TOOMAS TOOMSOO

Transcranial brain sonography in
the Estonian cohort of Parkinson's disease

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

291

TOOMAS TOOMSOO

Transcranial brain sonography in the Estonian cohort of Parkinson's disease

Department of Neurology and Neurosurgery, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia.

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy (medicine) on the June 19, 2019 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

Supervisors: Professor Toomas Asser, MD, PhD
                 Rector of the University of Tartu;
                 Department of Neurology and Neurosurgery, Institute of Clinical
                 Medicine, University of Tartu, Tartu, Estonia.

                 Professor Daniela Berg, MD
                 Head of Department of Neurology, Christian–Albrechts-University of
                 Kiel, Kiel, Germany

                 Professor Pille Taba, MD, PhD
                 Head of Department of Neurology and Neurosurgery, Institute of
                 Clinical Medicine, University of Tartu, Tartu, Estonia.

Reviewers: Assoc. Professor Eve Õiglane-Šlik, MD, PhD
                 Department of Pediatrics, Institute of Clinical Medicine, University of
                 Tartu, Tartu, Estonia

                 Assoc. Professor Pilvi Ilves, MD, PhD
                 Head of Department of Radiology, Institute of Clinical Medicine,
                 University of Tartu, Tartu, Estonia

Opponent: Professor Per Odin
                 Head of Division of Neurology Department of Clinical Sciences Lund
                 University, Lund, BMC F12, 221 84 Lund, Sweden

Commencement: Room No. 204, Ülikooli St. 18, Tartu, on October 14th, 2019, at 15.00

Publication of this dissertation is granted by the University of Tartu.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

1. Toomsoo, T., Liepelt-Scarfone, I., Kerner, R., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2016). Substantia Nigra Hyper-echogenicity: Validation of Transcranial Sonography for Parkinson Disease Diagnosis in a Large Estonian Cohort. J Ultrasound Med, 35, 17–23.

2. Toomsoo, T., Randver, R., Liepelt-Scarfone, I., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2017). Prevalence of Depressive Symptoms and Their Association with Brainstem Raphe Echogenicity in Patients with Parkinson's Disease and Non-PD Controls. Psychiatry Res Neuroimaging, 268, 45–49.

3. Toomsoo, T., Liepelt-Scarfone, I., Berg, D., Kerner, R., Pool, AH., Kadastik-Eerme, L., Rubanovits, I., Asser, T., Taba, P. (2018). Effect of Age on Substantia Nigra Hyper-echogenicity in Parkinson's Disease Patients and Healthy Controls. Ultrasound Med Biol, *45,* 122–128.

Applicants contributions:
Papers I–III: Toomas Toomsoo was involved in the design of the study, assessment of patients, data collection, partially data analysis, and writing the manuscripts.

ABBREVATIONS

αsyn	Alpha synuclein
AAN	American Academy of Neurology
ADL	Activities of daily living
AUCA	Area under the curve
BDI	Beck Depression Inventory
CBD	Corticobasal degeneration
CI	Confidence interval
CT	Computed tomography
DA	Dopamine
DaTscan	Tradename of ^{123}I Ioflpane manufactured by GE Healthcare
DLB	Dementia with Lewy bodies
DTI	Diffusion-tensor magnetic resonance imaging
HYS	Hoehn-Yahr Stage
LB	Lewy body
LDED	Levodopa equivalent dose
LN+	Lentiforme nucleus hyperechogenicity
MDS	International Parkinson and Movement Disorders Society
MDS-UPDRS III	Movement Disorders Society Unified Parkinson's Disease Rating Scale section III
MHz	Megaherz
MIBG-123	I 123 iodine-meta-iodobenzyl guanidine myocardial scintigraphy
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MSA	Multisystem atrophy
NICE	National Institute for Health and Care Excellence
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PDQ-39	Parkinson's Disease Questionnaire
PIGD	Postural instability and gait disorder
PET	Positron emission tomography
PSP	Progressive supranuclear palsy
QSBB	Queen Square Brain Bank
RBD	Rapid behaviour disorder
ROC	Receiver operating curve
RR	Relative risk

SE	Schwab and England Activities of Daily Living Scale
SN	Substantia nigra
SN+	Substantia nigra hyperechogenicity
SPECT	Single-photon emission computer tomography
SWEDD	Scans Without Evidence of Dopaminergic Deficit
SWI	Susceptibility weighted sequence
TCS	Transcranial sonography
THI	Tissue harmonic imaging
UKPDSBB	United Kingdom Parkinson's Disease Society Brain Bank

1. INTRODUCTION

Sporadic Parkinson's disease (PD) is the most widely recognized and second most common movement disorder (Fahn, 2010). It is known as an older person's disease, affecting 2–3% of the population 65 years of age or older (Poewe et al., 2017). PD is characterized by a selective, dynamic, progressive loss of dopaminergic neurons in the substantia nigra (SN).

PD clinical features are defined by both motor (bradykinesia, along with resting tremor and/or rigidity) and non-motor symptoms (e.g. constipation, apathy, fatigue, olfactory dysfunction, REM sleep behavior disorders (RBD), depression and cognitive decline) which become more severe as the disease advances. Treatment of PD is based on pharmacological substitution of striatal dopamine, in addition to non-dopaminergic approaches to address both motor and non-motor symptoms and deep brain stimulation for those developing medically untreatable L-DOPA-related motor complications.

The majority of PD cases are idiopathic, which means that there is not one specific cause but most probably several mechanisms have contributed to the pathogenesis including a genetic vulnerability, metabolic abnormalities, environmental factors and possibly infections (Hernandez et al., 2016). The effectiveness of establishing a clinical diagnosis of PD by a PD specialist has been reported to be over 90% in clinicopathological studies (Hughes et al., 2002). The new Movement Disorders Society (MDS) criteria for the diagnosis of PD (Postuma et al., 2015a) have been shown to lead to even better diagnostic accuracy (Postuma et al., 2018). Since Becker's 1995 study, much research has focused on the substantia nigra hyperechogenicity (SN+) and the diagnostic accuracy of transcranial sonography (TCS) in distinguishing PD patients from healthy controls. In more than 90% of PD patients, TCS reveals a characteristic SN+, which has been reported to be stable during the course of the disease. SN+ has been proposed to be a risk factor for developing PD (Berg et al., 2005; Berg et al., 2012; Walter et al., 2007) or a subclinical stage of the disease (Walter, 2004). Reported cut-off values for the discrimination between normal echogenicity and SN+ with different contemporary ultrasound systems are known in the literature (Walter et al., 2014b). Even so, the sensitivity and specificity of TCS in PD diverge widely due to ethnicity, representative size and diverse ultrasound devices. The normal ranges of SN+ need to be established for each lab because of some potential investigator dependency.

The first aim of this study was to validate SN+ in a large Estonian cohort. About 10% of the total number of Estonian PD patients were enrolled in the study. The importance of the study lies in the fact that validated SN+ data creates a valuable basis for future scientific research in the Estonian cohort.

The severity of depressive symptoms is an important factor in modulating the clinical aspects of PD. With an average prevalence of about 40%, ranging from 27% to 76%, depression is one of the most common non-motor manifestations of PD (Ji Won Han et al., 2018; Kadastik-Eerme et al., 2016;

Slaughter et al., 2001; Reijnders et al., 2008). The onset of depression during the late stages of PD has been argued to be mainly related to a broader monoaminergic deficit in combination with a higher occurrence of cognitive impairment (Braak et al., 2004). Depressive symptoms often remain untreated in PD patients (van der Hoek et al., 2011), underlining the need to recognize early biomarkers associated with depression in PD. The level of affective disorders was directly related to the anatomical changes in the brainstem raphe (Walter, 2007b). In healthy individuals, the mesencephalic part of the midline raphe is visualized as a continuous hyperechogenic line which is located in the middle of the mesencephalon. Raphe echogenicity should only be classified as reduced if TCS from both sides shows reduced echogenicity under adequate imaging conditions. Decreased echogenicity of the mesencephalic raphe has been found in 40%–60% of patients with PD having depression (Becker et al., 1997; Berg et al., 1999; Walter et al., 2007b). Therefore, the second aim of this study was to describe the prevalence and severity of depressive symptoms in Estonian PD patients and their possible associations with brainstem raphe echogenicity.

A negative correlation between age of disease onset and SN+ was previously reported (Walter et al., 2007a). An association between age of disease onset and SN+ area has not yet been convincingly demonstrated. On the other hand, the first two studies on TCS in PD showed no correlation between the size of the SN+ and age (Berg et al., 2001), but in patients with RBD (Iranzo et al., 2007) were demonstrated an increasing prevalence of SN+ with age in elderly individuals. Ageing may represent various stressors within SN+, weakening and/or causing loss of neuron function and their ability to react to further damages associated with the disease progression. Moreover, it can be speculated that an enlarged SN+ at the onset stage of PD results from active neuroinflammatory processes, which gradually diminish with the progression of PD and the decrease in the number of dopaminergic cells. Therefore, the study's third aim was to evaluate whether SN+ values are different on the respective sides mirroring the asymmetric character of the disease and to evaluate SN+ in healthy controls.

2. LITERATURE REVIEW

2.1. PD epidemiology in Estonia

The age-adjusted prevalence of PD in Estonia was 152 per 100,000 population, 159 for urban and 139 for the rural group, 154 for men and 153 for women in 1996 (Taba et al., 2002). The age-specific prevalence increased from 22 per 100,000 individuals in the age group 40–49 years up to 1,232 per 100,000 individuals in the age group 70–79 years. The mean age of PD patients was 71.4 years, the mean age at onset of the symptoms was 66.9 years (Taba et al., 2002) and the most recent data shows the same age specific prevalence (Taba et al., 2002; Kadastik-Eerme et al., 2018). But the overall disease prevalence in Estonia has increased from 152 per 100,000 individuals in 1996 to 197 per 100,000 individuals in 2013 (Kadastik-Eerme et al., 2018). When comparing the prevalence rates with other studies of Caucasian populations in Europe, the results are except for slightly similar (Kadastik-Eerme et al., 2018) but not significantly higher prevalence rates in the urban population in Estonia (Kadastik-Eerme et al., 2018).

2.2. Natural history of PD

The process of neurodegeneration in PD begins long before the onset of clinical motor symptoms, resulting in substantial cell loss by the time a diagnosis can be made. The period between the onset of neurodegeneration and the development of motoric disease would be the ideal time to intervene with disease modifying therapies.

The pathological hallmark of the disease is the presence of Lewy bodies (LB) within the SN and other parts of the brain, containing intracellular aggregations of misfolded αsyn. The anatomical origin of the disease process, however, is likely to be further afield, perhaps even outside of the brain (Braak et al., 2003; Braak et al., 2008). Braak's hypothesis suggests that neuro-degeneration is initiated in the lower brainstem and anterior olfactory structures before ascending to the basal ganglia and cortical areas in a characteristic sequence. In keeping with this, non-motor symptoms such as hyposmia, autonomic failure and sleep disturbances commonly emerge many years before motor parkinsonism (Postuma et al., 2016b). The importance of developing reliable methods for diagnosis during this pre-motor phase is underlined by the observation that >50% of nigral dopaminergic neurons and up to 80% of nigrostriatal synaptic activity have been lost by the time the motor phenotype emerges (Fearnley et al., 1991; Fernandez et al., 2011). Postmortem and neuroimaging studies suggest that degeneration within the basal ganglia may commence up to 7 years prior to diagnosis (Hawkes, 2008; Morrish et al., 1998), but prodromal symptoms such as hyposmia and REM sleep behaviour

disorder can emerge decades earlier in some cases (Iranzo et al., 2013; Schenck et al., 2013a,b). Techniques such as TCS of the SN have great potential to facilitate early diagnosis.

2.3. TCS as preclinical marker and an approach to early diagnosis

A few years ago, a task force of the Movement Disorders Society, building on previous systems (Berg et al., 2013; Obeso et al., 2017; Postuma et al., 2018) divided early PD into the following 3 stages:

(1) Preclinical: neurodegeneration is present but without measurable symptoms or signs – biomarker diagnosis required (Postuma, 2015b). By definition, patients are not just in an at-risk state (e.g., young persons with highly penetrant gene mutations).

(2) Prodromal: symptoms/signs are present, but they are insufficient to diagnose clinical PD.

(3) Clinical: this implies the presence of parkinsonism. Most markers of prodromal PD directly reflect symptoms and signs; some can be detected clinically, whereas others need biomarker confirmation (e.g., polysomnography). In PD, the SN has abnormally high SN+ on ultrasound imaging (Gaenslen, 2008). Whether this hyperechogenicity is a risk marker or a prodromal marker is unclear. Hyperechogenicity can be detected at an early age and does not progress with disease duration, suggesting that it is a risk marker (Iova et al., 2004).

In any case, it seems that SN+ may be a valuable tool to detect an increased vulnerability for PD. In one population-based study, SN+ was associated with a 20-fold increase in the risk of PD (Berg et al., 2012). In comparison with other neuroimaging procedures, ultrasound is inexpensive, but requires specialist training, and cannot be used for some people owing to insufficient bone window.

2.4. Pathology and pathogenesis

2.4.1. Pathophysiology of PD

Historically, PD has been considered a 'motor' disease linked to degeneration of midbrain dopaminergic neurons. Characteristic features of PD include neuronal loss in specific areas of the SN and widespread intracellular protein αsyn accumulation. Although neither the loss of pigmented dopaminergic neurons in SN (Titova et al., 2017) nor the deposition of αsyn in neurons is specific for PD, these two major neuropathologies are specific for a definitive

diagnosis of PD when applied together (Poewe et al., 2017). The multi-focal and multi-neurotransmitter driven pathology of PD has been emphasized by the landmark work of Braak and colleagues (Braak et al., 2003) who suggested that a 'bottom-up' six-stage pathological process could account for most cases studied neuropathologically (Titova et al., 2017; Braak et al., 2003). This work has proposed that αsyn pathology critically resulting in neuronal LB deposition and cell death spreads from regions interfacing with the 'environment'. In this model, regions including the olfactory bulb and the enteric nervous system with its connections to the medulla through the dorsal motor nucleus of the vagus nerve act as a 'conduit' for a spreading pathology perhaps mediated by a prion-like process (Titova et al., 2017). Such a model allows both dopaminergic and non-dopaminergic populations to be differentially affected and in so doing may permit insights into the motor and non-motor symptoms. Whilst by no means perfect, this concept of a multi-neurotransmitter, multi-organ (brain and peripheral nervous system) disorder is now well established in the literature with supportive pathological and biomarker driven projects (Titova et al., 2017). Inflammation and neuronal death in PD are clear evidence for activation of both the innate and adaptive arms of the immune system. In the brain, the cells primarily responsible for innate immunity are the resident microglia, involved in constant surveillance of the brain microenvironment. Postmortem studies of PD-affected brains and PD model systems consistently demonstrate striking microglial activation (Obeso et al., 2017; McGeer et al., 2008).

2.5. PD symptoms

2.5.1. PD motor symptoms

PD is an extremely heterogeneous disorder which is defined by the presence of classical motor features, including the hallmark presence of bradykinesia in all patients, rigidity and rest tremor in the majority. The unilateral onset of rest tremor is a phenomenon we still use today as an essential element of clinical diagnostic criteria for PD (Gibb et al., 1988; Obeso et al., 2017; Postuma et al., 2015b). The other types are clinically characterized by a postural and action tremor, which is mainly associated with tremor at rest, but the action tremor often has a higher frequency and may be disabling in daily activities. A further high-frequency action tremor is clinically associated with rigidity (Deuschl et al., 2008; Obeso et al., 2017). Parkinsonian tremor is the most specific sign of PD and likely occurs in more than 90% of PD patients at some time during the course of the disease (Obeso et al., 2017). However, it shows no correlation with the progression of rigidity and akinesia scores(Obeso, 2017). PD tremor may not be as reliably responsive to dopamine replacement therapy as other typical motor signs. Although in many cases there is a good and reproducible response to Levodopa, there is also a significant percentage of patients who do not respond significantly enough to make treatment worthwhile.

Postural reflex disturbances include flexed postures of the trunk and limbs as well as postural instability, which generally occur later in the disease progression and are no longer considered essential diagnostic features.

Bradykinesia, impaired posture and balance, and rigidity are the criteria on which we rest a clinical diagnosis of PD today. Sometimes a patient may speak softly, quickly, slur or hesitate before talking. Patient speech may be more monotonous, lacking the usual inflections. There may be changes in writing, such as increased difficulty and writing appearing small.

As the disease progresses, the clinical picture becomes a complex of levodopa-related motor complications, non-dopaminergic motor features such as speech and swallowing problems, freezing of gait and falls, and increasingly disabling non-motor symptoms including autonomic failure, psychiatric disturbances, and dementia.

2.5.2. Non-motor symptoms in PD

Today a large variety of nonmotor symptoms are considered an integral part of the disease, and there is strong evidence that some of these, such as hyposmia, constipation, or rapid eye movement (REM) sleep behavioral disorder and depression may even be the earliest manifestations of the disease, occurring years before any of the defining motor features are present (Obeso et al., 2017; Pont-Sunyer et al., 2015).

The initial studies (Szeto et al., 2015) highlighted that whilst tremor dominant patients are relatively spared from non-motor symptoms, the non-tremor dominant subgroup is more associated with cognitive impairment and mood disturbance. Indeed, a more recent study has identified a differential expression of mild cognitive impairment across these subgroups, with the highest frequency observed in the non-tremor dominant cluster, which was also associated with a higher prevalence of freezing of gait, hallucinations, daytime somnolence, and rapid behaviour disorder (RBD) compared with other subgroups (Szeto et al., 2015).

Several medical records have demonstrated diagnosis of depression often preceding the diagnosis of PD; estimates of the predictive value are similar, with an relative risk (RR) of 1.5–2.5 (Postuma et al., 2016b). Depression and anxiety are commonly comorbid in PD, so they will be considered together. Phobic anxiety and anxious personality traits have been associated with a 1.5-fold increase in the risk of PD (Postuma et al., 2016b). These low relative risks imply that predictive value is low. Many studies document that both mood disorders are associated with higher PD risk, but the RR is low, ranging from 1.5 to 2.5, with resulting low specificity (Berg et al., 2015). The lead time is unclear; it may be biphasic with an early tendency toward anxiety (e.g., the putative Parkinson personality) combined with a second episode of depression emerging soon before PD onset (Gustafsson et al., 2015; Emre et al., 2004). In

striking contrast to clinical markers, evidence for blood or cerebrospinal fluid variables as prodromal markers is extremely limited, although this may change in the near future for specific types of PD (Vilas et al., 2016).

2.6. Neuroimaging in PD

Neuroimaging in PD includes morphological imaging using magnetic resonance imaging (MRI) and TCS and functional techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT) to probe different aspects of the neurobiology of PD. Brain imaging is not routinely recommended in all patients with PD (Ransohoff et al., 2016).

2.6.1. MRI as the structural imaging

MRI has been extensively used in the imaging of parkinsonian syndromes. Besides the conventional MRI sequences, recent advanced techniques including diffusion weighted imaging with tensor imaging, magnet resonance morphometry, magnetisation transfer imaging and magnet resonance spectroscopy have also been used in the diagnosis of these group of disorders. Imaging is helpful to confirm the diagnosis of parkinsonian syndromes, classify them into various subtypes, rule out the alternative differential diagnosis and determine the severity of brain changes (Savoirado, 2003).

The conventional T1 and T2 weighted MRI, especially at 1.5 Tesla strength, are often normal in PD. The MRI is more often used to rule out other conditions like demyelination, vascular insults and normal pressure hydrocephalus. The advanced cases may show hyperintense signal changes on T2 weighted images in bilateral substantia nigra as the only finding (Savoirado, 2003). Some advanced MRI imaging may be helpful methods to differentiate parkinsonian syndromes from PD.

Traditional structural imaging does not generally reveal significant reproducible changes in PD. Reduced size and signal intensity of the SN were reported in PD patients using neuromelanin-sensitive imaging with a high diagnostic accuracy. Both techniques may be used in clinical practice as these changes can be detected by simple radiological reading. A combination of measures, for example, increased iron content and reduced fractional anisotropy, changes in nigrosome-1 containing area or neuromelanin imaging, may result in better separation of PD patients from control subjects as compared with each technique separately (Peran et al., 2010). High-resolution spin echo T1-weighted images are sensitive to neuromelanin and show the SN as an area of high signal intensity (Castellanos et al., 2015). Recent results using functional MRI at rest have also shown that the average connectivity in the basal ganglia may distinguish patients with PD from healthy controls (Obeso et al., 2017; Szewczyk et al., 2014).

