

Supplement

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Supplement

**Proceedings of the
4th International Medical Olympiad
3-5 November 2017**

Comments from the participants of the 4th International Medical Olympiad

"Let me congratulate you for the organization of the 4th Olympiad; it confirms every time its high scientific value".

Prof. Giuseppe Rubini

"I congratulate you and your team of Medical Olympic Association for the organizing 4th Medical Olympiad which was a great success. The participation in different sessions followed by healthy discussion was a worthy experience".

Prof. Devinder Dhawan

"It was nice meeting you and your team in Thessaloniki. This was one of the best Conferences; I had ever attended in my entire life".

Prof. Suresh Kumar Sharma

"Our colleagues have arrived in Belgrade with extraordinary impressions from your Meeting".

Prof. Vera Artiko

"At the 4th International Medical Olympiad we enjoyed friendly atmosphere, warm hospitality of the organizers, lessons from the distinguished speakers and connections established with other colleagues".

Prof. Slobodanka Beatovic

"I 'd like to thank you once again for the great organization of the 4th Olympiad".

Prof. Boris Ajdinovic

"It was a great pleasure to participate to the Congress that had a high scientific level".

Prof. Barbara Palumbo

"My congratulations for the very successful 4th International Medical Olympiad which reminded me of the Athens Academy of the 5th century b.C".

Prof. emer. S. Baloyannis

"The 4th International Medical Olympiad was an extraordinary experience for me among distinguished scientists".

Dr. Kostas Dervenis

"This conference will be unforgettable. Fantastic event and great knowledge, experience and specialists from all wide world".

Dr Agata Pietrzak

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Effect of hypoparathyroid on bone mineral density of lumbar spine in postmenopausal women with differentiated thyroid carcinoma

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Keywords: Hypoparathyroid -Differentiated thyroid carcinoma -Bone mineral density

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*Yongli Bai, Yanlei Huo and Fei Yu contributed equally to the paper.

Abstract

Objective: To investigate the effect of hypoparathyroid on bone mineral density in postmenopausal women with differentiated thyroid carcinoma (DTC). **Subjects and Methods:** Postmenopausal women with postoperative DTC, and undergoing thyroid residual ablation or for metastases treatment were collected and followed for two years. They were divided into hypoparathyroid group (PTH<15pg/mL) and a normal cognitive group (PTH>15pg/mL). Bone mineral density (BMD) at the lumbar spine was analyzed using dual-energy X-ray absorptiometry (DXA) at baseline, 6, 12 and 24 months. All patients had calcium and active vitamin D supplementation. **Results:** The thyroid cancers included 211 papillary carcinomas, 14 follicular carcinomas. The majority of them were retired from previous work (157/225, 70%). There were 45 DTC patients in hypoparathyroid group and 180 patients in PTH normal group (postmenopausal controls). They are comparable in age, TSH suppression, BMD at baseline. There is no significant difference in BMD of lumbar spine between hypoparathyroid group and postmenopausal controls at baseline 6, 12 and 24 months follow-up which were 1.03 ± 0.14 and 1.04 ± 0.18 ($t=0.4$, $P=0.69$), 1.04 ± 0.13 and 1.01 ± 0.19 ($t=1.25$, $P=0.21$), 1.06 ± 0.15 and 1.02 ± 0.16 ($t=1.16$, $P=0.26$), 1.06 ± 0.21 and 1.01 ± 0.17 ($t=0.93$, $P=0.29$), respectively. Areal BMD was increased by 2.9% in hypoparathyroid group in the lumbar spine at 12 and 24 months follow-up, while decrease of 2.9% in postmenopausal controls. No increase in BMD at lumbar spine was found in postmenopausal controls. **Conclusion:** Transient hypoparathyroid increased BMD at lumbar spine by DXA in postmenopausal DTC patients compared to postmenopausal controls.

Introduction

Hypoparathyroid is one of the major complications of total thyroidectomy which is characterized by deficient parathyroid hormone (PTH) and hypocalcaemia [1-3]. PTH is a key hormone involved in the maintenance of serum calcium levels and systematic regulation of bone resorption [4]. Patients with permanent

hypoparathyroid were found to have higher bone mineral density (BMD) than age- and sex-matched controls [5-6]. However, there were conflicting reports for the effects of PTH on BMD [6]. Structural abnormalities by histomorphometric analysis of iliac crest bone biopsies showed increased cortical, trabecular width and cancellous bone volume, and reduced dynamic skeletal indices [2-3]. We hypothesized that patients with hypoparathyroid may have altered BMD in postmenopausal women with differentiated thyroid cancer (DTC). The effect of surgery induced hypoparathyroid on BMD in postmenopausal patients with DTC was investigated in the study.

Methods

Patients

This investigation was a cross-sectional study with postmenopausal women with DTC. Between 2009 and 2015, from all the patients with DTC requiring ¹³¹I treatment or regular follow-up, postmenopausal women were included according to the following criteria: (1) history of total thyroidectomy and ablative radioiodine treatment for DTC and the histological results were papillary or follicular thyroid cancer; (2) Treatment with calcitriol and calcium at least 1 month after thyroidectomy; (3) BMDs were tested at least twice (at study entry and/or one during 0.5-1 year prior to the study). The exclusion criteria were as the following: (1) metabolic osteopathy; (2) malabsorption syndrome; (3) rheumatic disease; (4) severe liver or renal diseases; (5) other malignant tumor diseases; (6) distant metastasis originated from DTC or other malignancies; (7) Grave's disease; (8) ischemic heart disease or arrhythmia; (9) use of medications that might affect bone metabolism estrogen, glucocorticoids, and bisphosphonate, bisphosphonates, denosumab, SERMS, teriparatide, strontium ranelate, diuretics, lithium; (10) Subjects were excluded if they had ever been treated with PTH (1-34) or PTH (1-84).

The diagnosis of permanent hypoparathyroid was established by the concomitant presence of low circulatory levels of calcium and PTH after thyroidectomy, as well as by the requirement of continued calcium and active vitamin D treatment to maintain serum calcium levels in the low-normal range.

This study was approved by the Institutional Review Board of Hospital Research Ethics, and all subjects gave written informed consent.

Biochemical test

Serum thyroid stimulating hormone (TSH) was measured using a time-resolved immunofluorometric assay (Anytest, sym-bio lifescience co., ltd, Shanghai, China). The normal reference range of TSH is 0.3-4.6µIU/mL for TSH in our laboratory. PTH was tested using chemiluminescent microparticle immunoassay (Maglumi 2000, Shenzhen new industries biomedical engineering co., ltd, Shenzhen, China). The normal reference range of PTH was 15-65pg/mL. Calcium was measured by automated techniques with the normal ranges of 2.19-2.56.

Assessment of vertebral BMD

Areal BMD was measured at the lumbar spine, L1-L4 using dual-energy X-ray absorptiometry (version 13.20; enCORETM 2009, GE Healthcare) according to the manufacturer's protocol. The normative database of BMD was Asian population data provided by manufacturer. BMDs were analyzed on the same

regions of interest as those used for lumbar spine BMD. Subjects were measured on the same densitometer, using the same software, scan speed, and technologist. Measurements were performed at baseline for all subjects, 6-, 12- and 24- months after TSH suppression. The region of interest was automatically generated by the Lunar DXA system and adjusted by the technologist as necessary. Short-term in vivo precision error (root-mean-square standard deviation) was 0.029g/cm² for L1-L4 (1.3%).

Statistical analysis

Values with normal distributions are expressed as mean±standard deviation. To analyze the significance of intergroup differences, the χ^2 test for categorical data and the unpaired or paired t test for continuous variables. Paired t test was used to compare the BMD at baseline and follow-ups.

Results

Patients' clinical characteristics

Out of 232 postmenopausal DTC patients treated in our hospital, 7 patients were excluded for bone metastases of DTC in 4 patients and bisphosphonate treatment for osteoporosis in 3 patients. Finally, 225 patients met the inclusion criteria and were included. Patients were divided into a hypoparathyroid group (PTH<15pg/mL) and a normal cognitive group (PTH>15pg/mL). There were 45 DTC patients in hypoparathyroid group and 180 patients in PTH normal group (postmenopausal controls). The calcium, PTH were different between the two groups with the other parameters are similar, see Table 1.

The thyroid cancers included 211 papillary carcinomas, 14 follicular carcinomas. The majority of them were retired from previous work (157/225, 70%). T1N0-1M0 was observed in 19 (19/45) DTC patients, 8 (8/45) had T2N0-1M0, 4 (4/45) had TxN1M0, 4 (4/45) had TxN1M1 (lung metastases), 5 (5/45) had T3N0-1M0, 5 (5/45) had T4N0-1M0-1. In the 180 DTC patients with normal parathyroid, 85(85/180) had T1N0-1M0, 15 (15/180) had TxN0-1M1, 35 (35/180) had T2N0-1M0, 26(26/180) had T3N0-1M0, 19 (19/180) had T4N0-1M0-1.

Table 1. Patients' clinical characteristics with hypoparathyroid and normal parathyroid function

	Hypoparathyroid	Normal parathyroid	t	P
No. of patients	45	180		
Age(years)	54.45±8.02	55.88±7.80	-1.082	0.28
Height (cm)	161.80±6.44	161.26±5.55	0.553	0.581
Weight (kg)	62.41±8.40	63.00±10.93	-0.334	0.739
Serum Ca(2.19~2.56mmol/L)	2.10±0.17	2.26±0.15	-6.114	0.00
PTH (15~65pg/mL)	6.71±4.53	43.75±8.96	-2.738	0.007
TSH (0.3~5.0mIU/L)	0.17±0.03	0.24±0.03	-1.106	0.27
Bone density (g/cm ²)	1.05±0.13	1.05±0.18	-0.297	0.767
T score of lumbar spine	-0.53±1.08	-0.45±1.54	-0.213	0.831
TSH suppression (%)	77.8%(35/45)	66.1%(119/180)	2.27	0.132

*No, number; Ca, calcium; TSH, thyroid stimulating hormone; PTH, parathyroid hormone.

Biochemical evaluation

Serum calcium concentration was typically normal as a result of supplementation with calcium and active vitamin D. However, serum calcium was slightly lower than the lower normal range in 7 patients even with sufficient supplementation with calcium and active vitamin D. TSH was in the normal range, it was reduced in 154 (154/225) subjects indicating overtreatment with levothyroxine, with 35/45 (77.8%) subjects having a TSH value <0.3mIU/L in DTC patients with hypothyroid, and 66.1% (119/180) in DTC patients with normal parathyroid, see Table 1.

Comparisons of lumbar spine BMD

There is no significant difference in BMD of lumbar spine between hypoparathyroid group and postmenopausal controls at baseline 6, 12 and 24 months follow-up which were 1.03±0.14 and 1.04±0.18 (t=0.4, p=0.69), 1.04±0.13 and 1.01±0.19 (t=1.25, P=0.21), 1.06±0.15 and 1.02±0.16 (t=1.16, P=0.26), 1.06±0.21 and 1.01±0.17 (t=0.93, P=0.29), respectively. Areal BMD was increased by 2.9% in hypoparathyroid group in the lumbar spine at 12 and 24 months follow-up, while decrease of 2.9% in postmenopausal controls.

Discussion

PTH is a key hormone involved in the maintenance of serum calcium levels and systematic regulation of bone resorption [4]. In hypoparathyroid women, areal BMD was above average at the lumbar spine by DXA [5-9]. Our results agreed that in hypoparathyroid women, BMD increase by 2.9% at the lumbar spine by DXA compared with that at baseline. The increase in BMD in our study was lower than reported which was 10-32% in a small study (19 patients) [5]. The increase in BMD at lumbar spine may be due to the hypoparathyroid-provided protection against age-related bone loss by attenuating the high-turnover bone loss, lowering the remodeling activity and increasing mineralization following menopause [1, 4, 10-12]. All of our patients with postsurgical hypoparathyroid were treated with active vitamin D with calcium preparation to maintain a lower normal range of calcium for a long period. Therefore, the reduced PTH production, vitamin D treatment, and calcium supplementation might have contributed to the increased bone mass in these patients [5].

The postmenopausal controls had decreased BMD by 2.9% after 2 years follow up in the present study. Similarly, significantly decreasing of BMD in controls was observed in a couple of studies [5, 10, 14]. However, no significant differences in lumbar spine or total hip BMD were found in 16 postmenopausal women with hypoparathyroid compared with age, weight and height matched control [13]. The difference may be due to the age of included patients, the controls and the small samples. In the present study, the control was age-, gender and disease matched which was different from studies which was an only age matched control study in 33 postmenopausal patients [10], normal controls in 20 patients with postsurgical hypoparathyroid [5] and in 19 patients with postsurgical hypoparathyroid [6], age, weight and height matched control in 16 postmenopausal women with hypoparathyroid [13]. The weight-bearing exercise has been demonstrated to play an important role in skeletal microarchitecture [15]. While also not measured as part of this study, the majority of our hypoparathyroid DTC patients were retired and appeared to be less active after their diagnosis and a relative lack of weight-bearing exercise may have contributed to the difference [1]. The duration of hypoparathyroid may have different effects on BMD and bone strength. Transient hypoparathyroid was associated with increased BMD in postmenopausal women [16] as showed by our study. Longer duration of hypoparathyroid may mitigate the adverse effects of age on estimated bone strength and ultimately in fracture susceptibility [13].

Hypoparathyroid may have a differential impact of hypoparathyroid on diverse bone sites, and the reported results are conflicting. Increased areal BMD by DXA at the spine was showed also by our study and hip by other studies [5, 7]. Cortical BMD was increased in the hypoparathyroid cohort compared to controls at both the radius and tibia [1, 5, 6-9]. However, it is reported that the increase in BMD was higher in the lumbar spine than in the proximal femur [7], whereas forearm BMD was not positively affected [8]. Postmenopausal hypoparathyroid women compared to postmenopausal controls had higher cortical density and lower cortical porosity at both the radius and tibia (Natalie 2016). The cortical BMD and bone strength in hip and radius was not measured in the present study.

DTC patients had several risk factors for osteoporosis, including TSH suppression, postmenopausal, inactive lifestyle, and others. The majority of patients were retired from their work in the present study. Estrogen deficiency following menopause is a major cause of osteoporosis, carrying an increased risk of bone fracture [4] which results in rapid bone loss in postmenopausal women [16]. A negative effect of long-term TSH suppressive treatment on the BMD of patients with DTC was reported in

postmenopausal women [17-20]. In our study, the age- (postmenopausal women) and gender- and disease- matched controls was used for comparison. And the extent of TSH suppression was not significantly different between the two groups. Thus, the effects of estrogen deficiency, TSH suppressive therapy and lifestyle in the present study population were negligible.

In conclusion, transient hypoparathyroid increased BMD at lumbar spine by DXA in postmenopausal DTC patients compared to postmenopausal controls. However, the limitations of the study are the retrospective design, lack of a BMD assessment in cortical bone (hip joint and radius).

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All authors declare that they have no conflicts of interest

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Co-chair persons: Professors D. Moraitou, M. Myronidou and M. Matovic

Deep brain nucleus targeting in Parkinson's disease and essential tremor by image guided surgery using neuronavigation system with tractography and volume of tissue of activated assessment

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Keywords: Deep Brain Stimulation -Image Guided Surgery -Neuronavigation -Volumetry -Tractography - Unified Parkinson's Disease Rating Scale

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Abstract

Objective: Deep Brain Stimulation (DBS) is an effective surgical approach for treatment of Parkinson's disease (PD), dystonia and essential tremor (ET). Traditionally, DBS is performed using frame-based stereotactic technique. Recently, image guided surgery (IGS) using neuronavigation has gained popularity in neurosurgical procedures. We aim to investigate whether DBS using neuronavigation is capable of improving patient's outcome and minimize its complications. **Subjects and Methods:** From February 2011 to October 2016, 20 patients with PD and 6 patients with ET were enrolled in the study. Patients aged between 18 to 70 years, were included and they underwent magnetic resonance imaging (MRI) for deep brain nucleus volumetry. Among PD patients, 14 cases underwent subthalamic nucleus (STN) implantation, while other 6 cases underwent globus pallidus internus (GPI) implantation. Furthermore, ventral intermediate nucleus (VIN) implantation was performed for ET patients, all with IGS using neuronavigation system. Patients were assessed by unified Parkinson's disease rating scale (UPDRS) for PD and tremor scores for ET in their follow-ups. Authors utilized Butson model for volume of tissue activated (VTA) assessment. In addition, detailed tractography was performed to evaluate white matter circuits radiating from deep nuclei. **Results:** PD patients with GPI volume of more than 600mm³ and less than 400mm³ were excluded from the study. Mean right and left GPI volume was 526±89.9mm³ and 488±84.1mm³, respectively. Calculated VTA based on Butson model revealed that 70% of cases who exhibited improved UPDRS of more than 50% in the 7th month of follow-up, had their VTA outside their defined GPI and STN boundaries with outer layer overlap. In contrast, 60% of cases who showed UPDRS improvement of less than 50% in same follow-up month, have their VTA inside defined GPI and STN boundaries. Moreover, ET patients experienced mean 51% and 72% improvement in tremor scores at mean 6.7 and 9.9 follow-up month, respectively. No surgery related complications were observed. Furthermore, tractography analysis revealed increased superior frontal gyrus and thalamus connection in patients with improved UPDRS. **Conclusion:** IGS using neuronavigation allowed more accurate deep nucleus targeting, minimized intra- and post-operative complications and improved clinical outcome in DBS candidate patients. Our study revealed that increased white matter connections with remote parts of the brain would suggest that isolated deep nucleus stimulation could not explain symptom recovery and that patients' specific white matter stimulation by tractography coupled with IGS should be in priority.

Introduction

In the 1970s, scientists utilized Deep Brain Stimulation (DBS) for treatment of patients suffering from chronic pain, unresponsive to medical therapy [1]. However, due to various results and poor electrode design, most of the scientists abandoned this method as an approach to chronic pain management in the 1980s [2]. Since then, DBS was recognized as an effective treatment for various movement disorders such as Parkinson's disease (PD), dystonia and essential tremor (ET), which has been developed in past 20 years [3]. Besides, DBS may be the procedure of choice in other refractory medical conditions, such as major depression and obsessive-compulsive disorders and Tourette syndrome, characterized by multiple motor tics and at least one phonic tic [4, 5]. Also, some studies have shown positive effects of DBS in patients suffering from refractory epilepsies, schizophrenia and narcolepsy [6]. Minimal tissue damage, being reversible, potential to be adjusted according to disease progression and symptom manifestation, as well as the possibility for bilateral installation, have made DBS as the procedure of choice in patients suffering from PD [7]. Although no definite theory is capable of explanation the exact mechanism of DBS in treatment of movement disorders, creation of a hyper polarization of basal ganglia cell membranes resulting in a loss of excitability [8]. Traditionally, DBS is performed using frame-based stereotactic technique, while recently, frameless image guided surgery (IGS) using neuronavigation technology has gained popularity in neurosurgical procedures [9]. We aim to investigate whether DBS using neuronavigation is capable of improving patient's outcome and minimize its complications and possible hidden neurological circuits responsible for symptom improvement in PD and ET patients.

Subjects and Methods

From February 2011 to October 2016, authors have entered patients from neurology department of Shohada Tajrish Hospital with confirmed diagnosis of PD in 20 patients and ET in 6 patients. Authors defined inclusion criteria as patients more than 18 years old and below 70 years, with confirmed diagnosis of their movement disorder disease, being either PD or ET. Also, patients underwent magnetic resonance imaging (MRI) for image processing as a primary frame for pre-operative setting as well as volumetry of their deep brain nucleus in basal ganglia, using volBrain software by an expert neuroradiologist blinded to study. Also, patients with previous history of DBS either by frame-based or frameless stereotactic surgery and intracranial surgery, presence of intracranial lesions or brain metastases, patients with decreased level of consciousness or with diminished ability to complete post-operative neuro-examination sheets, active infection and refusal to complete informed consent were excluded from the study. We have utilized image guided surgery using neuronavigation system for accurate targeting of basal ganglia using pre-operative computed tomography (CT) scan and MRI. Among PD patients, 14 cases underwent subthalamic nucleus (STN) implantation, while other 6 cases underwent globus pallidus internus (GPi) implantation. Furthermore, ventral intermediate nucleus (VIN) implantation was performed for ET patients, all with IGS using neuronavigation system. Patients underwent post-operative CT scan for evaluation of electrode location and thorough neurological examination. Authors planned to assess PD patient's symptom improvement by unified Parkinson's disease rating scale (UPDRS). Also, symptom improvements of ET patients were investigated using tremor score, both by an expert neurologist blinded to the study in follow-

up periods. Authors have assessed PD patients using UPDRS-III (motor examination) under following sections: speech, facial expression, rigidity, finger tapping, hand movement, pronation-supination movements of hand, toe tapping, leg agility, arising from chair, gait, freezing of gait, postural stability, posture, body bradykinesia, postural tremor of hands, kinetic tremor of hands, rest tremor amplitude and constancy of rest tremor [10]. Authors utilized Butson model for volume of tissue activated (VTA) assessment [11]. In addition, detailed MR-tractography was performed to evaluate white matter circuits radiating from deep nuclei under supervision of an expert neuro-radiologist blinded to the study. Written informed consents were obtained from the patients with ethical approval by Ethics Committee of Shohada Tajrish Hospital under the principles of the Helsinki Declaration.

Results

Demographic data and their movement disorder score, either PD using UPDRS-III or ET using tremor scale were recorded. Among PD patients, there were 11 males and 9 females, with mean age of 64.5 years (ranged between 49.3 to 70 years), while 4 males and 2 females comprise ET patients with mean age of 47.2 years (ranged between 33.2 to 61.7 years). We have utilized volBrain software as a screening tool for patient enrollment in PD series for their GPi nucleus, due to higher level of statistic bias. PD patients with GPi volume of more than 600mm^3 and less than 400mm^3 were excluded from the study. Volumetric analysis revealed mean right and left GPi volume was $526\pm 89.9\text{mm}^3$ and $488\pm 84.1\text{mm}^3$, respectively. We have performed pre-operative volumetry using MRI which revealed mean 142.64mm^3 and 136.83mm^3 for right and left STN, respectively. We have mounted pre-operative basal ganglia's images on neuronavigation system for reconstruction and 3D visualization of STN in candidates. Lead placement was performed based on pre-operative coordinates using image guided neuronavigation. Patients underwent image guided surgery using neuronavigation system, with no intra-operative and immediate or remote post-operative complications, assessed by post-operative thorough neurological examination and CT scan. These patients, who underwent STN and GPi implantation, experienced mean 40% and 47% reduction in UPDRS III in a mean of 5.3 months follow-up, respectively. Furthermore, further reduction was observed at a mean of 9.5 months follow-up, with mean 54% and 67% reduction in UPDRS III for STN and GPi implantation, respectively. Calculated VTA based on Butson model revealed that 70% of cases who exhibited improved UPDRS III of more than 50% in the 7th month of follow-up, had their VTA outside their defined GPi and STN boundaries with outer layer overlap. In contrast, 60% of cases who showed UPDRS III improvement of less than 50% in same follow-up month, have their VTA inside defined GPi and STN boundaries. Moreover, ET patients experienced mean 51% and 72% improvement in tremor scores at mean 6.7 and 9.9 follow up month respectively. Interestingly, pre-operative MR-tractography analysis revealed increased superior frontal gyrus and thalamus connection in PD patients with improved UPDRS III.

Discussion

PD is one of the most common neurological disorders affecting approximately 1% of people aged more

than 60 years [12]. Loss of pigmented dopaminergic neurons of the substantia nigra pars compacta and lewy bodies are two major pathophysiologic mechanisms behind PD, with dopamine neuron loss occurs mostly in the ventral lateral substantia nigra [13]. PD patients usually manifest their motor symptoms when approximately 70% of dopaminergic neurons are lost, characterized by resting tremor, rigidity and bradykinesia. Although classic PD patients with mentioned manifestation in their 60s and 70s do not require neuroimaging or laboratory studies for diagnosis, iodine-123-labeled fluoropropyl-2beta-carbomethoxy-3beta-4-iodophenyl-nortropane (FP-CIT 123) (Ioflupane, DaTscan) single-photon emission tomography (SPET) for differentiation of diseases depending on their loss or presence of dopaminergic neurons [14]. The American Academy of Neurology have described following as predicting factor for poor prognosis in PD progression: older age at onset and initial rigidity and/or hypokinesia, being male and presence of associated comorbidities and decreased response to dopaminergic therapies [15]. While ET is the most common movement disorder, diagnosed by Movement Disorder Society with following criteria: 1) Bilateral, largely symmetrical, postural (occurring with voluntary maintenance of a position against gravity) or kinetic (occurring during voluntary movement) tremor involving hands and forearms that is visible and persistent, and 2) Possible additional or isolated tremor in head but absence of abnormal posturing [16]. Despite recent advances in understanding movement disorder neuropathology, exact mechanism of ET is yet to be known. However, several mechanisms have been explained: increased blood level of Harmaline (a heterocyclic amine which is a potent tremor-producing neurotoxin) and increased glucose uptake in medulla using fluorine-18-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG PET) scan [17]. Fifty to 70% of ET patients shows familial transmission, thus following susceptibility loci have been identified: 3q13 (EMT1), 2p25-22 (EMT2) and 6p23 (identified in two families) [18, 19]. It has been shown that DBS is responsible for symptom improvement in patients suffering from movement disorders, even irrelevant to their pathophysiology (associated with loss of dopamine neurons in PD and not associated with loss of dopamine neurons in ET) which its exact mechanism of action in symptom improvement is yet to be explained. The UK National Collaborating Centre for Chronic Conditions described following items as STN and GPi stimulation in PD patients: The presence of motor complications refractory to medical therapy, absence of significant comorbidities in a biologically fit individual, absence of significant mental health problems and response to levodopa [20]. Ondo et al have found 83% reduction in tremor in 14 ET patients following unilateral thalamic deep brain stimulation [21]. Based on previous documents, authors plan to perform DBS with more accurate technique, image guided surgery using neuronavigation system. Butson et al designed a human DBS model to provide precise predictions of VTA both from electrical and anatomical point of view. The Butson model is capable of predicting VTA as a function of electrode design and stimulation parameter setting. Both electrode placement and stimulation parameter setting interact together to define the therapeutic window for stimulation without stimulation induced side effects [22]. Authors utilized Butson model for VTA assessment and evaluation and analysis of results in PD patients underwent DBS using neuronavigation for possible hidden relationship. Interestingly, we have understood that PD patients, who show improvement in their UPDRS III score, have their VTA outside GPi and STN boundaries. Since efforts were not successful in complete explanation of DBS in symptom improvement, there are some studies in literature focusing on possible role of white matter tracts in DBS, unlike initial hypothesis of possible role of gray matter of deep brain nuclei in symptom improvement of movement disorders [23-25]. Thus authors plan to investigate and compare white matter tract circuits using MR tractography originating from and ending to our targets, meaning deep brain nuclei. Interestingly, we have found that PD patients with improvement in their UPDRS III have increased superior frontal gyrus and

thalamus connections.

Conclusion

IGS using neuronavigation allowed more accurate deep nucleus targeting, minimized intra- and post-operative complications and improved clinical outcome in DBS candidate patients. Our study revealed that increased white matter connections with remote parts of the brain would suggest that isolated deep nucleus stimulation could not explain symptom recovery and that patients' specific white matter stimulation by tractography coupled with IGS should be in priority.

The authors declare that they have no conflicts of interest.

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Psychological consideration in patients with cerebral gliomas candidates for intra-operative radiation therapy based on tumor location

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Keywords: Intra-operative Radiation Therapy -Cerebral Glioma -Magnetic Resonance Spectroscopy (MRS) -Surgical Resection -DSM-IV criteria -Psychological Disorder

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Abstract

Objective: Intra-operative Radiation Therapy (IORT) is gaining popularity as an adjuvant option to surgical resection, in treatment of glioblastoma multiforme (GBM) for increasing survival rate, which a highly aggressive cerebral tumor with poor prognosis. The authors plan to investigate the effects of IORT combined with surgical resection on the psychological status of these patients based on tumor location. **Subjects and Methods:** From December 2013 to February 2017, we have enrolled 109 patients with high grade cerebral gliomas, documented by Magnetic Resonance Spectroscopy (MRS). Patients with previous history of brain surgery or radiation, altered mental status and psychological content and patients diagnosed with metastases were excluded. Demographic data, tumor volume based on pre-operative magnetic resonance imaging (MRI) and psychological status were recorded based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. The remaining 56 patients, were equally randomized into conventional (surgical resection-group A), and trial (surgical resection with IORT-group B) who underwent IORT using the 50kV INTRABEAM® system (Carl Zeiss Meditec AG, Germany). Psychological profiles of both groups were re-evaluated in the 3rd post-operative month. Results: Group A consisted of 18 males and 10 females with mean age of 54.4 years, while group B consisted of 16 males and 12 females with mean age of 57.8 years. Tumor volumetry revealed mean 84.03cc and 85.73cc for group A and B, respectively. (P value 0.12) Patients were classified based on glioma location on pre-operative MRI: a) left parietal lobe (6 in group A, 5 in group B); b) left temporal lobe (7 in group A, 5 in group B); c) right parietal lobe (5 in group A, 6 in group B); d) left fronto-temporal lobe (4 in group A, 6 in group B); e) left parieto-temporal lobe (4 in group A, 5 in group B); and, f) right frontal lobe (2 in group A, 1 in group B). Group B received mean 8.05Gy radiation for mean 11.2 minutes. Post-operative psychological in the 3rd month evaluation revealed the following in each class: a) Group A: 1 mild depression, Group B: 1 mild depression and 2 major depression; b) Group A: no disorder, Group B: 1 mild depression; c) no disorders in both groups; d) Group A: no disorder, Group B: 1 mild depression, 1 major depression and 1 Obsessive-Compulsive Disorder (OCD); e) Group A: 1 mild depression, Group B: 2 major depression; and, f) no disorders in both groups. Conclusion: Utilization of IORT is shown to improve survival rate of patients suffering from GBM. However, the psychological status is a major determining factor for the quality of life of these patients. Our study showed that IORT increased psychological disorders in patients with gliomas located in left parietal, left fronto-temporal and left parieto-temporal lobes and should

be considered in pre-operative strategy selection.

Introduction

Glioblastoma multiforme (GBM) is by far the most aggressive and most common malignancy of glial tumors, composed of heterogeneous mixture of poorly differentiated neoplastic astrocytes which usually affect cerebral hemispheres of adult men, instead of deep brain structures such as brainstem and spinal cord [1, 2]. Despite recent development in neurosurgery and oncology, only minimal development is achieved in treatment of GBMs and current therapies are classified as palliative, rather than cure. Patients with GBM usually die within 3 months without treatment, while median survival rate in 12 months is achieved with combination of surgical resection, chemotherapy and radiation therapy [3]. However, reports of cases with 40 months survival rate have been discussed in some studies [4, 5]. Strong data support the fact that radiation therapy will increase patient survival and local tumor control. Also, utilization of recent technologies such as Intra-operative Radiation Therapy (IORT) will enhance tumor control and patient survival by delivering a large dose of radiation to the target, and it is capable of minimizing radiation threat to surrounding healthy tissue by shielding nearby structures during surgery under direct visualization [6, 7]. Although recent studies have shown improved survival rate by combination of IORT with surgery compared to conventional surgical resection in patients diagnosed with GBM, authors plan to investigate the psychological status of these patients based on tumor location and weight the possible psychological outcomes associated with utilization of IORT compared to conventional surgical resection.

Subjects and Methods

Authors have enrolled 109 patients from December 2013 to February 2017 who were diagnosed by high grade gliomas based on their previous imaging data including pre-operative computed tomography (CT) scan and magnetic resonance imaging (MRI). Also, the severity of cerebral gliomas as high grade was investigated and documented by magnetic resonance spectroscopy (MRS) using choline/N-acetyl aspartate (NAA) ratio to classify these patients under high grade gliomas and GBMs [8]. Inclusion criteria defined as patients aged more than 18 years and less than 75 years with confirmed diagnosis of GBM who completed written informed consent for study enrollment. Patients with previous history of brain surgery or radiation, altered mental status and psychological content and patients diagnosed with metastases, as well as patients with significant comorbidities and active infection and history of neurodegenerative diseases were excluded. Authors documented demographic data and analyzed pre-operative MRI for tumor location and its boundaries, as well as tumor volumetry, with an expert neuroradiologist blinded to the study. Also, psychological status was recorded based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria under the supervision of an expert psychologist. Authors examined patients for depression and Obsessive-Compulsive Disorder (OCD) under DSM-IV criteria. Major depression defined as 5 or more symptoms, including one of the key symptoms, while minor depression referred as 2-4 symptoms which may or may not include a key symptom. Depressed mood and loss of interest are two key symptoms, while significant weight loss or gain or decrease or increase in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or

excessive or inappropriate guilt, diminished ability to think or concentrate and recurrent thoughts of death, recurrent suicidal ideation without specific plan, or suicide attempt or specific plan are among other symptoms. Also OCD was diagnosed under DSM IV criteria. After exclusion, remaining 56 patients were equally randomized into conventional (surgical resection-group A), and trial (surgical resection with IORT-group B) who underwent IORT using the 50kV INTRABEAM® system (Carl Zeiss Meditec AG, Germany). Post-operative follow up includes post-operative CT scan and thorough neurological examination, as well as thorough psychological examination in the 3rd post-operative month for assessment of the effect of combination of IORT with surgical resection compared to conventional surgery on psychological profile of patients based on GBM location. Author performed statistical analysis with SPSS (version 19, SPSS Statistics/IBM Corp, Chicago IL, USA) using Mann-Whitney, Student's t-test and Chi-square tests. Written informed consents were obtained from the patients with ethical approval by Ethics Committee of Shohada Tajrish Hospital under the principles of the Helsinki Declaration.

Results

Data gathering and statistical analysis was performed on patients in each group. Group A consisted of 18 males and 10 females with mean age of 54.4 years, while group B consisted of 16 males and 12 females with mean age of 57.8 years. Image processing was conducted using volBrain software, capable of measurement of volume, segmentation and structure asymmetry ratio at different scales as:

1) Intracranial cavity, 2) Tissue Volumes and CSF volumes, 3) Cerebrum, cerebellum, and brainstem volumes and 4) Lateral ventricles and subcortical GM structures (putamen, caudate, pallidum, thalamus, hippocampus, amygdala, and accumbens). Tumor volumetry revealed mean 84.03cc and 85.73cc for group A and B, respectively (P value 0.12). Also, pre-operative MRI was conducted for classification of tumor location for further investigation. Patients were classified based on glioma location on pre-operative MRI: a) left parietal lobe (6 in group A, 5 in group B), b) left temporal lobe (7 in group A, 5 in group B), c) right parietal lobe (5 in group A, 6 in group B), d) left fronto-temporal lobe (4 in group A, 6 in group B), e) left parieto-temporal lobe (4 in group A, 5 in group B) and f) right frontal lobe (2 in group A, 1 in group B).

Group A underwent conventional gross total resection (GTR) of cerebral gliomas, while Group B received mean 8.05Gy radiation for mean 11.2 minutes during the operation. Post-operative CT scan and thorough neurological examination revealed successful GTR in 92.8% of group A patients, while GTR was achieved in 89.2% of patients in group B (P value 0.18). No significant intra-operative and post-operative complications were observed in two groups. Post-operative psychological in the 3rd month evaluation revealed the following in each class: a) Group A: 1 mild depression, Group B: 1 mild depression and 2 major depression, b) Group A: no disorder, Group B: 1 mild depression, c) no disorders in both groups, d) Group A: no disorder, Group B: 1 mild depression, 1 major depression and 1 Obsessive-Compulsive Disorder (OCD), e) Group A: 1 mild depression, Group B: 2 major depression and f) no disorders in both groups.

Discussion

GBMs are the most frequent primary brain tumor and accounts for 12%-15% of all intracranial tumors and more than 50% of all astrocytic neoplasms [9]. GBMs are classified into two categories: primary and secondary. Primary GBMs account for more than 60% of all GBMs, which originate *de novo* without any malignant precursor. However, secondary GBMs arise from less malignant precursors like low grade astrocytoma (World Health Organization (WHO) grade II) and anaplastic astrocytoma (WHO grade III), which usually affect young adults (less than 45 years) [10]. Recent studies have shown genetic abnormalities responsible for primary and secondary GBMs, which have minimal overlap and constitute different entities. Loss of heterozygosity (LOH) on chromosome 10q is the most frequent gene alteration in both primary and secondary GBMs, along with P53 mutations, role of epidermal growth factor receptor (EGFR) gene, amplification or over-expression of MDM2 and platelet-derived growth factor-alpha (PDGF-alpha) gene comprise some of the most encountered genetic abnormalities [11, 12]. Compared to surgical intervention alone, it has been shown that addition of radiation therapy is capable of increasing relatively poor survival rate from 3-5 months to 8-13 months in these patients. Radiation therapy has the potential to induce remission phases and reduce target's mass. However, remission phases may not last long and most of the tumor recurrences appear 12 to 13 months later [13]. IORT was initially developed for interstitial radiosurgery of brain lesions; however, it was replaced with stereotactic radiosurgery (stereotactic ablative radiotherapy). In the TARGIT trials, INTRABEAM achieved local control in up to 80% of cases with delayed necrosis appearing in less than 5% [14, 15]. These interstitial irradiations were performed with a needle-shaped applicator that was mounted on a stereotactic frame and inserted into the center of a mass without prior resection. Before TARGIT trials, Takakura and Kubo have used IORT with spherical applicators in 55 patients with GBM which resulted in 2-year survival rate of 42% [16]. John Kalapurakal and his colleagues from Chicago's Northwestern Memorial Hospital published the most widely recognized study in 14 pediatric patients who underwent IORT for their recurrent primary brain tumor with 10Gy in 2 and 5mm depth or 12Gy in 2mm depth) using spherical applicators [17]. They have achieved 57% local control with best response in un-irradiated lesions, thus affecting our inclusion criteria. Although benefits of combination of IORT with conventional surgical resection (preferably GTR) in patients suffering from GBM is not a debate, authors have shown that utilization of IORT for treatment of GBMs will be limited under psychological point of view, thus affecting patients quality of life, by manifestation of newly hidden psychological problems, as minor and major depression and OCD, mostly in specified regions of brain.

Conclusion

Utilization of IORT is shown to improve survival rate of patients suffering from GBM. However, the psychological status is a major determining factor for the quality of life of these patients. Our study showed that IORT increased psychological disorders in patients with gliomas located in left parietal, left fronto-temporal and left parieto-temporal lobes and should be considered in pre-operative strategy selection.

The authors declare that they have no conflicts of interest.

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Diuresis renography and ultrasonography in children with antenatally detected hydronephrosis can support diagnoses and suggest related surgery treatment

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Abstract

Objective: Prenatal ultrasound (US) screening detects the hydronephrosis (HN)-dilatation of fetal renal collecting system in 1%-5% of all pregnancies. In most children, HN is detected by prenatal US screening between 18-20 gestational week. Pelvi- ureteric junction (PUJ) stenosis is the most common etiological factor of prenatal HN and requires postnatal follow-up. Diuresis renography plays important role in the follow-up by complementing morphological information obtained by US with the data about differential renal function (DRF) and drainage. We studied the association between ultrasound parameters and results of diuresis renography in first diagnosed PUJ stenosis and the predictive factors of pyeloplasty in order to evaluate the usefulness of diuresis renography in these children postnatally.

Patients and Methods: Children with antenatally detected HN attributed to presumed PUJ stenosis were investigated with mercapto-acetyltriglycine (MAG3) diuresis renography. Parents gave informed consent for the procedure. The inclusion criteria were: age up to 4 years, diagnosis of prenatal HN determined by US during pregnancy based on the antero-posterior diameter (APD) of renal pelvis and at least one post-natal US which confirmed diagnosis. Exclusion criteria were: APD of pelvis <10mm, previous surgical treatment of HN, vesicoureteral reflux excluded by micturating cystourethrography, and patients having any anomaly of the contralateral kidney. Sixty two patients 43 boys, 19 girls, median age 16 months were selected. They were divided into three groups based on the size of pelvis, three groups based on the calyceal size and two groups according to thickness of parenchyma. Renography was performed for 24 minutes after the iv. application of ^{99m}Tc MAG₃, 144 ten-sec images were applied. Furosemide was administered after 2 min. (F+2). Post-void static images were acquired at 60min. The non-commercial software developed by International Atomic Energy Agency was applied to process the studies. The criteria for pathological findings (poor or no drainage) were the renographic curve maintaining a plateau, Normalized Residual Activity (NORA) at 20. min.>1.62, Output efficiency (OE) at 20. min.<71%, postmicturating NORA >0.11. The DRF was considered normal within the range of 45%-55%. **Results:** Good drainage had 74% of children, partial drainage 11%, and poor 15%. There was a clear association between the size of pelvis, calyces, parenchyma thickness and drainage. There was also a clear association between the calyceal size, parenchyma thickness and DRF. Differential renal function was <45% in 18% of children. A relation between the type of drainage and DRF was not determined. Thus, 66.7% of those with poor drainage had preserved DRF. Seven out of nine children with poor drainage underwent pyeloplasty. The threshold for

pyeloplasty was the pyelon of 18mm and calyces of 10mm. The model of the multivariate logistic regression which included ultrasound parameters (APD of pyelon, calyces size and parenchymal thickness), drainage and DRF, which were significant predictors in univariate analysis, showed that only drainage was an independent predictor for the need of pyeloplasty. **Conclusion:** Antero-posterior diameter of the pyelon <15mm indicates a favorable course of congenital HN in most children. Pattern of drainage obtained by diuresis renography was the only independent predictor for the need of pyeloplasty.

Introduction

Antenatal hydronephrosis (HN)-dilatation of the fetal urinary collecting system is one of the most common congenital anomaly with a prevalence of about 1-5% of all pregnancies [1]. In the most children, HN is detected by prenatal ultrasound (US) screening between 18-20 gestational week. In this prenatal period it cannot be confidently estimated the percentage of dilatation which will develop into an obstruction and require surgical treatment. Also, we know that congenital anomalies of the kidney and urinary tract are now considered as major cause of renal failure in children. Because of all, it is necessary that postnatal diagnostic algorithm is reliable, efficient and economical [2]. But, international debate about which model of post-natal diagnostic algorithm should be applied in children with HN is still going.

Pelviureteric junction (PUJ) stenosis is the most common etiological factor of prenatal HN, while PUJ obstruction is the most common reason of clinically significant prenatal HN. Finding dilatation of renal pyelon and calyces, usually unilateral without dilatation of the ureter on the same side arises suspicion of this diagnosis. Retrospective studies suggest that the rate of surgical treatment is 38%-52% [3]. The standard surgical procedure is pyeloplasty (Hines and Anderson) [4, 5] with high reported success rate [6].

The lower limit of significant dilatation of the renal pyelon is antero posterior diameter (APD) 4mm (gestational weeks 16-20), and APD 7mm (gestational weeks 28-32) [7]. Additional ultrasound parameters are used to assess the severity of dilatation: calyceal dilatation, thickness of renal parenchyma, abnormalities of the ureter and bladder, the amount of amniotic fluid. Postnatal APD of renal pyelon is normally less than 10mm. The Society of Fetal Urology (SFU) introduced a five- point grading system to report dilatation of the collecting system based on parenchymal thickness, appearance of the renal pyelon and calyces for postnatal reporting of HN [8].

Diuresis renography plays important role in the follow-up by complementing morphological information obtained by US with the data about differential renal function (DRF) and drainage.

Renography with tubular radiopharmaceuticals (RF) ^{99m}Tc Mercurioacetyltriglycine (MAG3) and ^{99m}Tc ethylenedicycysteine (EC) is standard postnatal procedure for evaluation whether HN is obstructive nature [9, 10]. It is recommended that all infants with moderately to severely unilateral or bilateral HN (SFU 3-4, pelvis APD>10mm), and who have not vesicoureteral reflux (VUR) undergo renography [11]. Calculation of the injected activity is now straightforward through the European Association of Nuclear Medicine (EANM) Dosage Card Calculator [12, 13].

The aims of this study were: 1) to determine the association between US parameters (pyelon size, calyceal size, parenchymal thickness) and results of diuresis renography (pattern of drainage, DRF) in the case of presumed PUJ stenosis and 2) to determine the predictive factors of pyeloplasty in order to evaluate the usefulness of diuresis renography and US in these children postnatally.

