<-lme(a.diff~1, random=~1|ID, data=Data,
na.action=na.omit)
a.withinsd<-as.numeric(VarCorr(a.lme)[2,2])
a.betweensd<-as.numeric(VarCorr(a.lme)[1,2])
a.totalsd<-sqrt((as.numeric(VarCorr(a.lme)[1,1])+as.numeric
(VarCorr(a.lme)[2,1])))
a.mean<-summary(a.lme)$coefficients$fixed[[1]]
a.lower<-a.mean-(1.96*a.totalsd)
a.upper<-a.mean+(1.96*a.totalsd)


# Manual IAD vs same-slice automated IAD
s.lme<-lme(s.diff~1, random=~1|ID, data=Data,
na.action=na.omit)
s.withinsd<-as.numeric(VarCorr(s.lme)[2,2])
s.betweensd<-as.numeric(VarCorr(s.lme)[1,2])
s.totalsd<-sqrt((as.numeric(VarCorr(s.lme)[1,1])+as.numeric
(VarCorr(s.lme)[2,1])))
s.mean<-summary(s.lme)$coefficients$fixed[[1]]
s.lower<-s.mean-(1.96*s.totalsd)
s.upper<-s.mean+(1.96*s.totalsd)
```

# Chapter 6
# Integrative Discussion

## 6.1 Contributions to the field

This thesis primarily set out to further characterise the changes in laryngeal cartilage posturing and movement on CT imaging which occur in Parkinson's disease as it advances, while also paving the way for further study by validating the use of automated machine-learning algorithms. The use of dynamic laryngeal CT permitted the measurement and quantification of laryngeal posturing and movement, allowing us to build upon the foundation of understanding offered by the descriptive laryngoscopic studies conducted previously.

The works detailed in Chapter 3 and Chapter 4 uncovered numerous insights, the culmination of which reveal the laryngeal changes of Parkinson's disease to be primarily abnormalities of phonatory posturing and position. Firstly, we confirmed our previous findings[5] that the *IAD* is significantly reduced in pwPD when compared healthy controls, implying that the arytenoid cartilages lie closer together during vocalisation in pwPD. Yet, despite the reduction in *IAD*, the *GA* is increased in pwPD relative to healthy controls, which can be inferred to represent the presence of vocal fold bowing (see ***Figure 6.1***). We also demonstrated that increasing disease severity and duration is correlated with lower values of *IAD* and higher values of *GA*, which suggest that these laryngeal measures change as PD advances. Thus, our results support our hypothesis that there are progressive changes to the positioning of the arytenoid cartilages and vocal folds as the disease advances.

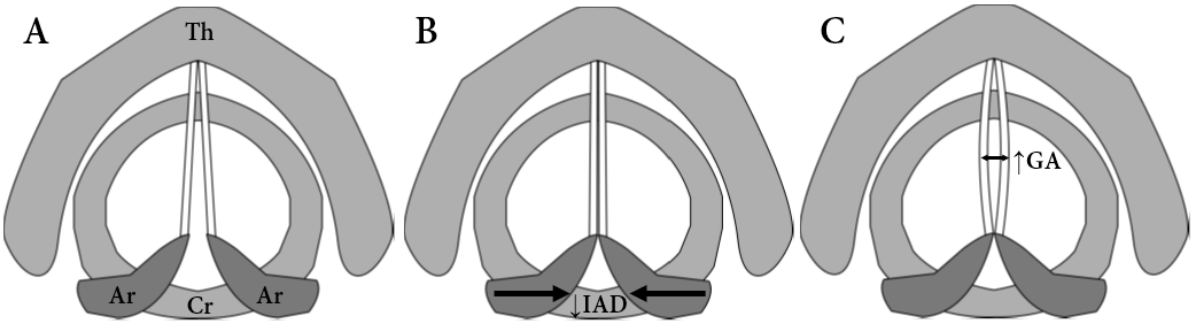


***Figure 6.1. Vocal fold bowing in Parkinson's disease.*** *Schematic diagrams illustrating the changes in the minimum IAD and GA, and their interpretation. A, normal larynx at adduction; B, reduction in IAD, with the arytenoid cartilages drawn closer together; C, increase in glottic area explained by bowing of the vocal folds. Th, thyroid cartilage; Cr, cricoid cartilage; Ar, arytenoid cartilage. Reproduced from Ma A, Thyagarajan D,[39] 2019.*

Our analyses of the minimum *IAD* and *GA* further support abnormalities of laryngeal posturing and positioning being primarily responsible for the voice changes of PD. We assessed the minimum values as these would occur when the vocal folds come closest together and the laryngeal adductors are maximally activated. We expected that abnormalities of arytenoid cartilage positioning would be most pronounced at this point. We found that the minimum *IAD* and *GA* both showed significant differences in pwPD when compared to healthy controls, and that the minimum *IAD* and *GA* showed more robust correlations to disease duration and motor severity than their mean values. This suggests that considering the remainder of the *GA* and *IAD* values throughout the vocalisation period, which represent their values whilst the arytenoid cartilages are in motion, do not provide additional utility in determining disease progression. In combination, these findings suggest that there are alterations to the positioning and posturing of the laryngeal apparatus in pwPD, rather than their movements.