2.6.2. MRI for differentiating between PD and atypical parkinsonism

In neurological practice sometimes an MRI brain scan might be valuable for patients with atypical features for PD. An MRI some of the time helps in patients with parkinsonian disorders – more established patients may have markers of progressive supranuclear palsy (PSP), multisystem atrophy (MSA), corticobasal degeneration (CBD) and vascular disease (Ramirez et al., 2006). Changes in PSP prevalent superior cerebellar peduncle and midbrain atrophy (Cebrian et al., 2014; Schapansky et al., 2014). Parkinsonian-type MSA is generally more prominent including atrophy, increased iron load, increased diffusivity and signal changes in specific brain regions (Obeso et al., 2017). In parkinsonian-type disorders, MSA can recognize specific abnormalities, for example the 'hot-cross bun' sign and decreased T2 signal of the putamen in MSA. However, their use in clinical practice remains limited because of the lack of normative databases and availability of these techniques in clinical centers (Obeso, et al. 2017).

2.6.3. Radiotracer imaging

The use of radiotracer imaging may play a relatively limited role in routine clinical diagnostics as it is sometimes difficult to be certain of a diagnosis, particularly in the early stages of a disease and may therefore be extremely useful for selecting patients to participate in trials of disease-modifying therapies, where a reliance on clinical assessment may result in the inclusion of approximately 15% of patients who do not have dopamine deficiency (Obeso et al., 2017). Radiotracer imaging as functional imaging in PD using a 123 I Ioflpane (DaTscan) tracer is outstanding in clinical practice, which leads to nigrostriatal dopaminergic denervation and loss of the typical pattern on a DaTscan (Gao et al., 2008; Ma et al., 2016). Dopaminergic PET/SPECT shows a 35% to 65% loss of innervation at diagnosis, implying that milder loss should be evident earlier (Obeso et al., 2017). Several studies document abnormalities in about 40% of patients with idiopathic RBD, which appear to progress over time (Bradley et al., 2010). Moreover, in the Parkinson At-Risk Study, patients with dopaminergic denervation had more hyposmia and constipation (Jennings et al., 2014). A follow-up, published in abstract form only, found that those who had innervation below 65% of expected values had a 20-fold increased risk of developing PD (Jennings, 2015). If confirmed, this would imply that dopamine transporter scanning is second only to idiopathic RBD in positive predictive value for PD (Jennings, 2015).

DaTscan imaging of presynaptic dopaminergic function shows a charac-teristic pattern of asymmetric involvement, with a rostral-caudal gradient in which the posterior putamen is maximally affected. However, although the preferential involvement of putamen over caudate is typical of PD, presynaptic

dopaminergic imaging will not reliably differentiate between PD and atypical forms of parkinsonism such as MSA and PSP.

DaTscan imaging utilizing [123] I-ioflupane has been affirmed by the U.S. Food and Drug Administration, the National Institute of Health and Care Excellence (NICE) and the American Academy of Neurology (AAN) for the DaTscan to differentiate essential tremor from parkinsonian disorders (Hirsch et al., 2009; Ransohoff et al., 2016).

123 Meta-iodobenzylguanidine (123 I-MIBG) cardiac scintigraphy is a technique used to evaluate post-ganglionic presynaptic cardiac sympathetic nerves in heart diseases through a false neurotransmitter analogous to norepinephrine. There is a significantly lower heart-to-mediastinum average count ratio in patients with PD than atypical parkinsonian disorders, as the degree of MIBG uptake reflects postganglionic sympathetic cardiac nerve damage in PD (Sampson et al., 2016). Patients diagnosed as having PD but who have a normal DaTscan are often referred to as having scans without evidence of dopaminergic deficit (SWEDDs). Patients with asymmetric rest tremor and a normal DaTSCAN represent a relatively common situation which can be misdiagnosed as PD. It has been shown that SWEDDs represent a very heterogeneous group of disorders: some of these patients have dystonic tremor (Batla et al., 2014; Obeso et al., 2017), whereas others develop an abnormal DaTscan at a later follow-up, raising the possibility of either benign tremulous PD or false-negative initial DaTscans (Batla et al., 2014; Obeso et al., 2017; Wile et al., 2016).

2.7. TCS as a diagnostic tool for PD

2.7.1. History of TCS in PD

TCS is a widely used method for the visualization of the brain parenchyma through the intact scull. The findings of early studies, implicating a prevalence of SN+ in more than 90% of PD patients (Becker et al., 1995; Berg et al., 2001), have been repeated by many others (Behnke et al., 2001; Walter et al., 2002; Okawa et al., 2007; Doepp et al. 2008; Fernandes et al. 2011; Bouwmans et al., 2013). Moreover, investigations of patients at different disease stages imply that there is no association between area of SN+ and disease severity. Therefore SN+ may rather be a vulnerability marker rather than a marker for disease progression in PD (Berg et al., 2005).

2.7.2. TCS in the clinical practice

TCS contrasted with other neuroimaging modalities can be performed without ionizing radiation with portable machines is noninvasive and is protected to movement artifacts. In the field of movement disorders, TCS has been built up

principally as a tool for the early diagnosis of PD (Berg et al., 2006; Walter et al., 2014b), moreover, the TCS may even be helpful for the preclinical identification of people at risk for PD (Berg et al., 2006). In the differential diagnosis of PD, the TCS has proven to be reliable and sensitive in detecting basal ganglia abnormalities, e.g. of the SN in PD and of lenticular nucleus (LN) in atypical parkinsonian syndromes (Walter et al., 2007a; Walter et al., 2014b).

According to a meta-analysis of five independent TCS studies (Walter, 2009), the finding of SN+ discriminates PD from atypical parkinsonian syndromes with a sensitivity of 92% and a specificity of 80% (Walter, 2009). Interestingly, SN+ was observed in about 9% of controls, 16% of essential tremor patients and in MSA and PSP as well as in DLB, PDD, and CBD cohorts to varying degrees (Fernandes et al., 2015). By combining age at disease beginning, asymmetry indices and SN+ , Walter and colleagues (Walter et al. 2007a; Walter et al. 2009) managed to differentiate PDD from DLB with a sensitivity of 96%, a specificity of 80%, and a positive predictive value (PPV) of 93% (Walter, 2014). Other findings detected between 66 and 88 percent of CBD patients have distinctly SN+, while only between 0–20% of the PSP patients presented with this characteristic (Chadery et al., 2018). 78% of atypical parkinsonism as well as 23% of PD patients demonstrated hyper-echogenicity of the lentiforme nucleus (LN+). A combination of LN+ with a normal echogenic SN supports the diagnosis of MSA and PSP (Chadery et al., 2018).

2.7.3. Causes of SN+ and predisposition

Postmortem ultrasound examinations compared with biochemical and histo-logical investigations of the SN disclosed that SN+ area correspond to an increasing amount of iron measured by spectroscopy and verified histologically (Berg et al., 2002). This association could also be confirmed in vivo by MRI investigations determining T2-relaxation times, which are used for the detection of elevated iron content in tissue (Berg et al., 2002). Here, patients with PD and increased area of SN+ showed decreased T2-relaxation time, corresponding to increased iron levels. Also, healthy subjects with SN+ had a significant decrease in T2-relaxation times when compared to subjects without this echofeature, although values were less reduced compared to PD patients. In normal conditions, iron is necessary for the maintenance of physiological function of neurons. However, excessive iron deposits in the brain may cause a cascade of events of oxidative stress and neuroinflammation that destroy neuronal phospholipid membranes, proteins and nucleic acids, leading to the degeneration and death of neurons (Shu yang et al., 2018). Thus, a healthy iron level in the brain is vital for maintaining a stable internal environment through a rigorous regulatory mechanism. Taken together, a good correlation for TCS, MRI and PET data could be shown, as healthy subjects with SN+ had a reduced T2-relaxation time as well as a reduced ^{18}F-Dopa uptake compared to healthy controls without this

echofeature. These changes were less obvious than in patients with idiopathic PD and SN+ (Behnke et al., 2005).

If TCS is suitable to detect preclinical changes of the SN, the question of whether there are any preclinical alterations in mutation carriers for mono-genetic PD is at hand. The first study by Walter showed that all symptomatic PD mutations carriers had either moderate or marked SN+. This was also true for asymptomatic carriers with abnormal PET findings and half of the aymptomatic carriers with normal PET findings displayed SN+, indicating, that TCS might be even more sensitive to identifying presymptomatic mutation carriers than PET (Walter, 2004). A predisposition for SN+ could also be shown for family members of patients with PD in a study comprising 58 first degree relatives of PD patients with almost half of them showing the same echofeature as their affected relatives. Several of these SN+ subjects showed signs of motor retardation, however, without a clinical picture of full-blown PD (Ruprecht-Dörfler et al., 2003).

2.7.4. SN+ pathogenesis in course of PD

The SN+ is typical in PD patients. However, the SN+ is not the same among all affected patients. A predisposition for this ultrasound characteristic, its association with a subclinical impairment of the nigrostriatal system and the fact that the signal alteration does not change with progression of the disease prove the need to establish the relationship between SN+ and the course of PD. The following subgroups of PD patients may be proposed: (1) those having a stronger predisposition documented by marked SN+ who might represent the more genetically determined PD-courses with earlier onset and slower progression and (2) those with smaller SN+ showing later disease onset and faster disease progression that might be mainly caused by other factors like exo- and endotoxins (Schweitzer et al., 2006). The relationship between SN+ and demographic information in PD patients is not clear yet.

Currently TCS is used for detecting subjects at risk for PD. Thus far, there is uncertainty regarding the exact moment at which the degenerative process starts, and how individuals at risk of reaching the symptomatic PD stages can be reliably identified in the earliest stages of the disease. In one recent study, comparisons of the demographic variables between the PD SN+ group and the PD non-SN+ group suggested that male sex, old age and late disease onsets were relevant to SN+ of PD patients. Although the reason for the gender difference was hardly explored, estrogen was thought to be involved (Shu yang, 2018). Estrogen was found to make female individuals less prone to iron accumulation by reducing iron levels (Shu yang et al., 2018). SN+ was associated with elevated unbound iron (Shu yang et al., 2018), thus female individuals more likely have lower SN+. Here, it was also found that older PD patients with late disease onset have larger SN+, which might be due to the increasingly accumulation of brain iron during the aging process. The

hyperechogenicity in SN was not related to disease duration, which was supported by another study observing no significant change in hyperechoic area even with an interval of 8 years between examinations (Shu yang et al., 2018). These results show that disease duration is not a definitive factor in iron accumulation and related SN+ in PD patients. In this investigation, echogenicity in SN was associated with disease severity, as indicated by the advanced Hoehn-Yahr Stage (HYS), and in their previous study, it was found that iron induced dopaminergic neurodegeneration through neuroinflammatoty mechanism indicated by the over activation of microglia and robust production of neuro-toxic factors (Shu yang et al., 2018), which might explain why the hyper-echogenicity revealed by the elevated iron level in SN was correlated with the rapid progression of PD reflected by advanced HYS.

The inter-individual variation of SN echogenicity has been suggested to be caused by a variable degree of local iron accumulation and abnormal iron-protein compounds but also by gliotic changes (Berg et al., 2008; Walter U. et al., 2014). Between the ages of 18 and 75, the distribution of SN echogenicity can be regarded as nearly constant (Mehnert et al., 2010), even though there are reports of a moderate increase during adult life, especially after the age of 80 (Walter et al., 2014). To rate SN echogenicity in an individual as normal or hyperechogenic, the 75th and 90th percentile of measures in the normal population are used as a reference (Berg., et al., 2008), and the larger bilaterally measured SN+ are used for classification as follows: the normal echogenic measured area is below the 75th percentile; the moderately SN+ measured area is between the 75th and the 90th percentile; and the markedly SN+ measured area is above the 90th percentile.

2.8. TCS in movement disorders: quality standards

2.8.1. Equipment

For TCS an optimized ultrasound system equipped with a 2.0 to 3.5 MHz phased-array transducer is used (Table 1).

Table 1. Recommended ultrasound system settings for TCS, is adapted from ref. Walter et al., 2014b, by thesis author Toomsoo, T.

Parameter	Settings
Image depth	14–16 cm, adapted as needed
Dynamic range	45–55 dB
Contour amplification	Medium to high
Time gain compensation	Adapted manually as needed
Image brightness	Adapted manually as needed
Post-processing parameters	Moderate suppression of low echogenic signals
Frequency	2.0–3.5 MHz, usually 2.5 MHz

Applying these parameters the highest image resolution was achieved at a depth of 5–9 cm which is the so-called focal zone of the transducer (Walter et al., 2014b). New contemporary systems achieve a higher image resolution in the focal zone of up to 0.7 to ~1.1mm thanks to tissue harmonic imaging (THI). (Walter et al., 2014b). The system-specific image processing technologies influence distinct measurements such as the assessment of echogenic areas of small brain structures. That is why normal ranges especially for SN echogenic areas need to be obtained separately for each ultrasound system. The application of THI rather than the conventional imaging mode increases the tissue contrast and can therefore enable an easier delineation of small echogenic structures, e.g. the SN.

2.8.2. Investigation procedures

The patient is placed in a supine position on an examination chair with a variably adjustable lean part. The investigation room should be darkened. The investigator sits behind the patient's head. For the usually performed transtemporal investigation, the TCS transducer is placed on the right temporal bone in front of the ear and parallel to the orbitomeatal line in order to obtain a standardized axial view of intracranial structures (Fig. 1A, B).
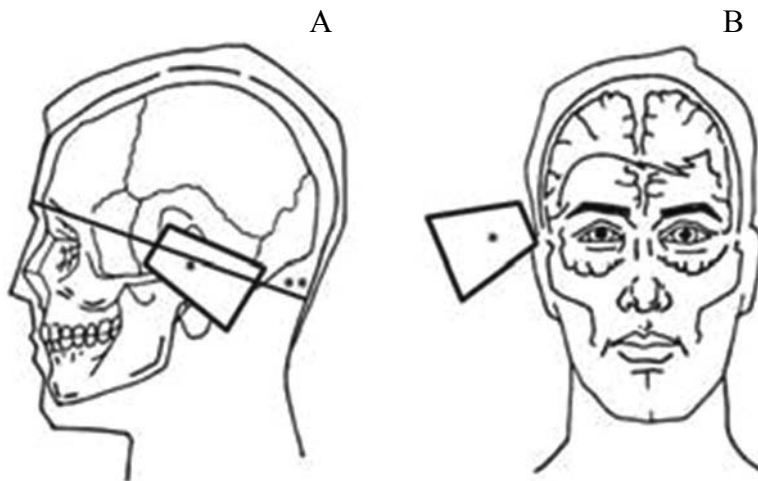
A　　　　　　　　　　　　　　　　　　　B

Figure 1A. TCS transducer is placed on the temporal bone in front of the ear and parallel to the orbitomeatal line. To scan the SN should be rather parallel to the orbito-meatal line. **Figure 1B**. The angle of the probe is too oblique, adapted from ref. Becker et al., 2001; Berg et al., 2006 Behnke et al., 2005, Berg et al., 2006 by thesis author Toomsoo, T.

An important precondition for obtaining valid TCS findings is the identification and keeping of the optimum bone window for insonation. For this, the transducer is moved near the anterior helix of the ear conch to find the position with the best available visualization of brain structures and the contralateral skull bone. Once the optimum position has been found, it is kept by pressing the transducer as well as the small finger/ulnar edge of the hand firmly against the patient's head throughout the whole examination. Even when applying optimum ultrasound systems settings, assessment of intracranial structures may not be or only partially be possible due to an insufficient transtemporal bone window which is found in 5 to 40 percent of patients depending on age, sex and geographic origin (Skoloudík et al., 2010). In neurodegenerative diseases transtemporal TCS is usually carried out at the mesencephalic scanning plane (Figure. 2).
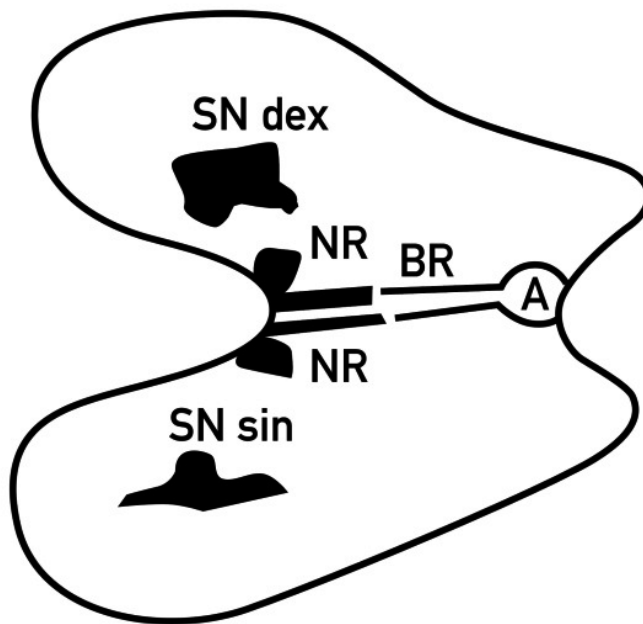
Figure 2. Sketched anatomy of visible brainstem structures on TCS is adapted from ref. Becker et al., 1997 by thesis author Toomsoo, T. Transtemporal TCS is usually carried out on standardized axial imaging planes. Abbrevations: SN dex, *substantia nigra* (right side), SN sin, *substantia nigra* (left side), NR, *nucleus ruber*, BR, brainstem *raphe*, A, *aquaductus.*

The best-validated method to grade SN echogenicity is the planimetric measurement of the SN echogenic signals on an axial plane (Berg et a., 2008, Skoludik et al., 2010, Walter et al., 2013, Walter et al., 2014).

2.8.3. Visualization and assessment of SN+

The visualization of midbrain structures (SN, raphe) on an axial imaging plane is essential for diagnosing movement disorders. Quality criterion of sufficient midbrain visualization: The butterfly-shaped midbrain transection surrounded by the highly hypoechogenic basal cisterns (cisterna ambiens, cisterna quadrigemina, cisterna suprasellaris) is completely displayed.

The SN echosignals may have a patchy, band-like or sometimes wide oval appearance (Figure. 3). Its appearance may slightly vary even in the same individual if using different transducer angulations or various ultrasound systems (Walter et al., 2013). The best validated method to grade SN echogenicity is the planimetric measurement of the SN echogenic signals on an axial plane (Walter et al., 2014). The reliability of TCS assessment of the SN mainly depends on two factors: (1) the investigator's experience and skill, which is associated with a potential subjective bias, and (2) the quality of the bone window (Berg et al., 2008; Školoudík et al., 2007). The influence of the latter may be reduced only slightly by lowering the insonation frequency, usually to 1.8–2.0 MHz, in the case of poor bone windows. Semiquantitative visual grading is less reliable (Walter et al., 2014b).

Because of potential interference with echosignals originating from structures of the basal cisterns, the echogenicity of the SN is assessed only ipsilaterally to insonation. Therefore, TCS of SN needs to be performed from both sides. The clearest, most compact view of the SN echogenic signals is visualized by very slight probe movements. The axial midbrain transection showing the echosignals of the ipsilateral SN in its largest extension is localized by slight tilting of the probe. If the SN is seen very clearly, the image is frozen immediately. After choosing the optimum frame using the cine mode if necessary, the midbrain is zoomed out two- to threefold. The SN echogenic signals are surrounded manually by the cursor using the trackball, resulting in automatic calculation of the echogenic area.

Quality criterion of the adequate visualization of the SN is when the echosignals of the SN are displayed at a typical anatomic location in the crus mesencephali and are well separated from the echosignals of the red nucleus and basal cisterns (Walter et al., 2014).

Several approaches for automated SN detection and the quantification of its echogenicity have been published recently, including principal component analysis based artificial neural networks (Blahuta et al., 2013), active contour segmentation algorithms (Sakalauskas et al., 2013), invariant scale blob detection (Walter et al., 2014), and 3-dimensional SN detection (volumetry) based on random forests. Novel technologies aiming to reduce investigator dependency, such as measuring the echointensity of the SN relative to the surrounding parenchyma, volumetry, semi-automated SN detection, or complex mathematical echo-signal analysis, have either failed or are not yet ready for clinical application (Chen et al., 2012; Walter et al., 2014).

The normal ranges need to be established for each ultrasound system because of some potential investigator dependency for each lab (Walter et al., 2013).

Figure 3. Visualization of midbrain SN+
SN+ dex (right side SN+ area, what was surrounded manually with the cursor and the planimetric area (cm^2 was calculated automatically; 0. 36 cm^2) and SN+ sin (left side SN+, 0.32 cm^2)

2.8.4. Visualization and assessment of brainstem raphe echogenicity

The assessment of the raphe is anatomically restricted to the lower midbrain with obligatory simultaneous visualization of the red nucleus. In early studies (Becker et al., 1995) 4 grades of echogenicity were applied as follows: (1) raphe not visible; (2) reduced or interrupted echogenicity; (3) normal, i.e. continuous line with an echogenicity similar to that of red nucleus; (4) increased echogenicity. The current consensus guideline recommends the discrimination of only two grades (normal vs reduced echogenicity, Figure. 4) (Walter et al., 2014). Raphe echogenicity should only be classified as reduced if TCS from both sides shows reduced echogenicity under adequate imaging conditions. Occasionally a healthy subject exhibits marked echogenicity of the red nucleus in its full anatomical extension. This finding is rated as a hyperechogenicity

even though the diagnostic implcations are unclear. Quality criterion of adequate assessability of the red nucleus: basal cisterns, SN and midline structures (raphe, aqueduct) are clearly displayed.