Patients and Methods

The study included children with antenatal HN who were referred by the competent pediatricians to MAG3 diuresis renography from January 2013 to January 2015. Parents gave informed consent for the procedure. The inclusion criteria were: HN attributed to presumed PUJ stenosis, age up to 4 years, diagnosis of prenatal HN determined by US during pregnancy based on APD of renal pelvis and at least one post-natal US which confirmed diagnosis. Exclusion criteria were: APD of pelvis <10mm, previous surgical treatment of HN, VUR excluded by micturating cystourethrography, and patients having any anomaly of the contralateral kidney. Sixty two patients 43 boys, 19 girls, median age 16 months with unilateral HN were selected. They were divided into three groups based on the APD of renal pelvis: group 1- mild HN (APD 10-14.9mm), group 2-moderate HN (APD 15-19.9mm) and a group 3-severe HN (APD \geq 20mm). According to the calyceal size children were divided into three groups: group 1 with no or mild calyceal dilatation (0-5.9mm), group 2-moderate dilatation of the calyces (6-10mm) and group 3 with severe calyceal dilatation (>10mm). Based on the thickness of renal parenchyma there were two groups of children: with normal parenchymal thickness and with reduced parenchymal thickness. Preparation for examination considered placement of intravenous cannula and adequate hydration of the child. Renography was performed in the posterior projection for 24 minutes (144 frames, each 10 seconds) after the iv. application of ^{99m}Tc MAG₃. The dose was adjusted according to respective guidelines [12, 13]. Furosemide was administered after 2 min. (F+2). Post-void static image (1 frame of 120 seconds duration) was acquired at 60min. Single head "Siemens Orbiter 7500" gamma camera with a low energy collimator was used. Drawing regions of interest (above the kidneys, heart, bladder) and computer processing of data within the software program the radiorenography curves and quantitative parameters of renography were obtained. The non-commercial software developed by International Atomic Energy Agency (IAEA) was applied to process the studies. Based on results of renography, children were divided into three groups: poor or no drainage, partial drainage and good drainage. The criteria for pathological findings (poor or no drainage) were the renographic curve maintaining a plateau, Normalized Residual Activity (NORA) at 20. min.>1.62, Output efficiency (OE) at 20. min.<71%, postmicturating NORA >0.11. Kidneys with partial drainage showed present, but very slow drainage and moderate retention of RF on postmicturating images. Kidneys with good drainage showed complete emptying during renogram, observed on sequential images and represented by the curve shape. Differential renal function was calculated by Rutland-Patlak analysis and was considered to be normal in the range of 45%-55%.

In order to monitor therapeutic strategy (surgical or conservative treatment) after renography (6 months to a year), data of children were collected from the medical records of children's urological clinics.

Statistical analysis

For assessing the results of the research descriptive and analytical statistics (SPSS version 20.0) was used. The default level of significance was $P<0.05$. Of the parametric tests chi-square or Fisher's test were used, according to the size group of subjects. Of the non-parametric tests, Mann-Whitney and Kruskal-Wallis tests were used. Logistic regression was used to predict the outcome.

Results

Distribution of children according to ultrasound parameters

Distribution of children according to the ultrasound parameters is presented in Table 1.

Table 1. Distribution of children according to ultrasound parameter

Ultrasound parameter		N (%)
APD of pyelon (mm)	10-14.9	33 (53.2)
	15-19.9	14 (22.6)
	≥20	15 (24.2)
Calyceal dilatation (mm)		62 (100)
	0-5.9	37 (59.7)
	6-10	18 (29.0)
	>10	7 (11.3)
Parenchymal thickness		62 (100)
	Normal	44 (71.0)
	Reduced	18 (29.0)
		62 (100)

Results of renography

The results of renography showed that 46/62 children had good or almost good drainage, partial 7/62, while 9/62 had poor drainage. Differential renal function was in the range 45%-55% in 49/52 children. In 3 children DRF was above normal, range 57%-68%. There was 7 kidneys with DRF<40%, range 18%-37% and 3 kidneys with DRF 40%-45%. It was not determined a relation between the type of drainage and DRF, P=0,156 (Figure 1). Thus, 66.7% of those with poor drainage have preserved DRF (Figure 2).

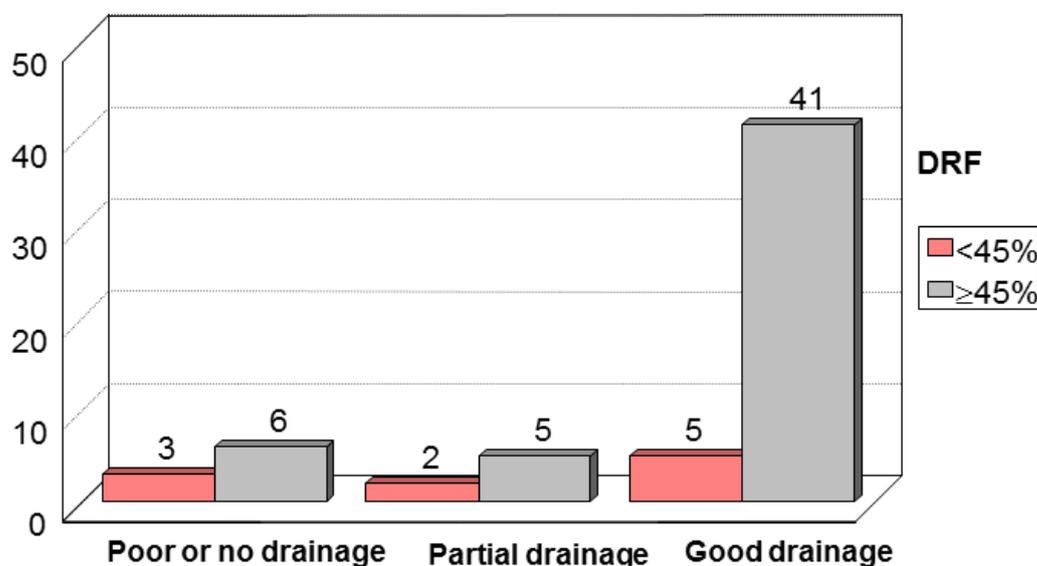


Figure 1. Association between drainage and DRF

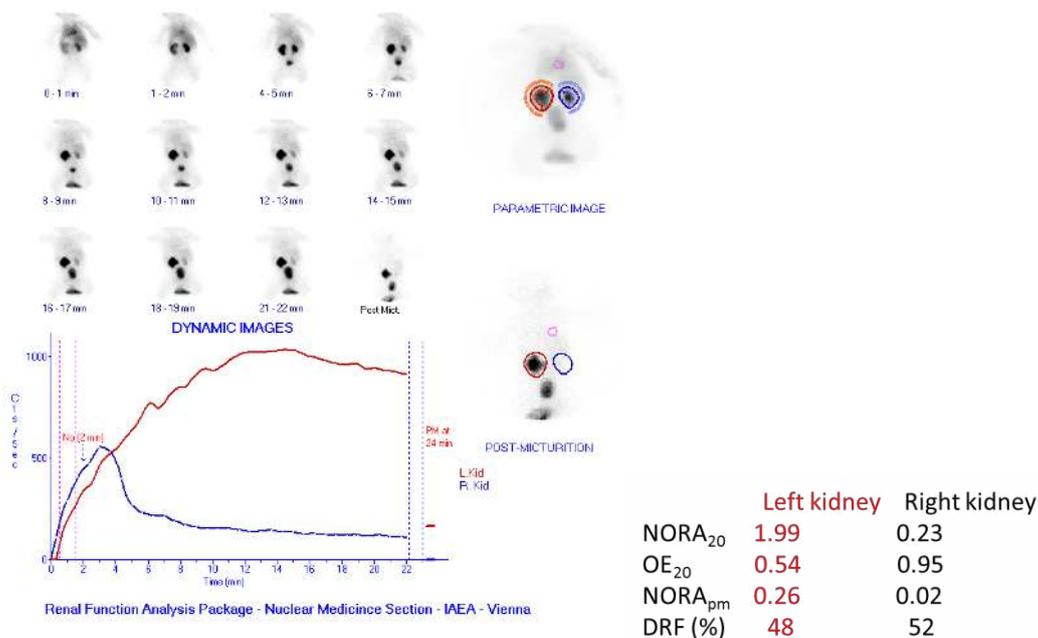


Figure 2. Girl age 2 months with prenatally detected PUJ stenosis on the left side, renal pelvis 24mm, calyces 12mm, reduction of parenchyma. Based on sequential images, renography curve and quantitative parameters of renography, obstruction could not be excluded, but DRF was preserved.

Degree of HN, calyceal size and parenchymal thickness compared with quality of drainage

There was clear relationship between the degree of HN (APD of pylon) and the type of drainage (Table 2). APD to 15mm was not indicate poor drainage, while in the group with APD>20mm, poor drainage was presented in 40% of children. In the group with calyceal dilatation >10mm, 71% of children had poor drainage. Calyceal size to 6mm does not show a pattern of poor drainage. In 87% of children with normal parenchymal thickness drainage was good, while in the group with reduced parenchymal thickness there was 39% children with good drainage.

Table 2. Ultrasound parameters in relation to drainage

Ultrasound parameter	Drainage N (%)			Total	P value
	Good	Partial	Poor		
APD of pylon (mm)					<0,001
10-14.9	32 (97.0)	1 (3.0)	0 (0)	33 (100)	0.009 10-14.9vs.15-
15-19.9	10 (71.4)	1 (7.2)	3 (21.4)	14 (100)	19.9
≥20	4 (26.7)	5 (33.3)	6 (40.0)	15 (100)	0.044 15-19.9vs.≥20 <0.001 10-14.9vs.≥20
Calyceal dilatation (mm)					<0.001
0-5.9	36 (97.3)	1 (2.7)	0 (0)	37 (100)	<0.001 0- 5.9vs.6-10
6-10	9 (50.0)	5 (27.8)	4 (22.2)	18 (100)	0.033 6-10vs.>10
>10	1 (14.3)	1 (14.3)	5 (71.4)	7 (100)	<0.001 0-5.9vs.≥10
Parenchymal thickness					<0.001
Normal	39 (88.6)	3 (6.9)	2 (4.5)	44 (100)	
Reduced	7 (38.9)	4 (22.2)	7 (38.9)	18 (100)	

Degree of HN, calyceal size and parenchymal thickness compared with DRF

Results showed a clear relation between the calyceal size and parenchymal thickness with DRF ($P < 0.05$), but not between degree of NH (APD) and DRF ($P = 0.105$). In the group of children with calyceal dilatation $> 10\text{mm}$, 57% had $\text{DRF} < 45\%$. In the group of children with reduced parenchyma 39% had $\text{DRF} < 45\%$ (Table 3).

Table 3. Ultrasound parameters in relation to DRF

Ultrasound parameter	DRF N (%)		Total	P value
	$\geq 45\%$	$< 45\%$		
APD of pyelon (mm)				
10-14.9	29 (87.9)	4 (12.1)	33 (100)	0.105
15-19.9	13 (92.9)	1 (7.1)	14 (100)	
≥ 20	10 (66.7)	5 (33.3)	15 (100)	
Calyceal dilatation (mm)				
0-5.9	33 (89.2)	4 (10.8)	37 (100)	0.007
6-10	16 (88.9)	2 (11.1)	18 (100)	
> 10	3 (42.9)	4 (57.1)	7 (100)	
Parenchymal thickness				
Normal	41 (93.2)	3 (6.8)	44 (100)	0.002
Reduced	11 (61.1)	7 (38.9)	18 (100)	

Relationship between quality of drainage, DRF, ultrasound parameters and pyeloplasty

Of the 62 children, pyeloplasty was done in 8 patients in the monitoring period 6 months to a year after the renography. There was a clear relation between the quality of drainage and decision about pyeloplasty ($P < 0.001$). Of the nine children with poor drainage in the seven was indicated pyeloplasty (Table 4). Also, pyeloplasty was indicated in a one child with partial drainage. Statistically significant differences was observed between children with normal DRF and impaired DRF ($< 40\%$) and decision of pyeloplasty ($P = 0.012$). In the group of patients with DRF less than 40% the pyeloplasty was performed in 3/7. The degree of HN (APD of renal pyelon), calyceal size and parenchymal thickness were clearly associated with decision of pyeloplasty (Table 4). The threshold for pyeloplasty was APD 18mm and calyceal dilatation 10mm. The model of the multivariate logistic regression which included ultrasound parameters (APD of pyelon, calyceal size and parenchymal thickness), drainage and DRF, which were significant predictors in univariate analysis, showed that only the drainage is independent predictor of pyeloplasty ($P < 0.05$, OR 2.9).

Table 4. Ultrasound parameters and results of renography in relation to pyeloplasty

Results of renography	Pyeloplasty		Total	P value
	Yes N (%)	No		
Drainage				
Good	0 (0)	46 (100)	46	<0.001
Partial	(100)			
Poor	1 (14.3)	6 (85.7)	7	
	(100)			
	7 (77.8)	2 (22.2)	9	
	(100)			
DRF				
<40%	3 (42.9)	4 (57.1)	7	0.012
≥40%	(100)			
	5 (9.1)	50 (90.9)	55	
	(100)			

Ultrasound parameter	Pyeloplasty		Total	P value
	Yes N (%)	No		
APD of pylon (mm)				
10-14.9	0 (0)	33 (100)	33	0.001
15-19.9	(100)			
≥20	2 (14.3)	12 (85.7)	14	
	(100)			
	6 (40.0)	9 (60.0)	15	
	(100)			
Calyceal dilatation (mm)				
0-5.9	0 (0)	37 (100)	37	<0.001
6-10	(100)			
>10	4 (22.2)	14 (77.8)	18	
	(100)			
	4 (57.1)	3 (42.9)	7	
	(100)			
Parenchymal thickness				
Normal	2 (4.5)	42 (95.5)	44	0.002
Reduced	(100)			
	6 (33.3)	12 (66.7)	18	
	(100)			

Discussion

Results of renography

Hydronephrosis due to obstruction is the major indication for renography in the postnatal period, particularly if PUJ stenosis is etiological factor. In the last few years new quantitative parameters of renography, NORA and OE are discussed. These are physiological parameters of drainage obtained on the basis of a mathematical model of renal function and can be calculated at any time of renogram, as well as on the late postmicturating acquisition. While NORA represents the residual activity in the kidney at a time, OE represents the percentage of eliminated activity by the kidney. These parameters allow more accurate assessment of drainage, they are not affected by the time of diuretic application, and kidney function has a much lesser impact on their values compared to the values of the traditional parameter of drainage ($T_{1/2}$).

Within the project of the IAEA, ten years ago began the development of a comprehensive non-commercial software for the analysis of dynamic renal scintigraphy (Software Package for the Analysis of Dynamic Renal Scintigraphic Studies). By this software which includes newly quantitative parameters (NORA, OE, DRF by Rutland- Patlak analysis) the interpretation of our results was carried out. "Cut off" values of these parameters are determined by quantitative validation study of the program published in 2014 developed after the first experiences with this software in our country [14]. The values of $T_{1/2}$ were also available, but how this parameter is not always confident enough in cases of poor drainage [15, 16], it was not used for final interpretation. We used the F+2 diuretic stimulation protocol and as a diuretic effect begins after 1 to 2 minutes, there was no impact on the value DRF.

In this study no connection between the quality of drainage and renal function was observed. Thus, 67% of children with poor drainage had preserved renal function ($DRF \geq 45\%$). Also one child with poor drainage had supranormal DRF ($>55\%$). Supranormal DRF occurs in big hydronephrotic kidneys [17]. Moon has found that supranormal DRF is predictor of UPJ obstruction and pyeloplasty [18], which was confirmed by surgical intervention in this child with supranormal DRF from our study.

Degree of HN, calycel size and parenchymal thickness compared with quality of drainage

Many studies have studied the association between prenatal ultrasound parameters and postnatal outcome of prenatal HN. Sidhu in the systematic analysis of 25 studies showed that the isolated HN resolves spontaneously or that is in stable condition in 98% of children with renal pelvis $APD < 12\text{mm}$ and in 51% of children with $APD > 12\text{mm}$ [19]. Lee in meta analysis, which included 1308 children concluded that the risk of post-natal pathology increases with the size of the prenatal APD of the renal pelvis, so that the risk of post-natal pathology increases of 11.9%, in mild HN ($APD 7-9\text{mm}$), 45.1% in moderate HN ($APD 10-15\text{mm}$) to 88.3% of children with severe HN ($APD > 15\text{mm}$) [20]. Our results showed that there is a strong association ($P < 0.001$) between the size of APD of renal pelvis and the renal drainage on renography. In the group of children with $APD 10-15\text{mm}$, 97% of children had a good drainage, so obstruction of hydronephrotic kidney was excluded, while one child presented with partial drainage. Thus, the results suggest that APD of renal pelvis up to 15mm has a favorable outcome in 97% of children. In moderate HN ($APD 15-19\text{mm}$), the risk of obstruction grows to 21.4%. In severe HN ($APD \geq 20\text{mm}$), the obstruction could not be excluded in 40% of children. Also in this group was not the small number of children with partial drainage, 33.3%. As already mentioned, the size of calyces is important predictor of postnatal outcome [21], so the idea of this study was to pay more attention to this ultrasound parameter. Grignon proposed five gradation of dilatation of the urinary tract, which beside APD of the renal pelvis includes calyceal dilatation and parenchymal thickness [22]. Also, SFU grading system of HN includes these ultrasound characteristics.

The results showed a clear correlation between the size of calyces and quality of drainage ($P < 0.001$). In the group of children without dilatation or with mild calyceal dilatation ($0-5.9\text{mm}$) for all patients obstruction of hydronephrotic kidney was excluded, one child had a partial drainage. The number of scintigraphic findings which could not exclude obstruction grows in the group with moderate calyceal dilatation ($6-10\text{mm}$) and occurs in 21.4% of children and in the group with severe dilatation ($>10\text{mm}$), more than two-thirds of the children had poor drainage and obstruction could not be excluded. With regard to parenchymal thickness a clear association with drainage ($P < 0.001$) was found. In the group of children with normal parenchymal thickness, 88.6% of children had a good drainage, while in the group of children with parenchymal reduction that number was 38.9%.

Degree of HN, calyceal size and parenchymal thickness compared with DRF

DRF is an important quantitative parameter of renography, highly reproducible [23] and very important for follow up of HN and for making decision of surgery. There are not many studies dealt with the association between prenatal ultrasound parameters and DRF, in particular related to calyceal dilatation and parenchymal thickness. Results of this study showed that there is no association between the size of APD of renal pelvis and DRF. Ulman in a study of 104 children in the follow-up period of 78 months concluded that in severe prenatally detected HN, DRF is often preserved [24]. Koff emphasizes the protective effect of HN, finding that a certain number of clinical and experimental studies suggesting that HN is not a pathological process, but it represents a compensatory mechanism designed to protect the kidney from a pressure and damage of its function [25]. Our results show that in the third group of children with the APD of renal pelvis ≥ 20 mm, 66.7% of children have preserved renal function. This can be explained by the extrarenal pelvis which does not make significant pressure on the parenchyma, and does not impair the RF uptake and DRF calculation, as well as study includes children younger age, so HN did not last long enough to disrupt renal function. As regards the calyceal dilatation and parenchymal thickness as an indicator of kidney function, there is a little literature data. The results of this study indicate that there is a strong correlation between the size of calyces and DRF ($P=0.007$). The group of children with prominent calyceal dilatation (>10 mm), more than half of the children, 57.1% have reduced kidney function. In the group of children with normal-sized calyces or moderate dilatation (0-5.9mm), about 90% have normal kidney function. Children with reduced parenchyma have an increased risk for impaired renal function, 38.9% of them, compared to the 6.8% of children with normal parenchymal thickness ($P=0.002$). A recent study by children's nephrologists and specialists of nuclear medicine also researched ultrasound and radionuclide parameters in children with prenatally diagnosed hydronephrosis caused by PUJ stenosis. The conclusion was that the strategy of postnatal follow-up of these children would be to postpone radionuclide procedures in children with APD of renal pelvis <30 mm, calyces dilation <10 mm and a normal parenchymal thickness [26]. Their results, the association of ultrasound parameters (APD renal pelvis, calyceal dilatation, thickness of parenchyma) and results of renography (drainage and DRF) agree to the results of the study, except for the association of APD of the renal pelvis and DRF, which has not been confirmed by this study.

Relationship between quality of drainage, DRF, ultrasound parameters and pyeloplasty

The results of this study show that in 8/62 children was done pyeloplasty. Univariate analysis revealed that the APD of the renal pelvis, the dilatation of calyces, parenchymal reduction, drainage and $DRF < 40\%$ were predictors of pyeloplasty. In multivariate logistic regression model of these variables pattern of drainage has remained an independent predictor of pyeloplasty ($P < 0.05$, OR 2.9). In one retrospective multivariate analysis APD of renal pelvis was the only independent predictor of pyeloplasty [27]. In another study $DRF < 40\%$ and the SPF 3-4 were significant predictors of surgery [28].

In the group of children who underwent pyeloplasty, threshold of pelvis was APD 18mm and calyceal size 10mm. Several studies have examined the "cut off" value of APD of renal pelvis for pyeloplasty. Dias in a study of almost 400 children suggested $APD > 16$ mm, with a sensitivity of 100% and specificity of 86% [29]. Coplete proved $APD > 15$ mm as a predictor of pyeloplasty, sensitivity 73%, specificity 82% [30]. The conclusion of a recent study with a follow-up period of 37 weeks was $APD > 24$ mm, sensitivity of 73.1% and specificity of 88% with a recommendation that the specificity can be increased by taking into account the DRF when making decision of pyeloplasty [31]. Also, in one more recent study in a ten years follow up period of children with pyeloplasty the mean preoperative APD of renal pelvis was 26mm, the

median age of operation was 15 months [32], in our study these values were following 22mm and 11.5 months.

It turned out that renography and its quantitative parameters are very helpful in making decision about surgical treatment. Thus, none of the children with excluded obstruction on dynamic scintigraphy was not considered for the surgery and in 77.8% of children with poor drainage pyeloplasty was performed ($P<0.001$). Our results confirmed the most frequently used "cut off" value of DRF ($<40\%$) for surgery ($P<0.05$). But in about 10% of the children with $DRF>40\%$, pyeloplasty was done. This decision could be explained by ultrasound worsening of HN, obstructive type of radiorenography curve or potential clinical complications.

It has already discussed that $T_{1/2}$ is not very reliable in assessing kidney drainage. Although this traditional parameter of drainage was not used for final interpretation of study, results confirmed that it is not predictor of pyeloplasty. In the context of discussion on the evaluation of prenatal HN, Gordon concluded that this parameter is unsuitable for recommendation for surgical treatment [33].

A limitation of this study in terms of monitoring the decision of pyeloplasty is a short follow-up period.

Conclusion

In summary, the present study shows the following:

Finding of poor drainage on renography is significantly more common with the increase of APD of renal pelvis, calyceal dilatation and reduction of parenchyma.

APD of renal pelvis to 15mm has a favorable course in the most children with PUJ stenosis.

There is a clear connection between the US parameters (APD of renal pelvis, calyceal dilatation, parenchymal thickness) and renography parameters (drainage, DRF) of hydronephrotic kidney and the decision of pyeloplasty.

Pattern of drainage is the only independent predictor for the need of pyeloplasty.

Radionuclide renography and its physiologically quantitative parameters of drainage are very useful in post-natal follow-up and therapeutical strategy in children with PUJ stenosis.

The authors declare that they have no conflicts of interest

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Honorary award to Professor Anastasios Tousimis

The detection of endocarditis, post implantation grafts, arteritis and other related disorders by ^{18}F -FDG PET/CT

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Keywords: ^{18}F -FDG PET/CT -SUVmax -Endocarditis -Vasculitis -Graft

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Abstract

Introduction: Positron emission tomography with computed tomography (^{18}F -FDG PET/CT) is a nuclear medicine diagnostic method which, unlike other technological modalities that assess anatomical features, detects increased glucose metabolism inside the cells, thus is very helpful in diagnosing cardiovascular infection and inflammation and also in therapy planning. **Aim:** Aim of this study was to assess the significance of ^{18}F -FDG PET/CT in detection of an active disease in patients with infection and inflammation of cardiovascular system. **Material and methods:** In this cohort retrospective study 73 cardiovascular patients (56.9±15.3 years; 33 male and 40 female) with persistent symptoms of inflammatory syndrome were referred to ^{18}F -FDG PET/CT in order to evaluate active disease. Biochemical blood analyses (erythrocyte sedimentation, CRP, leukocytic formula), CT, MRI, ultrasound were performed in all the patients. Out of 73 patients, 7 had a second ^{18}F -FDG PET/CT examination (62.1±12.3 years; 6 men and 1 woman) with a previous pathological PET/CT finding after which the therapy was changed. The degree of metabolic activity was analyzed visually and quantitatively using the maximal standardized uptake value (SUVmax). ^{18}F -FDG PET/CT findings were considered positive in case of higher focal glucose accumulation in projection of heart and diffuse uptake in blood vessels' wall than accumulation in surrounding tissue and liver. **Results:** Vasculitis was diagnosed in 36 patients (49,3%), endocarditis in 23 (31,5%) and graft inflammation in 14 (19,2%). The results were compared to the gold standard, biopsy of the blood vessel and histopathological verification during surgical treatment, or clinical follow up. Forty nine patients with the signs of an increased FDG uptake were considered true positive (TP) (SUVmax 5.7±2.9). In 21 patients ^{18}F -FDG uptake was physiological and they were considered true negative (TN). Two who used corticosteroid therapy which decreases inflammation, were false negative (FN), and only 1 false positive (FP) finding in the region of recent iatrogenic vein injury. Sensitivity of this method was 96.08%, specificity 95.45%, positive predictive value 98.0%, negative predictive value 91.3% and accuracy 95.89%. **Conclusion:** Our results indicate that ^{18}F -FDG PET/CT could be useful diagnostic method for the detection of signs of metabolically active disease in patients with persistent symptoms of infection and inflammation of cardiovascular system, as well as in monitoring therapy response.

Introduction

Infections and inflammation are common causes of cardiovascular disease occurrence. These processes are presented with different nonspecific symptoms and therefore it is challenging to the clinicians to make the right diagnosis. Vasculitis is a term for vascular disease, such as Takayasu arteritis, giant cell arteritis, polymyalgia reumatica, Wegener's granulomatosis and other types of vasculitis, which is characterized by blood vessels' inflammation of unknown origin in most cases [17]. Cardiac infections, such as infective endocarditis, is manifested by valves' insufficiency and abscess appearance and is usually caused by bacteria *Staphylococcus aureus*, *Streptococcus* sp. and *Enterococcus* sp. (also causes of vascular graft infections) [13]. Both, infections and inflammation, are manifested by persistent inflammatory symptoms of unknown origin: fever, weakness, chills, night sweats, weight loss, pulseless, muscle pain, fatigue [14]. These disorders are easy to treat, but are sometimes not easy to find and could lead to death [12]. The goal of the treatment is to suppress inflammation and immune system by using corticosteroid therapy (prednisone), immune suppressive drugs (methotrexate, azathioprine) and to eliminate the infective agent by various antibiotics [4]. Surgical treatment implies vascular graft and valves replacement. Diagnosis is made based on physical examination, blood analyses (erythrocyte sedimentation, C- reactive protein, white blood cell analysis) positive bacterial hemocultures, ultrasound, CT, angiography, and on the gold standard - histopathological finding of inflammation on biopsy [16]. However, biopsy of the cardiovascular system is an invasive method that is not always done or available in everyday practice. In addition, radiological modalities assess only anatomical features, which are not always precise in the evaluation of active disease.

Positron emission tomography with computed tomography with fluorine-18-fluorodeoxyglucose (^{18}F -FDG PET/CT) detects increased glucose metabolism inside the active inflammatory cells, thus could be very helpful in diagnosing active cardiovascular infection and inflammation [5]. Therefore, the aim of this paper was to prove the significance of ^{18}F -FDG PET/CT, as a noninvasive method, in detection of vascular and cardiac infection and inflammation. Also the goal was to assess the treatment management of the patients after first ^{18}F -FDG PET/CT examination and to follow-up of the therapy response.

Material and Methods

Study population

In this cohort retrospective study 73 cardiovascular patients (56.9 ± 15.3 years; 33 male and 40 female) with persistent symptoms of inflammatory syndrome were referred to ^{18}F -FDG PET/CT examination, to the National PET Center, Clinical Center of Serbia, Belgrade, from July 2011 to April 2017, in order to evaluate active disease. The majority of patients were sent under the diagnose or for the suspicion of: Fever of unknown origin (10), Endocarditis infectiva (19), Status post implatatio grafti + FUO (15), Takayasu arteritis (11), Vasculitis (9), Septicaemia (enterococcica, streptococcica) (6), polymyalgia reumatica (3).

The criteria for inclusion and indications for ^{18}F -FDG PET/CT examination were: suspicion of previous cardiovascular infection/inflammation recurrence, unexplained fever, positive hemoculture and inflammation factors (CRP, sedimentation), graft/ valvulae implantation. The criteria for exclusion were the presence of malignant disease and blood glucose level more than 11mmol/L.

Procedures

The ^{18}F -FDG PET/CT examination was performed on a 64-slice hybrid PET/CT scanner (Biograph, TruePoint64, Siemens Medical Solutions, Inc. USA). Biochemical blood analyses (sedimentation and CRP), CT, MRI or ultrasound were performed in all the patients. The ^{18}F -FDG PET/CT imaging was performed after intravenous injection of ^{18}F -FDG, following 1h of rest. Whole body acquisition, from the base of the skull to the mid thighs, was performed by low dose CT scans without contrast and 3D PET scans. Appropriate CT, PET and fused PET/CT images were displayed for analysis on the dedicated workstation. The degree of metabolic activity was analyzed visually and quantitatively using the maximal standardized uptake value (SUVmax), which was improved according to the patients' body weight and applied dosage. ^{18}F -FDG PET/CT findings were considered positive in case of greater focal glucose accumulation in projection of the heart and diffuse accumulation in the blood vessels' walls than the accumulation in surrounding tissue and liver.

The results of ^{18}F -FDG PET/CT were compared to the biopsy of the blood vessel during surgical replacement of the graft/ valve and histopathological finding or/and with the biochemical parameters and therapy response during clinical follow-up of 1 year after the PET/CT examination.

Statistical analyses

The results were showed as mean \pm standard deviation (SD). The ^{18}F -FDG PET/CT diagnostic output was evaluated by calculating specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

Results

Patients characteristics

Out of 73 patients referred to ^{18}F -FDG PET/CT examination, vasculitis (Figure 1) was diagnosed in 36 patients (49,31%), endocarditis (Figure 2) in 23 (31,5%) and graft inflammation (Figure 3) in 14 (19.17%).

Table 1. Diagnostic output of ^{18}F -FDG PET/CT during clinical follow-up

	Vasculitis	Endocarditis	Graft Inflammation	Total
True positive	28	10	11	49
True negative	7	12	2	21
False positive	0	1	0	1
False negative	1	0	1	2
Total	36	23	14	73

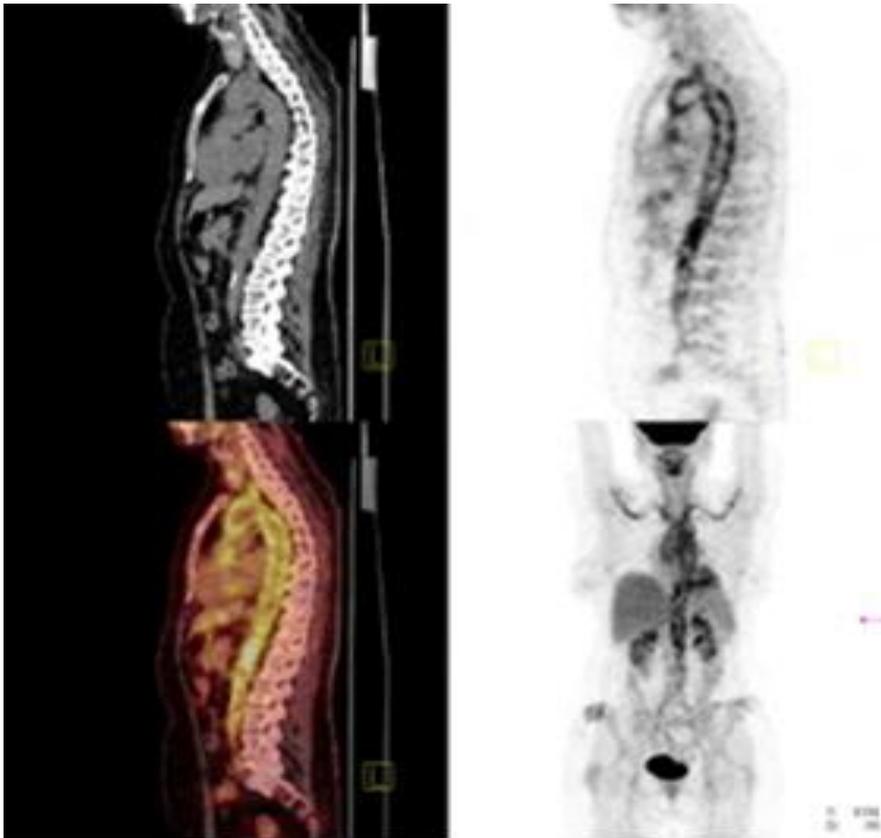


Figure 1. Sagittal PET/CT image: diffuse and increased ^{18}F -FDG uptake in thoracic and abdominal aorta; MIP (maximal intensity projection) image: diffuse and increased uptake in a. subclavia, a. jugularis

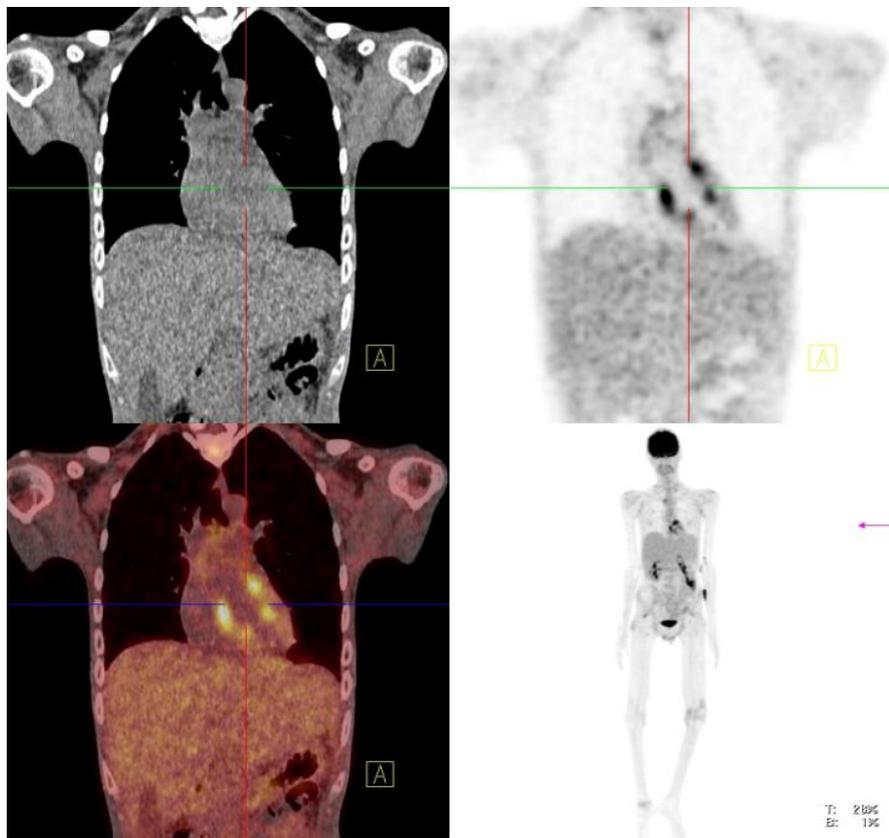


Figure 2. Coronal PET/CT: signs of an increased ^{18}F -FDG uptake in patient with endocarditis

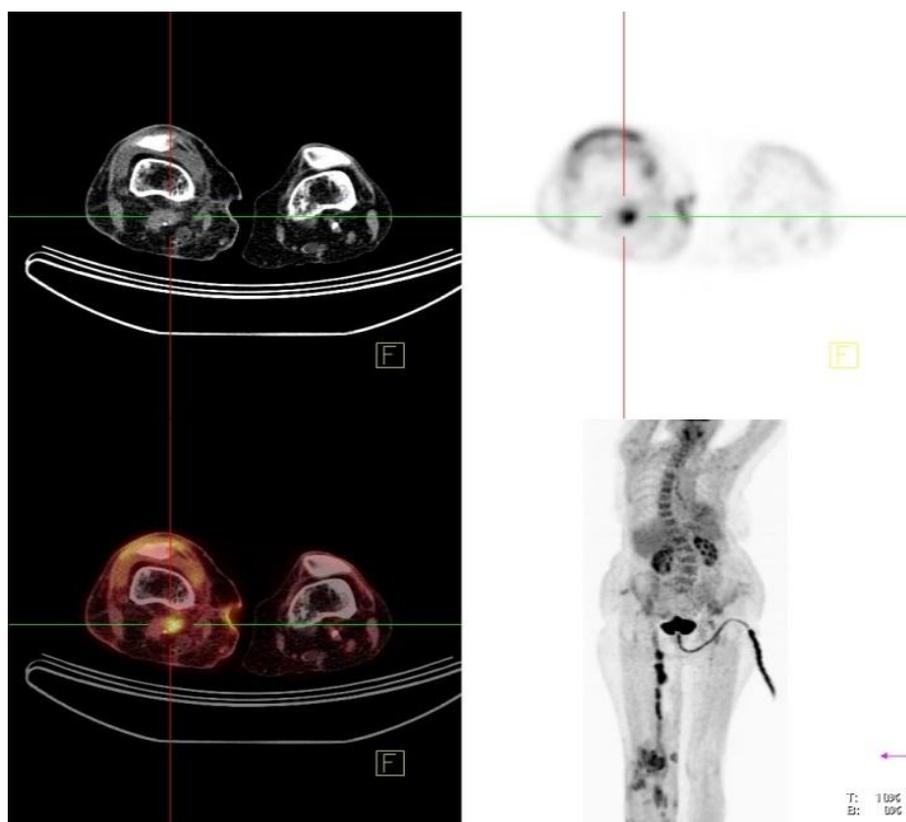


Figure 3. Transversal PET/CT: Increased ^{18}F -FDG uptake in projection of implanted right femoral graft.

Sights of an increased FDG uptake were discovered in 49 (47.1%) (21 men and 28 women), and they were considered true positive (TP) (SUVmax average was 5.7 ± 2.9) (Table 1). Febrility was manifested in 38 TP patients (77.5%) ($38.1 \pm 0.86^\circ\text{C}$), out of which 20 had elevated level of CRP ($95.3 \pm 28.7\text{mg/L}$). Other symptoms were nonspecific, such as exhaustion, fatigue, headache, weight loss, pulseless of the extremities.

Only 11 patients (47.8%) with endocarditis had positive hemoculture (6 staphylococcus, 3 enterococcus, 2 streptococcus). In 21 patients ^{18}F -FDG uptake was physiological and they were considered true negative (TN). Two patients who were using corticosteroid therapy which reduces inflammation and therefore also the accumulation of ^{18}F -FDG were false negative (FN). Only 1 finding was false positive (FP) due to the increased uptake of ^{18}F -FDG in the region of recent iatrogenic vein injury during an intravenous injection.

Therapy change and ^{18}F -FDG PET/CT follow-up

During 1 year of follow-up, 21 (58.3%) patients with vasculitis and 14 with endocarditis received a new treatment, corticosteroid and/or immunosuppressive therapy (Table 2). Three (27.3%) patients with a graft inflammation had a graft replacement and 7 patients with diagnosed endocarditis had a valve replacement (70.0%), with confirmed histological finding during surgery.

Out of 73 patients, 7 had a second ^{18}F -FDG PET/CT examination (62.1 ± 12.3 years; 6 men and 1 woman) in order to evaluate the therapy response. Physiological accumulation of previously active disease was found in 5 patients (4 men and 1 woman), whose therapy was changed after the first examination. In 2 patients ^{18}F -FDG PET/CT showed the recurrence of the disease after interruption of corticosteroid therapy. Overall sensitivity of this method is 96.08%, specificity 95.45%, positive predictive value 98.0%, negative

predictive value 91.3% and accuracy 95.89%.

Table 2. Therapy modification after ^{18}F -FDG PET/CT examination

	Corticosteroides	Antibiotics	Immunosuppressives	Surgery	None	Total
No change	9	7	3	0	0	19
New treatment	15	14	6	10	9	54
Total	24	21	9	10	9	73

Discussion

The results of this study show that ^{18}F -FDG PET/CT has a high sensitivity, specificity, accuracy, as well as positive and negative predictive values in detection of active cardiovascular inflammation/infection. Not all the patients with cardiovascular infection and inflammation have high level of CRP, symptoms such as febrility and proved infective agent. In our study, out of 49 TP patients, high fever was manifested in 38 (77.5%), 20 patients with fever had elevated CRP and only 11 patients with diagnosed endocarditis had positive hemoculture. In false negative (FN) patients, an uptake of radiopharmaceutical was decreased because of the utilization of corticosteroid therapy which reduces the inflammation and effects the accumulation by reducing it. Therefore, the sights of an active disease were not visible or had very low activity. The disease was discovered later on the second PET/CT examination, when the therapy was interrupted and the symptoms started manifesting again. In false positive (FP) patient, there was an increased diffuse uptake of FDG in the cubital vein due to the recent iatrogenic blood vessel injury during an intravenous injection.

The results obtained in other studies can relate to ours [2, 7, 9, 15]. Granados and coworkers [6] reported the sensitivity of 82%, specificity 96%, positive predictive value 94%, and negative predictive value 87% of ^{18}F -FDG PET/CT in their study. They concluded that ^{18}F -FDG PET/CT should be a method of choice in early detection of infective endocarditis in patients with implanted valves.

^{18}F -FDG PET/CT was especially helpful to the clinicians in therapy alteration and follow-up of it's response [3]. ^{18}F -FDG PET/CT is reported to be very useful in the evaluation of poor therapy response or suspicious relapse after the therapy interruption in patients with vasculitis [1].

Beside ^{18}F -FDG PET/CT, one of the nuclear medicine methods that is also used for discovering sights of an infection, in patients with endocarditis or vascular graft infection, is $^{99\text{m}}\text{Tc}$ -HMPAO-WBC SPECT/CT. There are several studies that have compared these two modalities [8, 16]. Erba's results show that $^{99\text{m}}\text{Tc}$ -HMPAO-WBC scintigraphy has a high specificity and can decrease the number of misdiagnosed cases of endocarditis [11]. In their study sensitivity was 90%, 94% negative predictive value, 100% specificity and 100% positive predictive value. Even though ^{18}F -FDG PET/CT had a high sensitivity, the possibility of false positive findings was higher due to the ^{18}F -FDG uptake in the heart region, for example in cases of primary cardiac tumors, metastases or inflammatory changes after interventions [11]. In order to improve specificity, ^{18}F -FDG WBC PET/CT is introduced in the detection of cardiovascular infection and

inflammation [18]. Yilmaz confirmed in his study higher diagnostic accuracy of ^{18}F -FDG WBC PET/CT than ^{18}F -FDG PET/CT [10]. However, this method is more time consuming and not suitable for clinical practice.

This study has some limitations. Only 10 out of 73 (13.7%) patients had a histologic confirmation of the disease, during surgical replacement of the graft (3) and valves (7). Biopsy is not always done in practice to verify cardiac or blood vessel inflammation/ infection. In those cases the results are compared to the level of CRP, white blood cell analysis, therapy performance or clinical condition of the patient during follow-up. Comparison between ^{18}F -FDG PET/CT and other radiological modalities such as CT, MRI, angiography, were not the subject of this study. Therefore, further prospective multicenter studies are needed to optimize clinical utility of ^{18}F -FDG PET/CT in patients with cardiovascular infection and inflammation.