We also demonstrated the utility of the *IAI*, an index which considers both the *IAD* and *GA*. The mean and maximum values of the *IAI* was significantly different between pwPD and controls, and it was more strongly correlated with the disease's motor severity than the mean or minimum values of the *GA* or *IAD* alone. As isolated abnormalities of the *GA* could potentially result from phenomena such as age-related vocal fold bowing (although this is partially accounted for the inclusion of age-

matched control participants), the combination of a reduced *IAD* and elevated *GA* may be more specific for PD. These findings imply that similar work in future would benefit from considering both the *IAD* and the *GA* in their analyses.

The findings of this thesis also allow us to draw inferences on the pathological underpinnings of the voice changes in PD. The alterations to the laryngeal apparatus in PD described in this body of work are quite distinct from those which occur elsewhere in the body. The arytenoid cartilage movements are not slowed, reduced in amplitude or asymmetric. Considered together, it may be that the voice changes of PD are primarily driven by disease mechanisms other than the dopaminergic deficiency which drives the cardinal features of the disease. This could explain the equivocal benefits to voice quality afforded by dopaminergic medications which were reported in the prior literature.

Lastly, we validated an automated method of measuring the *IAD* and found it to be accurate. Being able to employ such a tool will cut down on the laborious process of manually marking the arytenoid cartilages. This should increase the feasibility of using 4D laryngeal CT in larger-scale studies or clinical settings and serves to promote further research into this field.

Considered together, this body of work suggests that the changes which occur to the larynx in PD are not simply analogous to the elements of parkinsonism seen in the limbs. We did not find any evidence of slowing or reduction in amplitude of arytenoid cartilage movements, nor any asymmetry in the velocity of their movements. Instead, there are alterations to the posturing and positioning of the arytenoid cartilages and vocal folds in PD, and these progress in severity as the condition advances. These contributions provide guidance and a basis for further research in this field moving forward.

## 6.2 Limitations

While the inferences which can be drawn from this body of work are promising and introduce new concepts into the field, the limitations to the works presented in this thesis require careful consideration. The cross-sectional nature of the data set is a clear limitation to the results presented in Chapter 3, which investigated how these laryngeal measures change with increasing disease duration and severity. While we showed correlations between more advanced disease and changes in the laryngeal measures, we are unable to prove a causal relationship. Prospective studies are required to confirm whether these measures truly progress within individual pwPD as the disease advances. Nevertheless, given the cumulative risks of repeated radiation exposure incurred with serial imaging, beginning with a cross-sectional study was an ethically necessary step.

Although the data presented suggest that movements of the arytenoid cartilages in pwPD are not altered, there are caveats to this notion due to limitations of the study. We analysed movement of the arytenoid cartilages by measuring the Euclidean distance each vocal process moved between successive 100 ms timepoints. However, this is an oversimplification of arytenoid cartilage motion which opens and closes the vocal cords through a combination of sliding, rotational and pivoting movements. It could be that movement in one of these planes is altered, but compensations occur which result in the absence of difference in our measurements. Measurement of arytenoid cartilage movement across multiple planes would be of interest to clarify if movements are affected in any of these.

While one of our aims was to confirm the results of the prior study by Perju-Dumbrava et al,[5] the data set for the experiments performed in this thesis had a significant overlap with that prior study. To demonstrate reproducibility of our prior results, the experiment would need to be repeated with an independent cohort. Nevertheless, this was not performed as the primary purpose of the study was to determine how these measures changed depending on disease severity and duration. In this context, expanding the prior cohort by recruiting additional patients with more advanced disease was more ethically justifiable, particularly when considering the risks of radiation exposure.

### 6.3 Future directions

This thesis establishes a foundation upon which dynamic laryngeal imaging can be used to evaluate PD. Further work would stand to benefit by building upon the ideas brought forward by this body of work and addressing the limitations inherent to it.