Figure 4. Normal brainstem raphe (BR) echogenicity.
(arrows: BR)

2.8.5. TCS limitation

The first limitations of TCS in the evaluation of SN+ is the dependence of image quality on both the sonographer's experience and the quality of the bone window. In European populations, 4–15% of participates were found to have an insufficient temporal window (Mehnert et al., 2010; Dun-Hui, Li et al. 2016). However, the value rises to 15–60% in Asian populations (Izawa, et al.,2012; Dun-Hui, Li et al. 2016). This high recording failure rate in TCS application would mostly affect patients of advanced age with female gender (Okawa, et al. 2007) or patients with a small temporal window seen in Asian populations (Okawa et al., 2007). Sonographers without TCS experience could have problems with correctly imaging the SN. One of the possible reasons for worse visibility in female patients is osteoporosis, it was found in a prospective study that among the women bone mineral density was significantly lower in the PD patients than in the controls, and the decrease was greater in the subgroup with

more advanced disease (Raglione et al. 2011). Some new MRI–TCS fusion imaging with virtual navigation technology could be helpful in this case (Školoudík et al. 2014). Recently, high-resolution ultrasound systems with standardized settings or with automated segmentation technique were reported to reduce inter-observer and intra-observer variability (Sakalauskas et al., 2013), which may help improve TCS image quality and decrease the incidence of insufficient temporal window. Moreover, a novel approach using transcranial B-mode sonography, a 3-D ultrasound platform, was shown to be technically feasible and less dependent on sonographer experience or good bone windows (Plate,et al. 2012). These innovations and developments in ultrasound systems may effectively improve the application value and diagnostic accuracy of TCS.

2.9. Summary of the literature review

PD is the most common movement disorder. Clinical diagnosis of PD is reasonably easy in most cases but the distinction between different variants of parkinsonism may be difficult in early stages. TCS is a relatively young method which has evolved as an important diagnostic tool for not only PD diagnosis but also for atypical parkinsonian syndromes. SN+ is the hallmark for PD but how valid and helpful is it in clinical diagnosis of PD in a different community is the question. It's important to assess the validity of the SN+ in the PD group and in the healthy controls, because TCS as a method is easy applicable, noninvasive and easily available in everyday practice in Estonia. Yet each lab has to use its own cutoff value derived from the ultrasound system and assessment method used. Several studies showed that TCS is a well reproductible method and has high specificity for the diagnosis of PD.

Depression is a part of *PD* itself, resulting from changes in the chemistry of the brain. In my study, I decided to evaluate the role of BR in the occurrence or non-occurrence of the symptoms of depression and whether this can be assessed with TCS.

I know that age is the best PD progression marker but we need to study how SN+ reacts in different situations. Not only in longitudinal studies but also we need information from cross-sectional studies. It is not fully clear what the development of SN+ will be in the course of the disease of a patient. Most of the studies have confirmed that SN+ is a stable biomarker. Longitudinal studies would definitely provide a better overview of the stability of SN+, but it was necessary to establish whether different factors impact the stability of SN+ and in what way if this is the case.

3. AIMS OF THE STUDY

The primary objectives of the current study were:

1. To investigate the diagnostic accuracy for validation of SN + as assessed by TCS in a large sample of patients with PD in Estonia.

2. To describe the prevalence and severity of depressive symptoms in PD, and to analyze associations between brainstem raphe echogenicity and depressive symptoms of patients with PD compared to age- and education-matched healthy (non-PD) controls.

3. To analyze whether SN+ values are different on sides mirroring the asymmetric character of the disease.

4. PATIENTS AND METHODS

4.1. Study population and sample design

The study was prospectively conducted from January 2010 to January 2019 using a sample of 300 patients (aged 30–80 years), which accounts for 10% of PD patients in Estonia with PD according to the QSBB diagnostic criteria (Hughes et al., 1992; Lees et al., 2009). Patients were consecutively recruited from the Outpatient Clinic of the Department of Neurology of the East Tallinn Central Hospital and the Neurological Clinic of Tartu University with the same ultrasound maschine by one sonographer. Exclusion criteria included concomitant neurologic disorders affecting the central nervous system, other brain abnormalities and that made the diagnosis of PD unlikely. Therefore, all patients with PD underwent either CT or MRI before study enrollment. The control group consisted of 200 individuals who were similar in age and gender to the PD group with no history of central nervous system diseases. The control group comprised 141 individuals from older male and female choirs, 29 clinic staff members, and 30 neurologic patients with peripheral nerve disorders or musculoskeletal diseases or their family members. The control group did not undergo computed tomography or magnetic resonance imaging. From a total of 500 participants, 66 (13.2%) were exclude from the analysis because of insufficient temporal acoustic bone windows for TCS inspection.Thus, the study groups consisted of 266 patients with PD (88.7%) and 168 (84%) controls.

The study was approved by the Ethical Committee on Human Research of Tartu University. All participants signed a written informed consent form according to the Declaration of Helsinki.

4.2. Clinical examination

Patients underwent general neurological examination. The patients' history was assessed by a neurologist who was experienced in the field of neurodegenerative disorders. The neurological rating scales and other clinical tests were performed by 2 different neurologists who were blinded to TCS data. Patients with PD were taking their optimized dopaminergic treatment on the day of clinical evaluations. All investigations were performed within a single day.

Disease severity was scored using the Movement Disorders Society Unified Parkinson's Disease Rating Scale section III (MDS-UPDRS III), and HYS (Hoehn et al., 1967). To define the severity of PD-related motor impairment, the HYS and MDS-UPDRS III (Goetz., et al., 2012) were applied. Hyposmia was evaluated using the Sniffin' Sticks 12 smell test (Heinrich Burghart GmbH, Wedel, Germany). MDS-UPDRS III is the most widely used standardized scale to assess motor symptoms of parkinsonism. It is designed to monitor PD Shwab-England Scale (SE). HYS provides a global assessment of disease

severity, based on clinical findings and functional disability. The scale includes stages from 0 (no signs) to 5 (wheelchair-bound or bedridden unless aided) to indicate the relative level of disability.

In order to examine the cognitive function, the Mini-Mental State Examination (MMSE) was used (Folstein et al.,1975). Scores of 25–30 out of 30 are considered normal. Patients were assessed in the 'on' state and their medication dose was recorded as the levodopa equivalent dose (LDED) using the algorithm adapted from Brodsky and colleagues (Goetz et al., 2012).

4.2.1. Behavioral assessment

Depressive symptom severity was measured by the Beck Depression Inventory (BDI) (Beck et al., 1961). The BDI is regarded to be a valid, reliable, and responsive instrument to assess the severity of depressive symptoms in PD (Schrag al., 2007). Symptom severity based on BDI scores was categorized as being within normal limits (0–9), indicating mild (10–16) or severe depressive symptoms (>16).

Schwab and England Activities of Daily Living Scale (SE) is a means of assessing a person's ability to perform daily activities in terms of speed and independence, with 100% indicating total independence and 0%, indicating a state of a complete dependence (Schwab al.,1969).

Parkinson's Disease Questionnaire (PDQ-39) (Jenkinson et al., 1997) was used to assess the health-related quality of life of PD patients. The PDQ-39 is a self-administered questionnaire comprising of 39 questions related to eight key areas of health and daily activities, including both motor and non-motor disturbances in quality of life. It is scored on a scale of 0–100, with lower scores indicating better health and higher scores showing a worse quality of life.

4.3. TCS methodology

TCS examination was performed with a ultrasound machine Logic7 (General Electric, Healthcare, Waukesha, WI, USA) with a 1.8–3.6 MHz transducer (General Electric, Healthcare, Waukesha, WI, USA) which is also used for transcranial duplex sonography. The ultrasound examination was performed according to the international consensus guidelines (Walter et al., 2007a,b; Weise et al., 2009). The best visualization of the brain parenchyma was achieved when the imaging depth was set to 14–15 cm and the dynamic range between 40 and 60 dB. Image brightness was adjusted as needed (used the 'Gain'or 'Time Gain Compensation' button of the device. The best resolution was achieved at a distance of about 5–9 cm from the probe which is called the focal zone. Under optimal conditions, contemporary systems achieved an axial (horizontal) resolution of up to 0.7 mm in the focal zone. All TCS examinations

were performed by one experienced sonographer (thesis author) before clinical investigations.

The sonographer was unable to see the patient information, e.g. the onset of first clinical symptoms and the time of PD diagnosis. Ultrasound examination was performed in a darkened room. B-mode gain and lateral gain control were used to adapt image brightness. The mesencephalic brainstem was identifiable due to its butterfly shape. The image was frozen at the largest extension of SN. The SN+ at the anatomical site of the SN was marked and measured on the frozen image. The area of SN+ was encircled with the cursor and the size of the indicated area was calculated automatically (cm²). The measurement of the largest scope of SN was performed 3 times for both sides using gain/brightness and the mean value was calculated. The brainstem raphe imaging process was performed secondarily. The mesencephalic brainstem was identified by its characteristic butterfly shape (Figure 2). The image was frozen when the brainstem raphe was visible at its largest extension. After image freezing, the brainstem was zoomed two- to threefold. Echogenicity of the brainstem raphe was rated semiquantitatively, using a 3-point scale (2 – not visible; 1 – partially visible; 0 – fully visible, highly echogenic continuous line) (Bouwmans et al., 2016). To make sure that the best possible assessment of the brainstem raphe could be obtained, raphe echogenicity should only be classified as reduced if TCS from both sides shows reduced echogenicity under adequate imaging conditions.

4.4. Statistical methodology

All the studies included in this thesis were observational cross-sectional studies for which a large PD sample derived from two Estonian PD medical centers was used. The study data was analyzed with the statistical programs SPSS, MedCalc and open software R (version 2.15.0). Descriptive data were reported as the mean and the standard deviation for numeric data and frequency (percent) for categorical data.

Study I: Continuous data were expressed as the mean value and median value while identifying the cutoff value for different measurements. As the mean is more affected by the outliers, the median value of the measurements was also used. The logarithmic transformation was applied to SN+ value of the multiple measurements. Data for the area of SN+ were not normally distributed while evaluated by a quantile-quantile plot. For group comparisons of categorical data, a χ^2 test was performed. An independent paired t-test was used to compare numeric variables between the groups. For group comparisons the Mann–Whitney test was used. The cutoff value for SN+ was established by receiver operating curve (ROC) analysis, comparing patients with PD with controls. In addition, the 75th and 90th percentiles were defined for the control group. After determining the cutoff value for the different measurements, the following parameters were calculated: sensitivity, specificity, area under the curve (AUC), negative and positive predictive values, test accuracy, and the

number of false positive and false negative results. Furthermore, characteristics from different ROC curves were compared by a pair-wise comparison to examine whether 3-times repetition of SN assessment resulted in higher diagnostic accuracy than a single registration of SN+, while $p < 0.05$ was used as the criterion for statistical significance. To evaluate the normal distribution of the variable, the Kolmogorov–Smirnov test was used.

Study II: Depressive symptoms analysis with respect to PD patients and non-PD controls. Between two groups analysis with respect to covariates was performed with the Mann–Whitney test and MANCOVA. The assessment scores were compared with regards to the depressive symptom severity levels. The correlations of demographic values, scores on clinical rating scales, brainstem raphe echogenicity levels and depressive symptoms were performed using the Spearman's rank correlation and partial correlation.

Study III: 'Onset SN+' was defined as SN+ measured contralaterally to the side of the body that first manifested PD related motor disturbance. Conversely, the 'non-onset SN+' was measured ipsilaterally to the body side first experiencing PD motor symptoms. As regards the controls, this differentiation was not applied. Moreover, the average of both sides was calculated. Characteristics of onset and non-onset SN+ values were compared with the independent paired t-test, as these data were normally distributed (Kolmogorov–Smirnov test $p > 0.05$). To explain the changes in SN+, the correlation analysis was performed using candidate clinical parameters, and the Spearman coefficient was calcuated. For evaluating the association between the size of SN+ (onset vs non-onset side) with various clinical markers, only the PD group (266 patients) was included, because only in that group could the laterality of SN side be defined. Furthermore, the relationship between SN+ and significantly correlating clinical parameters was further characterized by the multiple linear regression analysis with the patient's age at study time, HYS (grading PD severity), and MDS-UPDRS III (measuring PD motor symptoms) as independent variables, and onset and non-onset SN+ as dependent variables. For the evaluation of a potential age effect on SN+ the healthy group was also included, assuming that age might be also a risk factor for developing the severity symptoms of PD. Both correlations were compared with the age to find out whether age or severity of PD symptoms were the main drivers of the SN+.

To determine whether SN onset side or the larger area is more relevant for evaluating PD patients, both correlations of the dependent samples were compared using the single-sided testing calculations (Eid et al., 2011).

5. RESULTS

5.1. Study population

The study groups consisted of 266 patients with PD and 168 controls, because of an insufficient temporal acoustic bone window for TCS inspection, 66 individuals (34 with PD, 32 healthy controls) were excluded from the analysis. The excluded subjects did not differ in mean age (69.8 vs 70.3) from the rest of the cohort. Among PD patients, an insufficient bone window was more noticeable in female patients than in males (25% vs 9%, p=0.96). The sex difference regarding bone window insufficiency was not statistically significant in healthy controls (12% of females, 6% of males). The age range of the patient group was 37–87, while the healthy control group was composed of 168 subjects between the ages of 50 and 86 (Table 2.).

Table 2. Characteristics of PD patients and healthy controls.

Characteristic	PD (n=266)	Controls (n=168)	P
Age at evaluation, y	69.3±9.19	70.4±7.79	0.56
Male, n (%)	140 (52.6)	88 (52.4)	0.96
Education, y	12.8 (2.85)	12.4 (2.52)	0.17
Disease duration, y	6.1±5.21	NA	
Neurological assessment			
MDS-UPDRS III ("on" state)	31.23±14.58	3.0±2.24	<0.001
HYS	2.66±0.80	NA	
1	28 (10.5)		
2	88 (33.1)		
3	113 (42.5)		
4	35 (13.2)		
5	2 (0.8)		
Behavioral assessment			
BDI	14.6 (8.3)	11.9 (6.0)	<0.001
MMSE	27.91 (2.84)	29.2 (1.31)	<0.001
Schwab-England	77.2 (14.60)	NA	
PDQ-39	29.2 (17.47)	NA	
Medication			
LDED, mg	418±366	NA	
Antidepressants, n	20 (7.5)	NA	
Neuroleptics, n	5 (1.9)	NA	

Data are presented as mean ± standard deviation (SD) and number (percent) where applicable.
MDS-UPDRS III indicates Movement Disorders Society Unified Parkinson's Disease Rating Scale, motor part; HYS (Hoehn Yahr Stage), BDI (Beck Depression Inventory); MMSE (Mini-Mental State Examination; PDQ-39 (Parkinson's Disease Questionnaire) LDED – levodopa-equivalent daily dose; NA – not applicable, P – p value.

5.2. Clinical examination

Based on the history given by the subjects, duration of PD ranged from 1 to 25 years (mean duration 6.1±5.21 years). The average total MDS-UPDRS III (Motor Examination part III) on state in the PD group was 31.23 ± 15.1 and in the controls 3.0±2.2. Sniffin' Sticks test result in the PD group was 5.6±0.19 and 8.6±0.18 in the controls.

The HYS was in the PD group 2.66±0.8. The levodopa equivalent daily dose in PD patients was 418 ± 366 mg. Only a small proportion of PD patients were using antidepressants at assessment time (n=20; 7.5%). Of those, 6.8% with mild and 13.7% with severe depressive symptoms were using antidepressants. In the PD group, 38.7% and 35.7% of patients had mild and severe depressive symptoms, respectively. In the control group, 26.8% and 28.6% were classified as having mild and severe depressive symptoms, respectively. The percentage of depressive symptoms in controls was significantly lower than in the PD group (p<0.001). The clinical profile of PD patients from no depressive symptoms to mild or severe depressive symptoms is based on BDI scores (Table 2).

PD patients who were using antidepressants had significantly more depressive symptoms (BDI mean of 21.9±9.41) than those who did not (BDI mean of 14.0±7.98) (p<0.001).

5.3. TCS results

5.3.1. Specificity and sensitivity of TCS in the study cohort

The study examined the significance of single and repetitive measurements of SN area in the diagnostic accuracy of PD. The single assessment cutoff value using the GE LOGIQ 7 system was 0.21 cm^2, with a sensitivity of 93.2% and specificity of 85.1% (Table 3). The rate of false-positives was 14.9% and the rate of false-negatives was 6.8%.

Optimal cutoffs were defined for the calculated multi-mean and multi-median values in order to establish whether an average of 3 repeated measurements of the echogenic SN area increased the diagnostic accuracy. The multi-mean cutoff value was 0.23 cm^2 (AUC, 0.962; p<0.0001), corresponding to a sensitivity of 88.7% and a specificity of 92.2%. The multi-median cutoff value was 0.23 cm^2 (AUC, 0.963; p<0.0001), with a sensitivity of 88.7% and a specificity of 90.5%. False-negative rate had a tendency to be lower in the case of multiple assessments compared with a single assessment. There was a high correlation between the results based on different measurement strategies. The coefficient of intraclass correlation was 0.991 for the single measurement versus multi-median and in the case of multi-median versus multi-mean the coefficient was 0.999. With regard to specificity, our data revealed that the multiple-assessment strategy was superior to the single assessment (p=0.0021). The statistical comparison of the multi-mean assessment with the multi-median assessment did not favor either of the two methods (p=0.18; Figure 5).

Table 3. Analysis of single and multiple measurements; cutoff of substania nigra hyperechogenicity (SN+) is 0.23 cm^2.

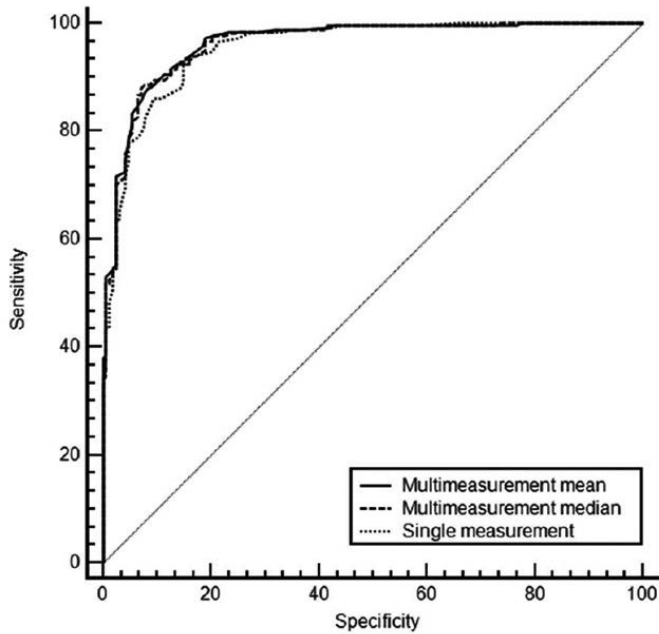| Parameter | Single measurement, first sonographic assessment in patients with PD (n=434) | Multiple measurements in patients with PD | | 75th percentile of controls, median (n=168) | 90th percentile of controls, median (n=168) |
		Mean (n=434)	Median (n=434)		
Cutoff (ROC), cm^2	0.210	0.226	0.225	0.184	0.222
Sensitivity, %	93.2	88.7	88.7	98.3	87.1
Specificity, %	85.1	92.2	90.5	77.1	92.2
AUC	95.6	96.2	96.3	96.4	96.4
Accuracy, %	87.6	89.6	89.4	91.7	88.2
Positive predictive value, %	90.8	91.4	93.7	87.0	94.7
Negative predictive value, %	88.8	87.3	83.5	96.3	81.6
False-positive, %	6.8	11.3	11.3	1.7	7.8
False-negative, %	14.9	7.8	9.5	22.9	12.9

Figure 5. Receiver operating curve (ROC) analysis: multi-mean and multi-median vs. single measurement.

5.3.2. Optimal cutoff vs 75th and 90th percentile scores

As statistically there was no difference between the SN+ multi-median and multi-mean values, in order to define the 75th and 90th percentiles in controls, only median values were used for the multiple assessments. The cutoff value for the 75th percentile was 0.18 cm^2 (AUC, 0.964; p<0.0001), whereas for the 90th percentile it was 0.22 cm^2 (AUC, 0.964; p<0.0001; Table 3).