Conclusion

^{18}F -FDG PET/CT is a hybrid diagnostic method that allows the assessment of both anatomical and morphological features and is therefore useful in detecting signs of metabolically active disease in patients with persistent symptoms of infection and inflammation of cardiovascular system. Also, ^{18}F -FDG PET/CT is helpful to the clinicians in understanding the intensity and expansion of the disease, as well as in planning a proper therapy, evaluation of a response of treatment.

The authors declare that they have no conflicts of interest.

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Honorary award to Professor George Sfakianakis

The association between Alzheimer's disease and cancer: Systematic review - Meta-analysis

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Keywords: Dementia -Inverse comorbidity -Cancer localization.

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Abstract

The objective of the present study was the quantitative assessment of the previously documented inverse relationship between Alzheimer's Disease (AD) and cancer (CA) by conducting a meta-analysis and evaluating systematic differentiations of the aforementioned relationship based on cancer localization. For the purpose of the study all available empirical data of the last decade, which met specific criteria, were included in the analysis by querying PubMed, Web of Science and Cochrane Library databases. Seven studies were included in the analysis, with a total sample of 18,887 (10,859 AD patients, 8,028 non-demented controls) participants to calculate cancer risk among AD patients, and 11 studies, with a total of 5,607,076 (1,853,318 cancer patients, 3,753,758 healthy controls) participants, were assessed to evaluate AD risk among cancer patients. The analysis revealed that AD patients appear to have a reduced risk of cancer, by 40% (RR 0.60, 95% CI 0.45 - 0.79), while cancer history was associated with a reduced risk of AD, by 15% (RR 0.85, 95% CI 0.77-0.92). Systematic differences were also identified based on site-specific cancer. Indications of heterogeneity and publication bias were present in the analysis. Our meta-analysis is only the fourth conducted on this subject, with newer evidence suggesting a mitigation of the inverse relationship. We emphasize the need for new studies to assess the inverse comorbidity hypothesis, especially in AD patients.

The association between Alzheimer's disease and cancer

Alzheimer's disease (AD) is a neurodegenerative disease that affects primarily persons over 65 years of age and it is the most common type of dementia [1]. AD patients experience impairment of their cognitive functions which gradually leads to autonomy loss. Despite the apolipoprotein E (ApoE) gene having been identified as the most important genetic risk factor for AD [2], the underlying cellular and molecular mechanisms responsible remain largely unknown [3, 4]. The most prevailing hypotheses have been focused on metabolic factors [5, 6], immunological factors [7, 8] as well as β -amyloid and tau pathology [9].

Demetriou et al. (2014), have suggested that in some cases (sporadic AD), an inverse metabolic reprogramming of mitochondria is observed, that is the reverse of mitochondrial reprogramming observed in cancer [10].

The Inverse Warburg effect hypothesis introduced a novel approach to studying the pathogenesis of AD into modern bibliography. According to this position, attention is centered on the comparison of mechanisms causing AD, to those that lead to the development of neoplasia in cancer [11-14]. Tabares-Seisdedos and Rubenstein (2013) introduced the hypothesis of an inverse comorbidity between some types of cancer and Alzheimer's disease. Inverse comorbidity, constitutes a situation where a lower-than-expected probability of disease is occurring in individuals who have been diagnosed with another medical condition [15]. Sánchez-Valle et al. (2017) observed an inverse relationship between AD and lung cancer [16] which is attributable to an oppositely mitochondrial metabolism regulation. Another study by White et al. (2013) supports a remarkable inverse relationship (HR 0.21, 95% CI: 0.051 - 0.87, P=0.031) between Alzheimer's disease and non-melanoma skin cancer (White, Lipton, Hall, & Steinerman, 2013), a very common cancer type in western cultures [17, 18].

In contrast, the co-occurrence of AD with some other diseases indicates a positive relationship. For example, AD has a high comorbidity with diabetes (RR 1.54, 95% CI: 1.33-1.79), obesity (RR 1.59, 95% CI: 1.02-2.50), hypertension (RR 1.31, 95% CI: 1.01-1.70) and hypercholesterolemia (RR 1.72, 95% CI: 1.32-2.24) [19, 20].

In each case, it is known that both cancer and AD are associated with age, as both have a higher instance in ages over 60 years and are also both associated with cellular dysfunction, whether it's the aberrant proliferation of cells observed in cancer, or the cellular degeneration and cell death observed in dementia [21].

Lately, there has been an increased research interest on the inverse relationship between AD and CA; the further understanding of this relationship could uncover valuable knowledge and benefit both the identification of AD pathophysiological mechanisms, as well as new therapeutic approaches. Even though studies so far suggest the presence of an inverse relationship [15], these findings should be interpreted with caution, as objections have been raised concerning methodological and attribution issues [22]. The appropriate statistical analysis and the synthesis of latest empirical data with the use of meta-analysis models could facilitate a quantitative evaluation of this phenomenon, as well as its critical assessment [23]. This is exactly the aim of the present study.

Methods

Search Strategies

The search for publications of relevant studies was performed in the electronic databases of PubMed, Web of Science and Cochrane Library, using (in the publication title) the combination of the following search terms; "Alzheimer*", "Dementia", "Cancer*", "Neoplasm*", "Carcinoma" and "Tumor", and limiting the publication span of the studies within the last decade (2007-2017), in an effort to only use the most recent bibliography. Moreover, the references used in the chosen publications as well as publications using the target articles in their bibliography were examined with the help of Google Scholar. The search returned among others, three meta-analyses, two of which were published in 2015 and one in 2014 [24-26]. Furthermore, one publication with relevant data was discovered [11].

Selection Criteria

The studies that were eventually selected had to conform to the following restrictions; (a) the study design had to be either an epidemiology and/or a case study; (b) the results had to be presented in the format of Relative Risk (Risk Ratio, RR), Odds Ratio (OR), Hazard Ratio (HR) or Standardized Incident Ratio (SIR) with a 95% Confidence Interval (CI); and (c) the diagnosis had to be stated clearly. Publications were excluded from the study for the following reasons; (a) the type of dementia was not clearly identified as AD; (b) absence of compatible statistical data and (c) studies that did not focus on human subjects. Finally, the publications selected were written primarily in English (Figure 1).

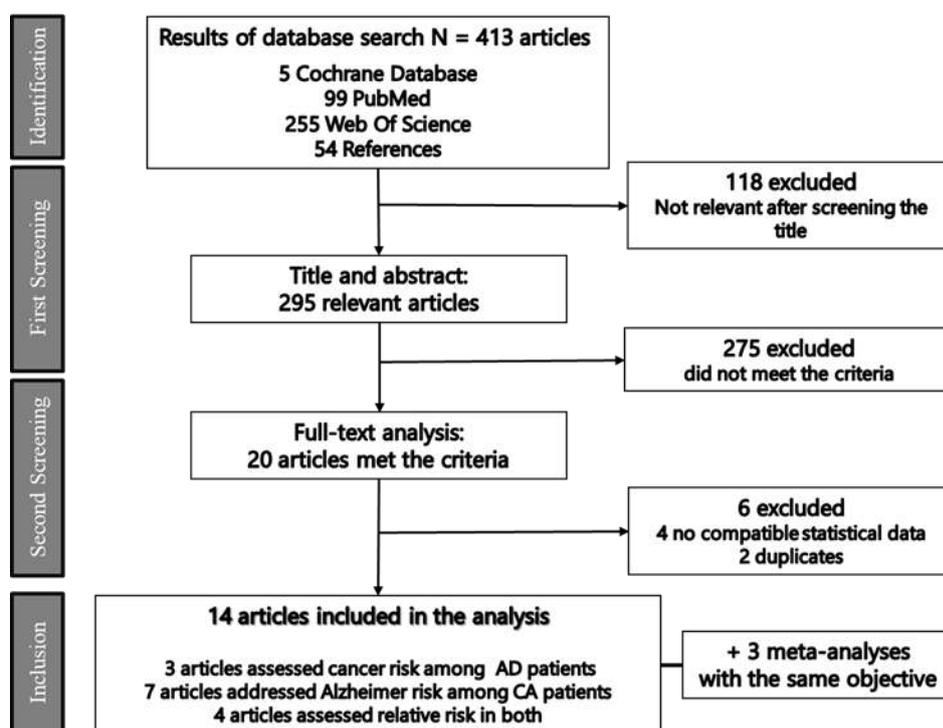


Figure 1. Diagram showing processes for study selection.

Data extraction

The following data were extracted from the selected publications; First Author; Publication Year; Study Design; Sample Size; Study Span and Study Duration; Follow-Up Duration, Diagnostic Criteria for Alzheimer's Disease and Cancer and results in RR, OR, HR or SID with a 95% Confidence Interval (CI).

Statistical Analysis

The pooled relative risk was calculated with the use of Open Meta Analyst 12.11.14 [27-29] and R software [28, 30]. The Cochrane Review Manager 5.3.5 Calculator was used for statistical data transformations [31, 32].

The Random-Effects Model; DerSimonian-Laird was the selected method for statistical analysis [33] with a 95% confidence interval, as the selected studies had variations in participants' profile, sample sizes, design and types of cancer studied [34]. Moreover, the heterogeneity factor (I^2) was used to quantify the heterogeneity between various studies. Heterogeneity was considered statistically significant for $P < .05$. Publication bias was assessed using the Egger's Test [35], with $P < .10$ considered as an indication of publication bias [36].

Due to the small number of studies used in the present analysis ($n_1=7$ and $n_2=11$), the design of Funnel Plots was avoided, since modern bibliography dictates the necessity for at least ten studies to be present ($n>10$), so that the effect of heterogeneity does not incur misleading conclusions [37]. In order to control the software reliability and the methods used for statistical analysis, we replicated the results of the previous meta-analysis studies on this subject; a) Ma et al. (2014); b) Shi et al. (2015); and c) Zhang et al. (2015).

The included studies that met the selection criteria were categorized into two groups. The first group of studies was focused on AD patients who were screened for cancer risk of developing cancer, in comparison with matched healthy controls. In the second group of studies, patients with a history of cancer were screened for the risk of developing AD. Statistical analysis was performed separately for each category of studies. Finally, an evaluation for the risk of developing AD based on cancer localization was performed using the identification of systematic differences.

Results

In our initial search, a total of 413 relevant publications were identified. From these, only 14 were eligible for inclusion in our analysis, based on the selection criteria (Figure 1). Three papers investigated the risk for cancer in AD patients [38-40], seven assessed the risk of AD in patients with cancer history [41-47] and four studies reported the relative risk in both, CA and AD patients [18, 48-50].

These studies were published between 2010 and 2017, had a span from 1992 to 2015 and took place in the United States of America, Taiwan, Spain, Italy, and Denmark. The participants were primarily individuals over 60 years of age. Both investigated diseases were defined based on the diagnostic criteria from the International Classification of Disease (ICD), the NINCDS-ADRDA for Alzheimer's disease, the Diagnostic and Statistical Manual of Mental disorders (DSM), while a subset of studies took into account pharmaceutical treatment or chemotherapy that the patients might have been subjected to. Tables 1 and 2 display detailed information on each group of studies. Among the studies that referred to AD patients ($n=7$), three were case studies and four were cohort studies, while among the studies that referred to cancer patients ($n=11$), four were case studies, six were cohort studies and one had a mixed study design. Two of these studies focused on non-melanoma skin cancer and one study on hepatocellular carcinoma.

Table 1. Characteristics of studies included in meta-analysis concerning cancer risk among Alzheimer's disease patients ($n=7$)

References	Year publ.	Design	N	N ctrl	N AD	Age	Country	Diagnosis criteria	Time period	Follow-up ^a	RR (95% CI)	P
Roe et al.	2010	C	2178	2107	371	≥65	USA	NINCDS-ADRDA	1992-2003	8.3	0.31 (0.12 - 0.80)	.01
Realmuto et al.	2012	S	378	252	126	≥71	Italy	NINCDS-ADRDA	2006-2010	-	0.75 (0.49 - 1.16)	.19
Driver et al.	2012	S	1308	981	327	≥65	USA	NINCDS-ADRDA	1986-2008	10	0.39 (0.26 - 0.58)	.00
Musicco et al.	2013	C	2832	-	2832	≥60	Italy	Medication hist.	2004-2009	6	0.79 (0.64 - 0.97)	.02
White et al.	2013	C	1034	958	76	≥70	USA	DSM	1993-2004	3	0.21 (0.05 - 0.86)	.03
Ou et al.	2013	C	6960	-	6960	≥40	Taiwan	Medication hist.	1995-2009	10	0.88 (0.80 - 0.97)	.01
Romero et al.	2014	S	4197	3730	467	≥65	Spain	NINCDS-ADRDA	1994-2007	12	0.45 (0.27 - 0.75)	.00

Note: C: Cohort study. S: Case-control. N: Total sample size. N ctrl: Control group sample size. N AD: AD patients sample size. ICD: International Classification of Disease. NINCDS-ADRDA: National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer Disease and Related Disorders Association. Medication hist.: Medication history for AD. ^a Years of follow-up (mean). -: No available data. RR: Relative Risk with 95% CI. N/M: Not mentioned.

Table 2. Characteristics of studies included in meta-analysis concerning AD risk among cancer patients (n=11)

References	Year		N	N ctrl	N CA	Age	Country	Diagnosis criteria	Time period	Follow-up ^a	RR (95% CI)	P
	publ.	Design										
Roe et al.	2010	C	3020	2122	898	≥65	USA	ICD-9	1992-2003	5.4	0.57 (0.36 - 0.90)	.01
Driver et al.	2012	S	1278	1102	176	≥65	USA	ICD	1986-2008	10	0.67 (0.47 - 0.95)	.02
Musicco et al.	2013	C	21451	-	-	≥60	Italy	ICD-10	2004-2009	6	0.64 (0.50 - 0.82)	.00
White et al.	2013	C	1102	-	-	≥70	USA	Selfrep/NMSC	1993-2004	3.7	0.69 (0.39 - 1.22)	.20
Frain et al.	2013	C	3499378	2728093	771285	≥65	USA	ICD-9	1996-2011	5.7	1.03 (1.01 - 1.05)	.00
Lai et al.	2014	S	13124	9843	3281	≥65	Taiwan	ICD-9	2000-2010	-	0.51 (0.19 - 1.37)	.18
Freedman et al.	2016	C/S	979816	142869	836947	≥65	USA	ICD-3	1992-2005	-	0.87 (0.84 - 0.90)	.00
Yarchoan et al.	2016	S	1289	888	401	P/M	USA	Selfrep	-	-	0.80 (0.69 - 0.94)	.00
Schmidt et al.	2017	C	1081097	864876	216221	≥18	Denmark	NMSC	1980-2013	10	0.95 (0.92 - 0.98)	.00
Bowles et al.	2017	C	4357	-	1339	≥65	USA	Medic. hist.	1994-2015	-	0.73 (0.55 - 0.97)	.02
Robles et al.	2017	S	1164	947	217	N/M	Spain	ICD-9	-	-	0.87 (0.87 - 1.46)	.58

Note: C: Cohort study. S: Case-control. N: Total sample size. N ctrl: Control group sample size. N CA: Cancer patients sample size. ICD: International Classification of Disease. NMSC: Non-melanoma skin cancer. Medic. hist.: Medication history. Selfrep: Self-reported history of cancer^a Years of follow-up (mean). -: No available data. RR: Relative Risk with 95% CI. P/M: Post-mortem. N/M: Not mentioned.

Risk of cancer among Alzheimer's Disease patients

Figure 2 (Forest Plot a) summarizes the risk of carcinogenesis among AD patients. Seven studies were included in the analysis, with a total sample of 18,887 participants (10,859 AD patients and 8,028 non-demented healthy controls). Pooled analysis showed that AD patients appear to have reduced risk of carcinogenesis when compared to age-matched healthy controls; RR 0.60, 95% CI 0.45 - 0.79, P<.001. However, both the heterogeneity and publication bias were statistically significant; $I^2=78.5\%$, P<.001 and Egger's test: P=.008. The study of Ou et al. (2013) had the most significant weight on the analysis (23%) while the smallest weight (3%) was observed for the study of White et al. (2013). The inverse relationship between AD and cancer was confirmed by the leave-one-out sensitivity analysis, which calculates to what degree the analysis results would be affected if one study was omitted; this was repeated for omitting all studies one at a time. The results ranged from RR 0.52, 95 % CI 0.34 - 0.80 to RR 0.63, 95% CI 0.47 - 0.84.

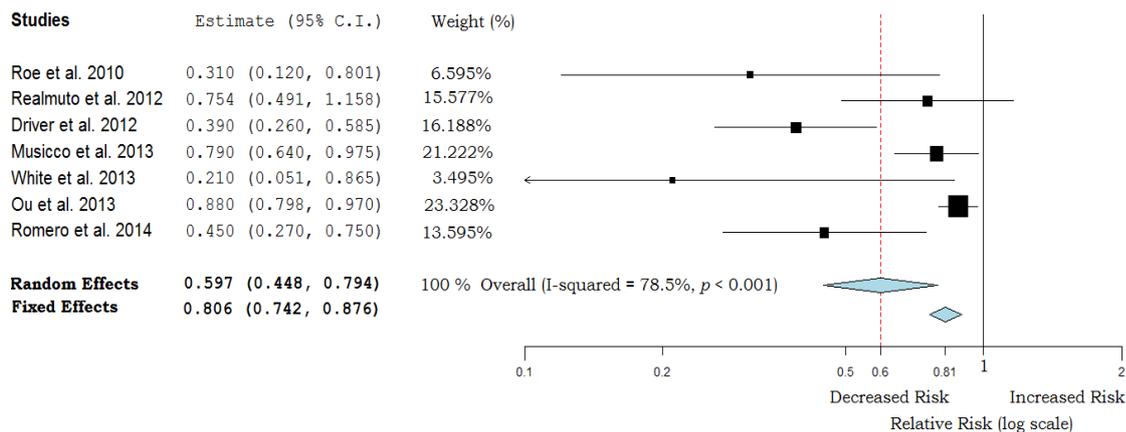


Figure 2. Forest plot (a) of studies analyzing risk of cancer among patients with Alzheimer's disease

Risk of AD development among patients with a history of cancer

Figure 3 (Forest Plot b) shows the analysis results for the risk of Alzheimer's Disease in individuals with cancer history. Eleven studies were included in the analysis, with a total of 5,607,076 participants (1,853,318 cancer patients and 3,753,758 healthy controls). Pooled analysis indicated that cancer patients have a reduced risk of AD when compared to age-matched healthy controls; RR 0.85, 95% CI 0.77 - 0.92, $P < .001$. Heterogeneity and publication bias were calculated and found to be significant; $I^2 = 90.7\%$, $P < .001$ and Egger's test: $P = .03$. The most considerable influence on the analysis was applied by three different studies, each weighing approximately 20%; Frain et al. (2013), Schmidt et al. (2017), and Freedman et al. (2016), while the smallest weight (0.8%) was carried from the study of Lai et al. (2014). The Leave-one-out Sensitivity analysis confirmed the inverse relationship with results ranging between RR 0.80, 95% CI 0.71 - 0.90 and RR 0.95, 95% CI 0.80 - 0.95.

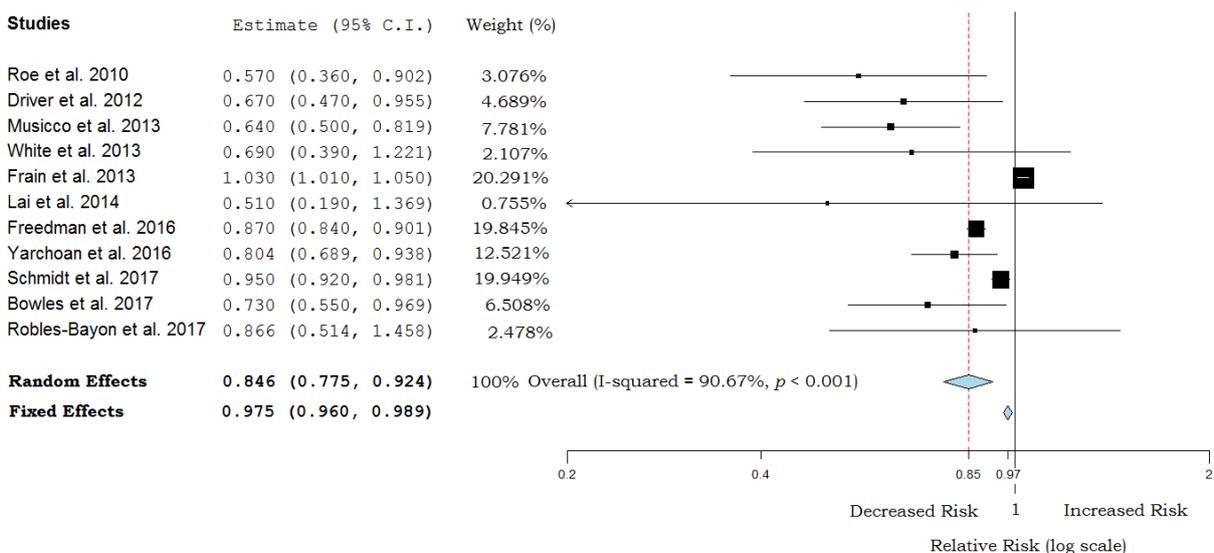


Figure 3. Forest Plot (b) of studies analyzing risk of Alzheimer's disease in patients with cancer history

Risk of Alzheimer's disease among patients with cancer history based on cancer localization

Table 3 demonstrates pooled results of the association between cancer history and AD morbidity, based on site-specific cancer. Results showed no significant relationship between prostate cancer and AD (RR 1.00, 95% CI 0.82 - 1.21). Moreover, malignancies located on the skin, urinary, colon, and the esophagus as well as lung cancer also seem to have a neutral relationship with AD. A significant inverse relationship was found between AD and stomach cancer (RR 0.82, 95% CI 0.69 - 0.96), renal cancer (RR 0.80, 95% CI 0.73 - 0.89) and hematological malignancies (RR 0.74, 95% CI 0.67 - 0.82). A considerable low risk was found for pancreatic cancer patients (RR 0.63, 95% CI 0.50 - 0.79), with data pooled from two substantial cohort studies (Frain et al., 2013; Freedman et al., 2016). Pooled results from five studies showed a non-significant relative risk (RR 0.95, 95% CI 0.83 - 1.01) for skin cancer patients. However, it should be noted that the analysis was based on data that referred to melanoma and non-melanoma skin cancer for which contradictory results are found in the bibliography up to date (see discussion).

Table 3. Relative Risk (RR) of Alzheimer's disease among patients with cancer history based on site-specific cancer

Site-specific cancers	References (n of studies)	Pooled RR (95% CI)	P	Heterogeneity		
				I ² %	Q test	P
Prostate	Frain et al. (2013), Freedman et al. (2016), Musicco et al. (2013), Robles & Gude (2017) (4)	1.00 (0.82 - 1.21)	.99	94	46.722	.00
Bladder	Frain et al. (2013), Freedman et al. (2016), Musicco et al. (2013), Robles & Gude (2017) (4)	0.99 (0.95 - 1.05)	.82	0	1.335	.72
Skin ^a	Frain et al. (2013), Freedman et al. (2016), Robles & Gude (2017), Schmidt et al. (2017), White et al. (2013) (5)	0.95 (0.83 - 1.01)	.51	83	22.927	.00
Colon	Frain et al. (2013), Freedman et al. (2016), Musicco et al. (2013), Robles & Gude (2017) (4)	0.94 (0.84 - 1.04)	.24	62	7.986	.05
Lungs	Frain et al. (2013), Freedman et al. (2016), Musicco et al. (2013), Robles & Gude (2017) (4)	0.85 (0.69 - 1.05)	.12	81	16.119	.00
Stomach	Frain et al. (2013), Freedman et al. (2016) (2)	0.82 (0.69 - 0.96)	.02	0	0.047	.82
Kidney	Frain et al. (2013), Freedman et al. (2016), Robles & Gude (2017) (3)	0.80 (0.73 - 0.89)	.00	0	0.384	.82
Esophagus	Frain et al. (2013), Freedman et al. (2016) (2)	0.76 (0.55 - 1.09)	.09	66	2.904	.17
Hematologic malignancies	Frain et al. (2013), Musicco et al. (2013), Freedman et al. (2016) (3)	0.74 (0.67 - 0.82)	.00	0	1.957	.38
Pancreas	Frain et al. (2013), Freedman et al. (2016) (2)	0.63 (0.50 - 0.79)	.00	0	0.642	.42

Note: Pooled relative risk with Random-Effects Model. Results are presented in descending order. ^aincludes both melanoma and NMSC. Q test: Cochran's Q. Heterogeneity $I^2 = 100\% \times (Q-df)/Q$.

Discussion

The inverse relationship between cancer and Alzheimer's disease was confirmed in the present meta-analysis; in particular, it appears that AD patients face approximately 40% reduced risk of developing

cancer, while persons with a history of cancer face almost 15% lower risk of developing AD.

Comparison with existing meta-analyses

The results of this meta-analysis should be considered with caution, as there is an apparent disparity when compared to the three previously published meta-analyses. In general, the most recent data appear to attenuate the power of the inverse relationship between the two diseases.

In particular, concerning the population with a history of cancer, the relative risk of Alzheimer's disease was not found equally low as in previous studies. In the meta-analysis of Zhang et al. (2015), the calculated risk for this population was RR 0.62, 95% CI 0.53 - 0.74, which translates to 38% lower risk of developing AD than healthy controls. These results were extracted from five studies using the fixed effects model. Similar conclusions were drawn by Ma et al. (2015); in their study, the relative risk was found to be RR 0.63, 95% CI 0.56 - 0.72, which constitutes a 37% reduced risk. In contrast, we found the relative risk of RR 0.85, 95% CI 0.77 - 0.92, which expresses only a 15% reduced risk of AD morbidity among cancer patients compared to the healthy controls.

It becomes apparent that results from previous meta-analyses converge significantly, in contrast to the present study; this can be explained by the fact that both meta-analyses from Zhang et al. and Ma et al. have shared virtually the same pool of studies in order to extract their conclusions. Essentially, four studies were used in both. Indeed, as noted by Hanson et al. (2016), one of the four studies used, namely the study by Musicco et al. (2013), held significantly more weight compared to the rest (49% and 76% respectively) and consequently, the results of both meta-analyses could be mainly reflecting the findings of that study (Hanson et al., 2016).

However, this notable disparity can be more satisfactorily explained by the use of more recent studies in the present meta-analysis, that were not yet available when the previous were conducted. Six novel publications were added to the pool of studies that were used to extract conclusions for the present body of work, with data mainly from cohort studies that weighed significantly in the calculation of results. On the other hand, the disparity of results between the present work and former meta-analyses was not observed in the calculation of cancer risk in AD patients, mainly due to the absence of recent studies and the use of about the same studies in the analysis. The relative risk in all meta-analyses so far, including the present, ranged between RR 0.50 and 0.60, which constitutes a significantly reduced risk of developing cancer for the AD population compared to healthy controls.

The most recent publication that was found focusing on AD patients was in 2014. By observing that, in conjunction with the attenuation of relative risk that was observed in the cancer patient sample, it is reasonable to assume that the risk of developing CA among AD patients might also have been influenced by the addition of large datasets from demographical studies. This possibility should stress the necessity for the undertaking of more research to study the risk of cancer development in AD patients. This would be essential in order to clarify the existence of the inverse comorbidity between the two diseases.

Relationship between AD development and cancer localization

Especially interesting are the results regarding the risk of developing AD in patients with a history of cancer, depending on the localization of the malignancy; notable differences were observed in the range of relative risk for different cancers (please, see Table 3).

However, caution should be exercised when interpreting these results; for instance, the considerable low risk found for pancreatic cancer patients could be attributed to the fact that pancreatic cancer has a more unfavorable prognosis, rather than to metabolic factors and tau pathology, which denote pathophysiological mechanisms of AD. In any case, it would be more appropriate to take into consideration the individual characteristics of each type of cancer, instead of regarding cancer as a single phenomenon.

Disparities were also observed in previous publications that referred to the localization of cancer as a factor for the development of AD. While White et al. (2013), denote a remarkably low risk for AD development in patients with a history of non-melanoma skin cancer (79% reduced risk compared to the control group), a novel study by Schmidt et al. (2017) calculated the risk for this population at only 5% lower compared to the control group (Schmidt et al., 2017; White et al., 2013).

In another study, it is claimed that the chemotherapy treatments that cancer patients receive can act protectively against AD. Frain et al. (2013) found a reduction of the risk for developing AD by 17-23% in patients that had undergone chemotherapy regimens and radiotherapy, compared to CA patients who did not (Frain et al., 2013). Similar findings were reported by Du et al. (2010), who calculated 9-37% less risk of developing AD in women that had undergone chemotherapy for breast cancer, when compared to patients that had not received corresponding treatment [51].

Nevertheless, the findings of the present meta-analysis provide strong indication that the risk of developing AD is variable in patients with a history of cancer, depending on the localization of the malignancy. This could potentially constitute an impetus for the study of AD pathophysiology through the investigation of the etiology for these variations.

The problem of under-diagnosis of cancer in AD patients

In the studies that were considered for this meta-analysis, the question of which disease appeared first is answered mainly with the application of follow-up study designs. However, in the more recent bibliography, it has been noted that there is a problem of under-diagnosis of cancer in patients that suffer from dementia, in which there appears to be a reduced amount of checks for the disease. This could however, be due to the difficulty of collaboration with patients suffering from dementia, as well as due to a tendency of physicians to avoid tests for cancer [52]. It is possible that the already inauspicious state, in combination with the usually advanced age of AD patients is a deterrent to perform tests and consequently potentially diagnosing cancer [53]. Therefore, a large percentage of the comorbidity of AD and cancer could remain undetermined and unregistered, thus strengthening the inverse relationship hypothesis. For instance, Jon Sánchez-Valle et al. (2017) report that patients suffering from AD face an increased risk of developing glioblastoma (the most aggressive type of brain cancer) than developing lung cancer (Sánchez-Valle et al., 2017). Could this finding be attributed to the commonality of brain imaging in AD patients, thus making it more likely for a brain tumor to be discovered, as opposed to a different type of cancer that is localized in other areas of the body that are less often checked?

Limitations

The considerable degree of heterogeneity between the studies that were used for analysis as well as the indications of publication bias should be taken into account when considering the results presented here. The reasons for the aforementioned heterogeneity could be attributed as much on the different study designs used in those studies (case-reports, cohort studies) as on differences in the sample population that

was used. For example, one of the studies focused on a group of war veterans, in which males were over-represented, while the fact that the sample consisted of war veterans was in itself a methodological issue when compared to other population samples. Moreover, the manner of diagnosis of disease varied between studies; for example, in some studies cancer history was solely based on self-reporting. A further limitation concerns the limited description of the characteristics of disease in some cases; a number of studies did not report medications of patients, the presence of an ApoE-ε4 allele (which is found in 40%-60% of AD patients) or the educational level of the participants. Finally, information was sometimes missing on the stage of cancer and/or the therapeutic regimen that was followed in each case.

The most prominent limitation was the limited number of available studies that were analyzed, especially ones focusing on patients with AD (n=7), which is mainly due to the fact that the inverse relationship between the two diseases has only been studied in recent years. It is therefore perhaps necessary to consider that there are unpublished data as well as “negative” studies that are under-represented in the bibliography. The aforementioned limitations should be taken under consideration before interpreting and generalizing the results of the present study.

Conclusions

The present meta-analysis evaluated the available current studies that investigated the hypothesis of an inverse relationship between cancer and Alzheimer’s disease. The negative comorbidity between the two diseases was confirmed, based on the data that was extracted from the available studies. Compared to older meta-analyses, an attenuation of the inverse relationship was noted, due to the fact that more recent studies were taken into account. The necessity for enrichment of the existing bibliography is supported, by further studies that take into account the characteristics of each disease and especially for the population of AD patients.

Moreover, we support that there needs to be a focus on the different types of cancer and their relationship with AD so that systematic differentiations can be determined. That could help with the approach of investigating the pathophysiology of AD. Limitations notwithstanding, this meta-analysis offers a novel approach to the study of the inverse comorbidity hypothesis between the two diseases and could therefore potentially contribute to the critical evaluation of this phenomenon.

The authors declare that they have no conflicts of interest.

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Self-report instruments of cognitive failures as screening tools for Subjective Cognitive Impairment in older adults

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Abstract

Background: The term “Subjective Cognitive Impairment (SCI)” is the most widely accepted term for cognitive complaints of otherwise apparently healthy older adults. It is presently clear that SCI might be a risk factor for the development of Mild Cognitive Impairment and dementia. As regards SCI measurement and potential diagnosis, several studies showed that SCI is a condition in which people score in the normal range on common tests but believe they experience cognitive decline. Hence, to assess the characteristic of the SCI subtle cognitive decline, self-report measures were developed to estimate ‘self-experience’ of minimal decline in cognition seem the most appropriate tools. In this vein, the present study aimed at examining the capacity of the Greek version of two self-report instruments of the aforementioned type to detect SCI in community dwelling older adults. **Materials and Methods:** The study sample consisted of 295 participants, who were allocated into four age-groups: young adults, middle-aged adults, older adults and older-old adults. The first three groups were gender and education-matched. The participants were examined via two objective tests of the Delis-Kaplan Executive Function System (D-KEFS) which is a neuropsychological battery designed to measure executive functions. In specific, they were tested via the D-KEFS Tower Test (TT) which mainly measures ‘planning’ function, and the D-KEFS Color-Word Interference Test (C-WIT) which primarily measures ‘inhibition’ and ‘switching’ functions. Both tests consist of four conditions. The participants were also asked to answer to: (a) the Cognitive Failures Questionnaire (CFQ), and (b) the Prospective and Retrospective Memory Questionnaire (PRMQ), which were designed to assess subjective estimations of everyday slips of actions and cognitive failures, and episodic memory slips in everyday life, respectively. As concerns the psychometric qualities of the two questionnaires, a single-factor structure of the Greek versions of the CFQ and the PRMQ was verified in a previous study via the application of Confirmatory Factor Analysis. **Results:** No age-group effects on CFQ score were found. Receiver Operating Characteristic (ROC) curve analyses were subsequently performed, using objective tests’ scores as test variables and CFQ classification based on the 75th percentile score, as state variable. ROC curves analyses using “C-WIT conditions’ 1, 2 time of completion” as test variables and CFQ classification, in older adult age-group, as state variable, showed that a CFQ score ≥ 47 is indicative of an early stage of objective cognitive impairment in older age. Cronbach’s α values, for the Prospective and Retrospective Memory Questionnaire ranged from .89 (young adults) to .93 (older adults). No age-group effects on PRMQ score were observed. ROC curves analyses were performed, using objective measures’ scores as well as CFQ score as test variables and PRMQ classification based on the 75th

percentile score, as state variable. These analyses using “C-WIT conditions’ 3, 4 time of completion” as well as CFQ score as test variables and PRMQ classification, in older adult age-group, as state variable, showed that a PRMQ score ≥ 43 is indicative of an early stage of objective cognitive impairment as well as of subjective estimations of general cognitive decline in older age. **Conclusion:** Self-report questionnaires of ‘everyday’ cognitive and memory failures seem to be associated with specific objective tests of cognition in aging. Hence, they are useful tools for detecting early cognitive impairment at least in older adults. Their administration together with objective cognitive tasks of high difficulty could substantially help for SCI screening. Given that there is also evidence that the experience of subtle impairment in cognition is related to increased likelihood of biomarker abnormalities indicative of AD pathology, the assessment of subjective estimations is revealed as a useful primary indicator of early AD effects on cognitive functioning.

Introduction

Identification of subjects at the early stages of dementia disorders, such as Alzheimer’s disease (AD), is crucial for pharmaceutical development and effective prevention of cognitive decline. During the last twenty years the term Mild Cognitive Impairment (MCI) was used to define persons at the transitional stage between normal aging and dementia. However, clinical trials for pharmacotherapy in patients with MCI were disappointing. Therefore, the concept of Subjective Cognitive Impairment (SCI) was introduced, in order for the AD spectrum to be extended to an earlier stage before MCI [1, 2].

In contrast to extensive efforts to standardize AD biomarkers and criteria for MCI, research on the quantification of SCI is limited [2].

The term ‘Subjective Cognitive Impairment (SCI)’ is the most widely accepted term used for cognitive complaints of otherwise apparently healthy older persons, in the absence of objective evidence of cognitive decline or psychopathology. In specific, SCI is defined as a self-experienced persistent decline in cognitive capacity compared to the subject’s previously normal status, when the subject had normal age-, gender-, and education-adjusted performance on standardized cognitive tests [3].

Although studies have demonstrated association between cognitive complaints and depression, anxiety, and personality, recent research also suggests that, in the absence of depression or objective cognitive dysfunction, SCI in older adults may be a harbinger of MCI and underlying dementia pathology. In particular, SCI has been found to be associated with AD biomarkers and neuroimaging markers, such as gray matter volume loss, cerebral hypometabolism and amyloid deposition [2]. Furthermore, SCI is predictive of incident dementia and is considered by some researchers to be the ‘very-very early stage’ of AD [3, 4].

As regards SCI measurement and potential diagnosis, several studies revealed that SCI is a condition in which people score within the ‘normal’ range on common tests but believe they experience cognitive decline [5, 6, 7]. Hence, to assess the characteristic of the SCI subtle cognitive decline, self-report measures were developed to estimate ‘self-experience’ of minimal decline in cognition and especially in memory seem the most appropriate tools [4, 8]. The Memory Complaint Questionnaire (MAC-Q) [9] and the Everyday Cognition Scale (ECog) [10] were the most commonly used assessments across different studies. The cognitive domain most often targeted was memory, followed by executive functions [4, 8]. However, more emphasis is needed to the examination of the psychometric qualities of the self-report instruments that could be used as diagnostic tools for SCI in community dwelling older adults, in order for the experts to select the most appropriate tools for their target population [4, 8, 10].

Aim of the study

In this vein, the present study aimed at examining the capacity of the Greek version of two self-report instruments of the aforementioned type -namely, the Cognitive Failures Questionnaire (CFQ) [11] and the Prospective and Retrospective Memory Questionnaire (PRMQ) [12] - to detect SCI in community dwelling older adults.

The main questions that this study will address are the following: a) is there any 'objective measure' of executive functions that could predict self-reports? b) Can these self-report instruments predict each other's performance? c) Which could be the cut-off scores for SCI in older adults?

Materials and Methods

Participants

The study sample comprised a total of 295 adults, that participated voluntarily in the study and were allocated to four age-groups: (i) young adults (n=106, mean age=22.8 years, SD=3.0, men=45, women=61), (ii) middle-aged adults (n=108, mean age=47.9 years, SD=5.1, men=45, women=63), (iii) older adults (n=53, mean age=69.9 years, SD=3.6, men=22, women=31), and (iv) older-old adults [n=28, mean age=83.5 years, SD=3.3, 64.3%=women, 42.9%=low educational level (0-9 years of schooling), Montreal Cognitive Assessment (MoCA): M=21.7, SD=4.4]. The first three groups did not differ in gender and educational level (EL): $\chi^2(2)=.019$, $P>.05$, and $\chi^2(4)=4.147$, $P>.05$, respectively. Low EL was not represented in these groups. Hence, their participants had at least 10 years of schooling. All were relatively to very satisfied with their life based on their answers to a single question in a 7-point Likert scale. Exclusionary criteria for potential participants were: the presence of uncorrected hearing or/and visual loss and any other severe physical, psychiatric and neurological disease. In order to include cognitively healthy community dwelling older adults as participants in the study, the existence of objectively measured general cognitive decline was examined: a score lower than '25' in the Montreal Cognitive Assessment (MoCA) is considered indicative of cognitive decline related to dementia symptomatology [13, 14]. Thus, a potential participant who scored less than 25, was excluded in order to ensure that cognitive decline was absent (MoCA \geq 25, M=26.8, SD=0.9). At this point it should be mentioned that this exclusionary criterion was not followed for the older-old adult group, since this group was included in the study as a secondary group in order to formulate a 'complete picture' of the performance in the self-report instruments in the total adult-age-range.

Instruments

Self-report measures

The Cognitive Failures Questionnaire (CFQ) [11]. CFQ is a 25-item questionnaire designed to measure everyday slips of actions and memory failures (e.g., bumping into people, forgetting names, etc.). For the purposes of a previous study [15], the 25 items of the CFQ were translated into Greek using forward - back translation and pre-testing of the instrument on the target population. Participants were asked to indicate the frequency with which they make such errors on a 5-point scale from 0 (never) to 4 (very often).

According to its constructors [11, 16], the CFQ assesses a single underlying general cognitive failures dimension. As concerns the psychometric properties of the Greek version of the CFQ, a single-factor structure was also verified by Moraitou and Efkliides [15] via the application of Confirmatory Factor Analysis (CFA): χ^2 (106, N=449)=260.46, P=.011, CFI=.985, SRMR=.036, RMSEA=.023. In the same study [15] the internal consistency of the Greek version of the CFQ was found to be excellent (Cronbach's α =.93). For the purposes of the present study an observed variable of cognitive failures was created as the sum of the scores on all specific items of the CFQ, which is considered as representative of the latent structural factor of the CFQ.

The Prospective and Retrospective Memory Questionnaire (PRMQ) [12]. PRMQ is a 16-item measure of prospective and retrospective memory slips in everyday life. For the purposes of a previous study [15], the 16 items of the PRMQ were translated into Greek using forward - back translation and pre-testing of the instrument on the target population. Participants had to rate how often each type of memory failure occurred, on a 5-point scale from 1 (never) to 5 (very often).

Of the PRMQ items, eight address prospective memory failures and eight address retrospective memory failures. Crawford and his colleagues [17, 18] found that responses to the PRMQ are best accounted for by a general factor of Self-Rated Episodic Memory and two specific factors corresponding to Self-Rated Prospective Memory and Self-Rated Retrospective Memory. This factor structure was also confirmed for the Greek version of the PRMQ via the application of CFA: χ^2 (88, N=464)=186.14, P<.001, CFI=.959, SRMR=.035, RMSEA=.049 [15]. The internal consistency of all the factors of the Greek version of the CFQ was found to be satisfactory: Cronbach's α =.84, for Self-Rated Memory; α =.84, for Self-Rated Prospective Memory; and α =.79, for Self-Rated Retrospective Memory [15]. For the purposes of the present study an observed variable of prospective and retrospective memory was created as the sum of the scores on all specific items of the PRMQ, which is considered as representative of the latent general factor of Self-Rated Episodic Memory.

Objective tests

Delis-Kaplan Executive Function System: Tower Test, Standard Form - D-KEFS - TT, SF [19]. In this test, participants have to make towers along three vertical pegs using five disks. The participant has to solve the problems using a specific minimum number of moves. The test consists of a wooden board with three vertical pegs, five wooden disks that vary in size and color (shades of blue), a timer, a recording and a stimulus booklet. The test comprises of nine problems of increasing difficulty. Each problem has a specific time to completion, 30'' for the first three problems, 60'' for the fourth, 120'' for fifth and sixth, 180'' for seventh, and 240'' for the eighth and ninth problem. There are two rules in this test which are given orally and on paper. The first rule is that only one disk can be moved at a time, and the second one is no disk must be placed on top of a smaller disk. In each problem the examiner slides the discs onto the pegs, in a specific order, and shows the participant the final position of the discs. The examiner records the total of moves, the total violations of rules, the completion time of the problem, and whether the problem was solved. If the participant exceeds the given time, the examiner interrupts the problem and marks it with 0. The examiner interrupts the administration of the test in case the participant has three consecutive failures (i.e. exceedance of given time, making a wrong tower, using fewer moves than the specified).

The scores - variables of D-KEFS TT used in this study are the following: a) the total number of administered problems, b) the total completion time, c) total achievement score which derives from the total scores of each correct solution (the scoring is also based on the number of moves). The range of raw

scores extends from 0 (zero problems solved) to 30 (all problems solved within the given time and with the minimum moves).

D-KEFS TT is a tool which measures complex executive functioning. Factor analysis showed positive loadings on spatial planning, learning rules, inhibition, and the ability to define and maintain cognitive sets. The validity of the tool has been studied by various studies in people with brain damage. A recent study showed that people with cardiovascular disease had achieved a lower overall score compared to control group [19-22].