Further research which adopts a prospective study design could establish whether these laryngeal measures truly change within an individual as PD advances. Prospective work would address the limitations inherent to the cross-sectional approach used in the studies performed as part of this thesis. As prospective studies which perform repeated imaging within an individual brings forth concerns of cumulative radiation exposure, future work could consider the use of ultrasound. Ultrasound can reliably detect the arytenoid cartilages and the movements of the vocal folds,[40, 41] and we have proposed this as an alternative which warrants further study.[42]

Future work with dynamic laryngeal imaging in PD should also consider focusing on abnormalities of arytenoid cartilage position, rather than their movement. As we were expecting arytenoid cartilage movements to be affected, we imaged participants during a repeated vocalisation task. However, we found that arytenoid cartilage movements were not significantly affected in pwPD. On the other hand, analysis of the laryngeal measures at the point of maximal vocal fold adduction (i.e. their minimum values) was at least comparable, if not superior, to assessing their values across the whole vocalisation period. Performing measurements only during a sustained phonation task – such as holding the phoneme /i/ ('eee') – brings the vocal folds to maximal vocal fold adduction and would have numerous benefits. Employing a more simple task would reduce some of the variability we observed in how participants performed the repeated vocalisation task. In particular, outlier measurements characterised by large *IAD* and *GA* values were introduced by participants who needed to take a breath between the five vocalisations of 'eee'. It would also reduce the amount of work involved in marking the relevant laryngeal measurements. Therefore, future work could consider only performing a sustained phonation task rather than repeated phonation.

Investigation into other laryngeal measures should also be considered. As the use of dynamic laryngeal imaging in PD is relatively novel, it is uncertain which measurements best determine its presence or severity. Based on the findings of this thesis, investigation into whether asymmetry in the

positioning of the arytenoid cartilages exists would be of particular interest. As we hypothesised that there would be asymmetry of arytenoid cartilage movement analogous to that seen in the limbs in PD, we only assessed the velocities of the left and right arytenoid cartilages. Accordingly, we did not perform measures which would allow us to detect asymmetry in the positioning of the arytenoid cartilages. As alterations to the posturing of the arytenoid cartilages appears to be the primary laryngeal abnormality in PD, and asymmetry of arytenoid cartilage positioning has already been described in laryngoscopic studies,[10] this would be a phenomenon worth evaluating. Imaging participants in both the 'on' and 'off' dopaminergic medication states would also be of interest. This could help to clarify the pathophysiological underpinnings of these voice changes and help determine whether dopaminergic therapies can improve the voice. With the amount of data captured by 4D laryngeal imaging, further work could also investigate for other novel parameters of laryngeal motion or posturing, but selection of these would need to be hypothesis-driven.

By avoiding the laborious process of manually marking the *IAD*, automated methods of measurement would enable the collection of larger data sets. In particular, future prospective studies would be capable of handling the large amounts of data which results from repeat imaging within participants. Machine-learning modules could also be developed for other laryngeal measures, which would make the study of a greater number of measurements more feasible. For example, to detect asymmetry in the positioning of the arytenoid cartilages, a mid-line feature point or line would also need to be marked. Assessing other planes of arytenoid cartilage movements, such as rotational and pivoting movements, could be facilitated by automated methods of measurement to mark additional feature points. Measurement of laryngeal structures other than the arytenoid cartilages may also yield additional information. Vocal tremor is a phenomenon observed in many pwPD[38] and involves oscillatory movements in the vertical plane of not only the arytenoid cartilages, but also the palate and the larynx as a whole.[43] Employing automated methods of measurement could enable the measurement of other laryngeal structures, or allow for study the arytenoid motion in multiple planes in future work.

Furthermore, our work only characterised the changes seen in patients with PD and healthy controls. Performing similar work on patients with atypical parkinsonian syndromes such as multiple system atrophy and progressive supranuclear palsy (PSP) would be of interest. Vocal fold bowing has been

identified in PSP[44] as well as multiple system atrophy[45], so dynamic laryngeal CT may also be able to serve as a marker of the presence of severity of these related diseases as well. On the other hand, these disorders have distinguishing vocal characteristics,[13-15] so there may be unique features on dynamic laryngeal imaging which could differentiate between these conditions.

### 6.4 Conclusion

This thesis set out to characterise the changes to arytenoid cartilage positioning and movement in PD using dynamic laryngeal CT. This was with the intention to detect the disease's presence and severity, while also serving to further our understanding of the changes to the voice which occur in the condition. I also set out to validate the use of automated measurement methods to improve the feasibility of undertaking further research into dynamic laryngeal CT, as well as its potential translation into clinical use. The works presented in this thesis have met these research aims. Through quantification of arytenoid cartilage dynamics, this body of work found that the voice changes of PD primarily relate to abnormalities of the position of the arytenoid cartilages, rather than their slowing of their movement. We found that these laryngeal measures not only indicate the disease's presence, but that they are also correlated with the disease's severity, suggesting that these laryngeal abnormalities progress as the disease advances. Automated measurement methods provide an accurate alternative to manual measurement of the *IAD*. The culmination of these findings further our understanding of the voice changes of PD and provide a foundation and direction for further study.

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