5.3.3. Severity levels of depressive symptoms in PD patients

The clinical profile of PD patients with no, mild and severe depressive symptoms based on BDI scores is presented in Table 4. Only a small sample of PD patients were using antidepressants at assessment time (n=20). Of those, 6.8% with mild and 13.7% with severe depressive symptoms were using antidepressants. PD patients who were using antidepressants had significantly more depressive symptoms. In the controls nobody used antidepressants.

36

Table 4. Patients' characteristics within depressive symptom severity levels.

	Within normal limits	Minor depressive symptoms	Major depressive symptoms	Significance (p-level)
UPDRS I	5.3 (3.8)	7.9 (4.5)	11.3 (5.6)	0.002[a], <0.001[b,c]
UPDRS II	7.7 (4.9)	10.7 (6.0)	15.2 (6.3)	0.01[a], <0.001[b,c]
UPDRS III	24.4 (13.2)	29.2 (12.3)	38.3 (14.9)	0.076[a], <0.001[b,c]
Hoehn and Yahr	2.3 (0.7)	2.5 (0.7)	3.1 (0.7)	0.089[a], <0.001[b,c]
Schwab-England	87.5 (9.4)	79.3 (11.3)	67.6 (15.0)	< 0.001[a,b,c]
MMSE	28.5 (2.5)	28.2 (2.3)	27.2 (3.4)	1.0[a], 0.025[b], 0.005[c]
BDI	6.0 (2.6)	12.3 (1.9)	23.4 (7.1)	<0.001[a,b,c]
PDQ-39	25.4 (20.1)	24.9 (13.2)	36.6 (17.3)	1.0[a], <0.001[b,c]

Results are given as mean standard deviation (SD):
[a] within normal limits vs. minor depressive symptoms;
[b] minor vs. major depressive symptoms;
[c] major depressive symptoms vs. within normal limits.

UPDRS I indicates Movement Disorders Society Unified Parkinson's Disease Rating Scale, part I; UPDRS II indicates Movement Disorders Society Unified Parkinson's Disease Rating Scale, part II; UPDRS III indicates Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III; HYS (Hoehn Yahr Stage), BDI (The Beck Depression Inventory); MMSE (The Mini-Mental State Examination; PDQ-39 (The Parkinson's Disease Questionnaire) LDED – levodopa-equivalent daily dose

5.3.4. Brainstem raphe echogenicity

In both PD patients and controls, higher BDI values were associated with reduced brainstem raphe echogenicity. In the PD group, there were statistically significant differences (p<0.001) between the patients' BDI scores of all three brainstem raphe visibility levels. In the control group, individuals with fully and partially visible brainstem raphe exhibited considerably fewer depressive symptoms than the ones whose brainstem raphe was not visible (p<0.001). As for the between-group (PD vs non-PD), significant differences in depressive symptoms were only evident in the 'partially visible brainstem raphe' subgroup (p<0.001). Based on the multivariate analysis, the effect of brainstem raphe echogenicity on depressive symptom severity scores in the patient group remained significant even after accounting for the HYS as a covariate (f1.5=15.1, p<0.001) (Table 5).

A direct correlation was discovered between brainstem raphe echogenicity and age (Spearman's rank correlation, r=0.132, p=0.006). Mean echogenicity scores in women and men did not seem to differ to that extent (women: 1.27 [SD 0.728], men: 1.18 [SD 0.763], p=0.205).

Table 5. Correlations of assessment scores with depression and brainstem raphe (BR) echogenicity levels.

	Patients' BDI correlation coefficient (r)	Controls' BDI Correlation coefficient (r)	Patients' brainstem raphe, correlation coefficient (r)	Controls' brainstem raphe, correlation coefficient (r)
Age at assessment	0.207 (p=0.001) ***	0.191 (p=0.013)*	0.181 (p=0.003)**	0.037 (p=0.633)
Education in years	0.066 (p=0.285)	0.009 (p=0.905)	0.053 (p=0.394)	0.002 (p=0.978)
MMSE	−0.217 (p<0.001) ***	−0.212 (p=0.006)**	−0.087 (p=0.159)	0.021 (p=0.788)
PD duration in years	0.240 (p<0.001) ***	NA	0.143 (p=0.02)*	NA
Schwab-England Scale	−0.550 (p<0.001) ***	NA	−0.293 (p<0.001) ***	NA
HYS	0.400 (p<0.001) ***	NA	0.195 (p=0.001) ***	NA
PDQ-39	0.293 (p<0.001) ***	NA	0.257 (p=0.001) ***	NA
UPDRS I	0.457 (p<0.001) ***	NA	0.317 (p<0.001) ***	NA
UPDRS II	0.496 (p<0.001) ***	NA	0.316 (p<0.001) ***	NA
UPDRS III	0.374 (p<0.001) ***	NA	0.194 (p<0.001) ***	NA

Higher BDI scores indicating severe depressive symptoms had a direct correlation not only with older age, but also longer disease duration, loss of independence in daily activities, more noticeable motor dysfunction and cognitive impairment (see Table 2 for details). Except for the MMSE, demographic and clinical variables indicating more severe disease stages were also directly associated with brainstem raphe echogenicity. Moreover, brainstem raphe echogenicity was significantly correlated with the BDI total score in both the PD (r=0.59, p<0.001) and control groups (n=0.66, p<0.001). Using partial correlation analysis and controlling for age, PD duration and HYS, the direct relationship between BDI score and raphe echogenicity level remained significant (r=0.5, p <0.001).

5.3.5. SN+ values are different on the respective sides mirroring the asymmetric character of Parkinson's disease group and in the control group

The average mean SN+ values of both sides were considerably higher in the PD group (0.33 ± 0.10 cm^2) than in the control group (0.16 ± 0.05 cm^2, p<0.001) (Table 2). The size of the left and right side SN+ (left side 0.35 ± 0.11cm^2; right side: 0.36 ± 0.13cm^2, p=0.31) were not statistically different among PD patients. Yet it was discovered that onset SN+ (0.35 ± 0.12 cm^2) of the contralateral SN of the initial motor symptoms side appeared to be on average 17.6% larger than non-onset SN+ in PD patients (mean 0.30 ± 0.10 cm^2, paired t test, p<0.00, Figure 6)
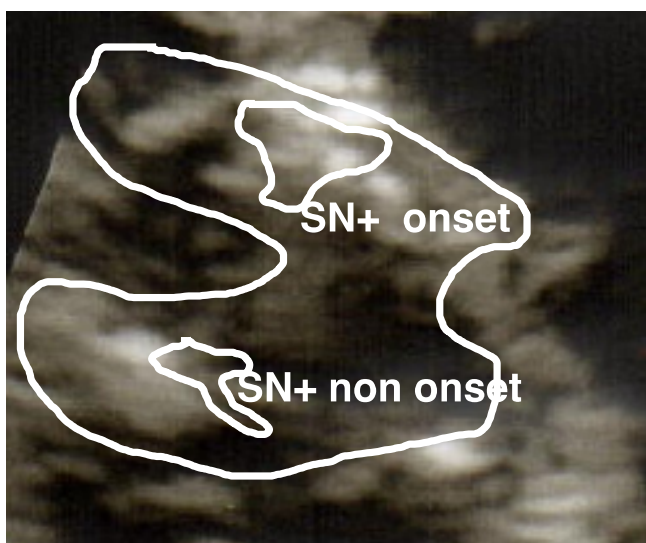

Figure 6. Sonographic image of substantia nigra hyperechogenicity (SN+) in PD patient.
Sonographic image of corresponding midbrain axial sections in PD patient. The butterfly-shaped midbrain was encircled for better visualization. Marked bilateral SN+ of the subject was encircled for computerized measurement. SN+ onset 0.31 cm^2 and SN+ non-onset 0.26 cm^2.

5.3.6. The effect of clinical and demographic parameters on SN+ in PD patients and healthy controls

A significant negative correlation was found between onset SN+ and PD patient's age at study time (r_S = –0.199, p<0.01) and the HYS (r_S = –0.162, p<0.01), and MDS-UPDRS III score (r_S = –0.139, p < 0.05) (Table 6). No significant correlation between non-onset SN+ side and any of the assessed clinical parameters was found. Multiple linear regression analysis with predictors (1) age at study time, (2) HYS and (3) MDS UPDRS III were used with the result of a significant model (p=0.0015). We discovered that the age at study time had a noticeable correlation with a change in the area of onset SN+ (p=0.017).

The regression model of PD patients showed that the age at study time and HYS have a negative correlation with the size of onset SN+. If the model included the non-onset SN+ as dependent variable with the same predictor variables, the overall model fit indicated an unstable prediction model (p=0.17).

Table 6. Spearman correlation coefficients (r_s) of SN+ for the onset and non onset side and clinical markers in PD (n=266).

	Onset SN+ Correlation coefficient (r)	Non onset SN+ Correlationcoefficient (r)
Age at study time	–0.199**	–0.076
HYS	–0.162**	–0.077
LDED†	–0.139*	–0.031
MDS UPDRS III	–0.132*	–0.068

*p<0.05; **p<0.01
HYS – Hoehn Yahr Stage
LDED – levodopa equivalent daily dose
MDS UPDRS III – the Movement Disorders Society Unified Parkinson's Disease Rating Scale part III
† Correlations for LDED are reported without 0 values

Subsequently, the relationship between age and SN+ was investigated, including the healthy controls. It was discovered that whereas SN+ in healthy controls increases with age (p=0.0004), in PD patients SN+ contralateral to the initially affected side decreases (p=0.0049) (Figure 7). The data suggest that the relationship with age and SN+ depends on whether the individual has PD. The higher age is associated with an increase in SN+ in healthy individuals (p<0.0001), while in PD patients it showed a decrease in onset SN+ (p=0.0049). Among the patients with visible SN (n=266) there were 67 patients out of 266 (25% of the cohort) who did NOT have a higher onset SN area and for 9 patients the area was the same on both sides.
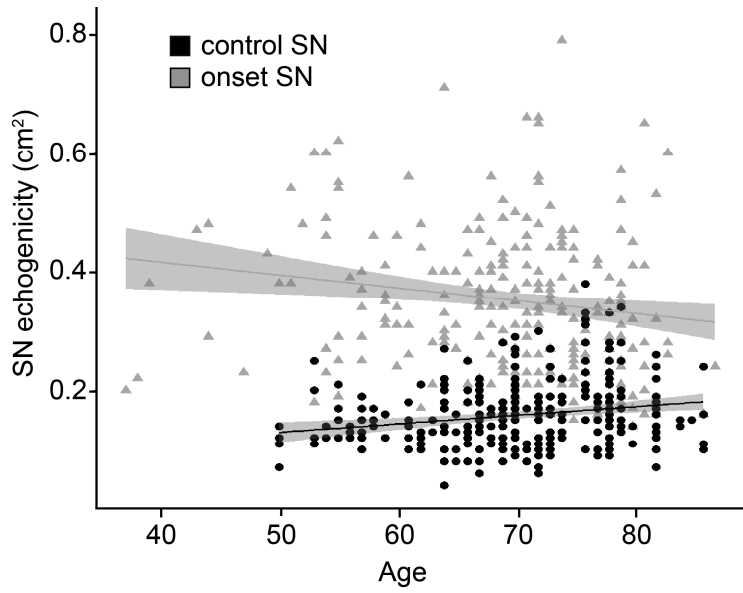
Figure 7. The effect of age on SN+ in PD symptom onset and healthy controls. The higher age is associated with an increase in SN+ in healthy individuals (p<0.0001), while in PD patients it showed a decrease in onset SN+ (p=0.0049).

6. DISCUSSION

The present study was the first research project using TCS investigation in the Estonian cohort of Parkinson's disease and healthy subjects.

Although TCS for movement disorders has been in use since 1995, we performed the first investigations of our patients in Estonia in 2008. By 2010 we had enough experience to start investigating PD patients on the scientific level.

Clinically, the most striking feature of TCS in its application in PD is the visualization of the SN with its different sizes and echogenicities. It has been abundantly shown that individuals with PD have an increased area of hyperechogenicity at the anatomical site of the SN compared to controls (Walter et al., 2004; Walter et al., 2007a), a finding unique for TCS. I investigated 266 Estonian PD patients and 168 healthy controls whose age and education matched the patient group.

The main limitation of the TCS is the lack of bone window in about 10% of the Caucasian population (Gaenslen et al., 2008; Walter et al. 2007a). This rate seems to be increasing with advancing age and may reach to up to 25% especially in elderly women (Walter et al. 2007a). It has been speculated that osteoporosis may play a role in the deterioration leading to an increased dispersion of the ultrasound beam. On the other hand, in Asian subjects, an insufficiency of temporal bone window occurs in 15 to 60 percent of patients (Kim et al., 2007; Okawa et al., 2007).

Our results correspond to the findings of other studies. Although an impenetrable temporal bone window is a clear limitation of the method, we showed that the percentage of individuals with an insufficient bone window among Estonians (13.2% and 25% of female PD patients) is similar to the rate found in other European countries (Berg et al., 2008; Školoudík et al. 2010). Higher failure rates for transcranial sonography due to an insufficient bone window have been reported among Japanese, Taiwanese, South American, Australian, and South Korean populations. These differences may be caused by lower bone mineral density in these populations due to lower dietary calcium intake compared with Filipino and white populations (Okawa et al., 2007; Go et al., 2012; Kim et al., 2007; Fernandes et al. 2011).

One possible reason for worse visibility in female patients is osteoporosis (Raglione et al., 2011), though we did not study the reason for differences in bone windows specifically. In a prospective study, it was found that among women bone mineral density was significantly lower in the PD patients than in the controls, and the decrease in bone window visibility was greater in the subgroup with more advanced disease (Raglione et al., 2011).

The aims of our study were:
1) To find an optimal SN+ cut off for our Estonian PD patients to continue research in this field, and to compare the SN+ diagnostic accuracy of PD patients in Estonia to patients in other countries.

2) To describe the prevalence and severity of depressive symptoms in PD, as well as to analyze possible associations between BR echogenicity and depressive symptoms in an Estonian sample of patients with PD compared to age- and education-matched healthy (non-PD) controls.
3) To study associations of SN echogenicity values measured in daily clinical practice with clinical or demographic charactreristics of PD patients.

6.1. Diagnostic accuracy of SN+

Each lab has to use its own cutoff value derived from the ultrasound system and assessment method used (Bowmans et al., 2013). For example, for the Sonoline Elegra system (Siemens AG, Munich, Germany) and the MyLab25 Gold system (Esaote SpA, Genoa, Italy), a cutoff value of 0.20 cm^2 has been suggested in German and Filipino populations (Berg et al., 1999; Go et al., 2012). For the GE LOGIQ 7 system, an even higher cutoff value (0.24 cm^2) for PD diagnosis has been reported in Austria and in Germany (Stockner et al., 2007; Van de Loo et al., 2010).

The first goal of this study was to validate SN+ in a large Estonian PD patient cohort and healthy controls. Our results correspond to the findings of other studies. The single assessment cutoff value using the GE LOGIQ 7 system was 0.21 cm^2, with a sensitivity of 93.2% and specificity of 85.1%. The rate of an enlarged SN area (false positive) was 14.9% and the rate of exhibited SN areas (false-negative) was 6.8%. The multi-mean cutoff value was 0.23 cm^2, corresponding to a sensitivity of 88.7% and a specificity of 92.2%. The multi-median cutoff value was 0.23 cm^2 (AUC, 0.963; p < 0.0001), with a sensitivity of 88.7% and a specificity of 90.5%. The false-negative rate was lower in multiple assessments compared to a single measurement. There was a high concordance between the results based on different measurement strategies: the coefficient of intraclass correlation was 0.991 for the single versus multi-median measurement, and in the case of multi-median compared to multi-mean the coefficient was 0.999.

Our study's cutoff value using the GE LOGIQ 7 system was ≥0.23 cm^2, the same cutoff value that has previously been calculated in other studies with the same ultrasound device, confirming the validity of the method itself (Stockner, et al., 2007; 2012).

The sensitivity and specificity of SN+ are still a matter of debate. The sensitivity and specificity for the diagnosis of PD based on the detection of SN+ by image analysis were 87% and 92%, respectively (Školoudík et al., 2013). In another study, applying 3-dimensional quantification of SN echogenicity sensitivity and specificity for the diagnosis of PD of up to 91% and 73% were reported (Plate et al., 2012).

The normal ranges need to be established for each different US-S and because of some potential investigator dependency also for each different lab. In general, the size of the enlarged area in the region of the SN in patients with PD

is compared to that of a representative control group (Van de Loo et al., 2010). Moreover, variations in cutoff values are also caused by the fact that different algorithms have been applied to define the cutoff value for SN+. Most studies calculated the 75th or 90th percentile of the enlarged SN area in a reference group as the critical value for SN+ (Bouwmans et al., 2013; Mahlknecht et al., 2013). In other studies, the cutoff value represented the first or second standard deviation below the population mean value in control partipants (Berg et al., 2010; Mahlknecht et al., 2013; Walter et al., 2002). To rate SN echogenicity in an individual as normal or increased (SN+), the 75th and 90th percentile of measures in the normal population were used as by Berg (Berg et al., 2008). The larger of bilaterally measured SN+ sizes is used for classification as follows: (1) normal echogenic: measured area is below the 75th percentile; (2) moderately hyperechogenic: measured area is between the 75th and the 90th percentile; (3) markedly hyperechogenic: measured area is above the 90th percentile.

We use of 75th and 90th percentiles of controls as an option to find the optimal SN+ cutoff, as statistically there was no difference between the SN multi-median and multi-mean values in order to define the 75th and 90th percentiles in controls; only median values were used for the multiple assessments. The cutoff value for the 75th percentile was 0.18 cm^2, whereas for the 90th percentile it was 0.22 cm^2.

Although it makes little sense statistically as it only fixes the specificity at 75th or 90th, I suggest another option: increasing the number of so-called categories, using two cutoffs, cutoff A and cutoff B, correspondingly. Thus, if the measurement result is X and $X > B$, the person is ill (specificity 100% or almost 100%), but if $X < A$, the person almost certainly has no illness (sensitivity 100%), but if $A < X < B$, additional investigations are needed.

I created a model 'what is your probability to become ill from a PD?' where X is an average measurement result, and A is SN+ with 100% sensitivity, and B is SN+ with 100% specificity. According to the formula, A is 0.14 cm^2 and B is 0.34 cm^2 in our group, and in such a case we can conclude that if the measurement result is 0.34 or higher, the person is ill (specificity 100%), and if the measurement is 0.14 or lower, the person does not have the illness (sensitivity 100%). This is a rather simple calculation, but helpful for the diagnosis of PD in clinical practice when the measurement result is in the range between 0.14 cm^2 and 0.34 cm^2. Still, additional investigations are needed on other progressive parkinsonian disorders (MSA, PSP, CBD). In cases of MSA, SN+ occurred in 15% cases (Behnke, 2005; Fujita, 2016; Okawa, 2007; Walter, 2003), but in contrast to PD, SN+ seems far less frequent in PSP with an average rate of 28% (Berg, 2018) In CBD patients had SN+ mean prevalence 67% (Sadowski, 2015).

This study had methodological limitations. Although the person performing transcranial sonography had no knowledge of the exact state and progression of the disease, he was not blinded to the diagnosis of PD. Therefore, the clinical investigation was performed after transcranial sonography.

In summary, the importance of this study in many areas. On the basis of our subjects we can say that the optimal cutoff diagnostical value 0.23 cm^2 is found on the basis of several consecutive measurements. The results of our study revealed the significance of repetitive measurements of the SN area for the diagnostic accuracy of PD: we verified that 3 repetitive measurements of the SN echogenicity on TCS increase the diagnostic accuracy of the disease.

6.2. The associations between brainstem raphe echogenicity and depressive symptoms in the Estonian cohort

In the present study, 72.3% of patients with PD with severe depressive symptoms (n = 68), and 89.6% of controls with severe depressive symptoms (n = 43) both showed significant BR hypoechogenicity whereby the raphe was completely not visible.

The present study demonstrated that depressive symptoms severity is an important factor modulating the clinical aspects of PD. We showed a direct correlation between brainstem raphe echogenicity and BDI score in PD patients and healthy controls, but with considerably greater reduction of brainstem raphe echogenicity in PD patients with depressive symptoms in comparison with non-PD controls with the same symptoms. However, the percentage of people exhibiting severe depressive symptoms and hypoechogenic brainstem raphe was higher among the controls. This phenomenon could be explained by the fact that the neurodegenerative process in PD has an effect on the brainstem raphe in the majority of people whose depressive symptoms are above normal levels, whereas in the non-PD control group, the higher occurence of self-reported depressive symptoms may be explained by a well-preserved awareness of mental health problems. TCS can be used to assess brainstem raphe as an important marker for depressive symptom severity in both PD patients and healthy individuals. As depression has a major effect on the quality of life, sufficient therapy is mandatory.