Delis-Kaplan Executive Function System: Color - Word Interference Test, Standard Form - D-KEFS CWIT, SF [19]. This test is based on Stroop's experiment (1935) and measures the ability to inhibit a dominant and automatic verbal response. The participants have to inhibit an overlearned verbal response (e.g., reading the printed words) to generate a contradictory response (e.g., naming the color of the word). The test includes four conditions: a) naming the three basic colors (green, red and blue), b) reading words that are written in black color and are naming colors, c) naming the dissonant ink colors in which the words are printed (inhibition), and d) switching between naming the color of the words and reading the words (inhibition/switching). The first two conditions that measure speed of naming and reading speed of the participants measure basic cognitive abilities and their completion is a prerequisite to proceed with the third and fourth condition which measure executive functioning: mainly inhibition and cognitive flexibility, respectively. There is a time limit for each condition, 90'' in the first two conditions and 180'' for in following two. The examiner interrupts the test when the participant cannot complete the task within the given time.

There are two training sessions in each condition to ensure participants understood the task. In the first condition, the participant has to name the colors of squares (green, red, and blue). In the second condition, he/she has to read black-colored words naming the above three colors. The third condition comprises of color words written in incompatible color than the one they name. The participant has to name the color of the word instead of reading the word. Finally, in the fourth condition the participant has to 1) name the color of the word and 2) read the framed word instead of naming its color. The instructions for these tasks display at the top left side of the stimulus booklet given to the participant. The test has discontinue rules. The examiner administers the third condition only if the participant succeeds to finish the first two. The examiner also interrupts the administration of the test in case participants show difficulty in the training sessions or conduct three consecutive uncorrected errors.

In this study, the examiner recorded the time to completion which must not exceed the time limit, and the total of uncorrected errors for each condition (two variables).

Plenty of clinical studies examined the validity of D-KEFS - CWIT and showed that people with frontal lobe epilepsy, cardiovascular disorder, chronic kidney failure, AD, and Huntington's disease have low performance in the third and fourth condition [23, 24].

Procedure

The above tools were merged to create a battery. Two different versions of this battery, regarding the order of administration, were designed to avoid order effects. The administration was individually and lasted around one hour for young adults and around two hours for older adults. In most cases of older adults, the administration was completed within two sessions. The examiner had to inform the participant about the content and the objective of the research. After participant's written consent, the examiner could start the administration. The examination took place in a quiet and comfortable environment.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All the participants were informed about the procedure and the aim of the study, and their written informed consent was taken.

Results

The ability of objective neuropsychological tests (D-KEFS TT & CWIT) to distinguish between older adults reporting a low and a high level of cognitive failures measured via CFQ

As regards the internal consistency of the CFQ for the specific groups of this study, Cronbach's α values ranged from .87 to .92, indicating an excellent level of reliability. In relation to potential age effects on the CFQ performance, no age-group effects on the total score on CFQ were found: (a) for young adults: $M=37.5$, $SD=14$, total score range=9 - 69; (b) for middle-aged adults: $M=34.5$, $SD=14.3$, total score range=10 - 73; (c) for older adults: $M=38.7$, $SD=16$, total score range= 6 - 72; (d) for older-old adults: $M=35.5$, $SD=14$, total score range= 8 - 62.

With reference to D-KEFS TT performance of the four groups of the study, young adults, middle-aged adults, and older adults didn't differ on the total number of TT problems administered. However, as regards the age-group effects on TT total completion time and total achievement score, older adults were found to need more time to complete the TT and had a lower TT achievement score, compared to young adult group ($P=.03$ and $P=.002$, respectively). Older-old adults had significantly worse scores ($P<.05$ to $.001$), compared to the other three age-groups, as concerns all TT variables: $F(3, 291)=40.207$, $P<.001$; $F(3, 291)=24.220$, $P<.001$; $F(3, 291)=6.189$, $P<.001$.

In relation to D-KEFS CWIT performance of the four groups of the study, all groups were found to differ with each other ($P<.005$ to $.001$) in the CWIT completion time of the fourth condition designed to measure cognitive flexibility, $F(3, 283)=43.304$, $P<.001$. As expected, given the increased difficulty of this condition compared to the rest three conditions of the D-KEFS CWIT, older-old adults displayed significantly higher score ($P<.001$), compared to the other three age-groups, in the number of uncorrected errors they made, $F(3, 283)=18.908$, $P<.001$. All groups were also found to differ with each other ($P<.03$ to $.001$) in the CWIT completion time of the third condition designed to measure specifically inhibitory control, $F(3, 287)=64.472$, $P<.001$. On the other hand, in the first and the second conditions of the D-KEFS CWIT, which measure lower-order cognitive functions, older adults and older-old adults differed from the younger groups and with each other ($P<.01$ to $.001$) in completion time: older-old adult group displayed the worst performance, older adult group the next one, while middle-aged adults' performance didn't differ from that of young adults: $F(3, 294)=62.308$, $P<.001$, and $F(3, 294)=43.865$, $P<.001$, for the 1st and the 2nd condition respectively.

To examine if there are any objective measures of executive functions that could predict self-reported cognitive failures and everyday slips of actions in older adults, *Receiver Operating Characteristic* (ROC) curve analyses were subsequently performed, using Tower Test's scores and Color Word Interference Test's scores as test variables and CFQ participant classification as state variable. Specifically, the three D-KEFS TT variables of the total number of administered problems, the total completion time, and the total achievement score, as well as the D-KEFS CWIT variables of the number of uncorrected errors and the completion time for

every one of the four conditions of the test, were inserted as test variables into the ROC curve analyses. As regards the state variable inserted into these analyses, based on the CFQ score (=47) at the 75th percentile of performance as regards the main group of interest, namely the older adult group, all participants were divided into two groups: group 0=those reported a low level (<47) of cognitive failures; group 1= those reported a high level (\geq 47) of cognitive failures. The area under the ROC curve (AUC) was used as an index of discrimination performance. For each measure, the ability to distinguish different groups was assessed by examining whether the AUC was significantly different from 0.

With reference to the D-KEFS TT, from all variables the 'total completion time' score was the only able to discriminate to some extent older adults reporting a low and a high level of cognitive failures, AUC=0.67, P=.04, 95%CI: 0.50-0.82. This distinguishing ability was not found for the younger age-groups.

As regards the D-KEFS CWIT, the 'total completion time' scores for the conditions examining lower-order cognitive functions (1st and 2nd) were the best able to discriminate older adults reporting a low and a high level of cognitive failures: AUC=0.81, P<.001, 95%CI: 0.69-0.93 and AUC=0.80, P<.001, 95%CI: 0.68-0.93, respectively (see Figure 1). The conditions designed to measure executive functions (3rd and 4th) appeared also able to distinguish between the two groups but not to the same extent: AUC=0.73, P=.006, 95%CI: 0.56-0.90 and AUC=0.74, P=.006, 95%CI: 0.58-0.90, respectively.

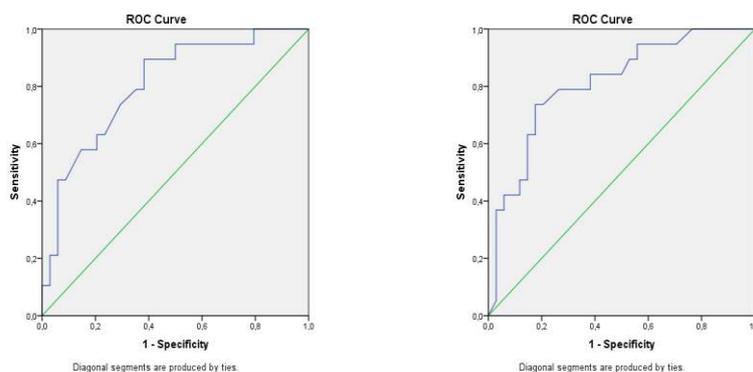


Figure 1. Receiver Operating Characteristics (ROC) curves computed to assess the ability of the completion time score in the 1st and 2nd conditions of the D-KEFS Color - Word Interference Test to discriminate between older adults reporting a low and a high level of cognitive failures

The ability of objective neuropsychological tests (D-KEFS TT & CWIT) in distinguishing between older adults reporting a low and a high level of memory complaints measured via PRMQ

In relation to the internal consistency of the PRMQ for the specific groups of the study, Cronbach's α values ranged from .89 to .93, indicating an excellent level of reliability. As regards potential age effects on the PRMQ performance, no age-group effects on the total score on PRMQ were found: (a) for young adults: M=36.1, SD=10.2, total score range=19 - 64; (b) for middle-aged adults: M=35.3, SD=9.5, total score range: 20 - 63; (c) for older adults: M=37.9, SD=11.4, total score range: 17 - 64; (d) for older-old adults: M=39.5, SD=11.1, total score range: 22 - 67.

ROC curve analyses were subsequently performed, using the three D-KEFS TT variables of the total number of administered problems, the total completion time, and the total achievement score as test variables. As regards the state variable inserted into these analyses, based on the PRMQ score (=43) at the 75th percentile of performance as concerns the main group of interest, namely the older adults, all participants were divided into two groups: group 0 = those reported a low level (<43) of memory complaints;

group 1= those reported a high level (≥ 43) of memory complaints. The findings showed that the D-KEFS TT variables were not able to distinguish between the two groups.

In the next step, ROC curves were constructed using the two D-KEFS CWIT variables of the number of uncorrected errors and the completion time for every one of the four conditions of the test as test variables, and group classification according to PRMQ score as state variable. The completion time of the D-KEFS CWIT conditions designed to measure executive functions (3rd and 4th) appeared able to some extent to distinguish between the two groups according to memory complaints, and this was true for the older adult age-group and not for the younger ones: AUC=0.79, P=.001, 95%CI: 0.66-0.92 and AUC=0.74, P=.01, 95%CI: 0.58-0.89, respectively.

The ability of the self-report instrument 'Cognitive Failures Questionnaire' to distinguish between older adults reporting a low and a high level of memory complaints measured via PRMQ

In order to examine whether the self-report instrument of cognitive failures (CFQ) could be able to discriminate between older adults reporting a low and a high level of memory complaints [PRMQ classification (< 43 , ≥ 43) based on the 75th percentile of performance of older adults], ROC curve analyses were performed, using the CFQ score as test variable and PRMQ classification as state variable. The findings showed that the CFQ score was able to distinguish between the two groups according to memory complaints and this was found not only for older adults but for all adults and for the older-old adult group as well: AUC=0.87, P<.001, 95%CI: 0.75-0.98, AUC=0.89, P<.001, 95%CI: 0.84-0.94, and AUC=0.95, P<.001, 95%CI: 0.87-1.00, respectively (see Figure 2).

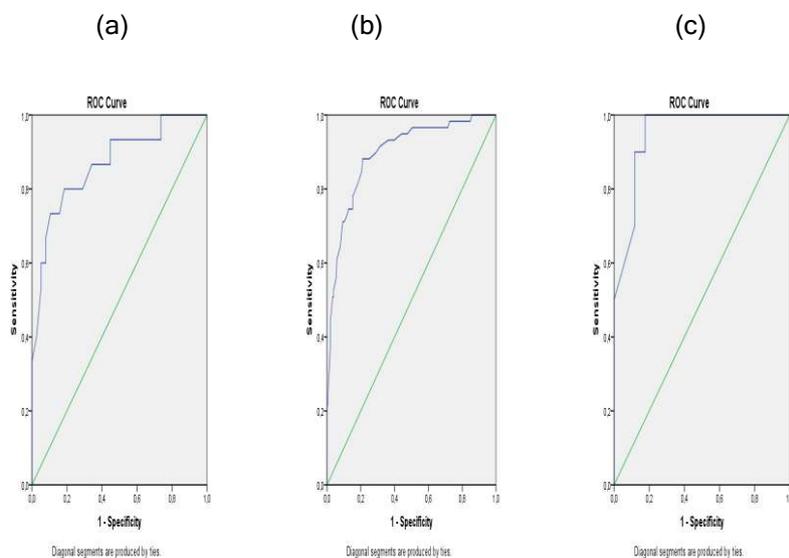


Figure 2. Receiver Operating Characteristics (ROC) curves computed to assess the ability of the Cognitive Failures Questionnaire score to discriminate between (a) older adults, (b) adults covering the age-range from young to older adulthood, and (c) older-old adults, who report a low and a high level of memory complaints as measured via the Prospective and Retrospective Memory Questionnaire

Discussion

Given that SCI is defined as a self-experienced persistent decline in cognitive capacity, compared with the person's previously normal status [1, 3], special emphasis is given by the experts on the field of dementia-related cognitive decline, on the development and/or adaptation of self report instruments - questionnaires about memory complaints, cognitive failures and everyday slips of actions potentially indicative of executive functioning decline. This interest can be explained by the need to find 'the most appropriate and valid tools' to measure and detect SCI in apparently healthy community dwelling older adults [2, 4, 25, 26, 27], in order to prevent dementia development and even reverse cognitive decline.

In this light, the present study aimed to examine the ability of two very well-known self-report instruments developed to measure cognitive and memory complaints, namely the Cognitive Failures Questionnaire [11, 15] and the Prospective and Retrospective Memory Questionnaire [12, 15], to detect SCI in community dwelling older adults. Both instruments have already been examined as regards main psychometric properties (internal consistency and factorial validity) and were found reliable and valid tools for use in the Greek population [15].

The main questions raised with regard to the usefulness of the two questionnaires to detect SCI, had to do with (a) the potential ability of 'objective neuropsychological tests' of executive functions to distinguish between persons reporting a low and a high level of cognitive failures and memory complaints, (b) the ability of the two self-report instruments to predict each other's performance, and (c) the identification of possible cut-off scores in the two questionnaires for SCI detection in older adults.

The Cognitive Failures Questionnaire (CFQ) as self-report instrument for SCI detection in community dwelling older adults

As regards the internal consistency of the CFQ [11], the findings of the present study verified previous findings [15] and showed that CFQ is a highly reliable tool that could be administered to adult population, from younger to very old persons. Moreover, the findings showed that the CFQ is a promising tool that can detect SCI in older adults, because it relates with some objective measures of cognition: interestingly, lower-order cognitive functions, measured as completion time of the first two conditions of the D-KEFS CWIT that examine speed of naming and reading speed, seem to better differentiate otherwise cognitively healthy community dwelling older adults reporting a low and a high level of cognitive failures, compared to higher-order cognitive control measured as completion time of the last two conditions of the D-KEFS CWIT and the D-KEFS TT. Taking into account that older adults were found to need significantly larger time to complete all conditions of the D-KEFS CWIT and the D-KEFS TT, compared to younger adult participants, it seems that processing speed might be the main cognitive function that is associated with self-reported cognitive failures and everyday slips of actions in older adults. This is absolutely compatible with theory and evidence supporting that age-related declines in processing speed underlie the widespread changes in cognition of older adults. In fact, slowed processing speed as a decrease in the rate at which people perform perceptual, motor, and cognitive tasks, is presented as a hallmark of cognitive aging. However, this slowing of processing speed has been associated with cerebral small vessel disease and declines in cerebral morphology [27-31]. Hence, the potential ability of processing speed in cognitive tasks to distinguish between older adults reporting a low and a high level of cognitive failures might reflect the

differential rate of dementia-related brain pathology development that is detected as SCI in the form of a high level of self-reported cognitive failures.

The Prospective and Retrospective Memory Questionnaire (PRMQ) as self-report instrument for SCI detection in community dwelling older adults

As regards the internal consistency of the PRMQ [12], the findings confirmed previous studies [15] and showed that CFQ is a highly reliable tool that could be administered to adult population at a broad age-range. The findings also showed that the PRMQ could function as a tool that can detect SCI in aging, because it relates with some objective measures of complex cognition: higher-order cognitive functions, measured as completion time of the last two conditions of the D-KEFS CWIT that examine inhibitory control and cognitive flexibility as combination of inhibitory control and rule/task switching, were revealed as the only objective tests which could differentiate to some extent otherwise cognitively healthy community dwelling older adults reporting a low and a high level of memory failures. Interestingly, these findings are consistent with previous studies on early Alzheimer's disease supporting that executive function deficits appear firstly in tasks requiring resolution of competing response tendencies (which requires inhibition of the predominant response) and cognitive flexibility, while episodic memory seems to be implicated in these deficits [32, 33, 34].

Furthermore, since the PRMQ includes items related to prospective memory, the findings of the present study appear in line with previous evidence indicating that executive functions differentially predict prospective memory performance in objective tasks. Specifically, it was found that the event-based prospective memory tasks require inhibition to avoid distraction from irrelevant tasks while the time-based prospective memory tasks require shifting of cognitive sets (rule/task switching) [35-39]. Hence, a high level of memory complaints in aging as a SCI index may reflect decline in complex cognitive mechanisms, and pathology which is developing in prefrontal brain networks, besides the general slowing of processing speed.

Beyond their differential associations with the objective neuropsychological tests, the findings of the present study indicated that the CFQ score can distinguish to a large extent, older adults reporting a low and a high level of memory complaints. The same is true for all adults in a broad age-spectrum. This means that even if the subjective reports on the two instruments reflect differential cognitive and brain networks' pathologies in aging, they also share a common cognitive background. According to our findings, this is made explicitly obvious in very old age, when the discriminative ability of the CFQ is excellent and general cognitive ability as measured via the MoCA is lower than the 'normal' score. On the other hand, the general absence of association between the objective tests and the self-report instruments in young and middle-aged adults, could lead to the conclusion that in younger adults both self-reported cognitive failures and memory complaints may reflect affective states rather than cognitive decrements [15].

In any case, the findings related to the discriminative ability of specific cognitive functions measured as completion time of the D-KEFS TT and CWIT conditions, showed that a cut-off score=47 for the CFQ and a cut-off score=43 for the PRMQ may be revealed useful to SCI detection in community dwelling older adults.

Limitations and future research

The restricted nature of the sample should be noted. Also, the cross-sectional design of the study and the small number of objective cognitive tasks that were used should be mentioned as limitations. It would be interesting to repeat the study using a large representative sample of the target population, in a longitudinal design. Besides the objective tasks, a series of similar to CFQ and PRMQ self-report instruments developed to detect SCI should be examined together with the aforementioned ones. Biomarkers related to SCI should be measured as well, in order to reveal potential associations between biological processes and cognitive failures in aging.

Conclusions

Self-report questionnaires of 'everyday' cognitive and memory failures seem to be associated with specific objective tests of cognition in aging. Hence, they are useful tools for detecting early cognitive impairment at least in older adults. Given that there is also evidence that the experience of subtle impairment in cognition is related to increased likelihood of biomarker abnormalities indicative of AD pathology, the assessment of subjective estimations is revealed as a useful primary indicator of early AD effects on cognitive functioning.

The authors declare that they have no conflicts of interest.

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A view of the lecture hall

A comparison of ¹⁸F-FDG PET/CT findings in HIV positive compared to HIV negative patients with recurrent cervical cancer

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Abstract

Objective: HIV-positive women with cervical cancer have higher recurrence and death rates with shorter time to recurrence and death compared with HIV-negative subjects. The objective of this study was to compare the recurrence patterns in HIV-positive women with invasive cervical cancer to their HIV-negative counterparts using ¹⁸F-FDG PET/CT. **Subjects and Methods:** We evaluated 40 HIV- seropositive and 79 HIV-seronegative patients with recurrent cervical carcinoma using ¹⁸F-FDG PET/CT. The PET/CT datasets were interpreted by two independent readers blinded to the HIV status of the patients. Areas of disagreement were resolved by consensus. Cervical cancer recurrence was confirmed by biopsy and histological examination of tissue, correlation with conventional imaging (CT and MRI) and by follow-up ¹⁸F-FDG PET/CT. **Results:** HIV-positive patients were 9 years younger than the HIV-negative patients at the time of diagnosis; mean age 39 years versus 48 years respectively. Initial treatment was comparable in both groups. Time to recurrence was shorter in HIV-infected compared with HIV-uninfected women (11 versus 24 months). The commonest sites of metastatic recurrence was in the lymph nodes. HIV-infected patients demonstrated significant higher recurrence in lymph nodes and lungs (P<0.05). No significant difference in the recurrence rate in liver or bone (P>0.05) between both groups. HIV-infected patients showed unusual metastases to brain, spleen and skin. **Conclusion:** By using the ¹⁸F-FDG PET/CT scan we showed that the time to recurrence is shorter among HIV seropositive patients with the commonest site of metastatic recurrence being in the lymph nodes. Nodal and liver metastases are significantly higher in HIV seropositive patients compared with seronegative patients.

Introduction

Cervical cancer has a high prevalence in developing countries with an estimate of close to 500,000 women worldwide and 233,000 deaths from the disease annually [1]. South Africa suffers from a dual burden of cancer of the cervix and human immunodeficiency virus (HIV) infection [2]. One in 41 women in South Africa has cervical cancer and 8 women die daily in the country as a result of this cancer. The HIV epidemic in South Africa has had a devastating effect on the carcinoma of the cervix and HIV infected women have been seriously disadvantaged due to the lack of a formally implemented screening strategy.

HIV positive patients are at increased risk of persistent human papilloma virus (HPV) infection,

pre-malignant lesions and cervical cancer compared with the uninfected. Mac Donald, 2014 showed in local data that cervical cancer occurred 10 years earlier in HIV infected women, at a more advanced stage when diagnosed, with more treatment related complications and at a higher risk of death than women who are HIV negative [3].

Recurrence is defined as cancer development at least 6 months after the treated disease has regressed [4]. An estimated 35 percent of patients with invasive cervical cancer develop persistent or recurrent disease following treatment. The recurrent cervical cancer rate is lower for those with early-stage disease. The majority of cancer recurrences occur within two years of treatment. The recurrence rate of uterine cervical cancer is reported to be 6.5% after surgery and 26.2% after radiation therapy alone [4]. Half of all cases of recurrent uterine cervical cancer are confined to the pelvic cavity however some cases show metastatic lesions in the lymph nodes, lung, bone, and liver [5].

Carcinoma of the cervix is staged according to the classification advocated by the International Federation of Gynaecology and Obstetrics (FIGO) [6]. The routinely used staging procedures include conventional imaging modalities, such as chest radiography, CT or MRI, and surgical staging [7-9]. Surgical assessment of para-aortic nodes, although considered the 'gold standard', is associated with certain morbidities and, hence, is not considered in the early stages of the disease.

Positron emission tomogram (PET) performed with the structural glucose analogue 2-fluoro-2-deoxy-D-glucose labeled with the positron emitter fluorine-18 (^{18}F) is a non-invasive molecular functional imaging modality. ^{18}F -FDG PET/CT is a valuable imaging modality in the management of many human solid tumors [10-12]. ^{18}F -FDG PET/CT has become an essential modality for staging and restaging and for assessment of response to therapy in the care of patients with cervical cancer [13-15].

The aim of this study was to compare the pattern of recurrent cervical cancer in patients with and without human immunodeficiency virus infection using ^{18}F -FDG PET/CT.

Subjects and Methods

Patients

We retrospectively reviewed patients previously treated for cervical carcinoma referred for ^{18}F -FDG PET/CT for the evaluation of the extent of disease recurrence. All patients had conventional imaging (CT or MRI) or ^{18}F -FDG PET/CT following completion of initial treatment confirming disease remission. Patients records were evaluated for their treatment history and HIV status. In HIV seropositive patients, the CD 4 count was recorded. Patients were excluded if no disease remission following treatment was confirmed, HIV status was not confirmed or previous treatment was unknown. Informed consent was obtained from all patients to allow the use of their clinical data for research purposes under a protocol approved in our institution. Ethics approval was obtained from the research ethics committee of the University of Pretoria.

PET/CT imaging

Patients were prepared in compliance with the guidelines of the European Association of Nuclear Medicine [16]. All patients had a minimum of six hours of fasting. Blood sugar prior to imaging was ≤ 11.0 mmol/L in all cases. The activity of ^{18}F -FDG injected was calculated based on weight using the formula: $[(\text{body weight} \div 10) + 1] \times 37 \text{ MBq}$. Imaging was acquired on a Biograph 40 Truepoint PET/CT scanner (Siemens

Medical Solution, Illinois, USA). Both oral and intravenous contrasts were given. For oral contrast, 30mls of gastrografin (Bayers, Isando, South Africa) in 1 liter of water was given over 1 hour prior to imaging. Intravenous contrast agent, 100mL Omnipaque 350 (GE Healthcare, Wisconsin, USA) was given with a scan delay time of 80 seconds. CT parameters were adjusted for patients' weight (120KeV, 40-150mAs) with a section width of 5mm and pitch of 0.8. Vertex to mid-thigh PET imaging was acquired in 3D mode at 3 minutes per bed position after 60 minutes of uptake. Computed tomography data were used for attenuation correction. Image reconstruction was done with ordered subset expectation maximization iterative reconstruction algorithm (4 iterations, 8 subsets). A Gaussian filter was applied at 5.0mm FWHM.

Image analysis and interpretation

Image were viewed on a dedicated work station equipped with a Syngo software. Images were displayed as PET, CT and fused PET/CT in axial. Coronal and sagittal views. Findings were evaluated and reported by two independent experienced nuclear medicine physicians who were blinded to the HIV status of the patients. Disagreements were resolved by consensus. Cervical cancer recurrence was confirmed by biopsy and histological examination of tissue, correlation with conventional imaging (CT and MRI) and by follow-up ¹⁸F-FDG PET/CT imaging demonstrating interval change in lesion.

Data Analysis

Description of Statistical analysis

Descriptive statistics such as mean, median, standard deviation and range were used depending on the nature of the data. Chi square test was use to evaluate for difference in rate of recurrence between the seropositive and seronegative group. Statistical analysis was done using STATA version 9/SPSS version 15.0.

Results

A total of 119 women (mean age=45±7.7 years, Range=30 to 80 years) were included in the study of which 40 (33.6%) were seropositive and 79 were seronegative. HIV-positive patients were 9 years younger than the HIV-negative patients with a mean age 39 years versus 48 years for HIV seropositive and HIV seronegative patients respectively. Among the seropositive patients, 25/40 patients were on HAART and 77.5% of all HIV seropositive patients had CD 4 count greater than 200cells/mm³. The CD 4 count distribution of the seropositive patients is shown in Table 1.

Table 1. Patient characteristics

Variable	Frequency	Percent
Age (years)		
Mean ± SD	45 ± 7.7	
Range	30 - 83	
HIV status		
Seropositive	40	33.6
Seronegative	79	66.4
HAART use		
Yes	25	62.5
No	15	37.5
CD 4 count (cells/ mm³)		
≤100	1	2.5
101 - 200	8	20.0
201 - 300	8	20.0
301 - 400	7	17.5
>400	16	40.0
Previous treatment		
EBRT	48	40.3
Chemotherapy	26	10.9
Surgery	13	21.8
EBRT + chemotherapy	21	17.6
EBRT + Surgery	7	5.9
Chemotherapy + surgery	2	1.7
EBRT + Chemotherapy + Surgery	2	1.7

Initial treatments patients included External beam radiotherapy EBRT 48 (40.3%), surgery 13 (10.9%) and chemotherapy 26 (21.8%). A total of 32 patients had multiple treatments including 21 patients who had a combination of external beam radiotherapy and chemotherapy. Table 1 shows the details of the prior treatment patients had before presenting with disease recurrence.

The prevalence of metastatic recurrence to the pelvic and para aortic nodes (Figure 1), mediastinal and supraclavicular nodes, as well as to the lungs were significantly higher among the seropositive group compared with the seronegative group ($P<0.05$), Table 2. Conversely, there was no statistically significant difference in the rate of recurrence to the liver and bones. Metastatic recurrence at the following sites were only seen among the seropositive group: brain (2 patients), spleen (two patients) and skin (1 patients), Table 2. Lymph nodes were the commonest sites of metastatic recurrence with 32 patients demonstrating isolated nodal metastasis. These 32 patients with isolated local recurrence included 18 seropositive patients and 14 seronegative patients.

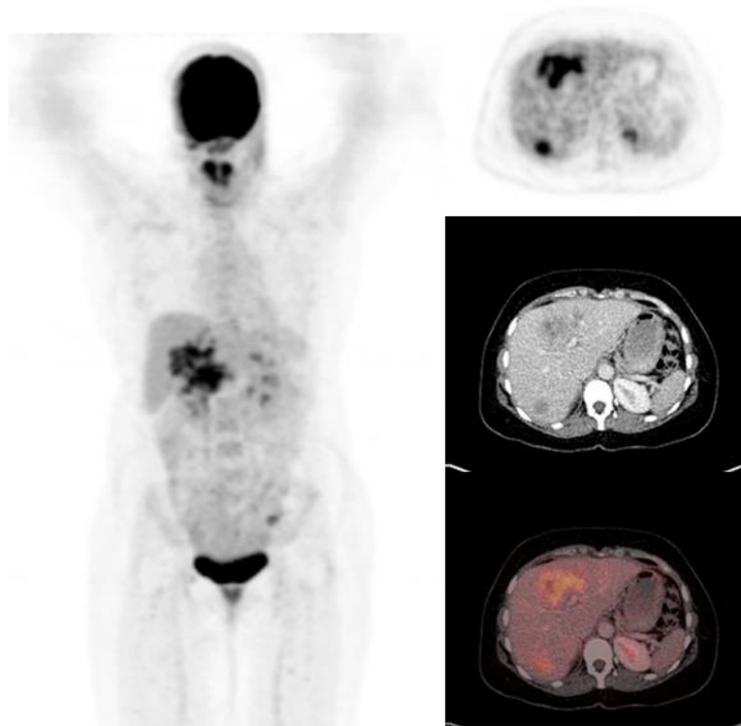


Figure 1. A 36 years old female initially with early-stage carcinoma of the cervix for which she had total abdominal hysterectomy plus pelvic nodal dissection. She is HIV-seropositive, CD 4 count >1000cells/mm³, viral load was undetectable. Recurrence was suspected based on new-onset vaginal bleeding. ¹⁸F-FDG PET/CT images show local recurrence as well as multiple liver metastases.

Table 2. Pattern of metastatic recurrence between HIV seropositive and HIV seronegative patients with carcinoma of the cervix

Site of recurrence	Seropositive n = 40 (%)	HIV Status Seronegative n = 79 (%)	Total n = 119 (%)	χ^2	P value
Pelvic+para aortic nodes	21 (52.5)	18 (22.8)	39 (32.7)	10.6423	0.001*
Mediastinal+supraclavicular nodes	23 (57.5)	17 (21.5)	40 (33.6)	15.4061	<0.001*
Lungs	11 (27.5)	7 (8.9)	18 (15.1)	7.1862	0.007*
Liver	5 (12.5)	3 (3.8)	8 (6.7)	3.2071	0.073
Bone	4 (10.0)	3 (3.8)	7 (5.9)	1.8453	0.174
Spleen	2 (5.0)	0 (0)	2 (1.7)		
Brain	2 (5.0)	0 (0)	2(1.7)		
Skin	1 (2.5)	0 (0)	1 (0.8)		

χ^2 : Chi square test; *: P value <0.05

The median time to disease recurrence was 11 months in the seronegative group (Figure 2). This is much shorter when compared with the time to recurrence in the seronegative group with a time recurrence of 24 months.

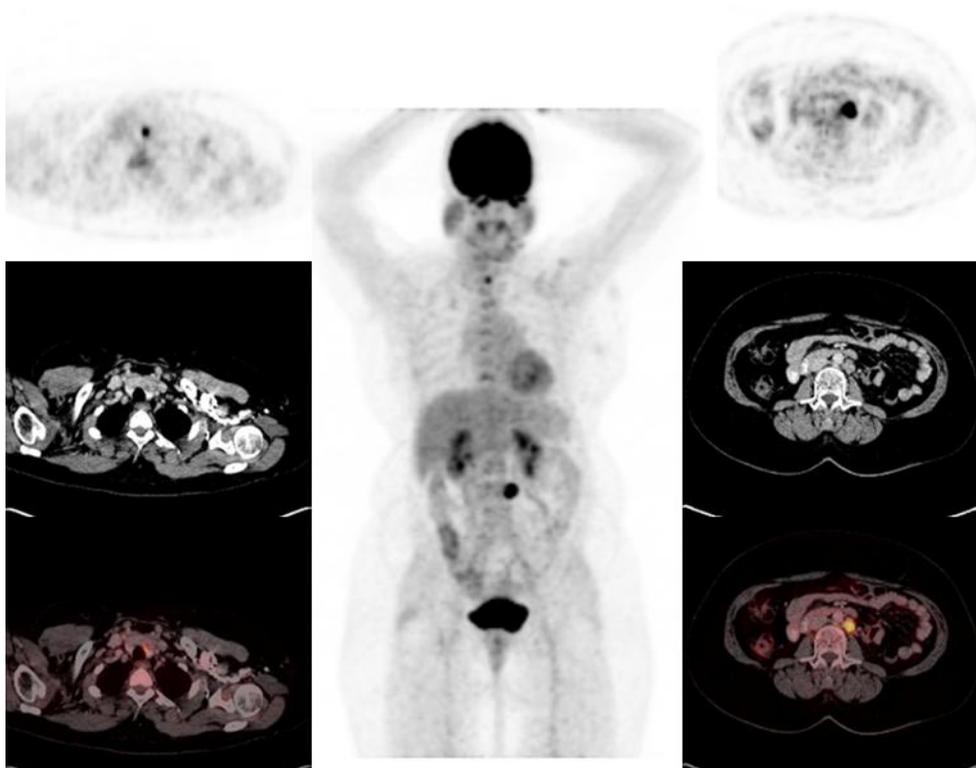


Figure 2. A 54 years old female with FIGO stage IIIB carcinoma of the cervix. She is HIV negative. Previously had chemotherapy and pelvic radiotherapy. ^{18}F -FDG PET/CT was obtained on suspicion of disease recurrence. Recurrence demonstrated in a left para-aortic node. No local recurrence is seen. Incidental ^{18}F -FDG -avid thyroid nodule confirmed to be papillary thyroid carcinoma.

The commonest initial treatment was external beam radiotherapy administered to 48 patients. These 48 patients included 13 seropositive and 35 seronegative patients. Among 13 patients who had initial surgical treatment, 38.5% (5/13) were seropositive while 61.5% were seronegative. Similar distribution was seen in the 26 patients who had chemotherapy only as the initial treatment (38.5% versus 61.5% for seropositive and seronegative groups respectively). The difference in the initial treatment modalities offered to the two groups of patients was not statistically significant, $P=0.675$. Table 3 shows the detailed distribution in the initial treatment offered to both groups of patients.

Table 3. Distribution of the initial treatment offered to the seropositive and seronegative patients.

Prior treatment	Seropositive n (%)	Seronegative n (%)	Total N (100.0%)	χ^2	P value
EBRT	13 (27.1)	35 (72.9)	48	2.3303	0.675
Chemotherapy	10(38.5)	16 (61.5)	26		
Surgery	5 (38.5)	8 (61.5)	13		
EBRT + chemotherapy	9(42.9)	12(57.1)	21		
EBRT + surgery	3(42.9)	4(57.1)	7		
Others	0(0.0)	4(100)	4		
Total	40 (33.6)	79 (66.4)	119		

χ^2 : Chi square; EBRT: External beam radiotherapy

Discussion

Carcinoma of the cervix is one of the acquired immunodeficiency syndrome (AIDS)-defining malignancies. In the presence of HIV infection, there is persistence of HPV infection, an oncogenic virus that has been recognised as a risk factor for cervical cancer. Also, HIV infection is associated with higher grade cervical intra-epithelial neoplasm, its rapid progression to frank cancer as well as its recurrence after conisation [17, 18]. We compared the pattern of recurrent cervical cancer between HIV-seropositive and HIV-seronegative women using ^{18}F -FDG PET/CT. Regional and distant nodes were the commonest sites of recurrent metastases. We have a significantly higher nodal recurrence among the seropositive patients compared with the seronegative group ($P < 0.05$). Similarly, a statistically significant higher rate of recurrence to the lungs was demonstrated (Figure 3) in the seropositive group compared with the seronegative group. In a study evaluating the role of ^{18}F -FDG PET/CT in women with suspected recurrent cervical cancer, regional (pelvic) and distant (retroperitoneal and mediastinal) lymph nodes were also the commonest sites of disease recurrence [19].

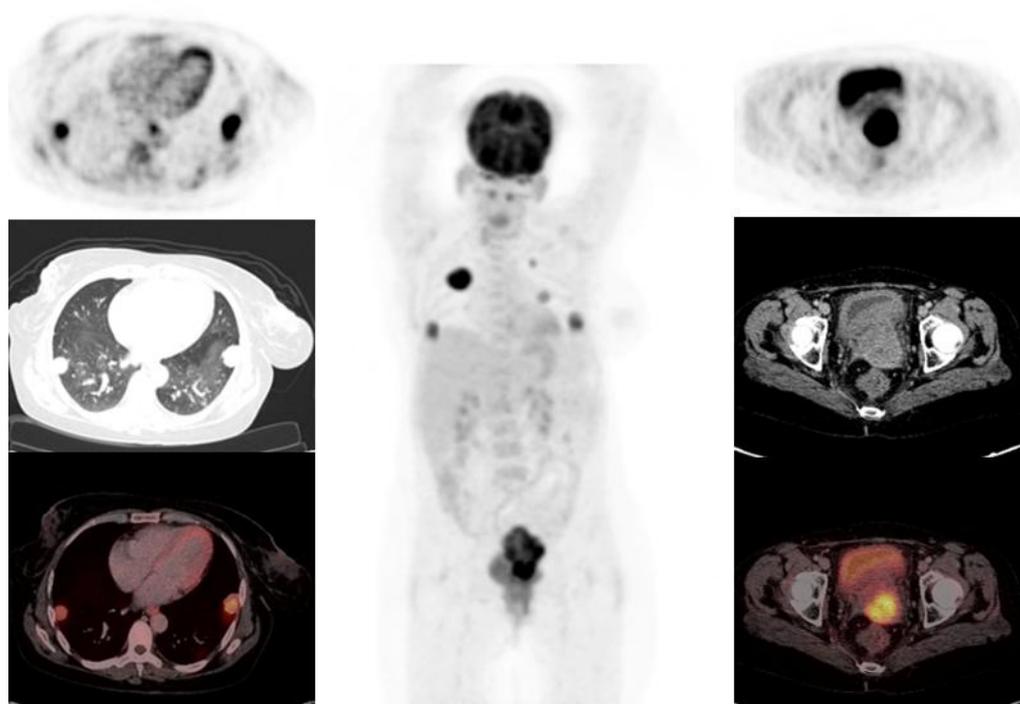


Figure 3. A 49 years old female previously diagnosed with stage IIIB carcinoma of the cervix. She is HIV-seropositive with a CD 4 count of $386\text{cells}/\text{mm}^3$. ^{18}F -FDG PET/CT was obtained for recurrence on account of new-onset vaginal bleeding and discharge. Images demonstrate local disease recurrence and bilateral pulmonary metastases.

HIV-associated malignancies have been found to be more aggressive and respond less optimally to therapeutic interventions in HIV-infected patients compared with when the same cancer is seen in HIV seronegative patients [3, 20]. The exact reason for this observation remained to be elucidated however, HIV-associated immunosuppression has been implicated in impairment in performance status which makes patient less likely to be able to withstand full course of treatment as well as make them prone to side effects of cancer treatment [21]. In our cohorts, the seropositive patients were younger than the seronegative patients indicating a younger age at diagnosis among HIV patients. Other authors from South Africa and

elsewhere have reported that HIV-infected patients are diagnosed 10 to 15 years younger than the non-infected patients [3, 22-24].

Another indication of more aggressive disease in our patient cohorts was the finding of a shorter time to disease recurrence among the seropositive group. The median time to disease recurrence was 11 months for HIV seropositive patients. This was more than double (24 months) for the seronegative patients. Most cases of cervical cancer recurrence within the first two years after initial treatment and the commonest sites site of early disease recurrence is the regional and distant lymph nodes. When patients are followed-up for longer, visceral metastases predominate in the long term, usually beyond five years [25].

Treatment option offered to patients in both groups were similar. Type of initial treatment offered to patients with cervical carcinoma is usually influenced by stage of the disease [26]. No statistical significant difference was seen in the treatment offered the HIV seropositive and HIV seropositive groups. This may suggest that the primary disease extent was comparable in both groups prior to treatment. Furthermore, we can deduce that the treatment option patients had initially was not a significant contributor to the differences in the pattern of recurrence seen between the seropositive and seronegative patients.

Our study is limited by its retrospective design. A larger prospective study will be needed to corroborate our findings.

In conclusion, HIV-infected patients are diagnosed with cervical cancer at younger age compared to non-infected patients. The time to recurrence is shorter among HIV seropositive patients with the commonest site of metastatic recurrence being in the lymph nodes. Nodal and liver metastases are significantly higher in HIV seropositive patients compared with seronegative patients.

The authors declare that they have no conflicts of interest.

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Sequential ^{18}F -FDG PET/CT imaging parameters for differentiating benign from malignant lymph nodes in head and neck carcinoma

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Keywords: ^{18}F -fluorodeoxyglucose positron emission tomography -Sequential examinations
-PET/CT -Head and neck region cancer

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Abstract

Objective: The aim of this study was to differentiate between benign and malignant head and neck lymph nodes by sequential imaging. **Subjects and Methods:** The total of 56 retrospectively analysed patients with suspected or histopathologically confirmed head and neck malignancy (nasopharyngeal cancers mainly; 28 patients), before any treatment, underwent sequential fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F -FDG PET/CT) examinations for staging purposes. Remaining 28 patients with physiologic and histopathologically confirmed inflammatory (of non-specified origin) lymph nodes were included into this analysis. Patients underwent sequential PET/CT scans 60 and 90min post injection (p.i.) of the ^{18}F -FDG. Semi-quantitative analysis of metabolic activity within lymph nodes was based on the standardized uptake value (SUV) evaluation. To compare the metabolic activity fluctuation over time, the retention index (RI) was used. For SUV value and RI cut-off evaluation, the receiver operating characteristic (ROC) analysis was performed. **Results:** The SUVmax value at 60min p.i. of physiologic, inflammatory and malignant (metastatic) lymph nodes were 1.09 ± 0.33 , 2.36 ± 0.60 and 6.31 ± 2.74 , respectively. The SUVmax value at 90min p.i. were: 1.01 ± 0.32 , 2.48 ± 0.61 , and 7.17 ± 2.91 , respectively, and there was statistically significant difference between physiologic and inflammatory and physiologic and the metastatic lymph nodes ($P<0.001$). The values of early and delayed SUVmax were significantly different between physiologic and inflammatory and physiologic and metastatic lymph nodes ($P<0.001$). The SUVmax, SUVmean values at 60 and at 90min p.i. between malignant and inflammatory lymph nodes were statistically insignificant ($P=0.33$). The RI at 60 and at 90min p.i. was: $-6\%\pm 16\%$ for physiologic, $6\%\pm 14\%$ for inflammatory and $15\%\pm 13\%$ for the metastatic lymph nodes. The SUVmax changes over time (the RI) were statistically significant for physiologic and metastatic and physiologic and inflammatory lymph nodes ($P<0.001$) and significant between malignant and inflammatory lymph nodes ($P=0.02$). **Conclusion:** Sequential delayed ^{18}F -FDG PET/CT examinations may increase specificity of this scan and provide information for the differentiation benign and malignant lymph nodes in the cases of head and neck cancer.

Introduction

The head and neck region cancer is one of most complex types of cancer because many kinds of cancer are there located. The most common histological type is squamous cell carcinoma (SCC) [1-3]. Fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F -FDG PET/CT) is useful in

identifying and monitoring the stage of the disease. Metabolic activity assessment in the regional lymph nodes is important because it is more crucial in non-enlarged organs than in the primary tumors. Furthermore, cancer cells infiltration of the lymph nodes may change the clinical management of the disease [4-6].

Fluorine-18-FDG PET/CT although very useful, is not tumor specific because it cannot differentiate between cancer and inflammation metabolically [7, 9]. The Warburg effect is the characteristic diagnostic means of ^{18}F -FDG PET/CT [10-15], because glucose uptake increases over time.

Some authors consider standardized uptake maximal value (SUVmax) greater than 2.5 as suggesting malignancy [16], while others disputed the importance of SUVmax values [17] or reported different cut-off SUV max values [18-24].

We decided to study the possible difference between: normal, inflammatory and metastatic lymph nodes by various metabolic indices of the ^{18}F -FDG PET/CT scan in the head and neck cancer.