In the current study, there was also a direct association between depressive symptom severity and hypoechogenic brainstem raphe in PD patients when taking into consideration the patients' age, disease duration and HYS. It is interesting to note that partially reduced visibility of the brainstem raphe was evident in 58.7% of controls with a normal BDI score and as high as 64.4% of controls suffering from mild depressive symptoms. In the PD group, the respective proportions were 23.5 and 53.4. Taken together, signs of mesencephalic brain pathology may be a radiological marker for depressive symptom severity in both patients and controls. Our findings are in concordance with previous reports that brainstem raphe echogenicity is altered in individuals with depression (Becker et al., 1994; Walter et al., 2007). The level of affective disorders was directly related to the anatomical changes of brainstem raphe.

Decreased echogenicity of the mesencephalic raphe has been found in 40 to 60 percent of patients with PD patients having depression (Becker et al., 1997; Walter et al., 2007a,b; Berg et al., 1999).

In only a small sample of our PD patients, antidepressants were used at the assessment time: in the PD group, 6.8% with mild and 13.7% with severe depressive symptoms, antidepressants were used. Patients who were using antidepressants had significantly higher BDI scores than those who were not. It has been shown that 50 to 60 percent of PD patients do not achieve an adequate therapeutic response following a course of antidepressants (Fava, 2003). Importantly, low echogenicity of the brainstem raphe is a common finding in 50 to 70 percent of patients with depression (Becker et al., 1994; Walter et al., 2007b) and is associated with significantly lower responsiveness to serotonin-reuptake inhibitors (Walter et al., 2007a).

The present study illustrated the significance of the finding that TCS of brainstem raphe echogenicity could be used as a non-invasive biomarker to improve detection of depressive symptoms in early PD stages where clinicians may not recognize affective disturbances in the context of PD phenomena. However, the sensitivity and specificity of brainstem raphe echogenicity as a predictor of depression in PD or controls is yet to be reported.

The level of affective disorders was directly related to the anatomical changes of brainstem raphe. Decreased echogenicity of the mesencephalic raphe has been found in 40 to 60 percent of patients with PD patients having depression (Becker et al., 1997; Walter et al., 2007a,b; Berg et al., 1999).

Higher severity of depressive symptoms has been associated with longer PD disease duration, more severe motor and cognitive impairment, and advanced stage of the disease. With an average prevalence of about 40%, ranging from 27% to 76%, depression is one of the most common non-motor manifestations of PD (Ji Won Han et al., 2018; Kadastik-Eerme et al., 2016; Slaughter et al., 2001; Reijnders et al., 2008). The onset of depression during late stages of PD has mainly been related to a broader monoaminergic deficit in combination with a higher occurrence of cognitive impairment (Braak et al., 2004). Depression may already occur before the onset of motor symptoms, suggesting that the neuropathological process of PD increases the risk of depression (Leentjens et al., 2003; Reijnders et al., 2008; Schuurman et al., 2002).

Regarding specific weaknesses of the study and subsequent recommendations for future studies, a psychiatric interview for diagnostic purposes in addition to various rating scales (both self- and clinician administered) of depression should be incorporated into the assessment procedure. A second brainstem raphe rater could be included in the study to strengthen the reliability of the imaging results. Taken together, our results must be seen as exploratory and must be interpreted with caution.

Increased clinician awareness of PD depression and application of available diagnostic and treatment interventions could eliminate significant suffering in PD and enhance quality of life. The early awareness and treatment of old age depression will benefit from further neuroanatomical, physiological, psycho-

logical, and pharmaceutical investigations. With this, it is vital to arrange the relevant therapy for depression and the patient's response to antidepressant treatment.

6.3. Asymmetry of clinical symptoms of PD, and the effect of age on SN+

The motor symptoms usually affect the body asymmetrically at first with the initially affected side remaining most prominently affected throughout the disease course (Djaldetti et al. 2006). The functional asymmetry in PD is also reflected in the SN pathology. The SN corresponding to the disease onset side (in the following onset SN+) experiences a more dramatic loss of dopaminergic neurons than the contralateral side (in the following termed non-onset SN+) (Kempster et al. 1989). It has been reported that PD patients with a more extended hyperechogenic signal had an earlier disease onset (Berg et al. 2001). Others, however, could not replicate this finding and argued that the enlarged SN hyperechogenicity on TCS is an important factor in determining the disease, but should be treated as one of many factors associated with the disease and disease onset.

In order to establish whether SN+ values are laterally different, the onset and non-onset SN+ were studied separately in the PD group and it was established that the area of SN+ on the right and left side were not statistically different. Our findings show that the area of SN+ is different in size depending on the side of PD symptom onset. Onset SN+ corresponding to the SN+ contralateral to the side with initial motor signs was on average 17.6% larger than non-onset SN+.

We found a correlation with a higher age in PD patients and a slight but significant decrease in SN echogenicity contralateral to the side of the initial motor symptoms. This is supported by a previous report that documented a negative correlation between age at disease onset and SN+ (Walter et al., 2007a). The underlying cause between age at disease onset and SN+ area has not yet been convincingly demonstrated. However, a post mortem investigation has reported a PD duration-related decrease in the density of intranuclear Marinesco bodies in the SN (Abott et al. 2017). Another hypothesis for the 'neurodegeneration' of the SN on the primarily affected side could be a disease duration-related cell loss and an accompanying decrease in echogenic molecules (e.g. iron). However, age may account for a number of stressors within SN, weakening and/or losing the neurons function and their ability to react to further damages associated with the disease process. Moreover, it could be speculated that an SN+ at the onset side of PD results from active neuroinflammatory processes, which gradually diminish with the progression of PD and the decrease of the number of dopaminergic cells.

In a cross-sectional study, we did not focus on the association between hyperechogenic area of SN and the disease duration because we did not know

the exact onset of PD (which in many cases is not even clear to the patients themselves) but age is a measurable, clear parameter.

Surprisingly, in our multiple regression analysis, the age failed to reliably predict the size area of SN+ ipsilateral to the side of the initial motor symptoms, though 42.5% of PD patients exhibited bilateral symptoms (HYS = 2). There could be several reasons why the non-onset SN+ demonstrates different area dynamics from onset SN+. Firstly, the extent of pathology of the non-onset side could appear later and to a lesser degree than the initially affected SN. Another reason might be the unilateral nature of the mechanisms triggering PD or at least that appear to be unilateral. For example, changes in the blood–brain barrier could allow pathogenic agents or toxic environmental factors to invade vulnerable structures preferentially on the more exposed side and thus limit the degenerative process mostly to one side (Berardelli et al., 2013). Furthermore, as the hyperechogenic area of the non-onset side is smaller than in the onset side, the changes may be harder to detect. It needs to be stressed, however, that it is currently unclear why PD symptoms generally occur asymmetrically and why the contralateral side becomes involved later on.

The present study also invites a comparison in the SN+ in healthy individuals and PD patients – while the area of the initially affected SN decreases in PD, in healthy individuals it increases. The correlation was not very strong, but still is statistically significant ($p < 0.01$). This may be affected by the fact that the statistically significant correlation exists, but we should treat the medical significance with caution due to the large cohort where more significant results could be achieved. Moreover, I found the line to have a downward slope ($r = -0.199$). Thus age appears to be a factor in predicting the SN+ and influencing the dynamics of PD in our study. This is corroborated by a study of the life-long changes in the SN in healthy individuals (Hagenah et al., 2010).

Our findings also reveal the effect of age on the area of hyperechogenicity at the anatomical site of the SN in healthy and diseased individuals – whereas the area of the primarily affected SN decreases in PD, it increases in healthy adults. We acknowledge that in PD-patients the hyperechogenicity of the onset side usually shows a larger area than the non-onset SN. In our Estonian PD cohort of 266 patients, this was not the case for 67 patients (25% of the cohort), who had a smaller SN area on the onset side, while for 9 patients the area was the same for both sides.

We found that older PD patients had a smaller hyperechogenic area than younger PD patients while in healthy people SN+ is positively associated with age. We do not know whether the triggering factor is younger age of PD onset or something else. This needs to be evaluated in further studies. In sum, however, we conclude that onset-side revealed a stronger correlation than only considering SN size irrespective of age and we may recommend to use onset-side as the best measurement for evaluating PD patients.

Findings of SN+ in PD patients expanded the research to subgroups such as clinical PD subtypes or genetic PD. Bilateral SN+ were more common in patients with an akinetic (rigid type compared to a tremor) dominant subtype

(Walter et al., 2007). Another study which reported larger SN sizes in non-tremor-dominant patients compared to tremor-dominant PD (Lauckaite et al., 2014). Accumulating evidence from independent studies prompted further efforts to understand the causes, consistency and significance of SN+ in PD. The side of the larger hyperechogenic area correlates with the clinically more affected contralateral side, indicating that SN+ size may be associated with clinical features or nigral pathology. Reports with regard to association between SN+ and disease specific features have been conflicting (Yilmaz et al., 2018). Although SN degeneration usually occurs bilaterally in PD, loss of dopaminergic neurons is more pronounced contralaterally to the initially clinically more affected body side (Kempster et al., 1989). As an enlarged SN+ seems to appear prior to motor symptoms, a smaller non-onset SN+ could be a sign of a delayed disease spread to the contralateral SN.

On the other hand, the first two studies on TCS in PD showed no correlation between the size of the SN and age (Berg et al., 2001), a finding that was supported in patients with RBD (Iranzo, 2007) which showed an increasing prevalence of SN+ with age in elderly individuals. With regard to the association between SN+ size and motor disability, measurements revealed no such correlations (Iranzo et al., 2010; Jesus-Ribeiro et al., 2016; Lobsien et al., 2012; Spiegel et al., 2006), contrary to the positive findings of others (Behnke et al., 2007, Sanzaro et al., 2014; Weise et al., 2009). A correlation between the SN+ size and disease duration or dopamine transporte uptake in functional imaging has also not been found in most studies (Lobsien et al., 2012; Spiegel et al., 2006). Nevertheless, more studies with longitudinal design are needed to elucidate this contentious issue.

These findings further underscore the importance of differentiating between the onset and non-onset SN+. Several authors have reported a lack of change of bilaterally averaged SN+ as a function of PD duration leading them to conclude that SN+ is just a trait marker for PD that does not reflect the disease progression nor the state (Mehnert et al., 2010; Walter et al., 2007a).

It is currently unclear whether the age-related increase in SN+ may overlap with PD pathophysiology or reflect an increased nigrostriatal vulnerability to PD (Berg et al., 2002). However, it is known that the SN is susceptible to iron accumulation (Bilgic et al., 2012), LB accumulation (Buchman et al., 2012) and neuronal loss (Ma et al., 1999), even in elderly individuals without clinically defined PD. This is perhaps a reflection of the high oxidative stress burden created by dopamine metabolism and the susceptibility to calcium overload in dopaminergic neurons (Reeve et al., 2014). We found that SN+ decrease in PD patients depended on the clinically first affected side, but it did not occur on the non-onset side, whereas in healthy people SN+ increases with age. This is my hypothesis that SN+ is higher in PD patients with a younger age of onset.

As a limitation, we are not able to eliminate some methodological problems. Although the person performing transcranial sonography had no knowledge of the exact state and progression of the disease, he was not completely blinded to the clinical symptomatology at study time. Therefore, the clinical investigation

was performed after transcranial sonography by another investigator. Secondly, >10% of the participants (and 25% of female PD patients) did not have a sufficient bone window and thus could not undergo this examination.

6.4. Future prospects

In daily clinical practice, widely varying SN+ areas in PD patients are visible that do not correlate with duration of the disease, but in PD with a long duration the SN+ occurs in a smaller area than in patients who were diagnosed with PD recently. The long-term study statistic is in progress, to show possible change in SN+ values during the course of PD. The same study group is included in repeated assessments 7 years later, and some of control group subjects became PD during this period.

The second part of the longitudinal study is the follow-up to identify if and in how many subjects among our primary healthy controls developed PD over the period of 7 years. By the 7th study year, we had 122 study control subjects available. The results of our performed studies are a good platform to move on to further steps in the field of ultrasound diagnostics in neurodegenerative diseases.

7. CONCLUSIONS

The present study was the first TCS study to validate SN+ as a biomarker for PD in a large Estonian cohort. Around 10% of PD patients in Estonia were involved in the study, which showed the following findings:

1. The Estonian population has the same percentage of SN+ as other white populations. The highest diagnostic accuracy was achieved by the use of multiple TCS assessments to define SN+ which was 0.23 cm^2 in the Estonian sample. Single measurements yielded higher sensitivity for the diagnosis of PD, while mean replicate measurements provided slightly higher specificity. This finding argues for performing multiple measurements to gain diagnostic certainty whenever possibl

2. The prevalence of mild (38.7%) and severe (35.7%) depressive symptoms in the patient sample was found to be significantly higher than in controls where mild depressive symptoms (26.8%) and severe depressive symptoms were 28.6%. BR echogenicity in both patients and controls was directly related to their total BDI score, although we found a significantly greater reduction of BR echogenicity in patients with PD and depressive symptoms compared to depressed non-PD controls. Only a small sample of PD patients were using antidepressants at assessment time (n=20). Of those, 6.8% with mild and 13.7% with severe depressive symptoms were using anti-depressants. In the controls nobody used antidepressants.

   In both patients and controls, severe depressive symptoms were related to reduced brainstem raphe echogenicity by TCS that allows to use brainstem raphe as a non-invasive ultrasound marker for diagnosing depressive symptoms in the early stages of PD when affective disorders may not be easily recognizable in the context of PD symptoms.

3. The size of SN+ is differentially affected depending on the side of disease onset. Onset SN+ corresponding to the contralateral side of the initial motor symptoms was on average 17% larger than the non-onset side measure. We found that elder PD-patients had a smaller hyperechogenic area than younger PD patients while healthy people SN+ is positively associated with age. The study did not detect similar interactions in the non-onset SN+.

   We conclude that onset SN+ is a better marker than the average measurement of both sides, hence onset SN+ should be taken into account while making the analysis, as this is much better describing the disease symptoms. We conclude that using onset-side gave us stronger correlation and we may recommend to use onset-side as the best measurement for evaluating PD patients. While we could say that larger measurement could be the second best option, as the correlation with the same negative trend exists taking into account the larger SN+. We may emphasize that evaluation of the "onset SN+" is probably more appropriate for PD analysis than the larger side.

8. REFERENCES

Abbott, R. D., Nelson, J. S., Ross, G. W., Uyehara-Lock, J. H., Tanner, C. M., Masaki, K. H., … Petrovitch, H. (2017). Marinesco bodies and substantia nigra neuron density in Parkinson's disease. Neuropathol Appl Neurobiol, 43, 621–630.

Adams, J. R. (2005). PET in LRRK2 mutations: comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation. Brain, 128, 2777–2785.

Batla, A., Erro, R., Stamelou, M., et al. (2014). Patients with scans without evidence of dopaminergic deficit: a long-term follow-up study. Mov Disord, 29, 1820–1825.

Beck, A.T., Ward, C.H., Mendelson, M.M., Mock, J.J., Erbaugh, J.J., (1961). An inventory for measuring depression. Arch. Gen. Psychiatry, 4, 561–571.

Becker, G., Struck, M., Bogdahn, U., Becker, T. (1994). Echogenicity of the brainstem raphe in patients with major depression. Psychiatry Res. Neuroimaging, 55, 75–84.

Becker, G., Seufert, J., Bogdahn, U., Reichmann, H., Reiners, K. (1995). Degeneration of substantia nigra in chronic Parkinson's disease visualized by transcranial color-coded real-time sonography. Neurology, 45, 443–454.

Becker, T., Becker, G., Seufert, J., Hofmann, E., Lange, K., Naumann, M., Lindner, A., … Reiners, K. (1997). Parkinson's disease and depression: evidence for an alteration of the basal limbic system detected by transcranial sonography. J. Neurol. Neurosurg. Psychiatry, 63, 590–595.

Behnke, S., Berg, D., Naumann, M. Differentiation of Parkinson's disease and atypical parkinsonian syndromes by transcranial ultrasound. (2005). J Neurol Neurosurg Psychiatry, 76, 423–425.

Berardelli, A., Wenning, GK., Antonini, A., Berg, D., Bloem, BR., Bonifati, V., Brooks D., Vidailhet, M. (2013). EFNS/MDS-ES recommendations for the diagnosis of Parkinson's disease, Eur J Neurol, 20, 16–34.

Berg, D., Becker, G., Zeiler, B., et al. (1999). Vulnerability of the nigrostriatal system as detected by transcranial ultrasound, Neurology, 53, 1026–1031.

Berg, D., Siefker., C, Becker., G. (2001) Echogenicity of the substantia nigra in Parkinson's disease an its relation to clinical findings. J Neurol, 248, 684–489.

Berg, D., Roggendorf, W., Schröder, U., Klein, R., Tatschner, T., Benz, P., Tucha, O., Becker, G. (2002). Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. Arch Neurol, 59, 999–1005.

Berg, D., Merz, B., Reiners, K., Naumann, M., Becker, G. (2005) A five year follow-up study of hyperechogenicity of the substantia nigra in Parkinson's disease. Mov Disord, 20, 383–385.

Berg, D., Behnke, S., Walter, U. (2006). Application of transcranial sonography in extrapyramidal disorders: updated recommendations. Ultraschall in Med, 27, 12–19.

Berg, D., Godau, J., Walter, U. (2008). Transcranial sonography in movement disorders. Lancet Neurol, 7, 1044–1055.

Berg, D., Marek, K., Ross, G. W. & Poewe, W. (2012). Defining at-risk populations for Parkinson's disease: lessons from ongoing studies. Mov. Disord, 27, 656–665.

Berg, D., Lang, AE., Postuma, RB., et al. (2013). Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. Lancet Neurol, 12, 514–524.

Berg, D., Postuma, RB., Adler, CH., et al. (2015). MDS research criteria for prodromal Parkinson's disease. *Mov Disord*, 30, 1600–1611.

Berg D., Adler C. H., Bloem B. R., Chan P., Gasser T., Goetz C.G…Postuma R.B. (2018). Movement disorder society criteria for clinically established early Parkinson's disease. Mov Disord. 33, 1643–1646.

Bilgic, B., Pfefferbaum, A., Rohlfing, T., Sullivan, EV., Adalsteinsson, E. (2012). MRI estimates of brain iron concentration in normal aging using quantitative susceptibility mapping. Neuroimage, 59, 2625–2635.

Boeve, B. F., Dickson, D. W., Duda, J. E., Ferman, T. J.,Galasko, D. R., Galvin, J. E., Goldman, J. G., Zabetian, C.P. (2016). Arguing against the proposed definition changes of PD. Mov Disord, 31, 1619–1622.

Bouwmans, A.E., Vlaar, A.M., Mess, W.H, Kessels, A., Weber, W.E. (2013). Specificity and sensitivity of transcranial sonography of the substantia nigra in the diagnosis of Parkinson's disease: prospective cohort study in 196 patients. BMJ Open, 3, e002613.

Bouwmans, A.E., Weber, W.E., Leentjens, A.F., Mess, W.H. (2016). Transcranial sonography findings related to depression in parkinsonian disorders: cross-sectional study in 126 patients. Peer J, 4, e2037.

Braak, H. (2003). Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging, 24, 197–211.

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., Del T, K. (2004). Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res, 318, 121–134.

Braak, H., Del, T. K. (2008). Invited article: nervous system pathology in sporadic Parkinson disease. Neurology, 70, 1916–1925.

Bradley, F. Boeve. (2010). REM Sleep Behavior Disorder: Updated Review of the Core Features, the RBD-Neurodegenerative Disease Association, Evolving Concepts, Controversies, and Future Directions. Ann N Y Acad Sci, 1184, 15–54.

Buchman, A. S., Shulman, J. M., Nag, S., Leurgans, S. E., Arnold, S. E., Morris, M C., Schneider, J. A., Bennett, D. A. (2012). Nigral pathology and Parkinsonian signs in elders without Parkinson disease. Ann. Neurol, 71, 258–266.

Castellanos, G., Fernández-Seara, M. A., Lorenzo-Betancor, O. (2015). Automated neuromelanin imaging as a diagnostic biomarker for Parkinson's disease. Mov Disord, 30, 945–952.

Cebrian, C. (2014) MHC-I expression renders catecholaminergic neurons susceptible to T-cellmediated degeneration. Nat. Commun, 5, 3633.

Chadery, C., Strafella, A. P. (2018). New Imaging Markers for Movement Disorders. Curr Neurol Neurosci Rep, 18, 22.

Chen, L., Hagenah, J., Mertins, A. (2012). Feature analysis for Parkinson's disease detection based on transcranial sonography image. Med Image Comput Comput Assist Interv, 15, 272–279

Deuschl, G., Bain, P., Brin, M. (2008). Consensus statement of the Movement Disorder Society on tremor. Mov Disord, 13, 2–23.

Djaldetti R., Ziv I., Melamed E. (2006). The mystery of motor asymmetry in Parkinson's disease. Lancet Neurol, 9, 796–02.

Dun-Hui, Li, Ya-Chao, He, Jun, Liu, Sheng-Di, Chen. (2016). Diagnostic Accuracy of Transcranial Sonography of the Substantia Nigra in Parkinson´s Disease: A Systematic Review and Metaanalysis. Sci Rep, 16, 20863.

Eid, M., Gollwitzer, M., Schmitt, M. (2011). Statistik und Forschungsmethoden Lehrbuch. Weinheim: Beltz.

Emre, M., Aarsland, D., Albanese, A. (2004). Rivastigmine for dementia associated with Parkinson's disease. *N Engl J Med*, 351, 2509–2518.

Fahn, S. Parkinson's disease: 10 years of progress, 1997– 2007. (2010). Mov Disord, 25(Suppl. 1), S2–14.

Fearnley, J. M., Lees, A J. (1991). Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain, 1991, 114, 2283–2301.

Fernandes, Rde. C., Rosso, A. L., Vincent, M. B. (2011). Transcranial sonography as a diagnostic tool for Parkinson's disease: a pilot study in the city of Rio de Janeiro, Brazil. Arq Neuropsiquiatr, 69, 892–895.

Fernandes, Rde. C., Berg, D. (2015). Parenchymal imaging in movement disorders. Front Neurol Neurosci, 36, 71–82.

Folstein, M., Folstein, S. E., McHugh, P. R. (1975). Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res, 72, 893–901.

Fujita, K., Vo, A., Sako, W., Peng, S., Mattis, P.J,. … Eidelberg, D. (2016). Parkinson's disease – related network topographies characterized with resting state functional MRI, 38, 617–630.

Gaenslen, A., Unmuth, B., Godau, J. (2008).The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study. Lancet Neurol, 7, 417–424.

Gao, L., Tang, H., Nie, K., Wang, L., Zhao, J., Gan, R., Huang, J., Zhu, R., … Wang, L. (2014). Cerebrospinal fluid alpha- synuclein as a biomarker for Parkinson's disease diagnosis: a systematic review and meta- analysis. Int J Neurosci, 125, 645–654.

George, S., Brundin, P. (2015). Immunotherapy in Parkinson's disease: micromanaging alpha-synuclein aggregation, J.Parkinsons Dis, 5, 413–424.

Gibb, W. R. G., Lees, A. J. (1988). The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease, J Neurol Neurosurg Psychiatry, 51, 745–752.

Goetz, C. G., Stebbins, G. T., LaPelle, N.R. (2012) MDS-UPDRS non-English translation program [abstract]. Mov Disord, 27(suppl 1), 304.

Go C.L., Frenzel A., Rosales R.L., et al. (2012). Assessment of substantia nigra echogenicity in German and Filipino populations using a portable ultrasound system. J Ultrasound Med, 31, 191–196.

Gustafsson, H., Nordstrom, A., Nordstrom, P. (2015). Depression and subsequent risk of Parkinson disease: a nationwide cohort study. *Neurology*, 84, 2422–2429.

Hawkes, C. H. (2008). The prodromal phase of sporadic Parkinson's disease: does it exist and if so how long is it? Mov. Disord, 23, 1799–1807.

Hernandez D. G., Reed X., Singleton A. B. (2016). Genetics in Parkinson disease: Mendelian vs. non-Mendelian inheritance. J Neurochem. 139, 59–74.

Hirsch, E. C., Hunot, S. (2009). Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol, 8, 382–397.

Hoehn, M. M., Yahr, M. D. (1967). Parkinsonism: onset, progression and mortality. Neurology, 17, 427–442.

Hughes, A. J., Daniel, S. E., Shlomo, Y. B., Lees, A. J. (2002) The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain, 125, 861–870.

Iova, A. (2004). Postnatal decrease in substantia nigra echogenicity. Implications for the pathogenesis of Parkinson's disease. J. Neurol, 251, 1451–1454.

Iranzo A., Santamaria J., Vilaseca I. (2007). Absence of alterations in serum sex hormone levels in idiopathic REM sleep behavior disorder. Sleep, 30, 803–806.

Iranzo, A., Valldeoriola, F., Lomeña, F. (2011). Serial dopamine transporter imaging of nigrostriatal function in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a prospective study. Lancet Neurol, 10, 797–805.

Iranzo, A., Tolosa, E., Gelpi, E., Molinuevo, J.L., Valldeoriola, F., Serradell, M., Sanchez-Valle, … Santamaria, J. (2013). Neurodegenerative disease status and post-mortem pathology in idiopathic rapid-eye-movement sleep behaviour disorder: an observational cohort study. Lancet Neurol, 12, 443–453.

Jenkinson, C., Fitzpatrick, R., Peto, V., Greenhall, R., Hyman, N. (1997). The Parkinson's Disease Questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. Age Ageing, 26, 353–357.

Jennings, D., Siderowf, A., Stern M. (2014) Imaging prodromal Parkinson disease: the Parkinson Associated Risk Syndrome Study. Neurology, 83, 1739–1746.

Jennings, D., Stern, M., Siderowf, A., Eberly, S., Oakes, D., Marek, K. (2015). Longitudinal imaging and phenoconversion in the PARS Prodromal Cohort, [abstract].30(suppl 1), 998.

Jesus-Ribeiro, Sargento-Freitas, J., Sousa, M., Silva, F., Freire, A., Januario, C.(2016). Substantia nigra hyperechogenicity does not correlate with motor features in Parkinson´s disease. Journal of the Neurological Sciences, 364, 9–11.

Ji, W. H., Yebin, D., Ahn, W. S., Kim, C., Min, S., Seomg, J. J., Yoo, S. S., Yung, B., Jong, M. K. (2018). Psychiatric Manifestation in Patients with Parkinson's Disease. J Korean Med Sci, 19, e 300.

Kadastik-Eerme, L., Muldmaa, M., Lilles, S., Rosenthal, M., Taba, N., Taba, P. (2016). Nonmotor Features in Parkinson's Disease: What Are the Most Important Associated Factors? doi: 10.1155/2016/4370674.

Kadastik-Eerme, L., Taba, N., Asser, T., Taba, P. (2018). The increasing prevalence of Parkinson's disease in Estonia. Acta Neurol Scand, 138, 251–258.

Kempster, P. A., Gibb, W. R., Stern, G. M., Lees, A. J. (1989). Asymmetry of substantia nigra neuronal loss in Parkinson's disease and its relevance to the mechanism of levodopa related motor fluctuations. J Neurol Neurosurg Psychiatry, 52, 72–76.

Kempster, P. A., Williams, D. R., Selikhova, M., Holton, J., Revesz, T., Lees, A. J. (2007). Patterns of levodopa response in Parkinson's disease: a clinico-pathological study. Brain, 130, 2123–2128.

Kim, J. Y., Kim, S. T., Jeon, S. H., Lee, W. Y. (2007). Midbrain transcranial sono-graphy in Korean patients with Parkinson's disease. Mov Disord, 22, 1922–1926.

Lang, A. E. (2011). A critical appraisal of the premotor symptoms of Parkinson's disease: Potential usefulness in early diagnosis and design of neuroprotective trials. Mov Disord, 26, 775–783.

Lauckaite, K., Rastenyte, D., Surkiene, D., Vaidelyte, B., Dambrauskaite, G., Saka-lauskas, A. (2014). Ultrasonographic (TCS) and clinical findings in overlapping phenotype of essential tremor and Parkinson´s disease (ET-PD). BMC Neurology, 12.https… //doi.org/10.1186/1471-2377-12-12

Lees, A. J., Hardy, J., Revesz, T. (2009). Parkinson's disease. Lancet, 373, 2055–2066.

Lobsien, E., Schreiner, S., Plotkin, M., Kupsch, A., Schreiber, SJ., Doepp, F. (2012). No correlation of substantia nigra echogenicity and nigrostriatal degradation in Parkinson's disease. Mov Disord, 27, 450–453.

Ma, S.Y., Roytt, M., Collan, Y., Rinne, J. O. (1999). Unbiased morphometrical measurements show loss of pigmented nigral neurones with ageing. Neuropathol. Appl. Neurobiol, 25, 394–399.

Ma, B. (2016). LRRK2 modulates microglial activity through regulation of chemokine (C-X3-C) receptor 1-mediated signalling pathways. Hum. Mol. Genet, 25, 3515–3523.

Mahlknecht, P., Seppi, K., Stockner, H. (2013). Substantia nigra hyperechogenicity as a marker for Parkinson's disease: a population-based study. Neurodegener Dis, 12, 212–218.

McGeer, PL., McGeer, EG. (2008). Glial reactions in Parkinson's disease. Mov Disord, 23, 474–483.

Mehnert, S., Reuter, I., Schepp, K., Maaser, P., Stolz, E., Kaps, M. (2010). Transcranial sonography for diagnosis of Parkinson's disease. BMC Neurol, 21, 10:9.

Monaco, D., Berg, D., Thomas, A., Di Stefano, V., Barbone, F:, Vitale, M., Ferrante, C., Bonanni, L., Nicola, M., Garzarella, T., Franciotti, R. (2018). Neurological Sciences, 39, 1887–1894.

Montgormery, E. (2013). Predictors of Parkinson's disease – not quite sound, Mov Disord, 28, 413–415.

Morrish, P. K., Rakshi, J. S., Bailey, D. L., Sawle, G. V., Brooks, D. J. (1998). Measuring the mutations in a cohort of patients with idiopathic REM sleep behavior disorder. Neurology, 86, 1072–1073.

Obeso, J. A., Stamelou, M., Goetz, C. G., Poewe, W., Lang, A. E., Weintraub, D., Burn, D., Stoessl, J., HM. (2017). Past, Present, and Future of Parkinson's Disease: A Special Essay on the 200th Anniversary of the Shaking Palsy. Mov Disord, 32, 1264–1310.

Okawa, M., Miwa, H., Kajimoto, Y. (2007). Transcranial sonography of the substantia nigra in Japanese patients with Parkinson's disease or atypical parkinsonism: clinical potential and limitations. Intern Med, 46, 1527–1531.

Peran, P, Cherubini, A., Assogna, F. (2010). Magnetic resonance imaging markers of Parkinson's disease nigrostriatal signature. *Brain*, 133, 3423–3433.

Plate, A., Ahmadi, S. A., Pauly, O. (2012) Three-dimensional sonographic examination of the midbrain for computer-aided diagnosis of movement disorders. Ultrasound Med Biol, 38, 2041–2050.

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A. E., Lang, A. E. (2017). Parkinson disease. Nat Rev Dis Primers, 23, 1–21.

Pont-Sunyer, C., Hotter, A., Gaig, C. (2015).The onset of nonmotor symptoms in Parkinson's disease (the ONSET PDStudy). Mov Disord, 30, 229–237.

Postuma, R. B., Iranzo, A., Högl, B. (2015a). Risk factors for neurodegeneration in idiopathic rapid eye movement sleep behavior disorder: a multicenter study. Ann Neurol, 77, 830–839.

Postuma, R. B., Berg, D., Stern, M. (2015b). MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord, 2015, 30, 1591–1601.

Postuma, R. B., Berg, D., Stern, M. (2016a). Abolishing the 1-year rule: how much evidence will be enough? Mov Disord, 31, 1623–1627.

Postuma, R. B., Berg, D. (2016b). Advances in markers of prodromal Parkinson disease. Nat Rev Neurol, 12, 622–634.

Postuma R.B, Poewe W, Litvan I, Lewis S, Lang A. E, Halliday G, Goetz C.G, Chan P, Slow E, Seppi K, Schaffer E, Rios-Romenets S, Mi T, Maetzler C, Li Y, Heim B, Bledsoe IO, Berg D. (2018). Validation of the MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 33, 1601–1608.

Raglione, L. M., Sorbi, S., Nacmias, B. (2011). Osteoporosis and Parkinson's disease. Clin Cases MinerBone Metab, 8, 16–18.

Ramirez, A. (2006). Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat. Genet, 38, 1184–1191.

Ransohoff, R. M. (2016). How neuroinflammation contributes to neurodegeneration. Science, 353, 777–783.

Reeve, A., Simcox, E., Turnbull, D. (2014). Ageing and Parkinson's disease: Why is advancing age the biggest risk factor? Ageing Research Reviews, 100, 19–30.

Reijnders, J. S. A. M., Ehrt, U., Weber, W. E. J., Aarsland, D., & Leentjens, A. F. G. (2008). A Systematic Review of Prevalence Studies of Depression in Parkinson's Disease. Mov Disord, 23, 183–189.

Ruprecht-Dörfler, P., Berg, D., Tucha, O., Benz, P., Meier-Meitinger, M., Alders, GL., Lange, KW., Becker, G. (2003). Echogenicity of the substantia nigra in relatives of patients with sporadic Parkinson's disease. Neuroimage, 18, 416–22.

Sadowski, K., Serafin-Krol, M., Szlachta, K., Friedman, A. (2015). Basal ganglia echogenicity in tauopathies. Journal of Neural Transmission, 122, 1635–1640.

Sakalauskas, A., Lukoševičius, A., Laučkaitė, K. (2013). Automated segmentation of transcranial sonographic images in the diagnostics of Parkinson's disease. Ultrasonics, 53, 111–121.

Savoiardo M. (2003). Differential diagnosis of Parkinson's disease and atypical parkinsonian disorders by magnetic resonance imaging. Neurol Sci, 24, S35–7.

Sauerbier,A., Jenner P., Todorova, A., Chaudhuri KR. (2016). Non motor subtypes and Parkinson's disease. Parkinsonism Relat Disord, 22, 41–46.

Schapansky, J., Nardozzi, J. D., Felizia, F., LaVoie, MJ. (2014). Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. Hum. Mol. Genet, 23, 4201–4214.

Schenck, C. H. (2013a). Rapid eye movement sleep behavior disorder: devising controlled active treatment studies for symptomatic and neuroprotective therapy – a consensus statement from the International Rapid Eye Movement Sleep Behavior Disorder Study Group. Sleep Med, 14, 795–806.

Schenck, C.H., Boeve, B.F., Mahowald, M.W. (2013b). Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16-year update on a previously reported series. Sleep Med, 14, 744–748.

Schrag, A., Barone, P., Brown, R. G., Leentjens, A. F., McDonald, W. M., Starkstein, S., Weintraub, D., Sampaio. (2007). Depression rating scales in Parkinson's disease: critique and recommendations. Mov. Disord, 22, 1077–1092.

Schwab, R. S., England Jr., A. C., Poskanzer, D. C., Young, R. R. (1969). Amantadine in the treatment of Parkinson's disease. JAMA, 208, 1168–1170.

Schweitzer, K. J., Hilker, R., Walter, U., Burghaus L., Berg, D. (2006). Substantia nigra hyperechogenicity as a marker of predisposition and slower progression in Parkinson's disease. Mov Disord, 21, 94–98.

Shu-yang, Yu., Chen jie, Cao., Li jun, Zuo., Ze jie, Chen., Teng hong, Lian., Fang Wang, Yang, Hu., Ying shan, Piao., Wei, Zhang. (2018). Clinical features and dysfunctions of iron metabolism in Parkinson disease patients with hyper echogenicity in substantia nigra: a cross-sectional study. BMC Neurol, 18. 9.

Školoudík, D., Fadrná, T., Bártová, P., et al. (2007). Reproducibility of sonographic measurement of the substantia nigra. Ultrasound Med Biol, 33, 1347–1352.

Školoudík, D., Walter, U. (2010). Method and validity of transcranial sonography in movement disorders. Int Rev Neurobiol, 90, 7–34.

Školoudík, D., Herzig, R., Blahuta, J. (2013). Comparison of automatic and manual transcranial sonographic morphometric measurement of the substantia nigra. Neurology, 80 (Meeting Abstracts 1), S39.006.

Školoudík, D., Jelínková, M., Blahuta, J., Čermák, P., … Herzig, R. (2014). Transcranial Sonography of the Substantia Nigra: Digital Image Analysis. American Journal of Neuroradiology, 35, 2273–2278.

Slaughter, J. R., Slaughter, K. A., Nichols, D., Holmes, S. E., Martens, M. P. (2001). Prevalence, clinical manifestations, etiology, and treatment of depression in Parkinson's disease. JNP 13, 187–196.

Spiegel, J., Hellwig, D., Möllers, M. O. (2006). Transcranial sonography and [123I] FP-CIT SPECT disclose complementary aspects of Parkinson's disease. Brain, 129, 1188–1193.

Stockner, H., Sojer, M., Seppi, K. (2007). Midbrain sonography in patients with essential tremor. Mov Disord, 22, 414–417.

Stockner H, Sojer M, Seppi K et al. (2012). Midbrain sonography in patients with essential tremor. Mov Disord, 22, 414–417.

Szewczyk-Krolikowski, K., Menke, R. A. L., Rolinski, M. (2014). Functional connectivity in the basal ganglia network differentiates PD patients from controls. Neurology, 83, 208–214.

Taba, P., Asser, T. (2002). Prevalence of Parkinson's disease in Estonia. Acta Neurol Scand, 106, 276–281.

Titova, N., Padmakumar, C., Lewis, S., Chaudhuri, K. Ray. (2017). Parkinson's : a syndrome rather than a disease? J Neural Transm (Vienna), 124, 907–914.

van der Hoek TC., Bus BA., Matui P., van der Marck MA., Esselink RA., Tendolkar I. (2011). Prevalence of depression in Parkinson's disease: effects of disease stage, motor subtype and gender. J Neurol Sci, 310, 220–224.

Van de Loo, S., Walter, U., Behnke, S., et al. (2010). Reproducibility and diagnostic accuracy of substantia nigra sonography for the diagnosis of Parkinson's disease. J Neurol Neurosurg Psychiatry, 81, 1087–1092.

Vilas, D., Shaw, L. M, Taylor, P. (2016). Cerebrospinal fluid biomarkers and clinical features in leucine-rich repeat kinase 2 (LRRK2) mutation carriers. *Mov Disord*. 2016, 31, 906–914.

Walter, U., Wittstock, M., Benecke, R., Dressler, D. (2002). Substantia nigra echogenicity is normal in non-extrapyramidal cerebral disorders but increased in Parkinson's disease. J Neural Transm, 109, 191–196.

Walter, U., Klein, C., Hilker, R., Benecke, R., Pramstaller, P. P., Dressler, D. (2004). Brain parenchyma sonography detects preclinical parkinsonism. Mov Disord, 19, 1445–1449.

Walter, U., Dressler, D., Wolters, A., Wittstock, M., Benecke, R. (2007a). Transcranial brain sonography findings in clinical subgroups of idiopathic Parkinson's disease. Mov Disord, 22, 48–54.

Walter, U., Prudente-Morrissey, L., Herpertz, S. C., Benecke, R., Hoeppner, J., (2007b). Relationship of brainstem raphe echogenicity and clinical findings in depressive states. Psychiatry Res. Neuroimaging, 155, 67–73.

Walter, U. (2009). Transcranial brain sonography findings in Parkinson's disease: implications for pathogenesis, early diagnosis and therapy. Expert rev Neurother, 9, 835–846.

Walter, U. (2013). How to measure substantia nigra hyperechogenicity in Parkinson's disease: detailed guide with video. J Ultrasound Med, 32, 1837–1843.

Walter, U., Blitzer, A., Benecke, R. (2014). Sonographic detection of basal ganglia abnormalities in spasmodic dysphonia. Eur J Neurol, 21, 349–352.

Walter, U., Školoudík, D,. (2014b). Transcranial Sonography (TCS) of Brain Parenchyma in Movement Disorders: Quality Standards, Diagnostic Applications and Novel TechnologiesTranscranial. Ultraschall in Med, 35, 322–331.

Weise, D., Lorenz, R., Schliesser, M., Schirbel, A., Reiners, K., Classen, J. (2009). Substantia nigra echogenicity: a structural correlate of functional impairment of the dopaminergic striatal projection in Parkinson's disease. Mov Disord, 24, 1669–1675.

Wile, DJ., Dinelle, K., Vafai, N. (2016). A scan without evidence is not evidence of absence: scans without evidence of dopaminergic deficit in a symptomatic leucine-rich repeat kinase 2 mutation carrier. Mov Disord, 31, 405–409.

Zecca, L., Gallorini, M., Schunemann, V. (2001). Iron, neuromelanin and ferritin content in the substania nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes. J Neurochem, 76, 1766–1773.

Zucca, F. A., Segura-Aguilar, J., Ferrari, E. (2017). Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. Prog Neurobiol, 155, 96–119.