Subjects and Methods

We studied retrospectively, 56 patients (Table 1), 19 women (mean age: 58 ± 10 years, age range: 52-83 years) and 37 men (mean age: 61 ± 7 years, age range: 51-83 years) with suspected or histopathologically confirmed not-treated before, head and neck cancers. They were: 21 nasopharyngeal cancers, 4 salivary glands cancers, 2 laryngeal and 1 tongue cancer and all underwent serial ^{18}F -FDG PET/CT examinations for staging purposes. All patients gave in writing their informed consent for participating in this study. Based on the ^{18}F -FDG PET/CT examinations, lymph nodes with higher ^{18}F -FDG uptake than local blood vessels were further evaluated with histopathology. The database consisted of not-treated (early diagnosed or planned for treatment) 28 patients with physiologic and histopathologically confirmed inflammatory (of not-specified origin) lymph nodes as well and 28 patients with malignant (metastatic) nodal findings. Patients underwent sequential PET/CT scans at 60 and 90min p.i. of the ^{18}F -FDG. Semi-quantitative analysis of the metabolic activity within the lymph nodes was based on the SUVmax and SUVmean values. To compare the metabolic activity fluctuation over time, the retention index (RI) was used.

All patients were hospitalized in the Greater Poland Cancer Centre, Poznan, Poland between 2014-2016.

Table 1 shows the patients' characteristics.

Table 1. Patients characteristics

Characteristic	Physiologic and inflammatory lymph nodes	Metastatic lymph nodes
	Value	
Number of patients	28	28
Avg age [years]	60	59
SD [years]	7	7
Range [years]	51-83	52-83
Number of women	9	10
Avg age [years]	58	57
SD [years]	7	9
Range [years]	52-74	52-83
Number of men	20	17
Avg age [years]	61	61
SD [years]	8	6
Range [years]	51-83	52-71

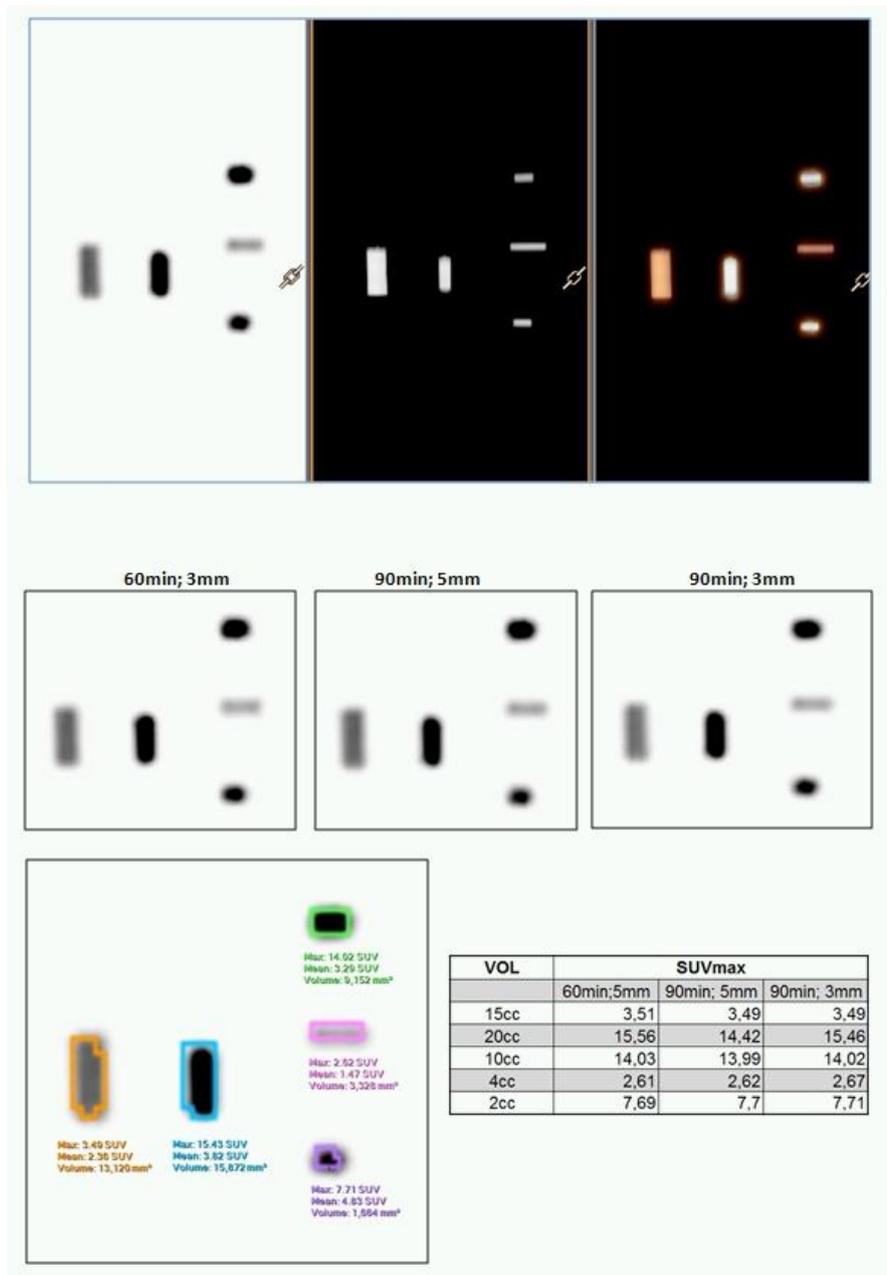
avg -average, SD- standard deviation

Study protocol

Whole body and head and neck ¹⁸F-FDG PET/CT scans were performed sequentially (PET/CT scanner Gemini TF 16, Philips, Cleveland) at 60 and 90min p.i. of the ¹⁸F-FDG with an activity up to 400MBq (range: 315-410MBq) without patient's head repositioning. Scans performed 90min p.i. were reconstructed with higher spatial resolution. According to the standard preparation protocol, patients fasted for at least 6 hours, had a low carbohydrate diet, avoided intensive exercises and cold environment for 48h before the scan. During the study, patient laid supine on PET/CT table during the up to 25-30min of scanning protocol. A low-dose unenhanced CT was performed before the PET acquisition with 120kVp and 100mAs with 16-slice CT scanner. Emission images were acquired for 1min 30s (1:30) per table [19-22] and the entire study time did not exceed 35-40 minutes.

Phantom study

The phantom study was performed. The phantom study consisted of four different acquisition simulating tumors of different volume and activity as shown on Figure 1. The phantom study showed no influence of technical factors (spatial resolution, time shift acquisition) on the SUV calculations.



The dataset analysis

We used the SUVmax, SUVmean values to obtain the lesions metabolic activity. The SUVmax values were calculated with the following equation [3, 16]:

$$SUV_{max} = \frac{\text{maximum tissue concentration} \left[\frac{MBq}{kg} \right]}{\frac{\text{injected dose} [MBq]}{\text{body weight} [kg]}}$$

We evaluated the SUVmax differences in the initial and delayed scans with the RI [9, 12, 23-26]:

$$RI = \frac{SUV_{max90min\ p.i.} - SUV_{max60min\ p.i.}}{SUV_{max60min\ p.i.}} \times 100\%$$

Methods of contouring

Figure 2. shows the semi-automatic method of contouring with 50% background cut-off used for findings in the PET/CT scans delineation (with the Siemens Fusion Viewer software):

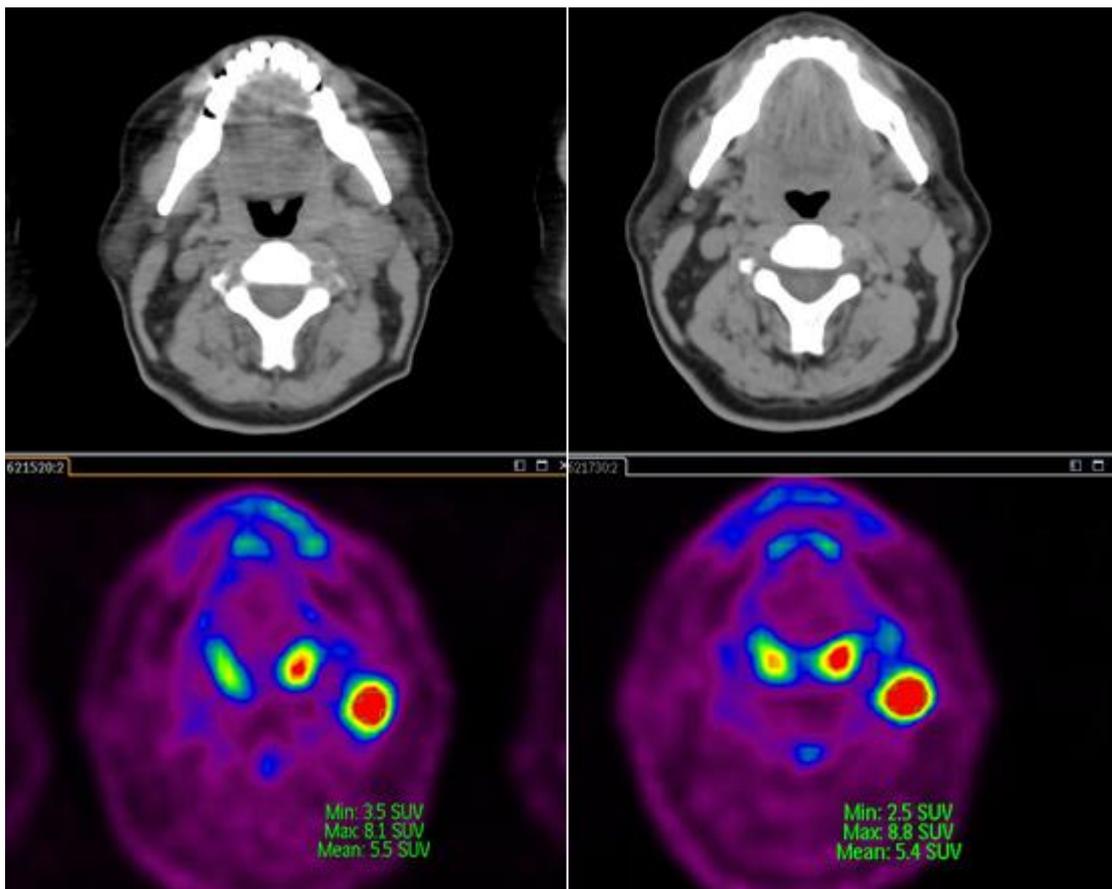


Figure 2. Malignant lymph node-90 (left) and 60 (right) min p.i of the ¹⁸F-FDG.

Statistical analysis

We compared several normally distributed values in the interval scales in two groups. The physiologic, inflammatory and metastatic lymph nodes in different groups of patients (comparison of independent values) were analyzed. We used the Students' t-test to compare the SUV values. The Pearsons' correlation coefficient was evaluated. The variables were compared considering as significant the statistically limit of $P < 0.05$. We used the Kruskal-Wallis test when needed (several groups of independent variables comparison).

We used the STATISTICA (StatSoft) commercial software for the statistical analysis.

Results

The authors compared several parameters in two groups: the SUVmax and SUVmean values at 60 and 90min p.i. of the ^{18}F -FDG: physiologic vs malignant lymph nodes, physiologic vs inflammatory lymph nodes, malignant vs inflammatory lymph nodes.

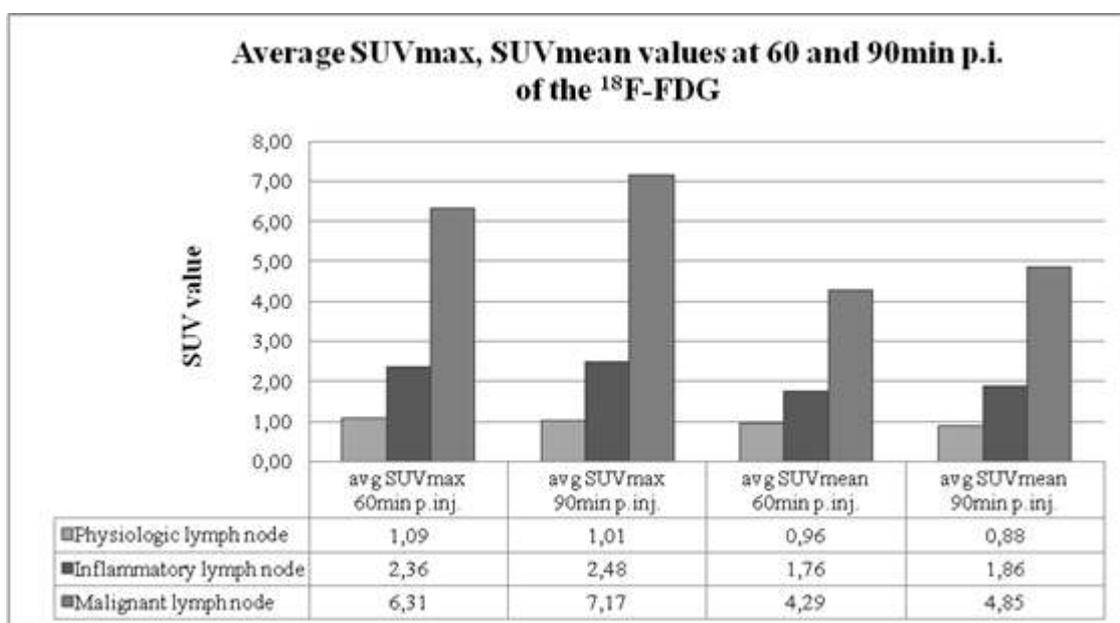
SUV results

SUVmax and the SUVmean 60 and 90min p.i. of the ^{18}F -FDG

The SUVmax values at 60min p.i. for physiologic, inflammatory and malignant lymph nodes were 1.09 ± 0.33 , 2.36 ± 0.60 and 6.31 ± 2.74 , respectively. The SUVmax values 90min p.i. were 1.01 ± 0.32 , 2.48 ± 0.61 , and 7.17 ± 2.91 , respectively. The differences between SUVmax values at 60 and 90min p.i. were statistically significant within physiologic lymph nodes ($P=0.04$) and metastatic lymph nodes ($P<0.001$), but insignificant within inflammatory lymph nodes ($P=0.06$).

The SUVmean values at 60min p.i. within physiologic, inflammatory and malignant lymph nodes were 0.96 ± 0.32 , 1.76 ± 0.46 and 4.29 ± 2.01 , respectively. The SUVmean values at 90min p.i. were 0.88 ± 0.27 , 1.86 ± 0.45 and 4.85 ± 2.08 , respectively (Figure 3). The differences between SUVmean values at 60 and 90min p.i. were statistically significant in physiologic ($P=0.02$), inflammatory ($P=0.02$) and the metastatic lymph nodes ($P<0.001$) as well.

Figure 3 shows the average SUVmax, SUVmean values at 60 and 90min p.i. of the ^{18}F -FDG in the lymph nodes.



avg = average, SUV = standardized uptake value, max = maximum, mean = mean

Figure 3. The comparison of SUV values within groups

According to the t-test results, the differences of the SUVmax values at 60 and 90min p.i. and the SUVmean at 60 and 90min p.i. between physiologic and inflammatory lymph nodes were statistically significant ($P<0.001$).

The SUVmax, SUVmean at 60 and 90min p.i. of the ^{18}F -FDG differences between physiologic and metastatic lymph nodes were statistically significant ($P<0.001$).

The SUVmax, SUVmean at 60 and 90min p.i. differences between inflammatory and metastatic lymph nodes were statistically insignificant (P=0.33).

Metastatic lymph nodes revealed the highest metabolic activity, observed as most intensive ^{18}F -FDG uptake over time. The SUVmax value changes over time were significantly different in every condition: when compared physiologic and malignant or physiologic and inflammatory lesions or malignant and inflammatory lymph nodes.

The ROC analysis-SUV value

For the ROC analysis purposes, the dataset has been organized in two groups: Group I-physiologic, inflammatory vs malignant lymph nodes, Group II-inflammatory and malignant lymph nodes.

To find the appropriate cut-off in Groups I and II patients, we used the ROC SUVmax value at 60 and 90min p.i. of the ^{18}F -FDG analysis (Figure 4-7). We evaluated the sensitivity and the specificity of the method (Table 2). To evaluate the reliability of the ROC analysis, we marked the Area Under the Curve (AUC) and the Youden Index (Table 3).

Group I: The SUVmax at 60 and 90min p.i. of the ^{18}F -FDG cut-off in all benign (physiologic, inflammatory) and malignant lymph nodes were 3.32 and 3.49 (P<0.05), respectively. The delayed phase of examination occurred as more sensitive and specific than initial scans analysis (Figure 4 and 5, Table 1, 2).

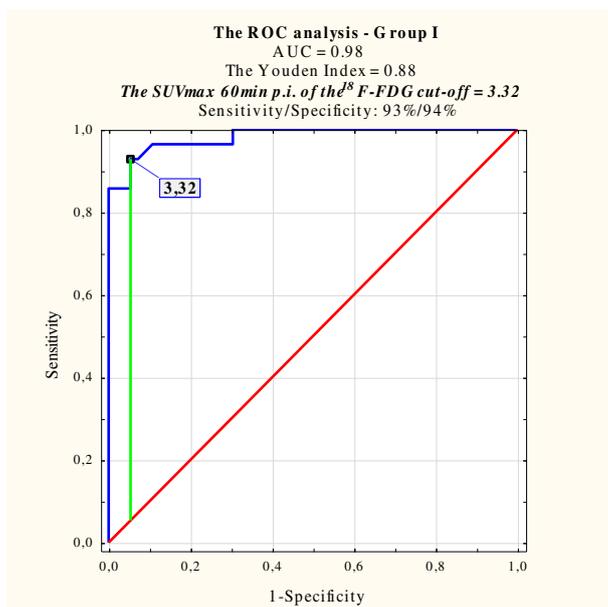


Figure 4. The ROC curve: SUVmax 60min p.i. of the ^{18}F -FDG-Group I

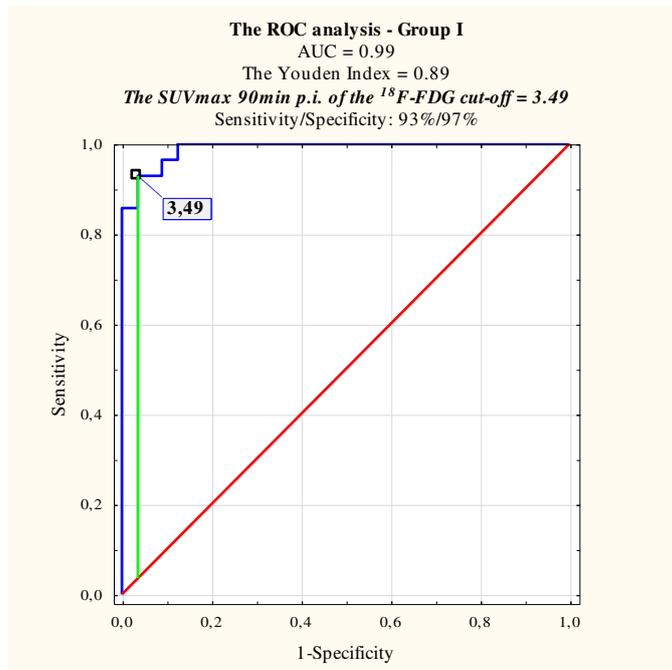


Figure 5. The ROC curve: SUVmax 90min p.i. of the ^{18}F -FDG - Group I

Group II: The SUVmax at 60 and 90min p.i. cut-off within inflammatory and malignant lymph nodes was 4.03 and 3.49 ($P < 0.05$), respectively. Sequential delayed ^{18}F -FDG PET/CT examinations increased the specificity of the method (Figure 6 and 7, Table 1 and 2). The sensitivity/specificity ratio on initial and delayed scans equaled 86%/100% and 93%/93%.

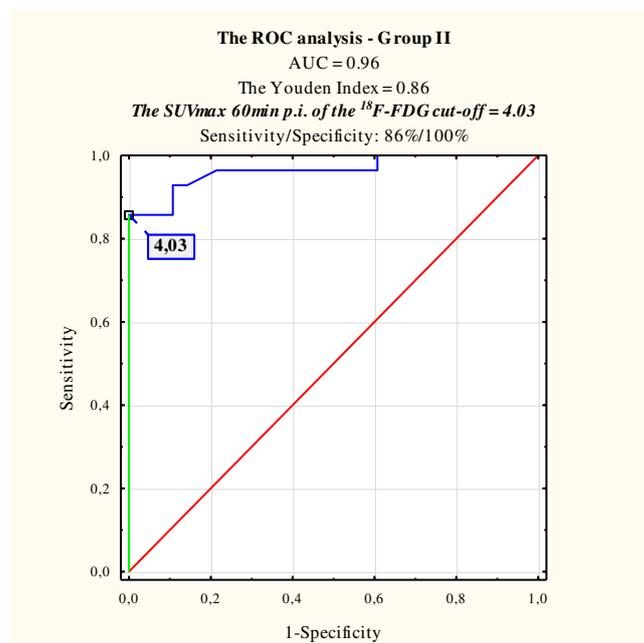


Figure 6. The ROC curve: SUVmax 60min p.i. of the ^{18}F -FDG - Group II

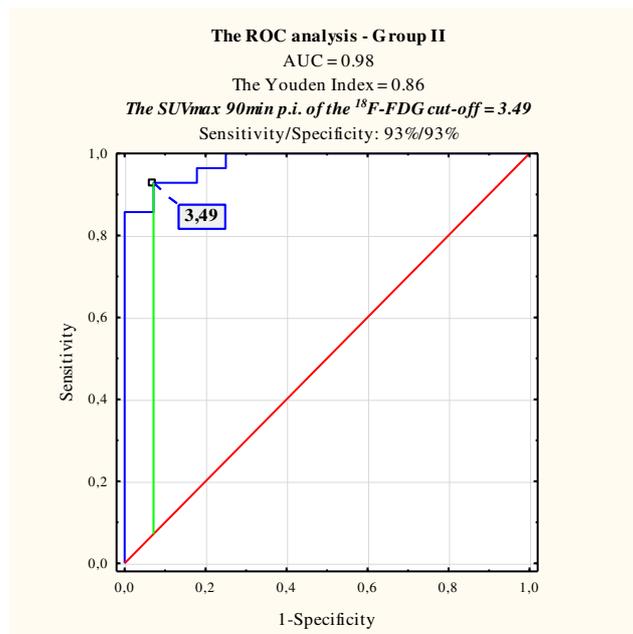


Figure 7. The ROC curve: SUVmax 90min p.i. of the ¹⁸F-FDG - Group II

Table 2 and 3 show the consisted data described above.

Table 2. The ROC analysis - ROC report data

The sensitivity and specificity of the method according to prognostic cut-off point (ROC report)

G I					
<i>SUVmax 60min p.i.</i>			<i>SUVmax 90min p.i.</i>		
Cut-off	Sensitivity	Specificity	Cut-off	Sensitivity	Specificity
3.32	93%	94%	3.49	93%	97%
G II					
<i>SUVmax 60min p.i.</i>			<i>SUVmax 90min p.i.</i>		
Cut-off	Sensitivity	Specificity	Cut-off	Sensitivity	Specificity
4.03	86%	100%	3.49	93%	93%

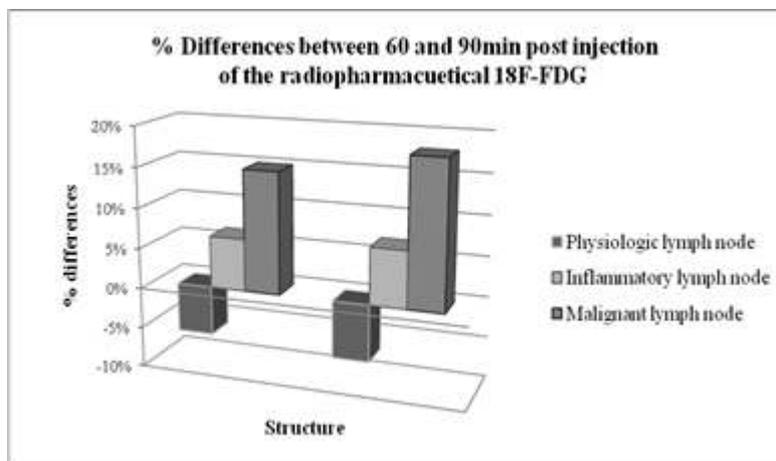
Table 3. The ROC analysis - test's reliability in the prognostic cut-off evaluation

The comparison of ROC analysis results - test's reliability

G I					
<i>SUVmax 60min p.i.</i>			<i>SUVmax 90min p.i.</i>		
AUC	Youden Index	Cut-off	AUC	Youden Index	Cut-off
0.98	0.88	3.32	0.99	0.89	3.49
G II					
<i>SUVmax 60min p.i.</i>			<i>SUVmax 90min p.i.</i>		
AUC	Youden Index	Cut-off	AUC	Youden Index	Cut-off
0.96	0.86	4.03	0.98	0.86	3.49

The Retention Index measurement

The percentage differences (RI) at 60 and 90min p.i. of the ^{18}F -FDG were $-6\% \pm 16\%$ for physiologic, $6\% \pm 14\%$ for inflammatory and $15\% \pm 13\%$ for the metastatic lymph nodes (Figure 8). According to Kruskal-Wallis test, the RI differences between physiologic, inflammatory and malignant lymph nodes were statistically significant ($P=0.03$).



avg=average, SUV=standardized uptake value, max=maximum, mean=mean

Figure 8. The SUV values changes over time diagram

According to the ROC analysis results, the 7% SUVmax changes over time suggest malignancy (Figure 9). Based on an ROC analysis the RI cut-off of 7% of the AUCs of SUVmax at 90min p.i. of 0.68 and the Youden Index 0.36 distinguished inflammatory from metastatic lymph nodes with the sensitivity and the specificity ratio 75%/61%.

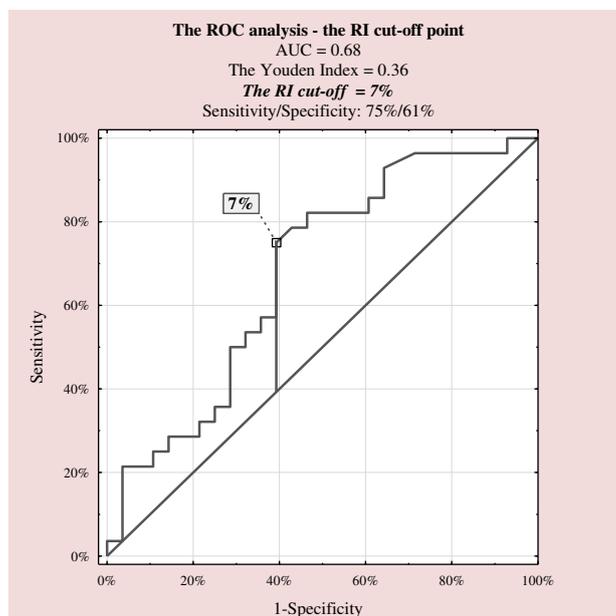


Figure 9. The ROC analysis: the Retention Index cut-off (inflammatory and malignant lymph nodes): 7% with sensitivity/specificity: 75%/61% (from final ROC report; X-axis: sensitivity, Y-axis: 1-specificity: $1 - 0.39 = 0.61$)

Discussion

Several studies have shown that increasing ^{18}F -FDG uptake over time commonly indicates malignancy, however it can also mark inflammation or other benign pathologies with high glucose utilization. Based on the Warburg Effect [9-16], only malignant lesions can be characterized by increasing glucose uptake over time, while in some conditions, inflammatory lymph nodes can be difficult to distinguish from malignancy.

The following metabolic indices were used for the PET/CT: Standardized Uptake Value (SUV), Metabolic Tumor Volume (MTV), Total Lesion Glycolysis (TLG). SUV value as an ^{18}F -FDG uptake indicator provides information about the highest ^{18}F -FDG uptake in single voxel within the tumor (SUVmax) or the average uptake of the whole tumor (SUVmean) [1, 3]. Volume-based parameters, such as MTV or TLG emerged from ^{18}F -FDG PET/CT to describe the biologic characteristics of malignant lesions in many different localizations and occurred as valuable in the head and neck region cancers management [1].

Performing delayed images might be useful in differentiating benign pathologies from malignancies according to the SUV value changes over time and helpful in detecting lesions which are unnoticeable on initial scans [9]. The SUV value is commonly used as a reliable prognostic marker [18] which depends on i.e. number and activity of neoplastic cells. This SUV value in the theory increases over time in malignant lesions and constantly decreases or shows no change when structure is non-malignant (physiologic or benign). Some pathologic but non-cancerous conditions can also be characterized by noticeable increase of the ^{18}F -FDG uptake, such as inflammatory processes, which determine significant issue in appropriate differential diagnosis. In general, the SUVmax value higher than 2.5 suggests malignancy [16], however available literature shows that inflammatory lesions may exceed this value and keep increasing tendency in delayed phase of the examination as well as malignant findings [7, 9]. Marking the most probable SUVmax value cut-off, distinguishing benign from malignant lymph nodes demands exercising several options such as semiquantitative SUV value analysis, RI calculation and the ROC or sensitivity and specificity curves delineation.

According to obtained results, the SUVmax at 60 and 90min p.i. of the ^{18}F -FDG higher than 3.0 suggests malignancy. The RI higher than 7% may also indicate that observed process can be malignant. In this study, the RI occurred as a useful tool, which can increase the sensitivity and specificity of the method. The cut-off value may apply to the similar study population, thus the number of examined patients could be too small to generalize the results.

Sequential delayed examinations seem to be more comfortable and help to avoid patients' repositioning, which may affect the results. According to obtained results in comparison to several authors, 60 and 90min p.i. of the ^{18}F -FDG protocol might provide appropriate clinical information and does not need additional, more delayed phases of the study.

According to literature the number of patients validates statistical tests reliability, thus the number of analysed patients may limit the investigation. Patients with either confirmed malignancy and suspected inflammatory lymph nodes were excluded from the analysis because of prediction, that there is a connection between malignancy and inflammatory occurrence. Nevertheless, clinical practice suggests the usefulness of delayed ^{18}F -FDG PET/CT examinations as helpful in benign from malignant lymph nodes distinguishing.

In conclusion, sequential delayed ^{18}F -FDG PET/CT examinations may increase specificity of this scan and provide information for the differentiation benign and malignant lymph nodes in the cases of head and neck cancer.

The authors declare that they have no conflicts of interest.

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Specific neck pain algometric measurements and their relation to heart rate and skin humidity

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Abstract

Cervical pain is very common in general population but only few methods have been used to evaluate it with objectiveness. There are only few studies which use algometer as a means of neck pain assessment. Studies have shown that algometer could be used in pain evaluation but more studies are necessary to support this. The main purpose of the study was the evaluation of algometer as a means of an objective measurement of pain threshold to people with non-specific neck pain. The study focused on the search of correlation between neck pain and pain pressure threshold (PPT) after recording the number of the minimum pain feeling in the sample with the pressure of the algometer at specific neck points. The study also aimed at searching the correlation of neck pain with heart rate and skin humidity. This is a part of a cross-sectional study which was held during a PhD study which assessed 185 randomly chosen people, 20-60 years old, who visited all the Public Centers in the County of Thessaloniki. The sample was separated in two groups according to the frequency of their neck pain, those who suffered frequently or almost every day from non-specific neck pain (neck pain group) and those with non-specific neck pain occasionally, rarely or never suffered from it (no pain group). Subjects were randomly chosen from people who visited the Public Centers for any reason. Neck pain was strongly associated with PPT ($P < 0.001$). Heart rate ($P = 0.216$) and skin humidity ($P = 0.14$) were not significantly related to neck pain. According to the results the algometer seems to be a useful tool as a mean of neck pain evaluation and algometry seems to enhance the idea of pain quantification. However more evidence is needed and more studies should be conducted in order to strengthen our results. Latter studies should be designed with more accuracy focusing on details in order to establish algometry as an objective method of pain evaluation.

Introduction

The main aim of the study was the evaluation of the algometer as a means of an objective measurement of pain threshold to people with non-specific neck pain. In this study the correlation of neck pain with heart rate and skin humidity was also investigated. The basic target of this protocol was to detect if there are

statistically significant differences in pain threshold between people who suffer from neck pain and those who do not. Additionally, these groups were tested on their differences in skin humidity and heart rate. For these purposes two devices were used, an algometer which was applied at six specific points at the neck area counting pain pressure thresholds and a smart watch which was put at the wrist area of the subject in order to estimate skin humidity and heart rate.

According to the International Association for the Study of Pain (IASP) neck pain is the pain in the region bounded superiorly by the superior nuchal line, inferiorly by an imaginary transverse line through the tip of the first thoracic spinous process, and laterally by sagittal planes tangential to the lateral borders of the neck [1]. Neck pain seems to be a frequent problem among people and a symptom which is influenced by many pathological conditions. Today, as the lack of exercise, the sedentary life and the use of computers are very common in population, neck pain symptom seems to be increased and this concerns the medical community [2]. The main causes of neck pain have postural or mechanical basis and affect about two thirds of people especially in middle age. This type of pain is called non-specific neck pain [3]. There are many different causes inducing neck pain but in this study the interest is focused on non-specific neck pain. Even though neck pain and radicular pain may have the same pathophysiology, they are not the same pathological entities [4]. Most patients with acute neck pain with or without radicular symptoms suffer by musculoskeletal or degenerative disorders and pain is usually restored automatically after a few days. Nevertheless, there is always the possibility of the existence of more severe situations which induce neck pain [5]. Pathologies such as mechanical irritation, inflammation, metabolic and psychological disorders, tumors and reflective pain from other parts of the body could induce pain at the neck area [6-9].

There are many epidemiological studies which have been carried out in many countries calculating the prevalence of neck pain and investigating its correlation with specific agents. In Greece there are only a few studies which have studied the epidemiology of neck pain. In Greece a rheumatologic study was conducted (8.740 people over 19 years old) and it was found that neck pain was caused in 4.8% of the cases by rheumatic diseases. Neck pain and low back pain are the fourth reason for visits at the Greek Health System and they are responsible for financial and social problems [10]. In another study 26.1% of the patients, selected in a Health Center of Crete, mentioned one episode of neck pain per year and those who suffered from neck pain mentioned restriction at their everyday activities (55.31%) [11]. In another study which was held in rural and urban Greece among 1000 participants 17.5% of them seemed to suffer from neck pain at least once during last month, 35.3% of these people followed a treatment for neck pain and 7.4% stayed bedridden for a while [12].

International literature indicates that the incidence of neck pain is rising and affects life in personal, social and financial level. The prevalence ranges from 4.8% to 79.5% with median 25.8%. Neck pain is more frequent in females, in people living at rural places and in high living status [13]. Neck pain seems to be more frequent in Scandinavian related to the rest of Europe and Asia, while another study suggests that its prevalence is 2.2% with higher incidence in middle aged females- with a median of 48.9 years of age- and 81% in Caucasians [14, 15].

Materials and methods

Sample

This is a part of a cross-sectional study which was held during a PhD study. The sample consisted of 185

randomly chosen people, 20-60 years old, who visited all the Public Centers in the County of Thessaloniki. The sample was people who visited Public Centers and were randomly chosen after their entrance. The sample was separated in two groups according to the frequency of their neck pain, those who suffered frequently or almost every day from non-specific neck pain (neck pain group) and those who faced non-specific neck pain occasionally, rarely or never suffered from it (no pain group). The target group of this study was 20-60 years old and was chosen because these years are the most active and productive years in comparison to younger or higher ages. People who visited the emergency rooms were excluded because their morbidity could affect the results of their examinations. Among the exclusion criteria were the presentation of fever, the consumption of painkillers, muscle relaxants and anti-inflammatory medicines in the last 24 hours. Elderly or children were also excluded due to the age effect on pain perception. Especially in elderly, PPT could also be affected by the process of aging [16, 17]. Additionally, people with reflections of pain in upper limbs were also excluded because our study involved non-specific neck pain.

Devices

The devices which were used in this study were the algometer Electronic Von Frey (Bioseb) and the empathy electronic watch/smarthphone which counted heart rate and skin humidity. The algometer calculated the pain pressure threshold (PPT). PPT is the minimal amount of pressure which causes a sense of pain. In this study when the subject felt pain said the word 'stop' and the examiner stopped pressing immediately. This was the critical point when the pressure turned to pain. The subject did not know the number which was shown at the screen of the device but only the examiner knew. The examiner put pressure at six points in the neck selected by the team of the study. The median of PPT from those six points was calculated and a number was formed. This number was the PPT for each person which was examined. The use of the algometer in other studies was very helpful and this experience helped the team to design the best option for the device application. The other device was the empathy electronic watch/smarthphone. This device was put on the wrist of the subject in order to calculate heart rate and skin humidity. The lack of experience in the use of the smartphone/watch was not helpful for the procedure of this study. However, the lack of studies and the absence of experience in the use of this device at such observational studies created an opportunity for a new field but also a big responsibility for the team.

Study design

At the entrance of the Public Centers there was a team of three health professionals who informed people for the study. People were chosen randomly some of them were excluded because they did not complete the selection criteria. The team asked them about their age, if they needed emergency care, if they had fever or consumed analgesic, muscle relaxant or anti-inflammatory drugs the last 24 hours. After the sample selection, people signed a consent paper and the questionnaire was distributed to them. Health professionals helped people to answer the questions giving them explanations whenever they needed.

Statistics

The statistical analysis was carried out by the statistical program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). We base our sample size calculation on the population of Thessaloniki and the neck pain prevalence. For 1.050.000 population at the County of Thessaloniki with accuracy $4.8\% \pm 2$ (as it is defined by the prevalence of neck pain in Greek population) and with significance level 5% 440 people were collected in total and 185 consented to be examined. The normality test was done through the tests of

Kolmogorov-Smirnov and Shapiro-Wilk. The normality was rejected in all cases. This is the reason why the previous tests were chosen. Mann-Whitney was used for the relation between quantitative and qualitative variables at the comparisons of the groups. Relations with a P-value (P)<0.05 were considered statistically significant.

Ethics

The study was approved by Ethics Committee of the Aristotle's University of Thessaloniki (number:2/27.3.2013). Every detail of the study was explained to patients and they participated to the study after signing a paper of consent. The study maintained the anonymity of the sample.

Pilot study

The calibration of the devices took place in a private room with the patient sitting on a chair which supported the back. After relaxing at the chair for 5 minutes, heart rate and skin humidity of the subjects were measured using an electronic watch. Afterwards the examiner pressed the six points of the neck which were chosen in order to measure pain threshold. A simple handheld pressure was applied by the examiner through a plastic thin stick which had the shape of a thick needle. The test points were examined symmetrically and bilaterally at both sides in neck area (Figure 1).

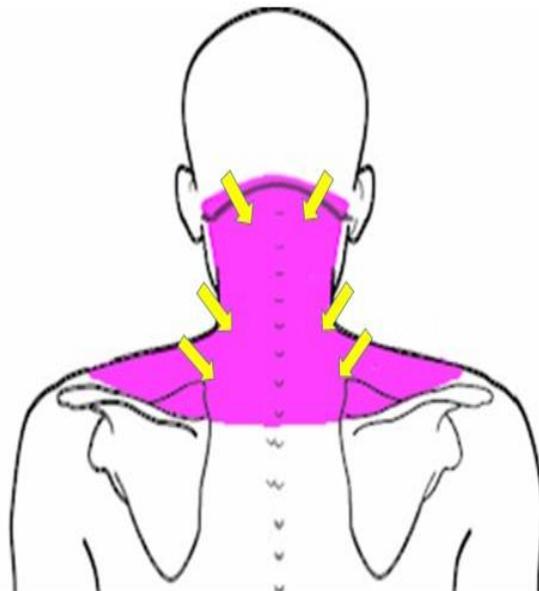


Figure 1. The six points of algometer measurements at neck

The examiner pushed the plastic stick with the purpose to irritate the point but it couldn't penetrate the skin because its nib was 1mm and had curved edge. The measurement was repeated 3 times at every point with 30sec interval for better perception of pain. The median of PPT was calculated for every person after the end of every examination. The procedure was explained to every person with details and many times because it was noticed that sometimes was not clear for the participants what they had to do.

Results

To search if there is a relation between algometer measurements and neck pain firstly normality is tested. Finding $P < 0.001$ normality is rejected and non-parametrical test Mann-Whitney is done. With the result $P < 0.001$ it is concluded that there is a statistically significant relation between neck pain and the median of algometer measurements and this relation is strong (Table 1).

The median at 'No Pain Group' is higher than the median of the algometer's measurements at 'Neck Pain Group'. In this study the algometer was used to measure pain threshold, which is the lowest intensity stimulation causing pain. 'Neck Pain Group' showed low values (median=88.8333) compared to 'No Pain Group' (median=98.3333) (Table 2). That means patients suffering from neck pain showed lower values of pain thresholds compared to the group which does not suffer from neck pain. Neck pain seems to be significantly connected to these measurements ($P < 0.001$) (Table 1).

Skin humidity was examined in 179 people and the median was 60.99% (Table 3). Normality test showed $P = 0.025$. After the non-parametrical test Mann-Whitney the relation between skin humidity and neck pain led to $P = 0.141$ (Table 1). This means that the relation between skin humidity and neck pain is not statistically significant ($P = 0.141$).

Heart rate was examined in 174 people. The average heart rate was 73.48 beats (Table 3). After normality test a p value less than 0.001 was found ($P < 0.001$). To test if there is a relation between heart rate and neck pain, the non-parametrical test Mann-Whitney was used and the result was $P = 0.216$ (Table 1). This result showed that the relation between heart rate and neck pain was not statistically significant ($P = 0.216$).

Table 1. Comparison between neck and no pain group (Mann-Whitney U Test)

Variables	Group	N	Sum of Ranks	Z-value	P-value
Median of Algometry Measurements	Neck Pain Group	105	8138.50	-4.508	<0.001
	No Pain Group	80	9066.50		
	Neck Pain Group	101	9595.00	-1.471	0.141
	No Pain Group	78	6515.00		
Skin Humidity	Neck Pain Group	98	8982.50	-1.238	0.216
	No Pain Group	76	6242.50		
	Neck Pain Group				
	No Pain Group				

P-value < 0.05 indicates significant associations

Table 2. Descriptive statistics

Median of Algometry Measurements	N	Median	Min	Max
Pain Group	66	88.83	16.22	259.67
No Pain Group	105	98.33	26.55	325.83

Table 3. Descriptive statistics

	MIN	MAX	MEAN	S.D
SkinHumidity	39	88	60.99	6.568
HeartRate	52	123	73.48	9.345

Discussion

As pain affects perspiration and heart rate through the nervous system it was hypothesized that neck pain could possibly correlate with pulse and skin humidity but such a case was not supported in this study. The results showed that heart rate ($P=0.216$) is not associated with neck pain. Up to now, only few studies have been conducted in order to investigate the association between the cardiovascular system and pain. In one of these it is stated that people with short duration of neck pain showed a rise in blood pressure during laboratory stressing ($P=0.25$) [21-25]. In another study subjects suffering from chronic muscle pain in the neck and shoulders showed reduced parasympathetic activation and increased sympathetic tone as an element in maintenance of chronic muscle pain [26]. From our results it was shown that there were not existing statistically significant differences between the two groups, with and without neck pain, regarding heart rate and skin humidity. However, the duration of neck pain in our sample was not determined and this might have affected our results. It was not clear if the neck pain group suffered from chronic pain or not. This group seemed to encounter chronic pain because the frequency of neck pain episodes was high but this does not mean that all of them suffered from chronic neck pain. In the definition of pain it is mentioned that chronic is the type of pain which lasts more than twelve weeks. Chronic and acute pain may share some common pathways but they have different mechanisms regarding the transmission of pain stimuli. Even if the neck pain group seemed more likely to suffer from chronic neck pain, it would have better if this had been predetermined from the beginning.

Another result of the study was that skin humidity did not show statistically significant relation with neck pain ($P=0.141$). As it has been mentioned perspiration process ruled by the autonomic nervous system could possibly have been affected by pain. However, this theory wasn't supported in our study. Except for the duration of pain which could have affected our results, another agent could be a confounder, namely weather humidity. During the examination existed different climate conditions and this could have affected the results of our study. Additionally carpal area is not rich of perspiration glands compared to other parts of the body. To our knowledge this is the first study to investigate the association of neck pain and skin humidity.

In this study it was shown that there exists an association between neck pain and pain pressure thresholds ($P<0.001$). In the 'Neck pain group' pain thresholds were lower (median PPT=88.8333) compared to the 'no pain group' (median PPT=98.3333). This means that the sense of pain was produced

by lower stimuli in the neck 'pain group' compared to the 'no pain group'. People without neck pain were expected to be less sensitive than the 'neck pain group' showing higher pain thresholds and this was in accordance with the results of the study. This result suggests that algometer is a reliable and objective tool for neck pain evaluation. According to literature, pressure algometry is a reliable and reproducible method which helps scientists to quantify local tenderness in clinical practice and research. Even if more sophisticated electrical devices with a strain or pneumatic pressure gauge have been developed, classic algometer remains a good choice being used in many protocols [18, 19]. The measurements have showed excellent reproducibility and validity when pressure thresholds were taken from muscles in opposite sides [20, 27]. Algometry is reliable with high responsiveness as a method of pain assessment in patients with knee osteoarthritis [27].

Studies have showed that algometry has excellent reproducibility and validity [26]. It is important that the examiner is familiar with the technique [22]. The intention of the team was to select as accurate measurements as possible, this is why a pilot study was designed as a first step and then six points were selected from the neck area. Until now pain scales such as NRS (Numerical Rating Scale) and VAS (Verbal Analog Scale) have been used in most studies in an attempt to quantify pain. These scales provide numbers from 0 to 10 and the patient had to choose a number to define pain intensity (0 for no pain and 10 for the worst pain ever felt) [28-32]. Using pain scales was the first step to express pain in numbers but algometry seems to be a more objective approach of pain. However, the main difference between algometry and pain scales being used for pain evaluation is that the number detected with algometer by the examiner can be hidden from the examined subject. At this study the pain threshold was unknown to the sample. This concealment of the results from subjects is an element which enhances algometer's objectiveness as a method of pain measurement.

For the accuracy of the results specific steps were designed. The protocol needed the examiner to press slowly and with stable rhythm the plastic stick. Algometry should be based on specific methodology and the equipment should be proper for reliable and valid measurements [33]. Pressure-pain threshold studies support the application of consistent manual force and previous familiarization with the method for excellent reliability [34]. According to clinical and experimental studies the electronic algometer could be recommended for the areas of human head and neck as it provides adequate evaluation of pressure pain thresholds [35]. In a study PPT seems to be decreased in patients with chronic mechanical neck pain compared with controls. This is not valid for acute pain up to now. These results support the different sensitization mechanisms for patients with acute and chronic mechanical neck pain [36]. In other studies neck points were tested three times each in people with neck pain in order to enhance accuracy and reliability in measurements [37-38]. The same was noticed for people with low back pain [39]. Additionally in another study all measurements were taken by the same examiner in order to keep good repeatability with an interval of 30 seconds after testing each point [40]. In a protocol where muscles were tested bilaterally at the back, it was shown that there were no statistically significant differences [41]. This technique was also followed by our study measuring six points bilaterally the neck area. Pilot measurements were very precious since they helped the team of the study to calibrate the devices. The experience from the pilot study showed that the pressure should be given with a slow and stable rhythm. An important limitation in this study was the familiarization of the examiner with the technique. The experience of the examiner is very important and it seems that experience was added in each use of the technique. The explanatory ability of the examiner and the mental level of the patient were also important factors. Lack of accuracy may occur in some measurements because of miscommunication between the examiner and the subjects. It

was important to explain many times that patients should say the word 'stop' when the sense of pressure turned to sense of pain. Some patients were confused or misunderstood these terms. For example there were many times when patients confused threshold with tolerance. The examiner should be capable of perceiving this misconception. Another consideration was whether conditions such as comorbidity, medication or menopause and menstruation could alter the results of the study. It was also not known if the type of pain was acute or chronic but the initial aim of the study was to capture the 'picture' of a sample and evaluate other features of neck pain regardless of its chronicity. However, the 'neck pain group' referred often or constant suffering from neck pain and this added a more chronic character to their pain in contrast with the 'no pain group' which referred occasionally, seldom or no pain attacks at the neck area.

These results showed also a prospective in application of algometry in therapeutic protocols for neck pain during follow up. This was also supported by some studies. In a study of healthy volunteers algometry seems to be a valuable tool for longitudinal assessment of a patient than for comparison between patients [42]. In another study with patients suffering from low back pain it was also shown that algometry can be used to measure the effects of interventions [39]. In a study with subjects suffering from myofascial syndrome, PPT also seemed to be a useful for the assessment of a treatment's effect, but not for diagnostic or screening purposes [43].

Algometry seems to be a very useful device which may be used at examination rooms in hospitals or private surgeries in the future. This device could probably be used to evaluate the analgesic result after each therapeutic session comparing pain thresholds in each examination (follow up). Familiarization with the method and experience by the examiner is required. Its simplicity in use and its low cost makes it an approachable method for doctors and other health personnel. This seems to be a method which could be used in Primary Health System by general practitioners or other medical specialties. However, the manipulation of the device needs experienced staff. The field of pain is a challenge for every health scientist. It is not easy to understand pain because of its complexed nature. This study tried to provide a new prospective to pain evaluation specifically neck pain.

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The authors declare that they have no conflicts of interest.

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Synchrotron-Fourier transform infrared maps of ovalbumin-induced murine chronic allergic airways disease: Correlation with conventional histology

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Abstract

Asthma is a chronic respiratory disease characterised by airway inflammation, remodeling and hyperresponsiveness. The ability to replicate these asthma traits in the well-established ovalbumin induced chronic model of allergic airways disease is an important tool for asthma research and preclinical drug development. Here, spectra derived from focal plane array and Synchrotron-Fourier transform infrared maps were used to analyse biochemical changes in lung tissue from an ovalbumin-induced murine chronic allergic airways disease model. Analysis of the chemical maps resulted in distinct clusters and significant changes in the lipid and proteins regions of the spectra between the saline control and diseased lung tissue samples. Overall, the utilisation of conventional histological methodologies and Synchrotron infrared microspectroscopy has the ability to expand the characterisation of murine models of asthma.

Introduction

Asthma is a chronic inflammatory airway disease. It affects 300 million people and results in approximately 250,000 deaths annually worldwide (GINA, 2012). The hallmarks of asthma comprise of airway inflammation, remodeling, and airway hyperresponsiveness (AHR) [1, 2]. Phenotypically, asthma is classified into various types including; allergic, non-allergic, aspirin-exacerbated, infection or exercise-induced, or alternatively by inflammatory profiles; eosinophilic, neutrophilic, or a combination of eosinophilic and neutrophilic or paucigranulocytic [3].

Effective management involves combination therapies of inhaled β_2 -agonists and corticosteroids [4]. However, there are subsets of people with severe asthma that are steroid insensitive [5]. New potential therapeutics are consistently being developed to alleviate some of the limitations of current asthma therapies. Such avenues include relaxin to target airway remodeling [6], statins [7] and stem cells [8] which are aimed to reduce inflammation of the airways without significant side effects in the lungs, and trefoil

factors aimed at targeting epithelial cell repair and anti-airway remodeling factors [9, 10].

Efficacies of these drugs are tested in animal models of asthma which attempt to model specific features of the disease. Typically these models are developed based on their inflammatory cell profiles; eosinophilic, neutrophilic or paucigranulocytic [11]. One of the most widely explored animal models of asthma is the ovalbumin (OVA)-induced chronic allergic airways disease model in BALB/c mice. This established model [12, 13] replicates the gross morphometric changes of airway eosinophilic inflammation, remodeling including epithelial thickening, goblet cell metaplasia, fibrosis and AHR (Figure 1, A) [14-16]. A standard timeline of this process, shown in Figure 1 (B), highlights the key time points of OVA sensitisation (days 0 and 14) and challenge (six weeks), to induce a chronic allergic T-helper 2 response. BALB/c mice represent an important strain for immunoglobulin E and T-helper 2 cytokines that include interleukin (IL)-4 and IL-5 production [12, 17].

The use of the OVA-induced model of allergic airways disease has been of particular importance in aiding our understanding of asthma. Basic tools used in this identification process include lung function tests, such as spirometry and peak flow analysis, as well as the addition of current β -agonists in the immediate measurement of air flow reversibility. Furthermore bronchial alveolar fluid (BALF) is used for inflammatory cell counts, and lung biopsies for histological analysis [18]. A novel avenue of disease characterisation is the identification of molecular fingerprints through focal plane array (FPA) and Fourier-transform infrared (FTIR) microspectroscopy.

Utilisation of FTIR tissue mapping as a diagnostic tool has been explored in numerous conditions including, cervical cancer [19], lung adenomatoid malformation [20] and colorectal cancer [21]. FTIR microspectroscopy mapping is an optical technique used to identify the structure and biochemical fingerprint of diseased tissue [20]. Similarly, FPA-FTIR microspectroscopy is used to identify larger areas of tissue, for a broader spectrum of biochemical maps. This study is to our knowledge first to utilise FPA-FTIR and Synchrotron-FTIR microspectroscopy to map tissues of an OVA induced chronic model of allergic airways disease. We examined lung tissue samples as a preliminary measure of the biochemical signatures of asthma. Overall, our aim was to investigate the potential novel biochemical markers associated between saline and OVA induced mice in a chronic model of allergic airway disease with the aid of Synchrotron-FTIR microspectroscopy tissue mapping.

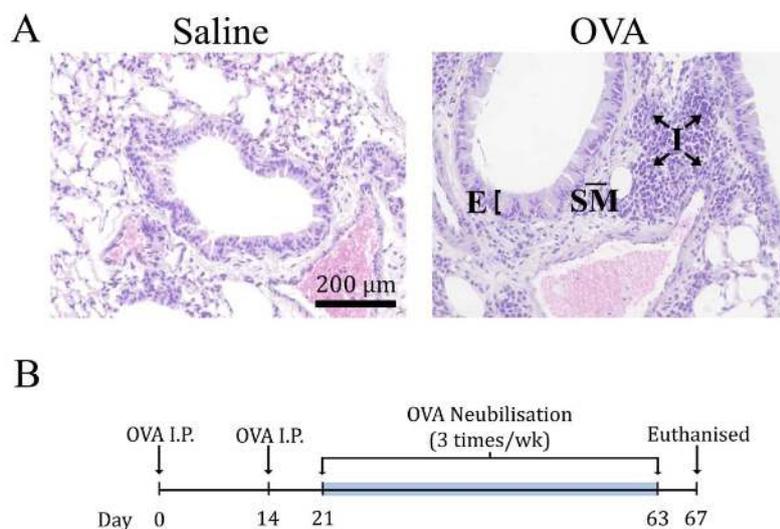


Figure 1. Typical histological analysis and timeline for establishing the ovalbumin-induced chronic allergic

airways disease model in mice. A haematoxylin and eosin (H&E) stain of OVA-sensitised mice displays the gross morphological changes that occur in the airways to replicate some of the key hallmarks of asthma (A). These include smooth muscle thickening (SM), epithelial thickening (E) and inflammation (I). This is typically established with mice first sensitised to OVA via an intraperitoneal (IP) injection on days 0 and 14, followed by nebulisation of OVA three times a week for six weeks, allowing for the development of the chronic allergic airway model to establish. Mice are commonly culled within 72 hours of last nebulisation treatment (B).

Methods

Animal model

The OVA-induced chronic allergic airway response model was implemented in six-week old female BALB/c mice at Monash University, Clayton, Australia. Ethics approval was granted by Monash University Animal Ethics Committee which complies with the Australian Guidelines for the Care and Use of laboratory Animal for Scientific Purposes.

Saline mice ($n=6$) were interperitoneally (IP) sensitised with 0.9% (500 μ L) saline solution (Baxter Health Care, NSW, Australia), and the OVA treated mice ($n=6$) were sensitised with 10 μ g Grade-V OVA (Sigma-Aldrich, MO, United States of America) and 400 μ g aluminium potassium sulphate adjuvant (AJAX Chemicals, Kotara, NSW, Australia) in 0.9% saline solution (Baxter Health care), on days 0 and 14. Starting at day 21, mice were nebulised, using an ultrasonic nebuliser (Omron NE-U07; Omron, Kyoto, Japan) three times a week for six weeks with 0.9% saline alone, or in combination with 2.5% (w/v) OVA (AJAX Chemicals, Kotara, NSW, Australia) for 30 minutes, depending on their treatment groups, as previously described [13, 22].

Methacholine-induced Airway hyperresponsiveness

Three days following the last nebulisation mice were anaesthetised with ketamine (IP: 200 μ g/g) and xylazine (IP: 10 μ g/g), prior to inducing airway resistance with methacholine. Measurements were recorded using Fine Pointe (Buxco Electronics, NC, USA) as a change of airway hyperresponsiveness (AHR) from baseline to corresponding increasing doses of methacholine (Sigma-Aldrich) every three minutes using an invasive plethysmograph nebulizer (Elan, Buxco Electronics, Troy, NY, United states of Amercia).

Tissue collection

Following invasive plethysmography lung tissue was fixed in 10% neutral buffered formalin overnight and embedded in paraffin ready for histological methodologies.

Histological assessment

Tissues (3 μ m) were sectioned with a Leica RM 2135 microtome (Leica Biosystems, Wetzlar, Germany) onto microscope slides (Mikro-Glass, Australia). Tissue sections were stained for haematoxylin and eosin (H&E), Masson trichrome, Gomori aldehyde fuchsin and silver impregnation for histological assessment. For each stain, five airways per mouse between 150-300 μ m in diameter, were imaged using an FSX100 widefield microscope (Olympus, Tokyo, Japan). The degree of inflammation amongst the different treatment groups from the H&E stain were identified using a scoring method, such that a score of 0

indicated no presence of inflammatory cells, 1 for 3 layers, 2 for 6 layers, 3 for 10 layers and 4 for more than 10 layers of inflammatory cells. Morphometric analysis obtained from the Masson trichrome stain, of epithelial and sub-epithelial thickness was determined amongst the different treatment groups using Image-Pro Plus 6.0 software (Media Cybernetics, Maryland, United States of America) and shown as a measurement per 100 μ m of basement membrane length.

Immunohistochemistry

The OVA-induced chronic model of allergic airways disease sections were cut and stained for α -SMA (1:150) (DAKO) by immunohistochemistry. Five airways per mouse were imaged on a brightfield setting with an FSX100 microscope (Olympus). Quantification of α -SMA was performed using Image-Pro Plus 6.0 Software (Media Cybernetics), by a count of surrounding fibroblasts per 100 μ m of basement membrane length.

Focal Plane Array (FPA)-Fourier Transform Infrared (FTIR) microspectroscopy

Paraffin-embedded tissue sections of the OVA-induced chronic allergic airway response model ($n=3$ per group) were cut (4 μ m) onto calcium fluoride (CaF₂) windows (Crystran, Dorset, UK), and deparaffinised by two consecutive washes of xylene (Sigma-Aldrich, St Louis, USA). The samples were stored in a desiccator prior to imaging (4x4 grid maps) with a FPA-FTIR microscope (Bruker Optik GmbH, Ettlingen, Germany), at the Australian Synchrotron, equipped with a liquid-N₂ cooled 64x64 element FPA detector and a 15x objective lens, coupled to a Vertex 70/70v FTIR spectrometer (Bruker). FPA-FTIR spectra, collected in the transmission mode within a 4000-800cm⁻¹ spectral region using 8-cm⁻¹ resolution, 64 co-added scans, Blackman-Harris 3-Term apodization, Power-Spectrum phase correction and a zero-filling factor of 2, operated through OPUS 7.2 imaging software (Bruker, Billerica, Massachusetts, USA). Spectra were first subjected to an atmospheric compensation water correction prior to band area integration of the lipid composition (3000-2830cm⁻¹) and protein (1695-1600cm⁻¹) regions.

Synchrotron-Fourier Transform Infrared (FTIR) microspectroscopy and analysis

Saline ($n=2$) and OVA ($n=2$) tissue sections were cut (4 μ m) using a microtome (Leica Biosystems) onto CaF₂ windows (Crystran), and deparaffinised by two consecutive washes in xylene (Sigma-Aldrich) before being air dried and stored in a desiccator. Tissue maps of samples were collected on the Fourier transform infrared (FTIR) beamline at the Australian Synchrotron, Clayton. A rectangular grid (25x25) area of 15,625 μ m² (aperture of 5 μ m), on a 36xIR objective were utilised for all tissue samples.

The hypercube of each tissue map was accessed using CytoSpec™ (Berlin, Germany), to manually separate the raw spectra into selected tissue sections; respiratory tract epithelium, subepithelium, and endothelium. The overall spectra were cut (3600-1000cm⁻¹), outliers were removed and data were exported into the Unscrambler® 10.1 software package (CAMO Software AS, Oslo, Norway) for spectral processing. Prior to multivariate principal component analysis (PCA), each tissue section data was converted to a 2nd derivative (25 smoothing points) using the Savitzky-Golay algorithm and corrected by extended multiplicative scatter correction (EMSC) at the spectral regions of 3033-2792cm⁻¹ and 1789-1008cm⁻¹. The average absorbance spectrums of each data for each tissue regions were also derived.

Statistics

Statistical analyses were performed with GraphPad Software Prism 6.02 (San Diego, California, United

States of America). Results were analysed by a two-way analysis of variance (ANOVA), with a post-hoc Bonferroni test. Statistical significance was determined at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. All results were displayed as means \pm standard error of mean (SEM).

Results

Airway inflammation, remodeling and collagen deposition

Morphological changes were found in the histological stains for inflammation, collagen deposition, and reticular fibrosis, as seen in Figure 2 (A). An inflammatory cell score (B, i) derived from the H&E stain was quantified in the airways, with a significant increase ($P = 0.0004$) of inflammation in the OVA sensitised group (2.97 ± 0.35) in comparison to the saline control (0.00 ± 0.0). Repeated OVA nebulisation induced a significant increase ($P < 0.0001$) in mean epithelial (B, ii, OVA: $19.0 \mu\text{m} \pm 0.44$, saline: $13.06 \mu\text{m} \pm 0.71$) and subepithelial (B, iii, OVA: $27.81 \mu\text{m} \pm 1.18$, saline: $13.53 \mu\text{m} \pm 1.26$) thicknesses in Masson's trichrome stained sections. Both the Gomori aldehyde fuschin and silver impregnation (Figure 2, A) stains were performed for the presence of reticular fibres, whereby minimal reticular fibres were present in the saline group in comparison to the OVA group. α -SMA was quantitated as a representation of myofibroblasts per $100 \mu\text{m}$ of basement membrane length (exclusion of the myofibroblasts found in the epithelium smooth muscle tissue). As the results in Figure 2 (B, iv) illustrate, there was a significant increase ($P = 0.0021$) in myofibroblasts per $100 \mu\text{m}$ basement membrane length in the OVA model, in comparison to the saline control group (OVA: 1.10 ± 0.16 , saline: 0.20 ± 0.05).

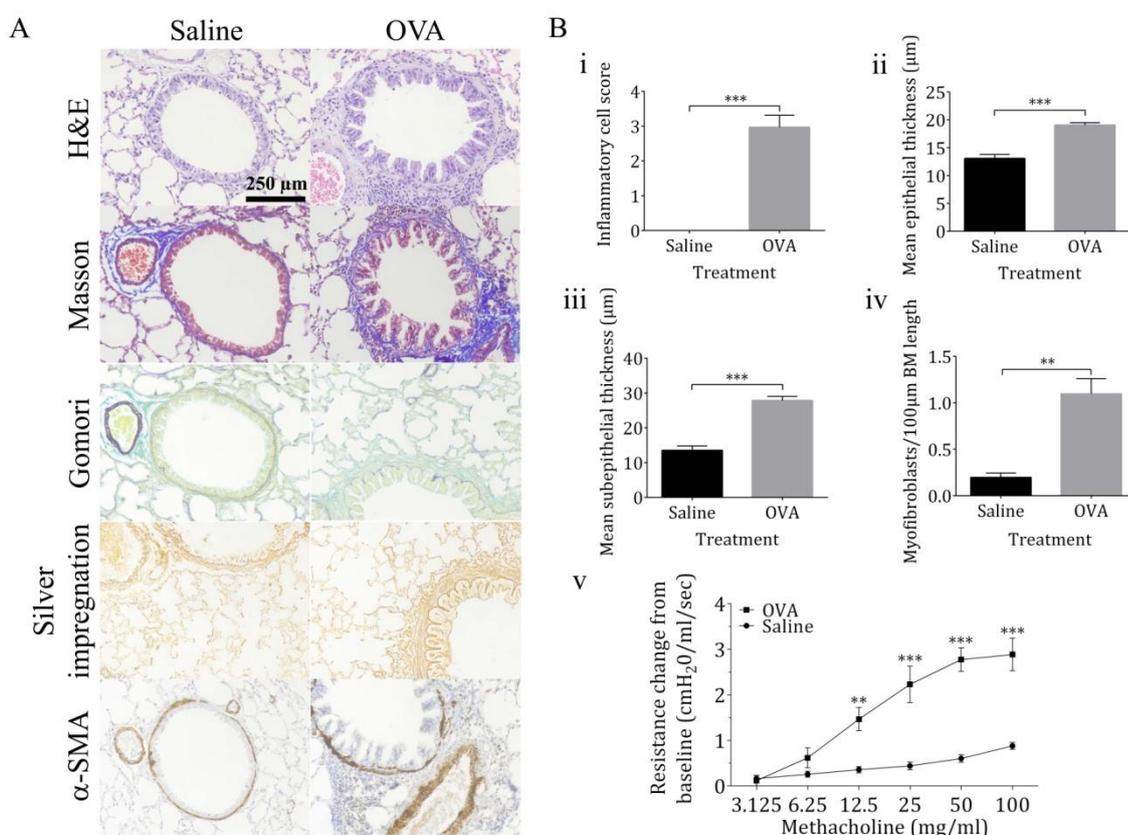


Figure 2. Histological analysis and methacholine induce airway hyperresponsiveness (AHR) of the murine chronic allergic airways model. The histological stains; haematoxylin and eosin (H&E), Masson trichrome, Gomori aldehyde fuschin, silver impregnation and immunohistochemically stained α -smooth muscle actin

(SMA) are represented for the saline ($n=6$) and OVA ($n=6$) control groups (A), acquired on the brightfield Olympus FSX100 microscope. Scale bars=100 μ m (x20 objective lens). Quantification of these stains (B) for an inflammatory cells score (i), mean epithelial (ii) and sub-epithelial thickness (iii) and myofibroblasts per 100 μ m of basement membrane (BM) length (iv) were analysed with Image-Pro Plus 6.0 Software (Media Cybernetics). AHR was induced by methacholine via invasive plethysmography and measure from baseline cmH₂O/mL/sec (v). A two-way ANOVA with a post-hoc Bonferroni test statistical analyses (** $P<0.001$) with a mean \pm standard error of mean (SEM) was performed.

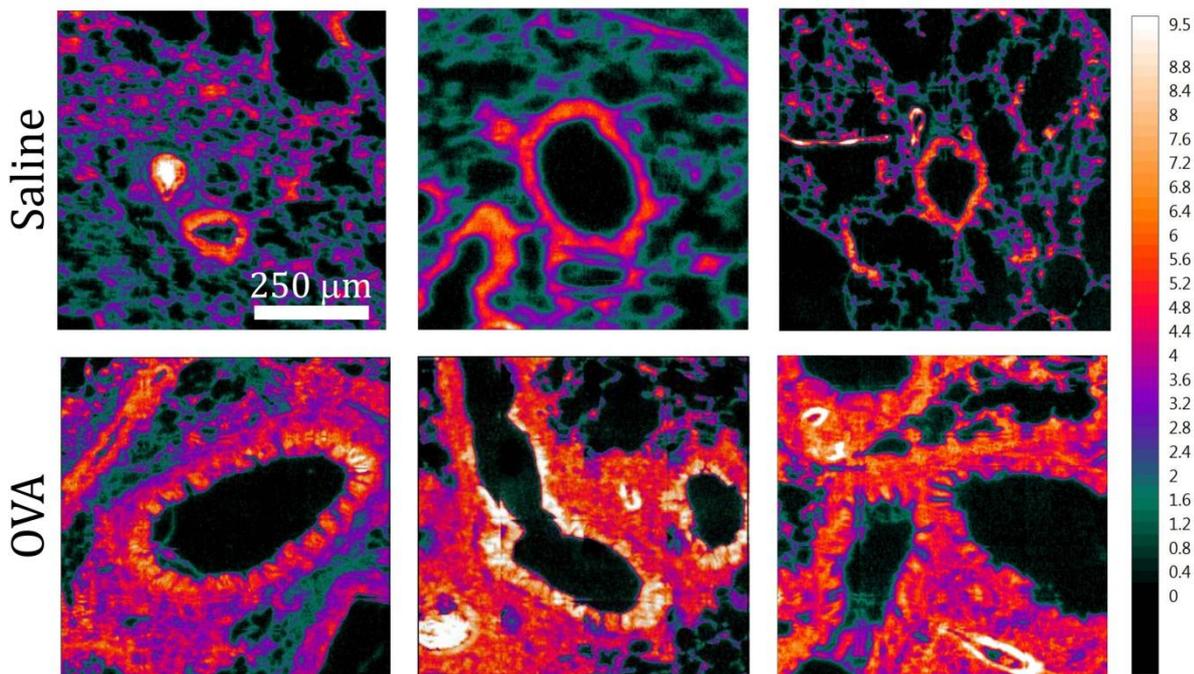
Airway hyperresponsiveness

The OVA challenged mice displayed increased airway resistance in response to methacholine, as seen in Figure 2 (B). In particular, AHR was significantly increased in OVA challenged mice compared to saline-challenged mice at the methacholine dose of 12.5mg/mL ($P<0.01$) and greater significance was identified with the respective higher doses ($P<0.001$).

FPA-FTIR and Synchrotron-FTIR microspectroscopy

Biochemical maps of the integrated lipid composition (3000-2800cm⁻¹) and protein (1695-1600cm⁻¹) regions obtained from the FPA-FTIR microspectroscopy are shown in Figure 3. These results are indicative of higher expression levels of both the lipid composition and proteins within these regions in the OVA induced mice as compared to the saline mice. Furthermore, Figure 4 illustrates the PCA analysis separation in the scores plot (iv) amongst both saline and OVA treated mice in the epithelium, subepithelium and endothelial tissue. According to the loadings (iii) and second derivative (ii) the separation of the epithelial cells amongst the two groups of mice is largely attributed to the amide I (~1654cm⁻¹) and amide II band (~1542-50cm⁻¹). Other heavily loaded peaks were found in the C-H stretching region included 2912, 2842, 2935 and 2867cm⁻¹. Respiratory subepithelium cells displayed strong clusters, primarily attributed to the amide I (~1656cm⁻¹) band from the PC-1 loadings. Stretching in C-H lipid region of the spectrum at point 2933, 2965 and 2840cm⁻¹ also contributed to this separation of the subepithelial cells. Lastly, endothelial cells which made up the smallest number of spectra collected, displayed separation of along the PC-1 loadings at the amide I band (1656cm⁻¹). Other major loaded peaks were found at 2935, 2869, 2912 and 2842cm⁻¹, in the lipid region in the saline and OVA tissue groups.

Lipids (3000 - 2830 cm^{-1})



Proteins (1695 - 1600 cm^{-1})

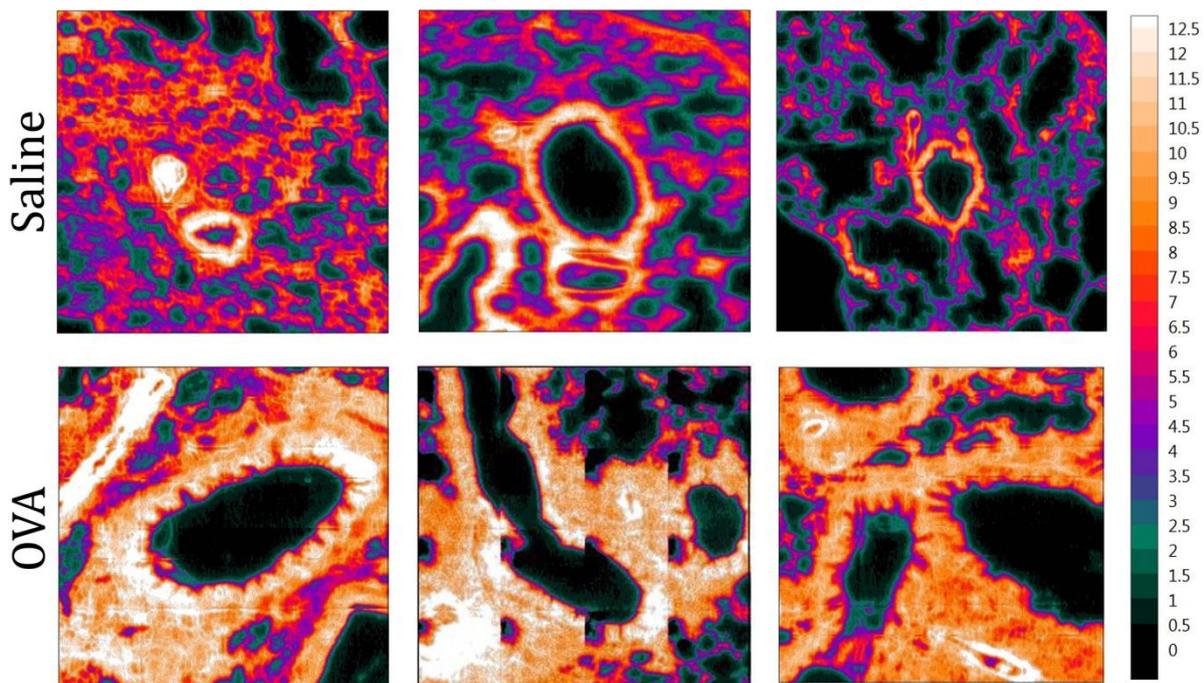


Figure 3. FPA-FTIR chemical mapping of the murine chronic allergic airways model. Integrated chemical tissue maps of saline ($n=3$) and OVA ($n=3$) challenged mice in the lipid composition (3000-2830 cm^{-1}) and protein (1695-1600 cm^{-1}) regions.

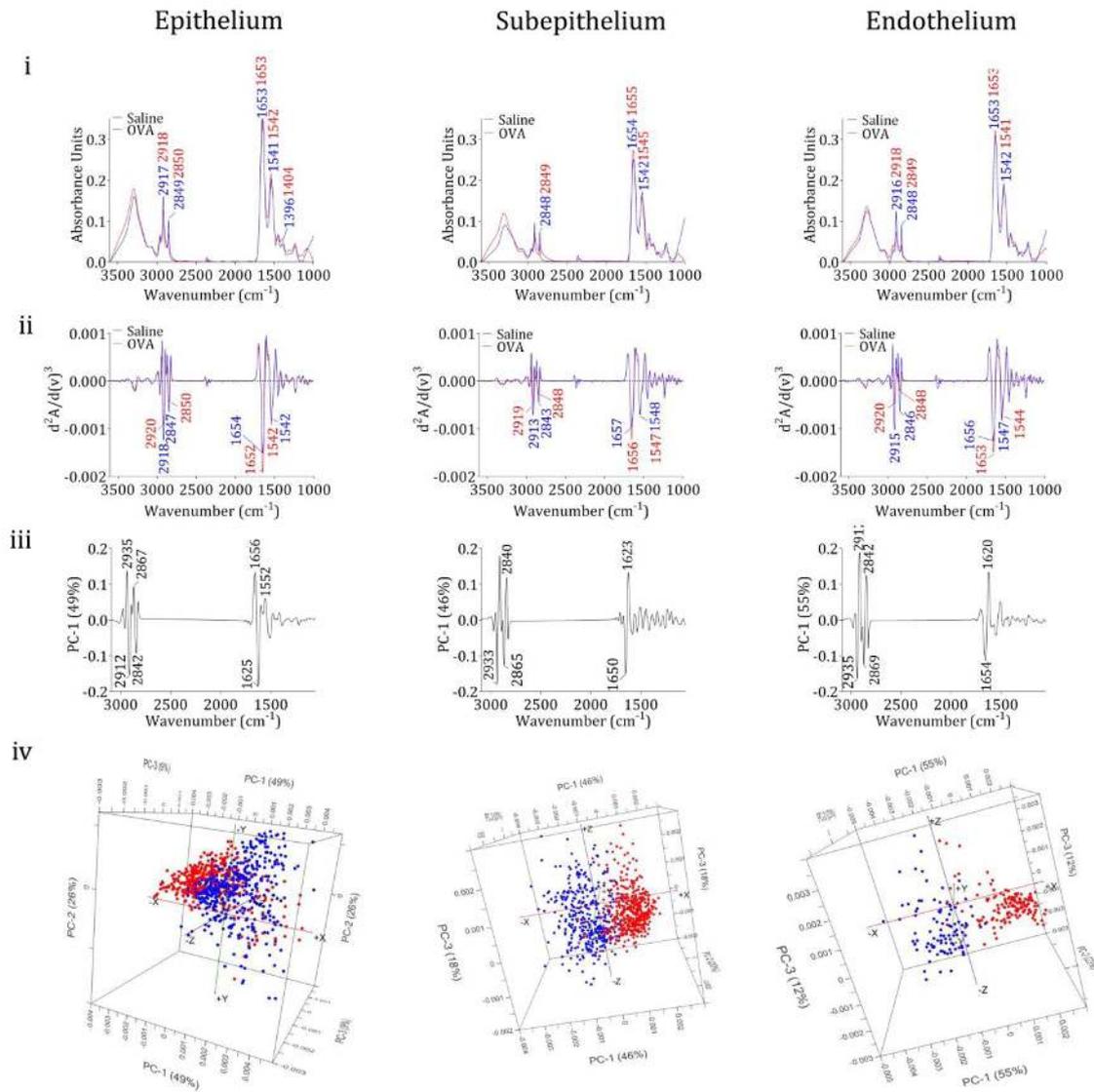


Figure 4. Synchrotron-FTIR tissue mapping of the murine chronic allergic airways model. Infrared spectral analyses of the epithelium (A), subepithelium (B) and endothelium (C) regions, within each of the saline ($n=2$) and OVA ($n=2$) groups, as displayed in the absorption spectrum (i), second derivative spectrum (ii), PC-1 loadings plot (iii) and PCA-scores plot (iv).

Discussion

Conventional histology and immunohistochemistry highlighted the hallmark characteristics of human asthma [23] in the OVA-induced chronic allergic airway model, consistent with previous studies [24, 25]. Of note are the significant increases in inflammation, mean epithelial and sub-epithelial thicknesses (Figure 2 B: i, ii, iii), of OVA-allergen exposed mice as compared to the saline group, essential features in the establishment of airway remodeling in asthma (Mauad, 2007, Yang, 2013). Additionally, airway resistance was measured with increasing doses of methacholine (mg/mL). Figure 2 (B: v) demonstrates that the OVA group responded with increasing resistance statistically different from the saline model, particularly at the final doses of methacholine. This is of particular importance as it has been suggested that airway

remodeling, collagen deposition and inflammation all contribute to AHR [23].

The sensitivity and accuracy of FPA-FTIR and Synchrotron-FTIR microspectroscopy was utilised to identify molecular information about the chronic allergic airways model. FPA-FTIR microscopy has the advantage of imaging larger map areas such that the overall identification of changes within the proteins and lipids can be determined following absorption corrections. As seen in Figure 3, the grid (4x4) maps allowed imaging of the entire airway and surrounding tissue areas. The results indicate that the OVA-induced tissue contains higher expression levels of C-H stretching associated with lipid composition and proteins within the respective $3000\text{-}2830\text{cm}^{-1}$ and $1695\text{-}1600\text{cm}^{-1}$ spectral integrated region. This highlights the vast differences seen in the lung airway between diseased and non-diseased tissue, particularly within the epithelial cells.

Synchrotron-FTIR infrared biochemical maps provided more detail in the absorption spectrum in conjunction with enabling PCA separation of the diseased and non-diseased tissue. Manual selection of spectra segregated in the airway epithelium, subepithelium and endothelium aids in the determination of the key biochemical markers within these regions. Figure 4 presents the airway epithelium average absorbance spectrum of approximately 400 spectra from each group in the OVA-induced chronic allergic airways model. Clear clusters of the saline and the OVA group can be seen in the scores plot (Figure 4: iv), however a large variability amongst the data, specifically in the saline mice is apparent. Strong peak loadings in the PC-1 plot (iii) were evident, indicating the key differences in the molecular composition within the saline and OVA groups. Of note is the negative loadings peaks of amide I band at 1656cm^{-1} [26] and amide II band at 1552cm^{-1} [27]. The assignment of the amide I band in combination with the positive loadings peak at 1625cm^{-1} , is indicative of the formation of proteins into the secondary structure of α -helices [26]. Other heavily loaded peaks were found within the C-H stretching lipid region [29, 30] of the spectrum at bands 2935 , 2896 , 2912 and 2842cm^{-1} . Although not identified as a significant peak in the PC-1 loadings, a higher absorption unit was found at the band 1404cm^{-1} in the OVA exposed mice in comparison to the saline group. This band in particular has been found to be associated with the deformation of methyl group bending modes of proteins [31].

Airway subepithelium is comprised of inflammatory cells, smooth muscles and fibrotic tissue. According to the scores plot of the airway subepithelium and endothelial tissue (Figure 4; iv) there was an apparent separation across the PC-1 plane of the saline and OVA groups with approximately 400 and 100 spectra selected in each group, respectively. Significant peaks were assigned to amide I band, with α -helix conformation due to the negatively loaded 1623cm^{-1} band, which predominates in the proteins associated with OVA sensitisation. Strong loaded peaks in the lipid region where C-H stretching occurs was also a contributor to the separation of the groups. However, limited detail in the biochemical fingerprint is apparent as compared to the gross morphometric changes in inflammation and fibrosis characteristic to that of asthma, as seen in our histological analyses (Figure 2).

FTIR tissue mapping has provided some insights into the biochemical fingerprint of an OVA induced chronic allergic airways model. However, there are some shortcomings with FTIR spectroscopy. Firstly, the DNA region (less than 1000cm^{-1}) of the IR spectrum is not reliable with the CaF_2 windows used in our experiment. These windows are optimal for FTIR imaging, however lack the sensitivity to lipid heads bands found below 1000cm^{-1} [20]. Considering this is the first study to investigate a chronic murine model of airways disease, our results have assisted in preliminary understanding of biochemical peaks associated with diseased and non-diseased tissues. Ultimately once these biochemical fingerprints are confirmed for lung tissue samples, analogous fingerprints may be associated with blood serum samples. This has already

been investigated with cancer patients [32-34]. Overall, we have extended analysis of the widely used OVA induced murine chronic allergic airways disease model to encompass analysis of spectra derived from FPA-FTIR and Synchrotron-FTIR maps. Analysis of chemical mapping identified significant changes in the lipid and protein regions which can be correlated with structural and biological changes observed with conventional histological and immunofluorescence methodologies. Although prolonged mapping times are necessary, infrared chemical mapping is an automated process and data analysis yields a plethora of biochemical information. Future studies will be aimed at extended the size and resolution of mapping to improve clustering and to include detailed analyses of the inflammatory process which is prevalent in the OVA induced murine chronic allergic airways disease model. An interesting approach would involve potentially developing predictive models based on biochemical markers in circulating blood cells in control compared to disease states. Finally, additional investigations will be aimed at exploring the biochemical basis accounting for the biological effects of compounds that can mitigate the allergic responses observed in this model of allergic airways disease.

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The authors declare that they have no conflicts of interest.

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Technetium-99m-dimercaptosuccinic acid renal scintigraphy can guide clinical management in congenital hydronephrosis

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Keywords: Renal scintigraphy -Congenital hydronephrosis -Clinical management

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Abstract

Objective. The purpose of this study was to evaluate damage of the kidney with technetium-99m-dimercaptosuccinic acid (^{99m}Tc-DMSA) scintigraphy in children with congenital hydronephrosis (CH) and the influence of other postnatal associated diagnoses on abnormal ^{99m}Tc-DMSA findings. **Subjects and Methods.** ^{99m}Tc-DMSA scintigraphy in 54 children (17 girls and 37 boys), aged from 2 months to 5 years (median 11 months) with 66 congenital hydronephrotic renal units (RU) (42 unilateral hydronephrosis-29 boys and 13 girls; 12 bilateral hydronephrosis-8 boys and 4 girls) was performed. Male/female ratio was 2,2:1, unilateral/bilateral hydronephrosis ratio was 4:1. Hydronephrosis classified into three groups according to ultrasound measurement of the antero-posterior pelvic diameter APD): mild (APD 5-9.9mm) was present in 13/66RU, moderate (APD 10-14.9mm) in 25/66RU, and severe (APD≥15mm) in 28/66RU. Simple hydronephrosis was present in 15RU, and the postnatal associated clinical diagnosis were vesicoureteric reflux (VUR) in 21, pelviureteric junction (PUJ) obstruction in 7, pyelon et ureter duplex in 11, megaureter in 11 and posterior urethra valves in 1RU, respectively. Static renal scintigraphy was performed 2 to 3 hours after intravenous (iv) injection of ^{99m}Tc-DMSA using a dose of 50μCi/kg (1.85MBq/kg; minimal dose: 300μCi). Four views (posterior, left and right posterior oblique and anterior) were obtained with a head gamma camera "Orbiter" filtered with high resolution parallel whole collimator. All images were stored in an Pegasys computer with a matrix size of 256×256. The relative kidney uptake (RKU) between the left and right kidney was calculated as an average number counts from anterior and posterior view. Renal pathology was defined as inhomogenous or focal/multifocal uptake defects of radiopharmaceutical in hydronephrotic kidney or as split renal uptake of <40%, and poor kidney function was defined as split renal uptake <10%. Descriptive and analytical statistics (SPSS version 20.0) was performed. Analytical statistics implied the non-parametric Mann-Whitney test for determination of statistically significant difference between the normal and pathological findings on ^{99m}Tc-DMS scan. The default level of significance was P<0.05. **Results:** Our ^{99m}Tc-DMSA scintigraphy findings in children with ANH were: decreased or enlarged kidney with inhomogeneous kidney uptake radiopharmaceutical in 22, irregular shape kidney with inhomogeneous accumulation of radiopharmaceutical in 3, connected (fused) kidney in 1 patient, and poorly or nonvisual kidney in 14RU respectively (total 40/66RU with pathological ^{99m}Tc-DMSA finding, 60,6%). Relative accumulation in hydronephrotic kidney was less or equal to 40% in 17RU, less than 10% in 14RU and inhomogeneous radiopharmaceutical uptake with relative accumulation over 40% was detected in 9RU. Regular kidney morphology with homogeneous accumulation of radiopharmaceutical (normal DMSA scintigraphy finding) were found in 26/66RU (39,4%). Statistically significant

correlation between the degree of the hydronephrosis (APD) and ^{99m}Tc -DMSA scan findings ($P<0.001$) and between the degree of the VUR and DMSA scan finding ($P=0.002$) was established. In our study, other associated diagnosis were not statistically correlated with pathological findings on ^{99m}Tc -DMSA scan due to low number of patients. **Conclusion:** On the basis of these results (60% pathological findings) we recommend DMSA scintigraphy in the evaluation renal pathology in children with congenital hydronephrosis. Greater number of patients is needed for the estimation of the associated diagnosis (other than VUR) influence on the renal parenchymal damage in children with CH.

Introduction

The widespread ultrasound (US) screening during pregnancy has resulted in increasing recognition of antenatal hydronephrosis (ANH) [1]. Depending on the diagnostic criteria and gestation, the prevalence of antenatally detected ANH ranges from 0.6% to 5.4% [2].

The causes of ANH vary from transient benign conditions-transit hydronephrosis, (resolves by birth or during infancy) to conditions that can significantly affect renal function. The outcome of ANH depends on the underlying etiology, so it is very important to determine these causes [3].

The definition and grading of ANH is based on anteroposterior pelvic diameter (APD) of the fetal renal pelvis [4]. It is an objective parameter, although it varies with gestation, maternal hydration and bladder distension.