Yilmaz, R., Berg, D. (2018). Transcranial B-Mode Sonography in Movement Disorders. Int Rev Neurobiol, 143, 179–212.

9. SUMMARY IN ESTONIAN

Transkraniaalne aju ultraheliuuring Eesti parkinsoni tõve haigetel

Parkinsoni tõbi (PT) on sageduselt teine liigutushäire. PT juhtivaks histopatoloogiliseks muutuseks on *Substantia nigra (SN)* degeneratsioon ning tsütoplasmaatilised inklusioonid-Lewy kehakesed- degenereeruvates rakkudes. PT kliinilistele avaldustele eelneb pikaajaline prekliiniline periood (Obeso et al., 2017). Kliiniliste sümptomite avaldumise hetkeks on dopamiini tase langenud 20%ni normaalsest (Poewe et al., 2017). PT diagnoosimine võib olla keerukas, millele viitab asjaolu, et patoanatoomilisel uuringul ei kinnitunud diagnoos kuni 25% haigetest (Hughes et al., 2002). Kuna haigus on hiiliva algusega ja selle esimesed sümptomid on sageli mittespetsiifilised, siis diagnoositakse PT tavaliselt mõne aasta jooksul pärast esmaste sümptomite tekkimist. Esmassümptomiteks võivad olla ka nn mittemotoorsed sümptomid, mis võivad esineda ka ilma PT-ta: nt lõhnatundlikkuse alanemine, kõhukinnisus, unehäired ja depressioon (Walter et al. 2014b, Titova, et al., 2017).

PT diagnoos on kliiniline; rutiinselt kasutatavaid radioloogilisi ega laboratoorseid diagnoosi meetodeid pole kasutusel nende hinna, samuti veel piisava tõenduspõhise teabe puudumise tõttu. Üheks innovaatilisemaks diagnostikameetodiks PT diagnostikas peetakse transkraniaalset aju ultraheliuuringut, mida esmaselt kirjeldas 1995. aastal Georg Becker, avastades keskaju leiduva *substantia nigra* hüperehhogeensuse (SN+) (Becker et al., 1995). Tänaseks on selgunud, et SN+ ei ole nähtav kõigil patsientidel. SN+ peetakse oluliseks bioloogiliseks markeriks varase PT esinemise hindamisel, kuivõrd seda on leitud üle 90% PT patsientidest (Berg et al., 2001). Sealjuures on ilmnenud, et SN+ on enamväljendunud kliinilisele sümptomaatikale vastaspoolel (Berg et al., 2001). Kuigi SN+ esineb mõlemapoolselt, on see muutus asümmeetriline ja korreleerub hästi kliinilise leiuga, erinevalt nt Lewy kehakeste haigusest, mille puhul SN+ on enamasti sümmeetriline. Samuti on leitud, et SN+ oli enam väljendunud monogeense pärilikkuse tüübiga juhtudel. SN+ võib erinevate geneetiliste mutatsioonide korral varieeruda (Walter et al., 2013).

SN kajarikkaks ehk hüperehhogeenseks muutumise bioloogiline alus pole teada. Püstitatud on hüpoteesid, mis seostavad SN+ dopaminergiliste neuronite hävimisega, SN rakkude arvu vähenemisega, SN muutunud mahu või koekoostise muutumisega. Kõige enam on leitud muutusi SN rauasisalduses. Seni on loom- ja surmajärgsetes inimuuringutes õnnestunud näidata vaid SNs raua suurenenud sisaldust SN+ korral (Berg et al., 2006). Arvatakse, et SN suuremat ehhogeensust põhjustab ferritiiniga sidumata rauakoguse suurenemine. See arvamus toetub haigete surmajärgsetele uuringutele, kus SNis leiti neuromelaniini vähenenud kontsentratsioon ja raua kogunemist ning näidati positiivset korrelatsiooni H- ja L-ferritiini hulga ning SNi ehhogeensuse vahel (Berg et al., 2006). Lisaks raua kogunemisele on SN rakkude degeneratsioonis oma osa ka teistel protsessidel, sealhulgas endo- ja eksotoksiinide toimel (Zecca

et al., 2004). PT haigete SNs esineb neuronaalsele degeneratsioonile järgnev gliiarakkude arvu suurenemine (Berg et al., 2010). Neuronite hukk ja gliiarakkude hulga kasv muudab neuronite ning gliiarakkude arvulist suhet ja võib seeläbi mõjutada koeomadusi, põhjustades hüperehhogeensust (Hagenah et al., 2010).

Uurimistöö eesmärgid

1. Uurida prospektiivselt transkraniaalse aju ultraheliuuringuga SN esinemist PT põdeval ulatuslikul Eesti patsientide rühmal ja võrrelda saadud tulemusi tervetel samaealistel inimestel, leides parima diagnostilise täpsusega SN+ (cm^2), mille abil edaspidi diagnoosida PT Eesti patsientidel.
2. Kirjeldada PT põdevatel Eesti haigetel depressiivsete sümptomite ilmnemist ja nende seost ajutüves asuvate *Raphe* tuumade ehhogeensuse esinemise põhjal, lähtuvalt transkraniaalse aju ultraheli uuringust.
3. Analüüsida ja kirjeldada, millistel tingimustel esineb SN+ asümmeetrilisus PT patsientidel ja milline on SN+ samaealistel tervetel uuritavatel.

Uuritavad ja meetodid

Uuringu kiitis heaks Tartu Ülikooli inimuuringute eetikakomitee. Kõik uuringus osalejad allkirjastasid teadliku nõusoleku vormi. Uuritavad leiti Ida-Tallinna Keskhaigla neuroloogiakeskuse ja Tartu Ülikooli Kliinikumi närvikliiniku neuroloogia osakonna andmebaasist.

Uuring oli prospektiivne ja läbilõikeline, kus osales kokku 300 Parkinsoni tõve patsienti, kes vastasid Ühendkuningriigi Parkinsoni Tõve Ajupanga diagnoosikriteeriumitele, ja 200 samaealisest tervet kontrollisikut, kes leiti Ida-Tallinna Keskhaigla Parkinsoni tõve patsientide lähedaste seast, vanemaealiste laulukoori liikmete ja nende abikaasade hulgast ning kaasabil, mõned uuritavad olid haigla personali seast ja mõned, kelle puhul polnud teada Parkinsoni tõbe, olid patsientidena ravil mõne muu mitte-neurodegeneratiivse haigusega (peavalu, neuropaatia). Parkinsoni haigetele oli eelnevalt läbi viidud neurovisualiseerimise uuring (kompuutertomograafia või magnetresonantstomograafia), kontrollgrupi liikmetele ei teostatud eelnevalt mingit aju visualiseerimisuuringut.

Haigeid ja kontrollgrupi liikmeid intervjueeriti ning hinnati nende kliinilist neuroloogilist leidu: parkinsonistlikku staatust, haiguse raskusastet, kognitiivset funktsiooni, meeleolu. Küsitleti haiguse tekkeaja kohta. Kui patsient seda ei teadnud, kontrolliti esmasümptomite tekkeaega ja haigestumist patsiendi haigusloost. Kõiki kliinilisi teste viis läbi üks uurija, kes ei olnud teadlik hiljem teostatava transkraniaalse ultraheli uuringu tulemustest.

Kliinilistest skaaladest kasutati järgmisi rahvusvaheliselt tunnustatud skaalasid: Parkinsoni tõve hindamise ühtlustatud skaala III osa (MDS-UPDRS

III), Hoehn Yahri skaalat (HYS), Schwab England (SE) igapäevaste tegevuste skaalat, mille abil hinnatakse inimese iseseisvust protsentuaalselt 0 −100 (100% on täiesti iseseisev, ei tunneta mingeid raskusi; 0% korral on säilinud vaid vegetatiivsed funktsioonid, vaimse seisundi miniuuringut (MMSE) ja Becki depressiooniküsimustikku, lõhnatundlikkuse hindamiseks kasutati Sniffin Sticks 12-osalist lõhnapulkade testi. Patsientidel arvutati antiparkinsonistlike ravimite raviannused, kasutades levodopa ekvivalent-doosi arvutusskeemi. Transkraniaalse aju ultraheli uuringu viis läbi teine uurija, kellel puudus informatsioon kliiniliste testide andmete kohta. Kõik uuringu osad, nii kliiniline intervjuu, patsiendi läbivaatus kui transkraniaalne aju ultraheli uuring viidi läbi samal päeval.

Transkraniaalne aju ultraheli uuring teostati pimendatud ruumis, patsient oli poollamavas asendis spetsiaalsel toolil. Ultraheli teostati ultraheliaparaadiga Logic 7, mil kasutati ultraheliandurit kiirgussagedusega 1.8–3.6 MHz (General Electric, USA). Kõikidel uuritavatel viis uuringu läbi üks uurija. Kõik uuringud viidi läbi ühesugust uurimismeetodit kasutades. Alustati kõrva ees asuva oimupiirkonna akustilise luuakna positsiooniga. Heaks uuringu teostamise orientiiriks oli orbitomentaaljoon, millega paralleelselt suunati ka ultraheliandur. Selle abil oli keskaju nähtav iseloomuliku hüpoehhogeense liblikakujulise struktuurina. Suurendusel oli keskajus lindikujulise struktuurina hästi nähtav SN, mida ümbritsesid hüperehhogeensed basaaltsisternid. Ajutüve sees keskjoonel asuvad hüperehhogeensed *Raphe* tuumad, mida kirjeldati kolme punkti skaalat kasutades; 2 – ei ole hinnatav; 1 – osaliselt hinnatav; 0 – täielikult hinnatav).

Ultraheli andurit mõne kraadi võrra kraniaalsele kallutades ilmusid hüpoehhogeensete struktuuridena kolmas ajuvatsake ja külgvatsakese eesmine sarv, olles ümbritsetud hüperehhogeense ependüümiga. Samuti asusid sellel tasandil taalamus ja basaalganglionid – sabatuum ja läätstuum, mis tervetel isikutel on madala ehhogeensuse tõttu praktiliselt eristamatud ümbritsevast valgeainest. Basaalganglione hinnati vastaspoolse oimuluu kaudu. SNi peetakse hüperehhogeenseks, kui see peegeldab ultrahelisignaali ebanormaalselt suure intensiivsusega ümbritseva valgeainega võrreldes või kui ehhogeense struktuuri pindala on suurem kui tervete isikute kontrollvalimis. SN leidmisel seisati ultraheliaparaadi ekraanile kuvatud pilt, suurendati seda 2−3 korda ja ümbritseti seejärel kursorjoonega kõige laiemas kohas. Selle alusel leiti ultraheliaparaadi arvutusliku mõõtmise tulemusel ristlõikepindala (cm^2). Samasugust uuringut teostati ühel patsiendil kolmel järjestikusel korral paremal ja vasakul poolel. Nii arvutati kõigi kuue mõõtmise keskmine, kolme mõõtmise keskmised paremal ja vasakul ning hinnati ühe mõõtmise tulemused. Enamiku seadmete puhul on normileiuks SN+ pindala alla 0,2 cm^2 ning väärtused üle 0,25 cm^2 liigitatakse selgelt hüperehhogeenseks. Kõigil uuritavatel hinnati kõiki kliinilisi parameetreid ja ultraheli mõõtmistulemusi samaaegselt ühe protokolli alusel. Vastavalt uuringu eesmärkidele saadi uuringutulemused statistilisi meetodeid kasutades.

Uurimistöö tulemused ja arutelu

Uuringust jäid välja isikud, kelle ultraheliuuringu tulemust ei olnud halva luuakna läbitavuse tõttu võimalik hinnata. Parkinsoni haigete grupist langes välja 34 isikut ja kontrollisikute grupist 32 isikut. Enam langes uuringus välja PT grupist naisi (25%); mehi (9%). Kontrollgrupist langes välja 12% naisi ja 6% mehi.

Kirjanduse andmetel võib sagedasemaks halva luulise läbitavuse põhjuseks olla osteoporoos või oimuluu paksus. Meie ei uurinud konkreetsete uuritavate väljalangemise põhjust. Seega jäi hinnatavate uuringugruppide suuruseks 266 Parkinsoni tõve haiget vanuses 37 kuni 87 aastat ja 168 tervet kontrollisikut vanuses 50 kuni 86 aastat.

Haiguse anamneesile toetudes leidsime, et uuringuhetkeks oli esmaste sümptomite tekkimisest möödunud 6,1±5,2 aastat, vahemikus 1–25 aastat. Keskmine MDS UPDRS III motoorne skoor oli haigetel 31,23±15,1 ja kontrollisikutel 3,0±2,2. Lõhnatesti 12 lõhnast tundsid haiged 5,6±0,19 ja terved 8,6±0,18. Parkinsoni haigete levodopa ekvivalentdoos oli 418±366 mg. Transkraniaalsel sonograafial leitud SN+ mõõtmistulemuste alusel leidsime, et parim diagnostiline täpsus haigete ja tervete grupi liitmisel oli SN+ 0,23cm^2, mis leiti kolme mõõtmistulemuse liitmisel paremalt ja vasakult. SN+ 0,23cm^2 andis parima sensitiivsuse 88,7% ja spetsiifilisuse 92,2% nn mitme mõõtmise keskmise arvutamisel. Pisut ebatäpsem oli SN+ (0,23cm^2) mitme mõõtmise mediaani arvutamisel, kuid mitte statistiliselt olulise vahega. Üksikmõõtmise SN+ 0,21cm^2 andis küll parema sensitiivsuse 93.2%, kuid halvema spetsiifilisuse 85.1%, mistõttu mitme mõõtmise ja üksikmõõtmise võrdluses oli mitme mõõtmise tulemus statistiliselt erinev (p=0,0021). Kuivõrd mitme mõõtmise keskmise ja mediaani vahel oluline statistiline vahe (p=0,18) puudub, on igapäevases töös kolme mõõtmise keskmise kasutamine piisav. Kasutades optimaalselt parimat diagnostilist SN+ 0,23cm^2, kasutasime sama näitajat 75 ja 90 protsentiilidele vastavalt tervetel isikutel ja leidsime, et 75 protsentiili näitaja juures 0,18 ja 90 protsentiili näitaja 0,22cm^2, suudab meie saadud tulemus 0,23cm^2 eristada haiged tervetest (AUC, 96,4; p<0.0001). Leidsime, et Eesti rahvastikul on võrreldes teiste riikide kirjeldatud üldrahvastikuga sarnane SN+ ning parimaks diagnostiliseks väärtuseks haigete ja tervete eristamisel Eesti rahvastikus on SNi pindala 0,23 cm^2.

Kasutades depressioonisümptomite raskust BDI skaala alusel ja *Raphe* tuumade ehhogeensuse hindamise skaalat, esines nende vahel oluline seos (p<0,001). Tervetel kontrollgrupi isikutel, kellel olid *Raphe* tuumad kas täie- likult või osaliselt nähtavad, leidus ka vähem depressiooni sümptomeid. Samas, kui võrdlesime PT ja tervete gruppi eraldi, ilmnes *Raphe* tuumade nähtavuse korral statistiline erinevus vaid osaliselt. Kõrgem BDI skoor kinnitas raskemate depressiivsete sümptomite olemasolu, mis omakorda oli otseselt seotud suurema vanusega, pikema haiguskestvuse ja raskema toimetuleku häirega. *Raphe* tuumade ehhogeensus oli statistiliselt olulisemalt seotud BDI raskema skooriga nii PT haigetel kui tervetel kontrollisikutel. Leidsime, et kõige tugevam oli

korrelatsioon BDI skoori, *Raphe* tuumade ehhogeensuse, vanuse, haiguse kestvuse ja HYS'ga (r=0,5, p<0,001). BDI alusel esines kergeid depressiooni-sümptomeid 38,7% ja raskeid 35,7% patsientidest ning kergeid depressiooni-sümptomeid 26,8% ja raskeid sümptomeid 28,6% kontrollisikutest. Vaid väike arv uuritavatest kasutas uuringu hetkel antidepressante (n=20; 7,5%). Kerge depressiooni sümptomitega oli 6.8% ja raske depressiooni sümptomitega 13,7% patsientidest. Neil uuritavatel, kes kasutasid antidepressante, olid depressiooni sümptomid väljendunumad

Kolmandas uuringus selgus, et SN+ on suurem Parkinsoni tõve haigete grupis (0,33 ± 0,10 cm^2) kui tervete kontrollisikute grupis (0,16± 0,05 cm^2, p<0.001). Statistilist erinevust ei ilmnenud aga vasaku ja parema SN+ vahel (vasakul 0.35± 0.11cm^2; paremal: 0,36± 0.13cm^2, p=0,31). Sealjuures esines oluline statistiline erinevus paaristesti alusel, (p<0,001) olles SN+ sõltuvalt esmaste haigusümptomitele vastaspoolses SNs. Nii oligi esmassümptomitega aju-poolel SN+ (0.35±0,12cm^2) ja mitte-esmassümptomite ajupoolel (0,30±0,10cm^2), erinedes kahe poole võrdluses 17,6%. Multiipelset lineaarregressiooni mudelit kasutades leidsime, et kliinilised parameetrid nagu patsiendi vanus, HYS, MDS UPDRS III osutusid statistiliselt oluliseks SN+, olles seotud esmaste haigus-sümptomite avaldumisega (p=0.0015). Taoline seos puudus mitte-esmas-sümptomitega SN+ (p=0,17). Lisaks uurisime, kuidas väljendub SN+ eakatel haigetel ja tervetel ning leidsime, et SN+ on erineva olemusega. SN+ suureneb tervetel vanuse tõustes ja väheneb esmaste haigussümptomitega seoses. Võimalik, et see on seotud efektiga, et mida kauem on haigus kestnud, seda enam SN+ väheneb ja vastupidi, mida vanemaks inimene elab, seda enam SN+ suureneb. Pole teada, kas selle suurenemise taga võib olla ka haigestumine PT.

Uurimistöö järeldused

Läbiviidud uuring oli esimene transkraniaalse aju ultraheliuuring Eestis, mis viidi läbi ulatuslikus PT patsientide ja tervete kontrollgrupi isikute valimis. PT patsientidest moodustas uuritavate hulk peaaegu 10%. Valideerisime parima diagnostilise väärtusena kasutatava SN+ eesmärgiga diagnoosida Eesti elanikel PT esinemist.
1. Eesti valimi SN+ ei erine teiste riikide valge rassi vastavate valimitega võrreldes. Parim diagnostiline väärtus, mis eristas terveid haigetest ja diag-noosis PT Eesti valimis, oli 0,23cm^2, sensitiivsusega 88,7% ja spetsiifi-lisusega 92,2%. Seejuures kasutasime mitme mõõtmise tulemuse aritmeetilist keskmist võrreldduna mediaanväärtuse või üksikmõõtmise meetoditega.
2. Transkraniaalse aju ultraheliuuringul hinnatav *Raphe* tuumade terviklikkuse määramine on hea meetod, et välja selgitada varjatud depressiooni sümpto-meid, kus patsient neid ei tunnista või hindab ennast valesti. *Raphe* tuumade terviklikkus välistab suure tõenäosusega depressiooni sümptomite olemas-olu.

3. SN+ asümmeetrilisus on seotud esmassümptomite tekkimisega ja meie uuringus on uuritavate vanus olulisim SN+ asümmeetria mõjutaja. SN+ suureneb tervetel vanuse tõustes. Esmaste haigussümptomitega seonduvalt SN+ väheneb. SN+ annab teavet haiguse kulu kohta ja võib olla parem biomarker, kuid vajab kinnitamiseks pikemaajalisi uuringuid.

AKNOWLEDGEMENTS

PUBLICATIONS

CURRICULUM VITAE

Name: Toomas Toomsoo
Date of birth: 20.10.1970, Tartu
Citizenship: Estonian
Phone: 620 7480
E-mail: toomas.toomsoo@itk.ee

Education:
2009–   University of Tartu, Faculty of Medicine, PhD studies in neurology
1997– 2001   University of Tartu, Faculty of Medicine, residency in neurology
1996–1997   University of Tartu, Faculty of Medicine, internship in general medicine
1990–1996   University of Tartu, Faculty of Medicine
1978–1989   Otepää Secondary School

Professional employment:
2005–   East Tallinn Central Hospital, Head of the Center of Neurology
2002–2005   East Tallinn Central Hospital, senior neurologist
2001–2002   East Tallinn Central Hospital, neurologist
                Eppendorf Klinik Hamburg, neurologist

Scientific work and professional organisations:
Fields: neurodegenerative disorders, Parkinson`s disease, tremors, headache, neuroimaging in Parkinson's disease
Membership: Estonian Society of Neurologists and Neurosurgeons, board member
                Estonian Movement Disorder Society, member
                Estonian Headache Society, board member
                European Academy of Neurology, member
                Movement Disorder Society, member
                German Neurological Society, member

Publications:
1. Toomsoo, T., Liepelt-Scarfone, I., Berg, D., Kerner, R., Pool, A. H., Kadastik-Eerme, L., Rubanovits, I., Asser, T., Taba, P. (2019). Effect of Age on Substantia Nigra Hyper-echogenicity in Parkinson's Disease Patients and Healthy Controls.*Ultrasound in Medicine & Biology*, 45, 122–128.
2. Toomsoo T., Pool AH., Liepelt-Scarfone I., Kadastik-Eerme L., Rubanovits I., Asser T., Berg D., Taba P. (2018). The effect of age on Substantia Nigra Hyperechogenicity in PD patients and healthy controls. Movement Disorders, 33, (Suppl 2), S688.
3. Vender K., Toomsoo T., Kalju I., Rubanovits I., Joost K., Taba P. (2018). The first case of Perry syndrome in Estonia. Movement Disorders, 33, (Suppl 2), S225.