ANH is present if the APD is $\geq 4\text{mm}$ in the second trimester and $\geq 7\text{mm}$ in the third trimester [3]. ANH is further graded as mild, moderate and severe depending on the size of the measured APD. While fetuses with minimal pelvic dilatation (5-9mm) have low risk of postnatal pathology, the APD $\geq 15\text{mm}$ at any gestation represents severe hydronephrosis and requires close follow-up [5-8].

Antenatal management includes antenatal ultrasound monitoring, which is usually repeated every 4-6 weeks, but its frequency depends on the gestation at which ANH was detected, as well as its severity and the presence of oligohydramnion. Almost 80% of the fetuses diagnosed in the second trimester show resolution or improvement of findings with the low likelihood of postnatal pathology [9]. Patients with persistence or worsening hydronephrosis in the third trimester show higher rates of postnatal pathology and require more frequent monitoring. Also, more frequent monitoring is required for fetuses with findings that suggest lower urinary tract obstruction. The controversy about the postnatal management of infants with the CH still exists. It is emphasized that an ultrasound in the first few days of life underestimates the degree of pelvic dilatation due to dehydration and a relatively low urine output. Despite this limitation, an early ultrasound, (24-48 hour after birth), is necessary in the neonates with suspected lower urinary tract obstruction, oligohydramnion, bilateral severe hydronephrosis, severe hydronephrosis in a solitary kidney [3].

The examination methods currently used cannot identify obstruction, but only reflect the consequences (decreased renal function, compromised drainage or increased pelvic dilatation). The only useful definition of obstruction is retrospective, defined as "any restriction to urinary outflow that left untreated will damage the kidney," or defined as "a condition of impaired urinary drainage that if uncorrected will limit the ultimate functional potential of a developing kidney" [10]. Diuretic renography is a cornerstone method for guiding the clinical management of asymptomatic congenital hydronephrosis [11].

Technetium 99m-dimercaptosuccinic acid renal scintigraphy (^{99m}Tc -DMSA) has been used in renal imaging to estimate the functional renal mass (damage the kidney) and relative renal function, especially in

pediatric patients. The radiation dose for ^{99m}Tc -DMSA scintigraphy has been estimated as 1mSv, regardless of the child's age [12].

Under physiological conditions, low molecular weight (LMW) proteins are excreted in the urine by glomerular filtration and then reabsorbed by proximal tubular epithelial cells through a process that is mediated by multi-ligand endocytic receptors: megalin and cubilin. The molecular size of secreted ^{99m}Tc -DMSA in the urine is 24-28kDa, which is well within the range of the LMW proteins. These findings suggest that ^{99m}Tc -DMSA is handled by kidneys in the same manner as LMW proteins-i.e. it is filtered in glomeruli and subsequently reabsorbed by proximal tubules via megalin-and cubilin-mediated endocytosis [13, 14]. Renal uptake depends on the binding of ^{99m}Tc -DMSA to the plasma protein a1-microglobulin, followed by glomerular filtration and megalin/cubilin-mediated endocytosis by proximal tubular cells [15]. Thus, the ^{99m}Tc -DMSA scintigraphy is a potential method for the evaluation of proximal tubule endocytic function in patients.

There is insufficient data in the literature on ^{99m}Tc -DMSA scintigraphy investigation of the renal parenchyma damage in children with CH.

The purpose of this study was to evaluate damage of the kidney with ^{99m}Tc -DMSA scintigraphy in children with CH and the influence of other postnatal associated diagnoses on abnormal findings.

Patients

Results of the ^{99m}Tc -DMSA scintigraphy in 54 children (17 girls and 37 boys), aged from 2 months to 5 years (median 11 months) with 66 antenatally detected hydronephrotic renal units (RU) (42 unilateral hydronephrosis-29 boys and 13 girls; 12 bilateral hydronephrosis-8 boys and 4 girls) are presented. Male/female ratio was 2,2:1, unilateral/bilateral hydronephrosis ratio was 4:1. Hydronephrosis classified into three groups according to ultrasound measurement of the APD: mild (APD 5-9.9mm) was present in 13/66RU, moderate (APD 10-14.9mm) in 25/66RU, and severe (APD \geq 15mm) in 28/66RU (Table 1).

Table 1. ^{99m}Tc -DMSA scan findings depending on the HN degree

			Finding		Total
			Norm.	Pathol.	
degree HN	5- 9,9	N	10	3	13
		%	76.9%	23.1%	100.0%
	10- 14, 9	N	4	21	25
		%	16.0%	84.0%	100.0%
	>15	N	12	16	28
		%	42.9%	57.1%	100.0%
Total	N	26	40	66	
	%	39.4%	60.6%	100.0%	

Simple hydronephrosis was present in 15RU, and the postnatal associated clinical diagnosis were vesicoureteric reflux (VUR) in 21, pelviureteric junction (PUJ) obstruction in 7, pyelon et ureter duplex in 11, megaureter in 11 and posterior urethra valves in 1RU (Table 2).

Table 2. Associated diagnoses

	N	%
Simple hydronephrosis	15	22.7
VUR	21	31.8
PUJ obstruction	7	10.6
Megaureter	11	16.7
Pyelon et ureter duplex	11	16.7
Posterior urethral valve	1	1.5
Total	66	100.0

Micturating cystoureterogram (MCUG) was performed in 27 children (54RU) and VUR was found in 21RU (39%) (16 on the left side (76%) and 5 on the right side), low grade VUR (I-II) in 4/21, medium grade VUR (III) in 4/21 and high grade VUR (IV-V) in 13/21RU, respectively.

Methodology

Static renal scintigraphy was performed 2 to 3 hours after intravenous (iv) injection of ^{99m}Tc labeled dimercaptosuccinic acid (^{99m}Tc -DMSA) using a dose of $50\mu\text{Ci}/\text{kg}$ ($1.85\text{MBq}/\text{kg}$; minimal dose: $300\mu\text{Ci}$). Four views (posterior, left and right posterior oblique and anterior) were obtained with a single head gamma camera "Orbiter" filtered with high resolution parallel whole collimator. All images were stored in a Pegasys computer with a matrix size of 256×256 . The relative kidney uptake (RKU) between the left and right kidney was calculated as an average number counts from anterior and posterior view. Renal pathology was defined as inhomogenous or focal/multifocal uptake defects of radiofarmaceutical in hydronephrotic kidney or as split renal uptake of $<40\%$, and poor kidney function was defined as split renal uptake $<10\%$. Descriptive and analytical statistics (SPSS version 20.0) was performed. Analytical statistics implied the non-parametric Mann-Whitney test for determination of statistically significant difference between the normal and pathological findings on DMSA scan. The default level of significance was $P<0.05$

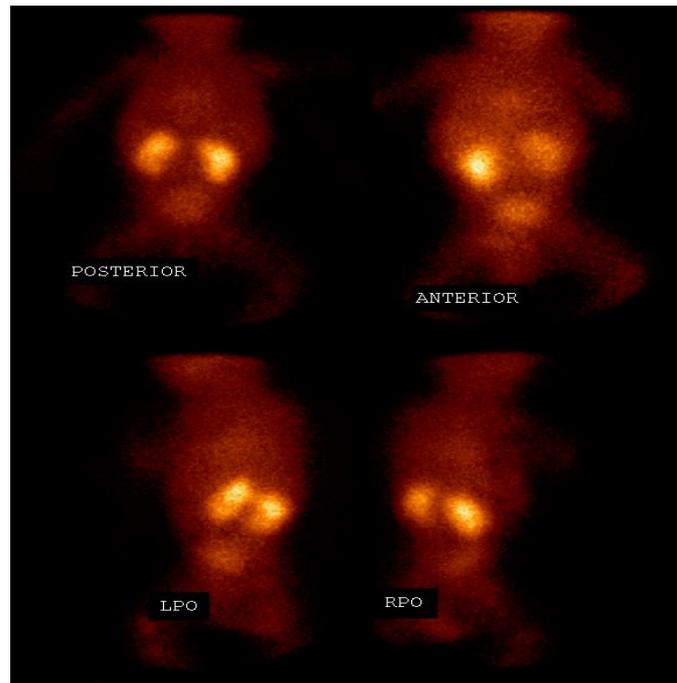


Figure 1. ^{99m}Tc -DMSA scan in a 7 months old boy with antenatally detected hydronephrosis. Cortical defects in superior and inferior right pole and diffusely reduced uptake in left kidney. Postnatal micturating cystourethrogram revealed bilateral vesicoureteral reflux of grade V.

Results

^{99m}Tc -DMSA scintigraphy findings in children with antenatal hydronephrosis were: decreased or enlarged kidney with inhomogeneous kidney uptake radiopharmaceutical was found in 22, irregular shape kidney with inhomogeneous accumulation of radiopharmaceutical in 3, connected (fused) kidney in 1 patient, and poorly or nonvisual kidney in 14RU, respectively (total 40/66RU with pathological ^{99m}Tc -DMSA finding, 60,6%).

Relative accumulation in hydronephrotic kidney was less or equal to 40% in 17RU, less than 10 in 14RU and inhomogeneous radiopharmaceutical uptake with relative accumulation over 40% was detected in 9RU.

Regular kidney morphology with homogeneous accumulation of radiopharmaceutical normal DMSA scintigraphy finding were found in 26/66RU (39,4%).

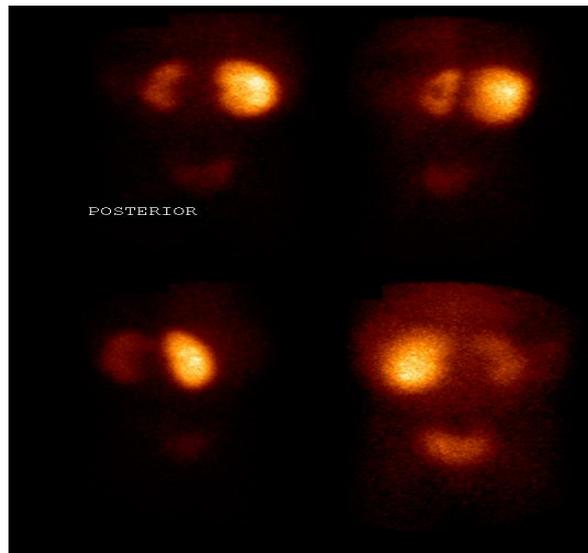


Figure 2. ^{99m}Tc -DMSA scan in a patient with perinatally detected mild dilatation of pelvis and vesicoureteral reflux of grade V on the left. Small scarred kidney with reduced and inhomogeneous uptake of tracer (relative kidney uptake 20%).

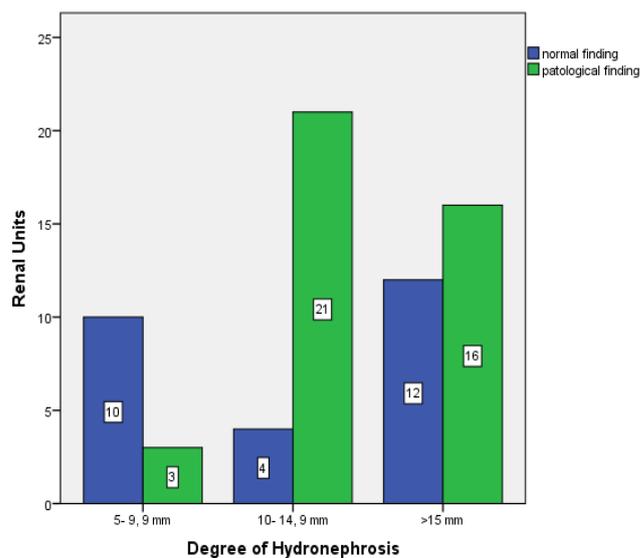


Figure 3. Statistically significant correlation between degree of the hydronephrosis and DMSA scan findings ($P < 0.001$).

Statistically significant correlation between degree of the hydronephrosis (APD) and ^{99m}Tc -DMSA scan findings ($P < 0.001$) (Figure 3) and between the degree of the VUR and ^{99m}Tc -DMSA scan findings ($P = 0.002$) (Figure 4) was established.

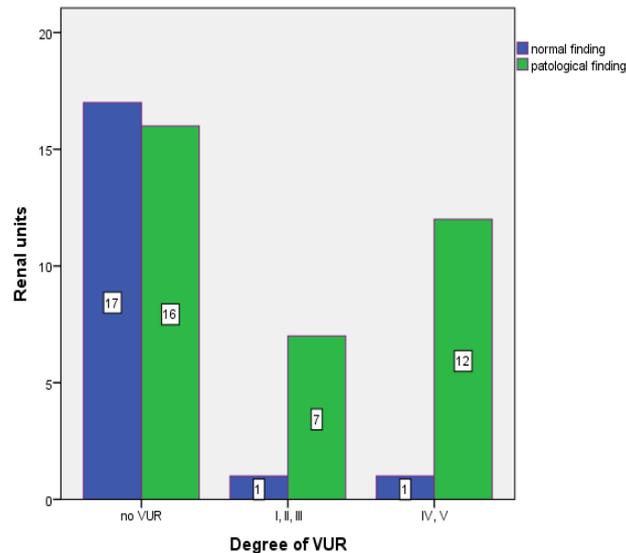


Figure 4. Significant correlation between pathological findings on ^{99m}Tc -DMSA scan and the degree of the VUR ($P=0.002$).

Statistically significant correlation between pathological findings on ^{99m}Tc -DMSA scan and associated diagnoses (other than VUR) could not be established. (Table 3)

Table 3. Postnatal associated clinical diagnosis and findings on ^{99m}Tc -DMSA scan

Parameters	Renal pathology, N (%)		Total	Relative accumulation of RF, N (%)			Total
	No	Yes		≤ 10	≤ 40	> 40	
VUR							
No	17 (53,3)	16 (46,7)	33 (100)	9 (20,0)	7 (15,6)	17 (64,4)	33 (100)
Yes	2 (4,8)	19 (95,2)	21 (100)	5 (23,8)	10 (47,6)	6 (28,6)	21 (100)
StenosisPUJ							
No	24 (40,7)	35 (59,3)	59 (100)	13 (22,0)	15 (25,4)	31 (52,6)	59 (100)
Yes	3 (42,9)	4 (57,1)	7 (100)	1 (14,3)	2 (28,6)	4 (57,1)	7 (100)
Megaureter							
No	23 (41,8)	32 (58,2)	55 (100)	13 (23,6)	11 (20,0)	31 (56,4)	55 (100)
Yes	4 (36,4)	7 (63,6)	11 (100)	1 (9,1)	6 (54,5)	4 (36,4)	11 (100)
Duplex system							
No	24 (43,6)	31 (56,4)	55 (100)	11 (20,0)	13 (23,6)	31 (56,4)	55 (100)
Yes	3 (27,3)	8 (72,7)	11 (100)	3 (27,3)	4 (36,4)	4 (36,4)	11 (100)

Discussion

Afshin, Safaei et al. [13] found that hydronephrosis was caused by PUJ obstruction in 44.5%, VUR in 22.2%, ureterovesical junction obstruction in 8.9%, posterior urethral valves in 8.9%, PUJ obstruction with VUR in 4.4% and non-VUR non-obstructive in 2.2% [11]. In our study in 22,7%RU (15/66) simple hydronephrosis was present, and VUR in 31.8% (21/66), megaureter in 16,7%, pyelon et uretr duplex in 16,7% (11/66), PUJ stenosis in 10,6% (7/66) and posterior urethral valve in 1,5% (1/66 RU) were detected

as an associated diagnosis. Small number in PUJ stenosis in our series is relatively unsuspected finding. They had (male:female ratio 1.8:1), and unilateral and bilateral hydronephrosis were seen in 73% and 27% of the cases, respectively. This finding is similar to our results (M:F ratio: 2.2:1; bilateral:unilateral ratio: 4:1) [13].

We demonstrated statistically significant correlation between degree of the hydronephrosis (APD) and ^{99m}Tc -DMSA scan finding ($P < 0.001$).

Lee RS et al. and Passerotti CC. et al. found that in children with a history of prenatal dilation of the urinary tract, the incidence of reflux ranges from 12% to 38%. In our group of patients VUR was present in 31,8% what is in concordance with literature data [14, 15].

VUR is the only uropathy in which the degree of dilatation of the urinary tract observed on the prenatal and postnatal US does not correlate with increasing risk of pathology. Moreover, there is poor correlation between VUR grade and severity of dilatation [16-19].

In our study VUR is the most common associated finding and is diagnosed on MCUG in 21/66RU (31,8%). In 19/21RU (90%) in whom on MCUG VUR was detected, DMSA renal scans was abnormal. Statistically significant correlation between the degree of the VUR and DMSA scan finding ($P = 0.002$) was established.

Other associated diagnosis-megaureter and duplex system was not statistically significantly associated with finding on DMSA scan.

We had only 7/66RU with pyelo-ureteric junction (PUJ) obstruction and we did not have statistically significant correlation between stenosis PUJ and pathological findings on DMSA scan.

In conclusion, although CH is mostly benign condition and has favorable outcome, it can also cause a significant morbidity. Renal parenchymal damage, estimated by ^{99m}Tc -DMSA scintigraphy, is a common finding in children with CH and is in statistically significant correlation with the degree of hydronephrosis. Renal parenchymal damage is common finding in children with CH and VUR and is in statistically significant correlation with the degree of VUR.

On the basis of these results (60% pathological findings) we recommend ^{99m}Tc -DMSA scintigraphy in the evaluation renal pathology in children with congenital hydronephrosis. Greater number of patients is needed for the estimation of the associated diagnosis (other than VUR) influence on the renal parenchymal damage in children with CH.

The authors declare that they have no conflicts of interest.

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The correlation of Evans index with CSF protein levels and ventriculomegaly

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Abstract

Objective: The role of ventriculomegaly and its interplay between various dementia entities and their pathogenesis, as well as its correlation to the levels of CSF protein markers, remains to be elucidated. Evans index (EI) is a well-established radiological marker in the diagnosis of idiopathic Normal Pressure Hydrocephalus (iNPH), assessing ventricular enlargement. However, there are no previous studies investigating the correlation between EI, age, beta amyloid (A β), tau and phosphorylated-tau (p-tau) proteins in the CSF. The aim of this study was to examine the correlation of the above CSF proteins, EI and age in patients with dementia. **Methods:** Sixty two (62) patients, with dementia and levels of A β , Tau and p-Tau in their CSF as well as age and the EI participated in this study. Multiple regression analysis was performed to investigate possible correlations between the aforementioned factors. A multiple regression was employed to predict A β values from age, tau and p-tau values in the CSF. The Durbin-Watson d was 1.942 so we can assume that there is no first order linear auto-correlation in our multiple linear regression data. **Results:** The variables statistically significantly predicted A β , F-value=4.429, P=0.011, R²=0.483. Specifically, out of all the variables, it was shown that only EI and age added statistically significantly to the prediction, P<.05. Specifically, these results are interpreted as: for every 0.01 unit increase in EI, it was predicted a -38.698 decrease in the A β value and for every 1 year increase in age, it was predicted a -17 decrease in the A β value. **Conclusions:** There seems to be a significant correlation between EI value, age and A β reduction in the CSF. No correlation was found between EI and tau and p-tau proteins in the CSF. This may indicate that A β levels in the CSF are affected significantly by ventriculomegaly and not as much by pathophysiological pathways characteristic for each dementia entity. On the other hand, tau and p-tau proteins could possibly play a crucial role in the differential diagnosis of iNPH and other dementia entities, as these proteins cannot be predicted by ventricular enlargement.

Introduction

Ventriculomegaly is a radiological finding presented in the MRIs and CT scans of many patients with neurological diseases and a particularly common one in patients with dementia symptoms. Despite its frequency, the pathophysiology related to ventriculomegaly, as well as the differential diagnosis of the dementia entities that present it, remain to be elucidated [1-3].

A neurological entity strongly associated with ventriculomegaly is idiopathic Normal Pressure Hydrocephalus (iNPH), a disorder of the elderly presented with Adams or Hakim's triad: impaired gait and

mobility, urinary urgency and incontinence, and mild cognitive impairment or dementia in the presence of ventriculomegaly [4-8]. It is considered as a treatable dementia form [6], although underdiagnosed [2, 9].

Here, it is worth noting, that hydrocephalus and ventriculomegaly are not two identical concepts. Ventriculomegaly is an increased intracranial content of cerebrospinal fluid resulting in the enlargement of the ventricular system, while hydrocephalus implies not only enlargement, but also raised pressure within the system [10]. Hence, every patient with hydrocephalus will consequentially present ventriculomegaly, while patients with enlarged brain ventricles do not necessarily have diagnosis of hydrocephalus.

The diagnosis of iNPH remains a rather difficult task. One reason for that could be that a great percentage of patients are presented with comorbidity of iNPH and other neurodegenerative diseases such as AD, PD and DLB [11, 12]. It has even been suggested that iNPH can be just a different manifestation of certain neurodegenerative phenotypes presented with ventriculomegaly [13]. For all the above reasons, an investigation on diagnosed iNPH patients meets a number of limitations and inaccuracies. In an attempt to tackle this problem, our research focuses on the enlargement of brain ventricles and not on specific diagnoses.

Evans index is an indirect radiological marker of the lateral ventricle size. It is a reflection of ventriculomegaly and it is commonly used in the diagnosis of iNPH. It is defined as the ratio between the maximal diameter of the frontal horns and the inner diameter of the skull (Figure 1), and is widely applied in both research and clinical practice [7, 14, 15]. A value of 0.3 or higher defines ventriculomegaly based on the most recent guidelines. Although, its linkage with ventricular volume is not well-established [16] and other ratios (e.g. frontal and occipital horn ratio (FOR)) have also been proposed, they never gained the same acceptance as EI.

Additionally radiological markers like periventricular hyperintensity, enlarged Sylvian fissure, modified cella media index and the mean width of the temporal horns, share the same importance with FOR and we chose not to include them in the study for this reason [16]. Only the measurement of the callosal angle has received wide acceptance in the scientific community and has been added to the guidelines for iNPH [5, 7]. It has also been suggested as valuable means in the differential diagnosis of AD and iNPH [17, 18]. However as the angle in itself doesn't represent volume size and it is described rather as a distinct sign of iNPH than a ventricular volume measurement, we also chose not to include it in our study [19].

Recently, improved hardware and software make it possible for the digital quantification of the brain volumes, including ventricles, with rather ease. These methods though can be rather costly and may require specially trained personnel, while the calculation of EI is rather easy, rapid and robust [16, 20, 21].

Beta- amyloid (A β) [22], total-tau and phosphorylated tau (p-tau) proteins [23] are widely used as markers for neuronal degeneration in many dementia diseases [24], such as AD [23, 25, 26], PD [27, 28] and iNPH and even in dementia free patients, in order to assess the possibility of developing dementia of Alzheimer's type [29, 30]. Our study uses these markers as indicators of abnormal neuronal processes, associated with the impact of ventriculomegaly in periventricular cells [31, 32]. It is suggested by a number of studies that these markers may be important in the differential diagnosis of iNPH and AD, which also shows their possible clinical significance [33, 34].

A β is considered a major pathological hallmark of AD [35, 36]. It is produced by the transmembrane Amyloid Precursor Protein (APP), which exists in the neurons of every normal brain.

In the normal brain, it can be enzymatically cleaved by insulin degrading enzyme (IDE) and neprilysin (NEP) [37], but in a brain with AD, this process does not take place and A β , aggregates producing neurodegenerative mature amyloid plaques [38]. As a result, its circulation changes and its turnover reduce in the CSF of AD patients [39]. In iNPH, decreased metabolism of the A β in the brain is expected to be indicated by low CSF levels, as well [3, 33].

Tau is a microtubule associated protein (MAP) located in the axon of the neuron cell [40, 41]. To date, the only established known function of tau, is the promotion of microtubule assembly and the stabilization of their structure [34, 42]. Postmortem biopsies of AD brain tissue revealed an atypical microtubule assembly caused by the hyperphosphorylation of tau protein [43].

In AD patients high levels of tau and p-tau proteins can be found in the CSF [39], contrary to iNPH, where tau and p-tau levels tend to be reduced in patients' CSF[33][3]. For this reason, tau and p-tau may be useful tools in the differential diagnosis of the two diseases [34].

On this basis, A β , tau and p-tau CSF protein markers, as well as age, were investigated in our study for possible correlation with ventriculomegaly, as evaluated by Evans' Index values.

Patients and Methods

Patients

Our initial sample consisted of 103 individuals selected from Greek Association of Alzheimer's disease and related disorders (Alzheimer Hellas), and "G. Papanikolaou" General Hospital. All individuals presented with symptoms of dementia in the presence of ventriculomegaly. Forty four (44) patients were excluded from further analysis as 28 of them were not tested for A β and 13 of them due to outlying values. Our final sample consists of 62 individuals at least 46 years of age. Dementia and ventriculomegaly were determined through clinical evaluation of an experienced neurologist, an extended neuropsychological battery including Mini-Mental State Examination (MMSE) scores and radiological findings.

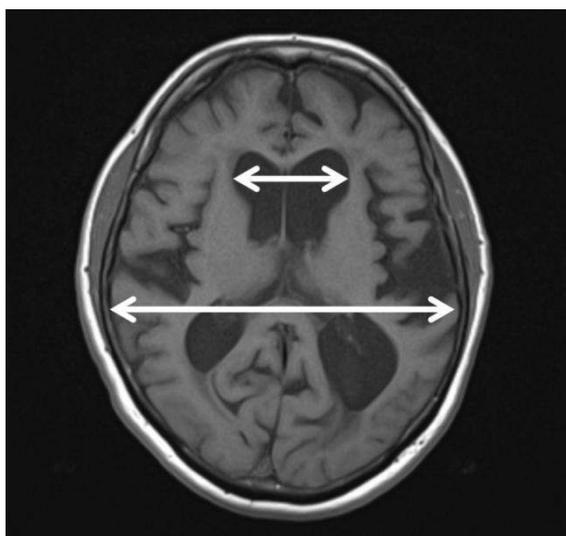


Figure 1. Magnetic resonance image showing EI.

Radiological Examination

EI was measured as the ratio of the maximal width of the frontal horns to the internal diameter of the cranium. All measurements were made from CT and MRI scans on transverse sections. The section with the largest horn width was selected. In the same slice, the largest internal diameter of the cranium was measured [44].

ELISA testing

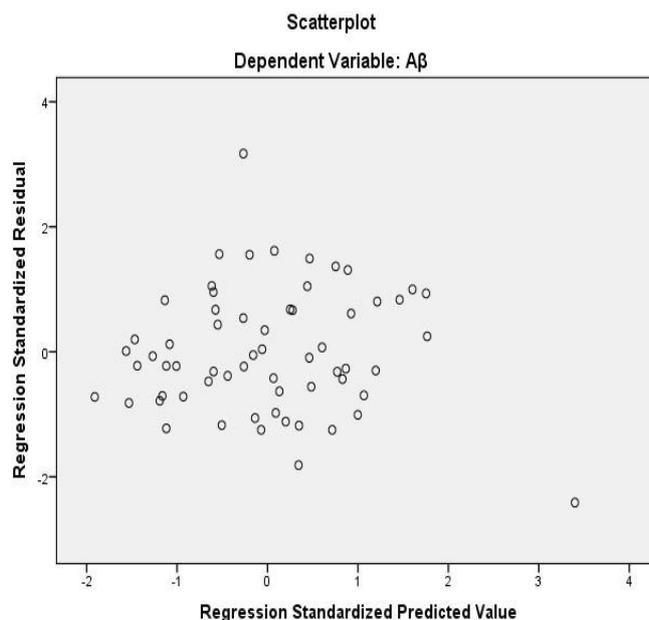
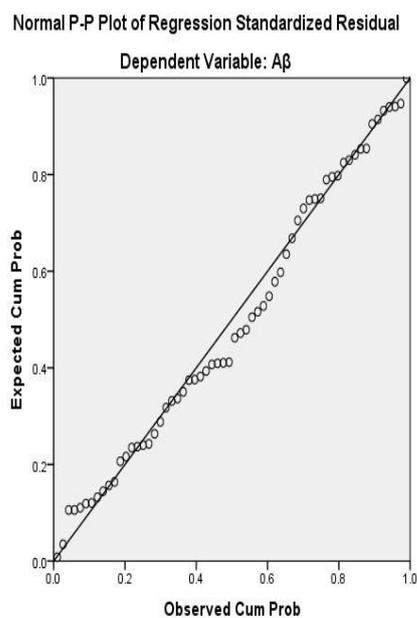
The lumbar puncture was performed early in the morning between L3 and L4. The collection of the CSF was performed with a G19 and rarely with G18 needle in a 10mL PP tube. After that the CSF was centrifuged (2000g for 10m) and aliquoted in PP tubes (each tube contains up to 500 μ L of CSF) for future measurements, and stored in -80°C. Levels of A β -42, p-tau and p-tau in CSF were measured by a solid-phase enzyme immunoassay (ELISA) distributed by INNOTEST, FUJIREBIO. All samples and controls were tested in duplicates and each assay was performed according to the instructions and guidelines of the manufacturer.

Statistical analysis

Mean and median values, dispersion, distribution and outliers were assessed using descriptive data, histograms and box plots. The relation between A β , age, Evans' index, tau and p-tau was examined using scatterplots and multiple linear regression analysis. The Durbin-Watson statistic was performed and produced a value of $d=1.942$. Consequently, we can assume that there is no first order linear auto-correlation in our multiple linear regression data. Statistical significance was assumed at $P<0.05$. Analyses were performed using SPSS 24.0 (SPSS, Inc., Chicago, IL, USA).

Results

In the total sample, EI ranged between 0.2067 and 0.4110 with the mean value of 0.3171 (SD=0.04468), age ranged between 46 and 95 with a mean value of 78.89 (SD=8,417) and A β ranged between 250 and 1300 with a mean value of 626.77 (SD=236.8). Additionally tau and p-tau values ranged from 65 to 1000 and 22 to 105, with mean values of 339.88 and 48.1 (SD=224.5, SD=18.82), respectively. The variables statistically significantly predicted A β , with the F-value=4.429, $P=0.011$ and $R^2=0.483$ supporting our claim. Specifically, out of all the variables, it was shown that only EI and age added statistically significantly to the prediction, $P<0.05$. Specifically, these results are interpreted as: for every 0.01 unit increase in EI, it was predicted a -38.698 decrease in the A β value ($P=0.02$) and for every 1 year increase in age, it was predicted a -17 decrease in the A β value ($P=0.04$). Tau and p-tau didn't correlate individually with A β .



Figures 2 and 3. P-P Plot diagram suggests that the normality assumption is met (left) and scatterplot of the standardized residuals (age and EI) against the predicted values of A β (right).

Discussion

A sample of 62 eligible patients has been assessed based on their CSF protein markers and EI, in this study. Based on our findings, there seems to be a notable correlation between A β and EI, although no such correlation was found between EI and tau and p-tau proteins. This finding validates our hypothesis that ventriculomegaly plays a vital role in A β regulation in the brain and thus in its turnover in the CSF. As tau and p-tau proteins are not predicted by ventriculomegaly, their levels in the CSF seem to be affected by the underlying pathophysiology of the specific dementia entity, which aligns with results of previous studies [34]. Thus, tau and p-tau proteins may prove to serve a crucial role in the differential diagnosis of iNPH and other dementia entities.

In the past, plenty of research has been conducted to investigate samples pertaining certain dementia entities and the correlation of each with CSF protein markers [39], including iNPH [3, 34]. However, there are no widely acknowledged diagnostic criteria for iNPH to date. Two separate expert committees have proposed different guidelines in order to approach the possibility of an objective and evidence-based diagnosis [5, 13]. The two groups of criteria are referred to as the Japanese guidelines and the American-European guidelines, respectively [45, 46]. However, the specificity and sensitivity of each has not been thoroughly investigated, while there are few bibliographic references on the subject [47][48]. In addition, most studies base their results on diagnoses attained by a neurologist, a fact that incorporates a certain level of inaccuracies and errors. This is exacerbated by the difficulty of the diagnostic effort. Moreover, the lack of knowledge in the pathophysiological pathways involved, hinders the efforts of a proposal for a pathognomonic test [32, 49, 50]. Furthermore, the large percentage of cases presenting comorbidity between iNPH and other dementia pathologies further complicates the task at hand, thus making a degree of uncertainty ever-present in diagnoses. Our study, tackles this risk by investigating the correlation of an objective radiological marker instead of specific

diagnoses with CSF protein markers, hence producing safer conclusions.

A future prospect of research would be an exhaustive analysis of the existing diagnostic criteria in a large sample towards the formulation of an improved set of guidelines. This would allow for the possibility of far more accurate conclusions on the correlation between CSF protein markers and the diagnosis of INPH. Other prospects would include different ways of estimating ventricular enlargement. Ventricular volume can be estimated with additional means to further increase the detail of the measurements made. By including FOR and Automatic Volumetry, one can improve the accuracy of the measurements. In addition to A β , tau and p-tau, which are considered currently major indicators of neuronal degeneration, the involvement of a variety of CSF biomarkers needs to be investigated concerning their correlation to ventricular enlargement in order to provide a deeper understanding of the pathophysiological changes engaged in the ventricular enlargement process and diseases associated with it. Examples of those are NFL, sAPP, interleukins 8 and 10. The understanding of the underlying mechanisms holds great value in the process of pinpointing new pharmaceutical targets.

Even though, we consider the results concerning the prediction of A β based on EI and age rather rigorous, our clinical study includes certain limitations and further research may resolve them while providing a clearer understanding on the topic. Our sample consisted of 62 individuals who met the inclusion criteria and a larger sample would increase the weight of our statistical analysis. To date, EI is the radiological marker which has gained the greatest acceptance for ventriculomegaly estimation in clinical practice, but more radiological markers are currently investigated in an attempt to a much more accurate estimation. To sum up, this study provides a demonstration of the importance of examining multiple quantitative measures, like biomarkers and radiological indexes in the understanding of the pathophysiology involved in ventriculomegaly associated dementia and hopes to serve as a stepping stone towards formulating improved diagnostic guidelines.

The authors declare that they have no conflicts on interest.

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Autologous free fat transfer in patients with velopharyngeal insufficiency

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Abstract

Aim: To report our initial experience and preliminary results of autologous free fat transfer to improve speech and hypernasality in patients with velopharyngeal insufficiency (VPI) as a sequela of cleft lip and palate repair. **Material-Method:** To date 2 patients with a mean age of 25 years were treated with this method. Both had initially received multiple procedures elsewhere for cleft lip and palate repair. We recorded the number of free fat transfer sessions, anatomical places of placement and volumes injected in-patient stay, occurrence or absence of complications and effectiveness of this operation in terms of clinical speech evaluation, functional velopharyngeal closure measurements and speech improvement percentage by an Ear, Nose and Throat (ENT) specialist. **Results:** Two autologous free fat transfer sessions per patient were performed. Mean hospitalization time was 1 day per operation. Following liposuction, autologous free fat was transferred to the following anatomical areas: a) Passavant's ridge, b) uvula, c) palatopharyngeal and palatoglossal folds. The volume of fat injected varied from 6.5 cc to 8 cc per session. Postoperative periods were uneventful for both cases in each session. On clinical examination, improvement in speech was noted as well as a reduction in hypernasality with an improvement in articulation and audibility of consonant words, which were also reported by the patients' relatives. This was confirmed by objective nasendoscopy velopharyngeal closure measurements, both during speech and deglutition. **Conclusion:** Augmentation pharyngoveloplasty with autologous free fat transfer in patients with velopharyngeal insufficiency is a safe and innovative alternative, particularly for small to medium degrees of structural velopharyngeal dysfunction.

Introduction

Velopharyngeal closure is a complex neuromuscular function that depends on the coordinated action of structures which elevate the soft palate and constrict both posterior and lateral pharyngeal walls and is important in the production of comprehensible speech. Velopharyngeal insufficiency (VPI) is a term used to describe any condition in which an individual is unable to completely close the velopharyngeal port, resulting in air leakage through the air passages during speech. As a consequence, speech production is abnormal and disordered, characterized by nasal emission, increased nasal resonance, decreased vocal

intensity and articulatory deficits which affect intelligibility and, subsequently, the individual's communication and psychological well being.

Velopharyngeal insufficiency can be secondary to anatomic deficits, neuromuscular disorders or articulation mislearning causes [1, 2]. It is commonly associated with a cleft palate, with a reported residual VPI incidence ranging between 20%-30% in patients who have undergone cleft lip and cleft palate repair, necessitating secondary treatment [2, 3].

The management of VPI includes nonsurgical treatment options such as speech therapy sessions alone or in conjunction with prosthetic rehabilitation. The use of prosthetic devices either in the form of palatal lift appliances [4] or pharyngeal obturator prostheses [5] is an accepted temporary or permanent treatment option especially in nonsurgical candidates.

The most commonly performed surgical procedures for restoration of velopharyngeal function include the superiorly or less commonly inferiorly based pharyngeal flap technique [4, 7] along with sphincter pharyngoplasty. Both are performed in order to decrease the residual velopharyngeal port, correcting central and lateral gaps respectively [12]. Furlow palatoplasty or double opposing Z plasty technique is an alternative procedure that is (re)gaining popularity in the field of surgical correction of VPI, improving velopharyngeal insufficiency by enhancing soft palate's length and movement [9]. However, all these treatment methods have potential risks and negative sequelae such as postoperative bleeding, obstructive sleep apnea and airway compromise [7, 13]. Augmentation of the posterior pharyngeal wall with the use of various alloplastic materials has also been proposed as treatment method for mild forms of VPI [18].

In recent years with the improvement in liposuction techniques and the refinement of lipotransfer procedures attributed to Coleman's atraumatic technique of fat harvesting and grafting [19] in autologous free fat transplantation is gaining an expanding role with many clinical applications in the fields of aesthetic, reconstructive and craniomaxillofacial surgery [1, 5, 14]. Therefore, autologous free fat transfer has emerged as a novel approach for the management of velopharyngeal insufficiency, especially in selected patients with mild to moderate forms of velopharyngeal closure defects [8].

Material and Method

To date, in our department 2 female patients with a mean age of 25 years (23 and 27 years respectively) were treated for velopharyngeal insufficiency with autologous fat transplantation. Both of them had been initially operated elsewhere with multiple procedures for cleft lip and palate repair. Of note, in the first patient, before proceeding to lipotransfer, we attempted to treat VPI with a pharyngeal flap, however the attempt was abandoned due to the highly fibrotic nature of tissues in the region. Both patients were preoperatively assessed by an ENT specialist and their VPI was evaluated by nasoendoscopy. We recorded the number of free fat transfer sessions, anatomical locations where fat grafts were placed, volumes of free fat injected, in-patient stay, occurrence or absence of complications and the effectiveness of this operation in terms of: clinical speech evaluation, functional velopharyngeal closure measurements and speech improvement percentage by the same observing ENT specialist.

Infiltration of the lateral surface of the thighs with Klein's solution followed by low pressure liposuction in order to minimize adipocytes' trauma was performed in both cases. The donor site selection

depended on the patient's pattern of lipodystrophy. Lipoaspirate was mechanically washed with Ringer's Lactate and the collected autologous material was purified through decantation. Purified fat grafts were then transplanted through a small cannula (18G) in the preselected anatomical areas and the submucosal plane. Volumes of fat grafts injected per anatomical location, session and patient are shown in Table 1.

Results

Two autologous free fat transfer sessions per patient were performed at an interval of 1 month and 2 months respectively. Mean hospitalization time was 1 day per operation. Autologous free fat was transferred via a small cannula into the following anatomical areas: a) Passavant's ridge, b) uvula, c) palatopharyngeal and palatoglossal folds. The volume of fat injected varied from 6.5 cc to 8 cc per session. Postoperative periods were uneventful for both cases in each session. After a six-month follow-up, on clinical examination, improvement in speech was noted as well as a reduction in nasal escape with an improvement in articulation and audibility of consonant words, which were also reported by the patients' relatives. These findings were confirmed by objective nasendoscopy velopharyngeal closure measurements both during speech and swallowing by the ENT specialist and nasoendoscopy evaluation at 1 and 3 months postoperatively.

Table 1. Volumes of fat grafts injected per anatomical location, session and patient.

		1 st Patient	2 nd patient
1 st session	<i>Posterior pharyngeal wall</i>	7.5 cc	5 cc
	<i>Uvula</i>	-	2 cc
	<i>Palatoglossal & Palatopharyngeal Folds</i>	-	-
2 nd session	<i>Posterior pharyngeal wall</i>	6 cc	3,5 cc
	<i>Uvula</i>	2 cc	-
	<i>Palatoglossal & Palatopharyngeal Folds</i>	-	3 cc

Discussion

The purpose of this paper is the preliminary report of speech improvement results for treating VPI with

autologous fat transfer to the velopharynx, a relatively new indication for this technique. *Filip et al*, reported their results after injecting fat into the velum, the posterior pharyngeal wall and the palatopharyngeal arches in nine patients (3 of them were noncleft patients). They found significant reduction of the median velopharyngeal distance and gap, as this was assessed by pre- and postoperative MRI during phonation. Furthermore, speech parameters were also evaluated and improvement in nasal turbulence, nasal emission was noted while hypernasality improved in 3 of the patients [15]. In another group of eleven patients, treatment with isolated posterior pharyngeal wall augmentation with autologous fat transplantation with graft volumes ranging between 5-22cc resulted in speech resonance improvement as assessed by nasometry and subjective hypernasality evaluation, while postoperative nasoendoscopy confirmed the achievement of complete velopharyngeal closure in seven patients [10]. Twenty five patients were treated with fat transfer into the posterior pharyngeal wall, along with injections into the velum, the peritonsillar arches in eighteen of the patients. In three patients additional fat transfer session was required. Postoperative evaluation of speech quality relied on Borele Maisonnay scores with patients demonstrating improved speech capabilities [16]. None of the above authors reported any complications after fat grafting in the velopharyngeal sphincter.

Conclusion

According to our preliminary results, augmentation of the posterior pharyngeal wall and velum with autologous free fat transfer in patients with velopharyngeal insufficiency is a safe and minimally invasive technique, although multiple sessions may be needed. Augmentation pharyngovelooplasty with autologous fat could serve as a viable alternative to surgical procedures, particularly in mild forms of VPI, or when surgical intervention is not considered an option. Therefore, the effectiveness of the technique relies apart from technical parameters, on the selection of the appropriate candidates.

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The authors declare that they have no conflicts of interest.

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The operation of the Day Care Centre of Alzheimer Hellas “Saint Helen” and of the Memory Clinic of Papanikolaou General Hospital from 2007 to 2017

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Abstract:

Objective: Nowadays, there is a rapid increase of neurocognitive disorders (ND), while Alzheimer disease (AD), is the most frequent type of neurodegeneration. Therefore, Day Care Centers (DCC) and Memory Clinics (MC) have been created in order to correspond to the rising needs of people with ND. The aim of the study was to record clinical and demographic characteristics of people with ND from the broader area of Thessaloniki. **Subjects and methods:** Data bases were collected and were analyzed in order to examine demographical characteristics of all visitors of the DCC of Alzheimer Hellas and of visitors of the Papanikolaou General Hospital, during the years 2007-2017. Six thousand and ninety six people (N=6.096) were totally classified as single visitors of the outpatients of MC of Papanikolaou General Hospital and of the DCC of Alzheimer Hellas. **Results:** Among the total number of visitors, 3.078 people received the diagnosis of Mild Cognitive Impairment (MCI), whereas 3.018 people met the dementia diagnosis criteria. As far as the MCI group is concerned, the majority of them were women (72.1%) while the mean age of MCI group was M (SD)=67.73(9.21) years. Their mean education was M (SD)=10.65 (4.59) years, while their mean score in general cognitive performance (MMSE) was M (SD)=27.03(2.18) points and their score of general functional performance (FUCAS) was M (SD)=44.32(2.49). In regards to the dementia group, the majority of them were also women (63.8%) with a mean age M (SD)=76.08 (8.60) years and a mean education M (SD)=7.35 (4.41) years. The MMSE score of the group was M (SD)=17.79 (6.53) and the FUCAS score was M (SD)=63.25 (18.12). Moreover, the vast majority of dementia type was AD (53.1%). **Conclusion:** Women are presented more frequently to primary care services and people with dementia have lower education and greater age. AD continues to be the most common type of dementia.