4. Milovidov, A., Matsalu, T., Kaarlõp, M., Toomsoo, T. (2018). Kiire kuluga dementsus, mis osutus Creutzfeldt. Jakobi tõveks. Eesti Arst, 97, 213–218.
5. Toomsoo T., Kadastik-Eerme L., Asser T., Taba P. (2018). Lõhnade eristamise erinevused Parkinsoni tõve patsientidel ja tervetel kontrollidel. Eesti Arst, 97(Lisa 2).
6. Kalju I., Toomsoo T., Vender K. (2018). Odor identification in PD patients and controls in Estonian cohort. Movement Disorders, 33, (Suppl 2) S753.
7. Toomsoo, T., Randver, R., Liepelt-Scarfone, I., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2017). Prevalence of depressive symptoms and their association with brainstem raphe echogenicity in patients with Parkinson's disease and non-PD controls. *Psychiatry Res Neuroimaging*, 268, 45–49.
8. Toomsoo, T., Liepelt-Scarfone, I., Kerner, R., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2016). Substantia Nigra Hyperechogenicity: Validation of Transcranial Sonography for Parkinson Disease Diagnosis in a Large Estonian Cohort. *J Ultrasound Med*, 35, 17–23.
9. Nõmm, S., Toomela, A., Kozhenkina, J., Toomsoo, T. (2016). Quantitative analysis in the digital Luria's alternating series tests. In: 2016 14th International Conference on Control, Automation, Robotics Vision (ICARCV) [Phuket, Thailand, 13–15th November 2016], Proceedings (1 –6). IEEE.10.1109/ICARCV.2016.7838746.
10. Nõmm, S., Bardõš, K., Mašarov, I., Kozhenkina, J., Toomela, A., Toomsoo, T. (2016). Recognition and analysis of the contours drawn during the Poppelreuter's test. In: 2016 15th IEEE International Conference on Machine Learning and Applications, ICMLA 18–20 December 2016, Anaheim, California, Proceedings (170 –175). Los Alamitos, Calif: IEEE Computer Society./10.1109/ICMLA.2016.0036.
11. Toomsoo, T., Asser, T., Taba, P. (2015). Transkraniaalne sonograafia Parkinsoni tõve diagnoosimisel. *Eesti Arst,* 94, 1–144.
12. Randver R., Toomsoo T., Bachmann T. (2015). Clinical and therapeutic applications of repeated transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) in patients with Parkinson's disease. Journal of the International Neuropsychological Society, 21, 126 –126.
13. Antsov, E., Silveira-Moriyama, L., Kilk, S., Kadastik-Eerme, L., Toomsoo, T., Lees, A., Taba, P. Adapting the Sniffin' Sticks olfactory test to diagnose Parkinson's disease in Estonia. *Parkinsonism Relat Disord*, 20,830–833.
14. Toomsoo T., Kaasik, A., Margus, S. (2014). The Journal of Headache and Pain 2014, 15 (Suppl 1), M12.
15. Toomsoo T., Rubanovits I. (2014). Transcranial brain sonography in patients with Wilson disease. Movement Disorders, 29 (Suppl 1), S429.
16. Toomsoo T., Rubanovits I. (2014). Transcranial sonography findings and coeliac disease. Movement Disorders, 29, (Suppl 1). S430.
17. Toomsoo, T., Taba, P. (2008). Treemorid. *Eesti Ars*t, 87, 424–430.

ELULOOKIRJELDUS

Nimi: Toomas Toomsoo
Sünniaeg: 20.10.1970, Tartu
Kodakondsus: Eesti
Telefon: 620 7480
E-mail: toomas.toomsoo@itk.ee

Haridus:
2009–   Tartu Ülikool, Meditsiiniteaduste valdkond, doktoriõpe
1997– 2001 Tartu Ülikool, Arstiteaduskond, residentuur
1996–1997   Tartu Ülikool, Arstiteaduskond, üldarstlik internatuur
1990–1996   Tartu Ülikool, Arstiteaduskond, arstiteaduse põhiõpe
1978–1989   Otepää Keskkool

Teenistuskäik:
2005–   Ida Tallinna Keskhaigla, neuroloogiakeskuse juhataja
2002–2005   Ida Tallinna Keskhaigla, neuroloogia osakonna vanemarst
2001–2002   Ida Tallinna Keskhaigla, neuroloog
             Eppendorfi Kliinik, Hamburg, neuroloog

Teadus- ja erialane tegevus:
Valdkonnad: neurodegeneratiivsed haigused, Parkinsoni tõbi, treemorid, peavalu
Liikmelisus: L. Puusepa nimeline Neuroloogide ja Neurokirurgide Selts, juhatuse liige
             Eesti Liigutushäirete Selts, liige
             Eesti Peavalu Selts, juhatuse liige
             Euroopa Neuroloogia Akadeemia, liige
             Liigutushäirete Ühing, liige
             Saksa Neuroloogide Selts, liige

Publikatsioonid:
1. Toomsoo, T., Liepelt-Scarfone, I., Berg, D., Kerner, R., Pool, A. H., Kadastik-Eerme, L., Rubanovits, I., Asser, T., Taba, P. (2019). Effect of Age on Substantia Nigra Hyper-echogenicity in Parkinson's Disease Patients and Healthy Controls.*Ultrasound in Medicine & Biology*, 45, 122–128.
2. Toomsoo T., Pool AH., Liepelt-Scarfone I., Kadastik-Eerme L., Rubanovits I., Asser T., Berg D., Taba P. (2018). The effect of age on Substantia Nigra Hyperechogenicity in PD patients and healthy controls. Movement Disorders, 33, (Suppl 2), S688.
3. Vender K., Toomsoo T,. Kalju I., Rubanovits I., Joost K., Taba P. (2018). The first case of Perry syndrome in Estonia. Movement Disorders, 33, (Suppl 2), S225.

4. Milovidov, A., Matsalu, T., Kaarlõp, M., Toomsoo, T. (2018). Kiire kuluga dementsus, mis osutus Creutzfeldt. Jakobi tõveks. Eesti Arst, 97, 213–218.
5. Toomsoo T., Kadastik-Eerme L., Asser T., Taba P. (2018). Lõhnade eristamise erinevused Parkinsoni tõve patsientidel ja tervetel kontrollidel. Eesti Arst, 97(Lisa 2).
6. Kalju I., Toomsoo T., Vender K. (2018). Odor identification in PD patients and controls in Estonian cohort. Movement Disorders, 33, (Suppl 2) S753.
7. Toomsoo, T., Randver, R., Liepelt-Scarfone, I., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2017). Prevalence of depressive symptoms and their association with brainstem raphe echogenicity in patients with Parkinson's disease and non-PD controls. *Psychiatry Res Neuroimaging*, 268, 45–49.
8. Toomsoo, T., Liepelt-Scarfone, I., Kerner, R., Kadastik-Eerme, L., Asser, T., Rubanovits, I., Berg, D., Taba, P. (2016). Substantia Nigra Hyper-echogenicity: Validation of Transcranial Sonography for Parkinson Disease Diagnosis in a Large Estonian Cohort. *J Ultrasound Med*, 35, 17–23.
9. Nõmm, S, Toomela, A., Kozhenkina, J., Toomsoo, T. (2016). Quantitative analysis in the digital Luria's alternating series tests. In: 2016 14th International Conference on Control, Automation, Robotics Vision (ICARCV): [Phuket, Thailand, 13–15th November 2016], Proceedings (1 – 6). IEEE.10.1109/ICARCV.2016.7838746.
10. Nõmm, S., Bardõš, K., Mašarov, I., Kozhenkina, J., Toomela, A., Toom-soo, T. (2016). Recognition and analysis of the contours drawn during the Poppelreuter's test. In: 2016 15th IEEE International Conference on Machine Learning and Applications, ICMLA: 18–20 December 2016, Anaheim, California, Proceedings (170–175). Los Alamitos, Calif: IEEE Computer Society./10.1109/ICMLA.2016.0036.
11. Toomsoo, T., Asser, T., Taba, P. (2015). Transkraniaalne sonograafia Parkinsoni tõve diagnoosimisel. *Eesti Arst,* 94, 1–144.
12. Randver R., Toomsoo T., Bachmann T. (2015). Clinical and therapeutic applications of repeated transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) in patients with Parkinson's disease. Journal of the International Neuropsychological Society, 21, 126–126.
13. Antsov, E., Silveira-Moriyama, L., Kilk, S., Kadastik-Eerme, L., Toom-soo, T., Lees, A., Taba, P. Adapting the Sniffin' Sticks olfactory test to diagnose Parkinson's disease in Estonia. *Parkinsonism Relat Disord*, 20,830–833.
14. Toomsoo T., Kaasik, A., Margus, S. (2014). The Journal of Headache and Pain 2014, 15 (Suppl 1), M12.
15. Toomsoo T., Rubanovits I. (2014). Transcranial brain sonography in patients with Wilson disease. Movement Disorders, 29 (Suppl 1), S429.
16. Toomsoo T., Rubanovits I. (2014). Transcranial sonography findings and coeliac disease. Movement Disorders, 29, (Suppl 1). S430.
17. Toomsoo, T., Taba, P. (2008). Treemorid. *Eesti Ars*t, 87, 424–430.

DISSERTATIONES MEDICINAE
UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos**. The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar**. Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik**. Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu**. Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar**. Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus**. Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar**. Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm**. On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross**. Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo**. Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik**. The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa**. Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk**. Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar**. Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi**. The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar**. Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang**. The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak**. Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki**. Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar**. Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel**. Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik**. Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk**. Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets**. Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik**. Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi**. Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo**. Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber**. *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla**. Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk**. Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf**. Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv**. Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi**. Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva**. Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin**. Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev**. Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik**. Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto**. Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar**. Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge**. Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg**. The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp**. Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots**. Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe**. Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv**. Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan**. Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik**. Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand**. Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi**. Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask**. Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson**. Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar**. Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl**. Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap**. Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg**. *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro**. Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi**. Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann**. Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke**. Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves**. Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda**. Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist**. Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik**. Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull**. Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos**. Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova**. Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda**. Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner**. *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi**. Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla**. Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas**. Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk**. Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen**. Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar**. Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep**. Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes**. Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu**. Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi**. Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa**. Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk**. Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.

84. **Helena Soomer**. Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.

85. **Kersti Oselin**. Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.

86. **Jaan Soplepmann**. Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.

87. **Margot Peetsalu**. Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.

88. **Kersti Klaamas**. Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.

89. **Pille Taba**. Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.

90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.

91. **Ingrid Kalev**. CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.

92. **Lumme Kadaja**. Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.

93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.

94. **Andres, Kulla**. Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.

95. **Mari Järvelaid**. Health damaging risk behaviours in adolescence. Tartu, 2004.

96. **Ülle Pechter**. Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.

97. **Gunnar Tasa**. Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.

98. **Tuuli Käämbre**. Intracellular energetic unit: structural and functional aspects. Tartu, 2004.

99. **Vitali Vassiljev**. Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema**. Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.

101. **Evelin Seppet**. Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.

102. **Eduard Maron**. Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.

103. **Marje Oona**. *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.

104. **Kersti Kokk**. Regulation of active and passive molecular transport in the testis. Tartu, 2005.

105. **Vladimir Järv**. Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.

106. **Andre Õun**. Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.

107. **Piibe Muda**. Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.

108. **Külli Kingo**. The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.

109. **Mati Merila**. Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.

110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.

111. **Tiia Ainla**. Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.

112. **Andres Sell**. Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.

113. **Tiia Tamme**. Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.

114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.

115. **Tiia Voor**. Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.

116. **Priit Kasenõmm**. Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.

117. **Eva Zusinaite**. Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.

118. **Piret Kõll**. Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.

119. **Tiina Stelmach**. Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell**. Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako**. Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtson**. Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld**. Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann**. Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar**. Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson**. Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus**. Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder**. Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru**. Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand**. Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo**. Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo**. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild**. Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik**. Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller**. Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik**. Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals**. Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus**. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab**. Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme**. Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin**. The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik**. Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu**. Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu**. Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik**. Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets**. The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits**. Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam**. Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots**. Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp**. The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull**. Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre**. Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link**. Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre**. Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak**. Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich**. Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep**. Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk**. Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Auto-immune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass**. L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai**. Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum**. Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova**. The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi**. Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi**. A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe**. Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm**. Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu**. Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont**. Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt**. Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru**. Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov**. Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg**. Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm**. Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg**. Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal**. Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass**. Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.

204. **Kai Truusalu**. Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.

205. **Oksana Jagur**. Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.

206. **Katrin Sikk**. Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.

207. **Kai Blöndal**. Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.

208. **Marju Puurand**. Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.

209. **Aili Tagoma**. Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.

210. **Liis Sabre**. Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.

211. **Merit Lamp**. Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.

212. **Erik Salum**. Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.

213. **Maire Karelson**. Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.

214. **Kuldar Kaljurand**. Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.

215. **Raido Paasma**. Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.

216. **Anne Kleinberg**. Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.

217. **Triin Eglit**. Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.

218. **Kristo Ausmees**. Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.

219. **Kristi Huik**. The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.

220. **Liina Tserel**. Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.

221. **Irina Kerna**. The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrid Liiv**. Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison**. Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress**. Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru**. Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu**. Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets**. The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome**. Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo**. Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin**. Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna**. Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase**. Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel**. Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere**. Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust**. Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman**. Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar**. Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson**. Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme**. High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part**. Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.

241. **Urve Paaver**. New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.

242. **Aleksandr Peet**. Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.

243. **Piret Mitt**. Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.

244. **Merli Saare**. Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.

245. **Kaja-Triin Laisaar**. People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.

246. **Eero Merilind**. Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.

247. **Jaanika Kärner**. Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.

248. **Kaido Paapstel**. Metabolomic profile of arterial stiffness and early biomarkers of renal damage in atherosclerosis. Tartu, 2016, 173 p.

249. **Liidia Kiisk**. Long-term nutritional study: anthropometrical and clinico-laboratory assessments in renal replacement therapy patients after intensive nutritional counselling. Tartu, 2016, 207 p.

250. **Georgi Nellis**. The use of excipients in medicines administered to neonates in Europe. Tartu, 2017, 159 p.

251. **Aleksei Rakitin**. Metabolic effects of acute and chronic treatment with valproic acid in people with epilepsy. Tartu, 2017, 125 p.

252. **Eveli Kallas**. The influence of immunological markers to susceptibility to HIV, HBV, and HCV infections among persons who inject drugs. Tartu, 2017, 138 p.

253. **Tiina Freimann**. Musculoskeletal pain among nurses: prevalence, risk factors, and intervention. Tartu, 2017, 125 p.

254. **Evelyn Aaviksoo**. Sickness absence in Estonia: determinants and influence of the sick-pay cut reform. Tartu, 2017, 121 p.

255. **Kalev Nõupuu**. Autosomal-recessive Stargardt disease: phenotypic heterogeneity and genotype-phenotype associations. Tartu, 2017, 131 p.

256. **Ho Duy Binh**. Osteogenesis imperfecta in Vietnam. Tartu, 2017, 125 p.

257. **Uku Haljasorg**. Transcriptional mechanisms in thymic central tolerance. Tartu, 2017, 147 p.

258. **Živile Riispere**. IgA Nephropathy study according to the Oxford Classification: IgA Nephropathy clinical-morphological correlations, disease progression and the effect of renoprotective therapy. Tartu, 2017, 129 p.

259. **Hiie Soeorg**. Coagulase-negative staphylococci in gut of preterm neonates and in breast milk of their mothers. Tartu, 2017, 216 p.

260. **Anne-Mari Anton Willmore**. Silver nanoparticles for cancer research. Tartu, 2017, 132 p.

261. **Ott Laius**. Utilization of osteoporosis medicines, medication adherence and the trend in osteoporosis related hip fractures in Estonia. Tartu, 2017, 134 p.

262. **Alar Aab**. Insights into molecular mechanisms of asthma and atopic dermatitis. Tartu, 2017, 164 p.

263. **Sander Pajusalu**. Genome-wide diagnostics of Mendelian disorders: from chromosomal microarrays to next-generation sequencing. Tartu, 2017, 146 p.

264. **Mikk Jürisson**. Health and economic impact of hip fracture in Estonia. Tartu, 2017, 164 p.

265. **Kaspar Tootsi**. Cardiovascular and metabolomic profiling of osteo-arthritis. Tartu, 2017, 150 p.

266. **Mario Saare**. The influence of AIRE on gene expression – studies of transcriptional regulatory mechanisms in cell culture systems. Tartu, 2017, 172 p.

267. **Piia Jõgi**. Epidemiological and clinical characteristics of pertussis in Estonia. Tartu, 2018, 168 p.

268. **Elle Põldoja**. Structure and blood supply of the superior part of the shoulder joint capsule. Tartu, 2018, 116 p.

269. **Minh Son Nguyen**. Oral health status and prevalence of temporo-mandibular disorders in 65–74-year-olds in Vietnam. Tartu, 2018, 182 p.

270. **Kristian Semjonov**. Development of pharmaceutical quench-cooled molten and melt-electrospun solid dispersions for poorly water-soluble indomethacin. Tartu, 2018, 125 p**.**

271. **Janne Tiigimäe-Saar**. Botulinum neurotoxin type A treatment for sialorrhea in central nervous system diseases. Tartu, 2018, 109 p.

272. **Veiko Vengerfeldt.** Apical periodontitis: prevalence and etiopathogenetic aspects. Tartu, 2018, 150 p.

273. **Rudolf Bichele.** TNF superfamily and AIRE at the crossroads of thymic differentiation and host protection against *Candida albicans* infection. Tartu, 2018, 153 p.

274. **Olga Tšuiko**. Unravelling Chromosomal Instability in Mammalian Pre-implantation Embryos Using Single-Cell Genomics. Tartu, 2018, 169 p.

275. **Kärt Kriisa.** Profile of acylcarnitines, inflammation and oxidative stress in first-episode psychosis before and after antipsychotic treatment. Tartu, 2018, 145 p.

276. **Xuan Dung Ho.** Characterization of the genomic profile of osteosarcoma. Tartu, 2018, 144 p.

277. **Karit Reinson.** New Diagnostic Methods for Early Detection of Inborn Errors of Metabolism in Estonia. Tartu, 2018, 201 p.

278. **Mari-Anne Vals.** Congenital N-glycosylation Disorders in Estonia. Tartu, 2019, 148 p.
279. **Liis Kadastik-Eerme**. Parkinson's disease in Estonia: epidemiology, quality of life, clinical characteristics and pharmacotherapy. Tartu, 2019, 202 p.
280. **Hedi Hunt**. Precision targeting of intraperitoneal tumors with peptide-guided nanocarriers. Tartu, 2019, 179 p.
281. **Rando Porosk**. The role of oxidative stress in Wolfram syndrome 1 and hypothermia. Tartu, 2019, 123 p.
282. **Ene-Ly Jõgeda**. The influence of coinfections and host genetic factor on the susceptibility to HIV infection among people who inject drugs. Tartu, 2019, 126 p.
283. **Kristel Ehala-Aleksejev**. The associations between body composition, obesity and obesity-related health and lifestyle conditions with male reproductive function. Tartu, 2019, 138 p.
284. **Aigar Ottas**. The metabolomic profiling of psoriasis, atopic dermatitis and atherosclerosis. Tartu, 2019, 136 p.
285. **Elmira Gurbanova.** Specific characteristics of tuberculosis in low default, but high multidrug–resistance prison setting. Tartu, 2019, 129 p.
286. **Van Thai Nguyeni.** The first study of the treatment outcomes of patients with cleft lip and palate in Central Vietnam. Tartu, 2019, 144 p.
287. **Maria Yakoreva**. Imprinting Disorders in Estonia. Tartu, 2019, 187 p.
288. **Kadri Rekker**. The putative role of microRNAs in endometriosis pathogenesis and potential in diagnostics. Tartu, 2019, 140 p.
289. **Ülle Võhma**. Association between personality traits, clinical characteristics and pharmacological treatment response in panic disorder. Tartu, 2019, 121 p.
290. **Aet Saar**. Acute myocardial infarction in Estonia 2001–2014: towards risk-based prevention and management. Tartu, 2019, 124 p.