Introduction

Epidemiological studies have shown that the world's population is aging rapidly. Nowadays, the elderly world population is nearly 900 million people and by 2050, this number is expected to grow reaching about 1.5 billion, representing 16% of the world's population [1]. The rapidly rising aging population is one of the reasons for the progressive increase of neurodegenerative disorders, such as Mild Cognitive Impairment (MCI) and Dementia, with dementia due to Alzheimer's Disease (AD), to be the most frequent reason of

cognitive impairment among the elderly [2]. Specifically, regional estimates of dementia prevalence in people aged 60 years and over, range nowadays from 4.6% in Central Europe to 8.7% in North Africa, though all other regional estimates are up to 6.6 percent [3]. The current prevalence of dementia worldwide has been reported to be approximately 5 to 6% for most regions of the world, and 8.5% in Latin America [4]. Around the world 35.6 million people were living with dementia in 2010 and this number will nearly double every 20 years reaching 74.7 million in 2030 and almost 131 million in 2050. The current estimation is around 47 million people worldwide [5].

As far as the Greek population is concerned there are not many epidemiological studies reporting the prevalence of Minor or Major Neurocognitive Diseases in Greece. The only available data are presented in studies conducted in the island of Crete and in the northern part of Greece, near the city of Thessaloniki.

More specifically, in 1999, evidence from a study in Pylaia, a suburb of Thessaloniki, had demonstrated that the estimated annual incidence rate for all forms of dementia in this suburb in Greece was 57 in 1.000 people aged more than 70 years. Precisely, 39.9/1,000 people met criteria for AD, 13.9 /1,000 people were diagnosed with vascular dementia, while 3.5/1,000 people presented with other forms of dementia [6].

Another study in Greece was held in the municipality of Alexander the Great, a rural area in the north of Greece. This study has shown that out of 678 residents who were finally examined, 26.3% were classified as Mild Cognitive Impaired without depression, 8.8% as Mild Cognitive Impaired due to depression, 5.9% met criteria for a depressive disorder without cognitive deficits, 2.4% were diagnosed with dementia and 56.6% had normal mental status. The observed prevalence for MCI with and without depression implies a total of 35.1% of all people aged over 65 with MCI in the study area. Accordingly, Mild cognitive impairment is more prevalent in Greece than dementia [7].

The last epidemiological study was carried out in Crete and indicated that among the elderly of Crete only 15.3% of the study sample met the criteria for MCI, while 2.0% was diagnosed with dementia and 7.2% presented with comorbidity; that is dementia with depression. Taking into account these data, dementia was less prevalent compared to global data and other Greek areas and MCI was more prevalent than dementia [8].

Until nowadays, there are not many studies in Greece to report data from clinical centers such as memory clinics or day care centers for dementia and related disorders, concerning the prevalence of AD and related dementias or the prevalence of MCI and other demographic data, such as the estimation of gender, age and education among these disorders. Therefore, the aim of the present study was to contribute to the existing epidemiological studies about dementia in Greece by demonstrating the prevalence of different types of dementia, gender, age, and education in a rural population, including people over 35 years age, in the broader area of northern Greece.

Materials and Method

Data Source

The particular study was a data based one. The sample was recruited from two different data bases of the Day Care Center of the Greek Association of Alzheimer Disease and Related Disorders "Saint Helen"

(Alzheimer Hellas) and of the Papanikolaou General Hospital of Thessaloniki. Saint Helen is one of the two Day Care Center's of Alzheimer Hellas in Thessaloniki, where people with minor or major cognitive deficits and also people with subjective memory complaints are visiting it in order to receive a diagnosis. The memory clinic of the Papanikolaou General Hospital of Thessaloniki, includes outpatients with minor or major cognitive deficits and also people with neurological diseases, such as strokes, Parkinson's disease, hydrocephalus etc. The study sample was recruited from September 2007 to September 2017.

Subjects

During the period from 2007-2017, out of 7.901 visitors, who visited at least one time either the day care center of Alzheimer Hellas or the memory clinic of the Papanikolaou General Hospital of Thessaloniki, 390 people were excluded from the study because they were healthy people with subjective cognitive impairment with or without severe depression or anxiety. Four hundred and sixty five (n=465) people were also excluded because there were people with other medical conditions that might secondarily lead to subjective cognitive complaints and/or cognitive impairment. Among others, the most commonly referred factors in this study were strokes, migraines, mental retardation and hydrocephalus and these cases constituted in total 5.9% of the study sample. Moreover, people who did not complete both the typical clinical neurological examination, and the routine check-up along with the expected neuroimaging procedure, or the entire neuropsychological assessment, could not receive a careful diagnosis, therefore nearly 416 cases remained undiagnosed (5.3%). Finally, there were 534 people with incomplete data, such as age or years of education, pharmaceutical treatment etc., therefore they were also excluded from the study. As a result, the total study sample which was analyzed included 6.096 visitors with neurocognitive impairment who were divided in two large groups, people with MCI (n=3.078) and people with dementia (n=3.018).

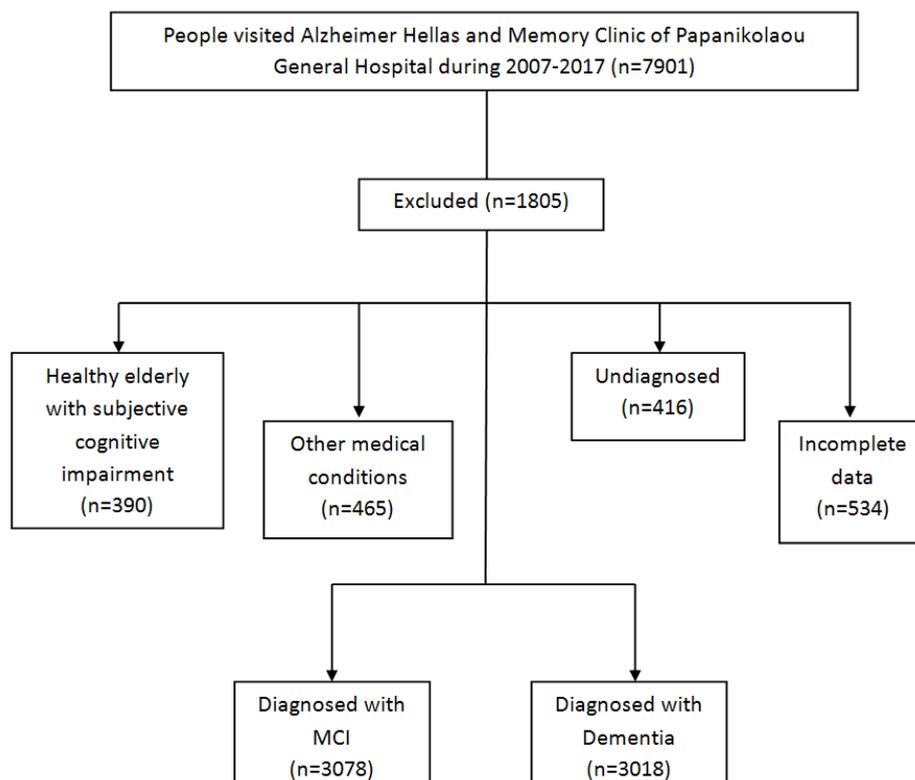


Figure 1. Chart Flow

Diagnosis

The participants in this study had either MCI or Dementia with or without depression/anxiety and psychosis, according to suitable criteria. The MCI diagnosis was established according to Petersen criteria [9], while the diagnosis of Dementia was based on DSM-5 criteria [10]. People with MCI with depression or anxiety and people with dementia with depression or anxiety, met the criteria above, but the presence of depressive or anxiety symptoms ($GDS \geq 6$ and $SAST \geq 23$) implied possible MCI or Dementia due to depression or due to anxiety.

In order to set the diagnosis, neurological examination, neuroimaging (computed tomography or magnetic resonance imaging) and blood tests, were carried out by specialized health professionals and experts in neurocognitive disorders. Moreover, in order to determine the diagnosis neuropsychological and also neuropsychiatric assessment was performed. Neuropsychological assessment included a battery of psychometric tests which assessed general cognitive function, activities of daily living, depression and neuropsychiatric symptoms. These tests were:

- a) The Mini Mental State Examination (MMSE) [11, 12], for the assessment of general cognitive function, while the Hindi Mental State Examination [13] was administered to people who had completed less than 4 years of basic education.
- b) Functional Cognitive Assessment Scale (FUCAS) [14], for general functional performance.
- c) The Geriatric Depression Scale (GDS) [15, 16], was used in order to assess the state of depression in our sample and
- d) The Neuropsychiatric Inventory (NPI) [17, 18] was used for the assessment of psychopathological symptoms.
- e) The Short Anxiety Screening Test (SAST) [19, 20], was used for the diagnosis of general anxiety disorder.

Statistical Analysis

Statistical analysis was performed using SPSS v20.0. Descriptive statistics were used to determine demographical data, such as age, gender, educational level and diagnosis of dementia and other neurocognitive disorders. Means and standard deviations were used in order to assess the general cognitive and functional state in our sample.

Results

Overall, 6,096 people were analyzed. Three thousand seventy-eight people ($n=3,078$) met the criteria for MCI and 3,018 people met the criteria for dementia. Out of 3,078 people with MCI, 531 were diagnosed with MCI due to depression or anxiety (8.7%), while 7 people (0.1%) met the criteria for MCI due to psychosis (Figure 2). Out of 3,018 people who were diagnosed with dementia, 169 (2.8%) met the criteria of dementia due to depression or anxiety, while 3 people met the criteria for dementia due to psychosis (0.1%). Therefore, in order to analyze the demographic characteristics of our sample, we categorized the sample into two different diagnostic groups. One group consisted of people with MCI and the other group

consisted of people with dementia.

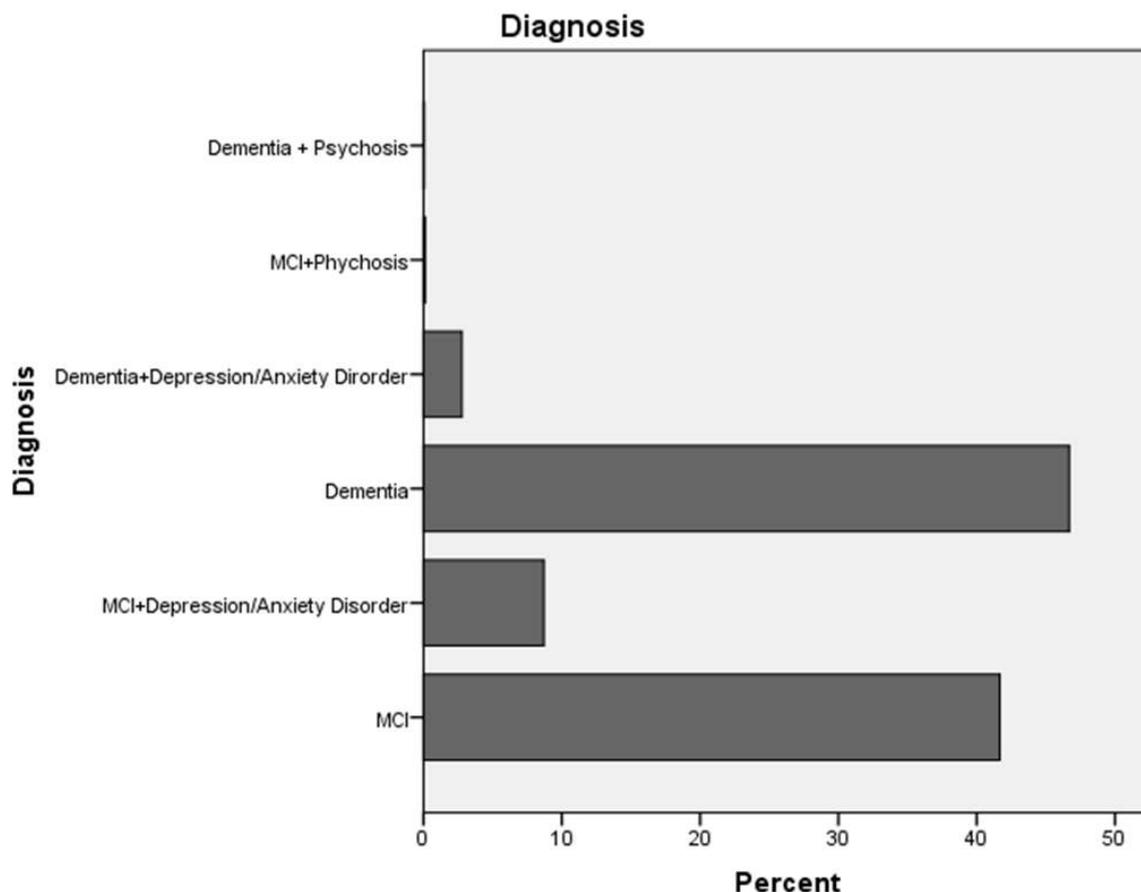


Figure 2. Baseline diagnosis (n=6.096)

MCI Group

As far as gender is concerned, in the group that consisted of people with MCI, descriptive analysis has shown that women predominate and represent 72.1% (2219 women vs 859 men) of the study sample. In regards to age, results have shown that the mean age of the MCI population was $M (SD)=67.73 (9.21)$ years. The majority of people who met the MCI diagnostic criteria were between 66 to 75 years age (41.5%), while 37.1% were people aged between 50 to 65 years. 17.8% were between 76-85 years, whereas very few were up to 86 years age (1.4%) or above 50 years age (2.2%). Concerning educational level, the mean education was $M (SD)=10.65 (4.59)$ years. The majority of the sample completed 7-12 years of education (35%), while 31.5% received education up to 13 years. 32.8% had education 2-6 years and only 0.6% had completed less than 1 year of basic education. Finally, the mean score of general cognitive performance according to the Mini Mental State Examination was $M (SD)=27.03(2.18)$ points and the mean score for the group's general functional performance was $M (SD)=44.32 (2.49)$ points (Table 1).

Table 1. Demographic Characteristics of people with MCI

	N	Percentage (%)
MCI group with or without depression /anxiety/psychosis		
Male	859	27.9%
Female	2218	72.1%
Years of Age		
35-50	66	2.2%
51-65	1118	37.1%
66-75	1251	41.5%
76-85	536	17.8%
≥86	43	1.4%
Years of Education		
0-1	18	0.6%
2-6	1008	32.8%
7-12	1076	35.5%
≥13	967	31.5%
General Cognitive Performance (MMSE) [(M(SD))]	67.73 (9.21)	
General Functional Performance (FRSSD) [(M(SD))]	44.32 (2.49)	

MMSE: Mini Mental State Examination, FUCAS: Functional Cognitive Assessment Scale

Dementia Group

In the group which consisted of people with dementia, in regards to gender, descriptive analysis has shown that women (63.8%) were more than men (36.2%). As far as age is concerned, results demonstrate that the mean age of the dementia population was M (SD)=76.08 (8.60) years. The majority of people with dementia were between 76 to 85 years age (49.4%), while 30.6% were between 66-75 years age. 9.8% were between 50-65 years age, whereas 9.5% were up to 86 years age. Finally a small percent (0.8%) was younger than 50 years age. Concerning the educational level of participants, the mean education of people who met the criteria of dementia, was M (SD) =7.35 (4.41) years. The majority of the sample had 2-6 years of education (60.7%), while 22.6% had educational level between 7-12 years. Only 12% had more than 13 years of education, whereas 4.8% had less than 1 year of education. The mean score of general cognitive performance according to the Mini Mental State Examination was M (SD)=17.79 (6.53) points and the mean score for the group's general functional performance was M (SD)=63.25 (18.12) points (Table 2).

Regarding the dementia type (Figure 3), the vast majority of dementia type is AD (53.1%), while frontotemporal dementia is reported to 2.9%. Mixed dementia is estimated to 2.8% and vascular is presented to 2.2%. Moreover, Lewy Body dementia is estimated to 2.0%. Fewer cases are presented with dementia due to Parkinson's disease (0.7%) and even fewer with frontal dementia (0.3%). Finally, 1.2% of the study sample is diagnosed with other types of dementia. A great percentage of 34.9% could not receive a careful diagnosis; therefore 1.052 people remained undiagnosed, concerning the type of dementia.

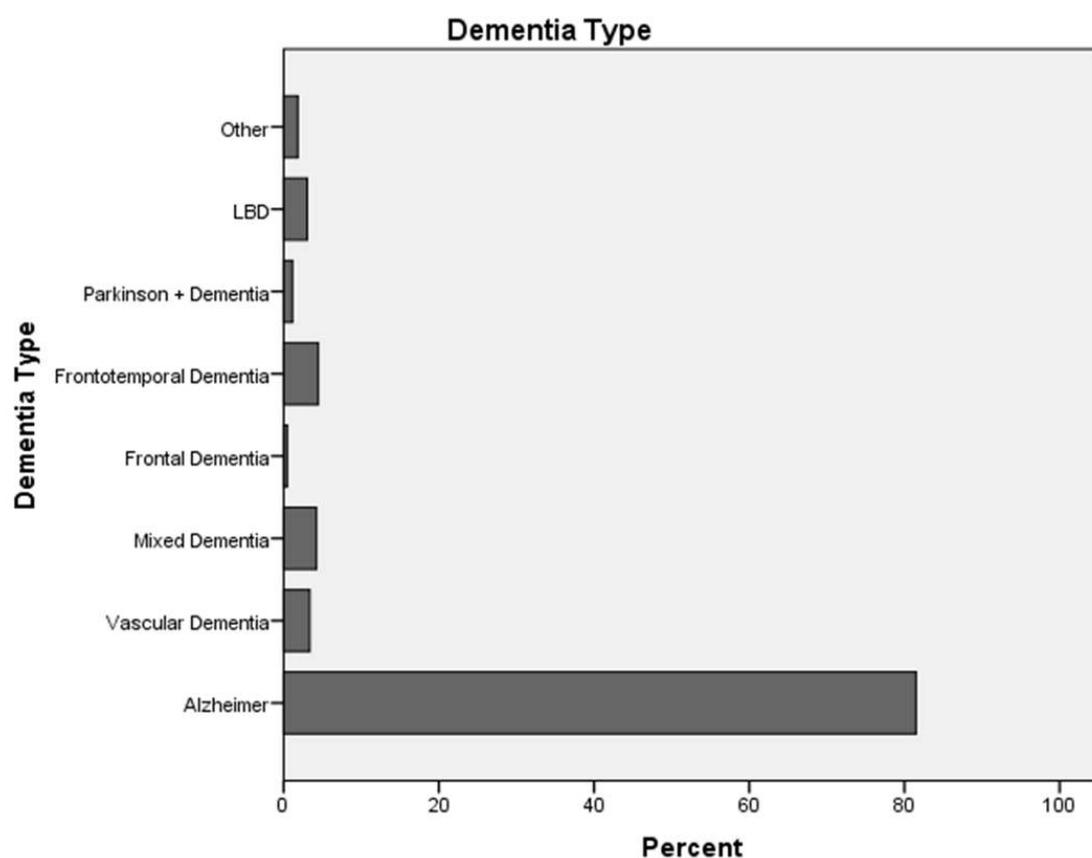


Figure 3. Type of neurocognitive disorder in people with dementia (n=3018).

Table 2. Demographic characteristics of people with dementia

	N	Percentage (%)
MCI group with or without depression/anxiety/psychosis		
Male	1094	36.2
Female	1924	63.8
Years of Age		
35-50	22	0.8
51-65	281	9.8
66-75	877	30.6
76-85	1416	49.4
≥86	272	9.5
Years of Education		
0-1	142	4.8
2-6	1793	60.7
7-12	667	22.6
≥13	354	12.0
General Cognitive Performance (MMSE) [(M(SD))]	17.79 (6.53)	
General Functional Performance (FRSSD) [(M(SD))]	63.25 (18.12)	

MMSE: Mini Mental State Examination, FUCAS: Functional Cognitive Assessment Scale

Discussion

The aim of the present data based study was to record clinical and demographic characteristics as well as

epidemiological data of people with ND from the broader area of Thessaloniki. Findings of our study are in agreement with other literature evidence which demonstrate that dementia is a growing health problem that occurs more often in women than men [21]. Furthermore, in comparison to people who met the diagnosis of MCI, those who met the dementia diagnosis, were characterized by less years of formal education (≤ 6 years) and greater age (≥ 76 years). In general, it is indicated that among other socio-economic factors, low education, advanced age and female gender are strong risk factors of dementia onset [22, 23]. More specifically, the above aspects are strongly linked with the concept of cognitive reserve theory [24]. Individuals with an advanced education have a larger repertoire of strategies to resolve complex tasks as well as redundant neural networks to carry-out the same activities. Nevertheless, high brain-reserve individuals are at risk of developing rapidly clinical symptoms once they have been diagnosed with dementia. Generally speaking, a higher educational level and occupational achievement along with a greater engagement in cognitive activities provides higher reserve against dementia and results in varying cognitive aging trajectories among individuals [25]. As a result, among women in the particular study, during the past decades it was usual to stop basic school training and consequently to have a low occupational history. This could provide some explanation why dementia appears more commonly in women.

Alzheimer's disease is the most common reason of cognitive dysfunction among the elderly. In the specific study it is confirmed that dementia due to AD continues to be the most common cause among others to provoke dementia and accounts for 53% of all dementia cases, whereas the observed prevalence in other studies ranges between 60%-80% [26]. Mixed dementia and frontotemporal dementia are less common than AD and normally account for 10% of all dementia cases. Similarly, in this study mixed dementia and frontotemporal dementia were the second most common types of dementia diagnosis.

Consistent with other studies, in our study it is observed that women either with MCI or with Dementia are presented more frequently to primary care services in order to receive early diagnosis [27]. This is potential to be associated with cognitive worry, a state which is related to exposure to mild cognitive deficits, or to experiencing a high number of depressive or general worry symptoms along with various memory concerns. Generally, elderly people with emotional distress are more likely to develop MCI compared to those without MCI. Prevalence and incidence of depressive symptoms or syndromes in MCI vary as a result of different diagnostic criteria and different sampling and assessment procedures. The prevalence of depression in individuals with MCI is higher in hospital-based studies (44.3%) than in population-based studies (15.7%) [28]. In our study, the number of people with co morbidity was relatively lower (8.7%) in comparison with rates from other literature evidence. Finally, in accordance with other studies [29], in our study it is confirmed that MCI subjects are principally female, younger than 75 years old and with lower education (\leq than 12 years).

Limitations

An important issue concerning the present study is that results demonstrating other specific domains in cognitive and functional state of subjects were not included due to differences in cognitive tests that are conducted in the DCC and in the MCPH. In regards to future study goals, as it is known from other literature evidence, the annual rate of progression to MCI in normal subjects has been estimated at between 1% and 4% annually. Also, subjects with MCI have an annual risk between 14% to 18% of developing dementia [30]. As a result, the conversion rate from SCI state to MCI and from MCI to dementia could be a future study goal.

Conclusion

Our study indicates that women who either meet the criteria of MCI or the criteria of Dementia are presented more frequent to primary care services, such as day care centers or memory clinics. Moreover our study contributes to other studies which support that people with dementia have lower education and greater age than people with MCI. Furthermore, according to our results, Alzheimer Disease continues to be the most common type of dementia.

The authors declare that they have no conflicts of interest.

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Mobile phone use for 5 minutes can cause significant memory impairment in humans

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Keywords: Mobile phone -Electromagnetic field -Cognitive function -Working memory -Cognitive impairment -Mild cognitive impairment.

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Abstract

Introduction: Concerns about the possible adverse health effects of mobile phones (MP) have increased along with the expansion of their use. A number of research papers have tried to address this issue. Although many investigations concluded that MP use does have negative consequences, in terms of cognitive function of the human brain, the results so far have been divisive. A number of studies reported impairment of cognitive function after exposure to mobile phone electromagnetic field (MP EMF), while others observed no effect or improved performance. The variance in the results may be attributed to methodological issues. The present article focuses on possible effects of MP use on cognitive function and more specifically on working memory processes. An emphasis is placed in the lack of a validated tool, a cognitive task, that can produce MP EMF effects on human cognition in a repeatable fashion. **Methods:** Sixty four (64) healthy participants as well as 20 with Mild Cognitive Impairment (MCI) were the experimental group, while 36 healthy individuals were the control group. A computerized list of 10 words was presented and the participants were asked to reproduce it. The words were presented very briefly in order to increase the difficulty and hence the sensitivity of the task. Three measurements were taken for the experimental group: a) before using the MP, b) immediately after using the MP for a duration of 5 minutes, c) 5 minutes after the second measurement with no usage of the MP in between. Three measurements of the memory task were also taken for the control group in the same time intervals with no usage of a MP. The effect of age and gender in the performance of the task was taken into account. **Results:** Healthy participants of the experimental group performed worst in the memory task after using the MP. While the third measurement (5 minutes after the 2nd measurement) was better than the second (after using the MP), but worse than the first (before using the MP). In contrast for the control group the second measurement was better than the first and the third even better than both previous ones. All differences were statistically significant. The reduction of the performance in the task after using the MP was even higher for the age group of 60-80 years old in comparison with younger age groups, as well as for the individuals with MCI in comparison to healthy participants. Age was significantly negative correlated with performance in the task, while gender showed no significant correlation. **Discussion:** MP use has a significant negative impact on working memory performance of human participants. The effect is apparent even for a 5 minute use of the MP. Working memory deficits are greater not only for the 60 years old and above participants but also for individuals with Mild Cognitive Impairment. These results are in agreement with previous studies on animals as well as humans on the effects of MP use on the brain. It is argued that low sensitivity of some of the cognitive tasks used until now and the lack of a validated tool in the form of a cognitive task may account for some of the variability in the literature so far. It is suggested that the experimental paradigm that was used in this study for an increased sensitivity measurement of cognitive function and working memory processes in particular may be used for the display of the effects of MP use on cognitive function and for the development of other tasks sensitive to it. Overall, it is

concluded that the development of certain restrictions on MP use is necessary for the protection of the brain health of the users.

Introduction

During the last few decades the development of new electronic devices has introduced an increasing number of appliances that emit electromagnetic fields. This has resulted in an increased intensity of electromagnetic radiation in the human being's daily environment [1]. Electromagnetic radiation of different frequency and intensity can produce adverse effects in human health when different organs and cellular structures of the body are exposed to it. An increasing number of experimental studies that demonstrate negative consequences in human health by electromagnetic fields [1-11], have appeared in the literature. However, the results have been inconclusive in terms of explaining how these fields affect human health, in defining the adverse effects that are produced and in recommending the guidelines that should be applied in order to protect human health [1]. Characteristic examples of devices that emit electromagnetic radiation and that are used in daily life include LCD monitors, automotive electronic equipment, the electrical machines used by physiotherapists, mobile phones and the internet wireless connection (Wi-Fi).

The present article focuses on the use of mobile phones which is continuously expanding and is considered necessary by the vast majority of people. Being one of the fastest growing technologies [12] mobile phone (MP) connections have increased from 12.4 million to 5.6 billion, in the last 20 years, affecting approximately 70% of the earth's population [13]. The MP transmits and receives electromagnetic waves in the frequency range of 900Hz-1800Hz. Electromagnetic radiation is emitted by its antenna and can pass through organic tissue and be absorbed by the body where it is converted to heat. The device is usually used close to the ear, thus increasing the absorption of radiation by the brain [12]. The amount of electromagnetic energy absorbed by the user's head can reach 45%-50% of that which is transmitted by the MP [14].

Concerns about the possible adverse health effects of MP have increased along with the expansion of their use. Health problems such as headaches, sleep disorder, memory impairment, concentration difficulties, dizziness, increase of seizures in children with epilepsy, brain tumors and high blood pressure have been reported as results of exposure to electromagnetic radiation [15]. The most frequent health complaints by MP users are headaches, fatigue, muscle pain, nausea and feeling ill. Electromagnetic radiation emitted by MP, in the frequencies that it is transmitted, can have biological consequences for the human organism [16]. These consequences and their severity depend on different parameters such as: the duration of exposure, the situation of the central nervous system and the immune system of the person exposed, the rate of absorption, the distribution of electromagnetic energy in different tissues of the human body and so forth [17].

Along with the increase in the concerns on the biological effects of MP use a number of research papers have tried to address the issue. Although many studies conclude that MP use does have negative consequences [1-11], the results so far have been inconclusive. A possible account for this may be the heterogeneity of different populations in terms of their sensitivity to electromagnetic radiation as well as individual differences in this respect [18]. The present article focuses on possible effects of MP use on cognitive functions of the human brain and more specifically on working memory function. Working memory refers to those cognitive processes that are responsible for short-term storage and process of information in

order to help more complex cognitive processing to take place [21, 22]. It has been demonstrated that electromagnetic energy emitted by MP can cause cognitive impairment and alter brain function in animal experiments [10, 11, 19]. Also, effects of these fields have been revealed in relation to sleep [8, 20] and EEG recordings [7] in human subjects without many controversies. However, in terms of human cognitive function the results so far have been divisive. A number of studies report cognitive impairments after exposure to MP electromagnetic field (EMF) [24-26] with a decrease in speed [27-33], accuracy [34, 35], attention and working memory performance [36]. While others observe no effect [37-42] or improved performance in speed and accuracy [43-49] and improvements in verbal memory and visuospatial working memory capacity [50]. Three studies using meta-analyses have produced inconsistent results with one concluding that there is an effect of MP EMF in human cognition in terms of attention and working memory [51] and the other two that there is no significant effect in cognition or psychomotor abilities [52, 53]. A recent critical evaluation of the research concludes that the results may vary due to methodological issues such as the cognitive tasks used, differences in sample size, criteria of inclusion, experimental design and exposure conditions to EMF [54]. The authors suggest that these parameters need to be sufficiently controlled and a standardized protocol in this field of research needs to be developed in order to obtain more consistent findings.

In the current study an emphasis is placed in the lack of a validated tool, a cognitive task, that can produce MP EMF effects on human cognition in a repeatable fashion. According to a recent critical evaluation of the literature the recognition of specific tasks that can reliably measure cognitive performance changes due to MP EMF exposure are needed [54]. This lack has been recognized as one of the most important factors of the inconsistency in the findings so far [54]. Different types of tasks have been used so far contributing, in this way, to the variety of the results in the literature. It is recognized that the identification of sensitive tasks to the effects of MP EMF are needed, that will produce replicable findings in order to detect and understand the consequences on brain and cognitive function.

A cognitive task was developed in order to evaluate the effects of MP EMF on cognitive function. The standard memory test that involves the presentation of a list of words and the reproduction of it was altered in order to become more sensitive by increasing its difficulty. The words were presented very fast in order to increase the load on working memory processes and to make the task more difficult by the interference effect. According to interference theory the stored memory is intact but unable to be retrieved due to competition created by newly acquired information [55]. By increasing the speed of the presentation of words the interference effect becomes larger, thus increasing the difficulty of the task. The task is based on the classic memory test of a word list with manipulation of the time of the presentation of the words. A similar experimental paradigm with very brief presentation of the word lists has been used in the studies on implicit memory [56].

The goal of the present study is the assessment of working memory performance after the use of a MP in humans. The first hypothesis is that performance on the cognitive task will be impaired after the use of a MP. This effect is investigated in healthy participants as well as individuals with Mild Cognitive Impairment (MCI). MCI is defined as the intermediate stage between normal aging and dementia and specific criteria are being used for the diagnosis of this condition [23]. The second hypothesis is that participants with MCI will present more impairment after the use of the mobile phone. The effect of age on memory and the use of a MP is also investigated. The third hypothesis is that age will be positively correlated with the impairment detected on memory by the use of the MP.

Methods

Participants

The study is a neuropsychological, psychometric evaluation of the cognitive impairment detected after the use of a MP in healthy individuals as well as participants with MCI. Sixty four (64) healthy as well as 20 participants with MCI were the experimental group, while 36 healthy individuals were the control group. The research methodology used is a within subjects design for the experimental and the control groups in order to detect the presence of cognitive impairment after the use of a MP.

Selection criteria for MCI participants:

- ▶ MCI diagnosis by an experienced neurologist based on the international criteria for the syndrome.
- ▶ Absence of a psychiatric or other neurological disorder.
- ▶ Detection of impairment in one or more cognitive functions by a neuropsychological evaluation.
- ▶ Intact perception in order to participate in the experimental process with no difficulty.

Experiment

A computerized list of 10 words was presented and the participants were asked to reproduce it. The words were presented very briefly in order to increase the difficulty and hence the sensitivity of the task. Three measurements were taken for the experimental group:

- ▶ a) before using the MP,
- ▶ b) immediately after using the MP for a duration of 5 minutes,
- ▶ c) 5 minutes after the second measurement with no usage of the MP in between.

Three measurements of the memory task were also taken for the control group in the same time intervals with no usage of a MP. The effect of age and gender in the performance of the task was taken into account.

Neuropsychological evaluation

The cognitive task used involved the presentation of a list of 10 words on the computer. Words were presented for 0.15 seconds each, the presentation lasted for 15 seconds and the participants were asked to reproduce as many words as they could by writing them down within 15 seconds immediately after the presentation. The task was applied three times for each group. In every application the same number of letters was included in order to control the difficulty between the different trials. Only one word was common between the two different applications of the task. The task was created by the experimenter with the help of computer software. The task is based on the classic memory test of a word list with manipulation of the time of the presentation of the words. A similar experimental paradigm with very brief presentation of the word lists has been used in the studies on implicit memory⁵⁶.

Statistical analyses

Data analyses was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago,

IL, U.S.A.) for Windows, version 20. The quantitative data were recorded as mean and standard deviation when they were normally distributed and as median and interquartile range (IQR) when they were not normally distributed. Qualitative data were recorded as frequencies. The requirements for repeated measures ANOVA were not met. Thus, the t-test was used for comparing performance on the memory tests within the same group when the distribution of the data was normal and the Wilcoxon Signed Rank test was used when the distribution was not normal. Also, Pearson's correlation test was used when the distribution of the data was normal, while Spearman's correlation test was used when the distribution was not normal. P was significant for $P < 0,05$.

Results

Descriptive statistics (Table 1) showed that healthy participants of the experimental group performed worst in the memory task after using the MP while the control group improved its performance. Comparison of performance on the different trials of the memory task for all groups according to the Wilcoxon Signed Rank Test (Table 2) further revealed that for the healthy participants of the experimental group the third measurement (5 minutes after the 2nd measurement) was better than the second (after using the MP), but worse than the first (before using the MP). In contrast for the control group the second measurement was better than the first and the third better than both previous ones. All differences were statistically significant. The reduction of the performance in the task after using the MP was even more apparent for the age group of 60-80 years old in comparison with younger age groups, as well as for the individuals with MCI in comparison to healthy participants. Performance of the MCI participants did not improve in the third measurement in comparison to the second as it did for the healthy participants of the experimental group. Age was correlated significantly negative with performance in the task, while gender showed no significant correlation. Improvement in the performance in trials two and three was higher for the age group of 20-40 years old of the control group. While the experimental group of the same age range did not show the same improvement in performance.

Table 1. Descriptive statistics for all the groups in each trial of the memory task (Median and IQR)

Memory Trial		1 st	2 nd	3 rd
Experimental group	Healthy	4 (1)	3.5 (1)	4 (2)
	MCI	3 (0.75)	2 (1.75)	2 (1)
Control group	Healthy	3 (1)	4 (1.75)	4 (2)

Table 2. Comparison of performance on the three different trials of the memory task for all groups according to the Wilcoxon Signed Rank Test.

	Healthy (exp. gr.)	MCI (exp. gr.)	Healthy controls
2 nd vs 1 st	P<0.001	P<0.001	P=0.088>0.05
3 rd vs 2 nd	P=0.009<0.05	No sig.	P=0.225>0.05
3 rd vs 1 st	P<0.001	P=0.004<0.05	P=0.015<0.05

Discussion

The results are in agreement with the studies that detected cognitive impairment after exposure to MP EMF [24-35] and more specifically with the studies that detected impairment in working memory [36]. Also, the findings are in agreement with the meta-analysis study that concludes that MP EMF exposure produces cognitive impairment [51].

It is argued that increasing the task's difficulty and thus sensitivity has allowed the cognitive impairment to become more apparent than what it would be with the typical cognitive tasks used before. In this way it has been attempted to address the lack of a validated tool, a cognitive task, that can produce MP EMF effects on human cognition in a repeatable fashion. It is suggested that the task used can produce replicable findings in detecting the consequences of MP EMF in brain function. Further research is needed to verify this. If this is the case then the variety of the results so far that is due to the different types of tasks used can be addressed.

The fact that the experimental group in the 3rd memory test does not improve its performance in comparison with the 1st trial suggests that MP EMF may impair the learning process. The control group improves its performance after every trial showing in this way that learning has occurred and every repetition of the memory test produces better results. For the experimental group the use of the MP does not only impair performance for the memory test following the MP use but seems to affect negatively the learning process and does not allow performance to improve as the memory test is being repeated.

The findings in this experimental paradigm showed that with an increase in age there is an increase in the impairment detected. Older people are more prone to the negative effect of MP EMF exposure in cognition. Thus, the argument that in old age it does not matter if one gets exposed is not valid. The fact that MCI and age affect the cognitive impairment detected after exposure to MP EMF may account for some of the variability of the results so far.

However, there are limitations in the study due to the methodology used. The control group did not perform a task similar to that of talking on the MP as the experimental group did between task trials 1st and 2nd. Thus, part of the impairment detected could be attributed on the interference effect of performing a task between the two trials of the memory test. Further research is needed to clarify this. Also, the effect of the education level or the IQ level was not explored. Furthermore, there is an indication in this experiment that

brain function does not recover fully 5min after MP EMF exposure. However, it is not clear in this experimental design if it recovers later, how long it takes and if there is a long term effect of MP EMF on cognition.

Apart from the limitations these research findings provide an indication that MP EMF exposure impairs cognitive function and possibly normal brain function in general. Further research is needed that would provide definite proof whether this is or is not the case. These results are in agreement with previous studies on animals [10, 11, 19] as well as on humans [24-36] about the effects of MP use on the brain, thus, raising the possibility that MP EMF exposure is not innocuous when it comes to the health of the Central Nervous System. It is necessary to further explore the issue in order to provide stronger evidence.

In relation to future research it is suggested that the experimental paradigm that was used in this study for an increased sensitivity measurement of cognitive function and working memory processes in particular may be used for the display of the effects of MP use on cognitive function and for the development of other tasks sensitive to it. A replication of the present experiment is needed with control on:

- ▶ IQ and/or level of education between the groups,
- ▶ the amount of EMF exposure

and, also, with having the control group perform a similar task to that of talking on the MP in the condition where the experimental group uses the MP.

Overall, MP industry seems to be ahead of scientific literature on the issue of MP use and health risks. It can be argued that billions should be spent on MP use health risks before billions start using MPs. It is needed to clarify through research the conditions under which MP use affects or does not affect brain function and health in general. MP industry needs to be guided by neuroscientific research in order to insure that MP use does not have negative effects on the health of the users. In the present study it is concluded that the development of certain restrictions on MP use may be necessary for the protection of the brain health of the users.

The authors declare that they have no conflicts of interest.

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Abstracts

Bone scintigraphy can diagnose osteoporotic vertebral compression fractures better than conventional radiography

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Keywords: Bone scintigraphy -Osteoporotic compressive fractures -Conventional radiography

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Abstract

Objective: Most difficult and very frequent complications of osteoporosis are vertebral compression fractures (VCF). Bone scintigraphy with ^{99m}Tc-phosphonates enables early detection of vertebral compression fractures in the first 72 hours of occurring. Typical scintigraphic findings is markedly increased radiotracer uptake in the linear pattern, throughout collapsed vertebral body. Bone scintigraphy is useful in follow-up of vertebral fractures healing, showing reduction of radiotracer uptake in fractured vertebrae. In patients with osteoporosis and suspicion of VCF, we detected compression vertebral fracture by bone scintigraphy and compare it with conventional radiography findings. **Patients and Methods:** Bone scintigraphy was done in 40 patients with osteoporosis and suspicion of compression vertebral fractures, 32 women and 8 men, mean age, 71 years. Three hours after iv. injection of 740MBq of ^{99m}Tc-DPD to the patients, a whole body scintigraphy was done. Standard radiographic views AP, lateral, and oblique were done in all patients. Results: Radiography findings were positive for vertebral compression fracture in 28 patients (70%), and with bone scintigraphy in 36 patients (90%). In one patient with healed-old vertebral fracture, with positive radiographic finding, scintigraphic finding was negative. Bone scintigraphy incidentally diagnosed bone metastases in 3 patients. **Conclusion:** Bone scintigraphy has a very high sensitivity for detection of vertebral compression fractures in osteoporotic patients. Conventional radiography showed a much lower sensitivity and could not differentiate acute from old vertebral compression fractures.

The authors declare that they have no conflicts of interest.

Risk stratification and staging in prostate cancer with prostatic specific membrane antigen PET/CT: A one-stop-shop

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Keywords: PSMA PET-CT -Risk stratification -Staging -Extrapelvic lymph node -Spleen

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Abstract

Objective: Current imaging modalities for prostate cancer (PC) had limitations for risk stratification and staging. Magnetic resonance imaging (MRI) frequently underestimated lymphatic metastasis while bone scintigraphy often had diagnostic dilemmas. Prostatic specific membrane antigen (PSMA) positron emission tomography-computed tomography (PET/CT) has been remarkable in diagnosing PC recurrence and staging. We hypothesized it can become one-stop-shop for initial risk stratification and staging. **Subjects and Methods:** Ninety seven PSMA PET-CT studies were re analysed for tumor node metastases (TNM) staging and risk stratification of lymphatic and distant metastases proportion. The histopathology of 23/97 patients was available as gold standard. Chi-square test was used for proportion comparison. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), over-estimation, under-estimation and correct-estimation of T and N stages were calculated. Cohen's kappa coefficient (κ) was derived for inter-rater agreement. **Results:** Lymphic or distant metastases detection on PSMA PET/CT increased significantly with increase in risk category. PSMA PET/CT sensitivity, specificity, PPV and NPV for extra prostatic extension (EPE), seminal vesicle invasion (SVI) and lymphatic metastases were 63.16%, 100%, 100%, 36.36% & 55%, 100%, 100%, 25% and 65.62%, 99.31%, 87.50%, 97.53%, respectively. Cohen's kappa coefficient showed substantial agreement between PSMA PET/CT and histopathological lymphic metastases (κ 0.734) however, it was just in fair agreement (κ 0.277) with T stage. PSMA PET/CT over-estimated, under-estimated and correct-estimated T and N stages in 8.71%, 39.13%, 52.17% and 8.71%, 4.35%, 86.96% cases, respectively. **Conclusion:** We found that PSMA PET/CT has potential for initial risk stratifications with reasonable correct estimation for N stage. However, it can underestimate T stage. Hence, we suggest that PSMA PET/CT should be used for staging and initial risk stratification of PC as one-stop-shop with regional MRI in surgically resectable cases.

The authors declare that they have no conflicts of interest.

Hematopoiesis is prognostic for toxicity and survival of ²²³Radium treatment in patients with metastatic castration-resistant prostate cancer

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Keywords: Hematopoiesis -Toxicity and survival -²²³Radium treatment -Prostate cancer -Metastatic castration

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Abstract

Objective: We evaluated the impact of pre-therapeutic hematopoiesis on survival, hematotoxicity (HT) and number of ²²³Radium (²²³Ra) treatments in patients with metastatic castration-resistant prostate cancer.

Subjects and Methods: Hemoglobin-levels (Hb), the number of platelets (Plts), leukocytes (Leuk), and survival data were collected in 56 patients treated with ²²³Ra. Pre-therapeutic hematopoiesis as well as adverse events during and after therapy were scored (grade 0-4) according to the CTCAE recommendations. The association of pre-therapeutic hematopoiesis, survival, HT and numbers of ²²³Ra cycles was analyzed. **Results:** Median survival in all patients was 69.9 weeks; 77% of patients had pre-existing impaired Hb (1.7% grade 3, 12.5% grade 2, 62.5% grade 1). 8/56 (14.3%) had impaired Plt (grade 1) Maximum toxicity (Tox) grades of patients during treatment were grade 4 (Hb 1.7%; Plt 1.7%), grade 3 (Hb 14.3%; Plt 7.1%; Leu 7.1%), grade 2 (Hb 33.9%; Plt 7.1%; Leu 23.2%), grade 1 (Hb 46.4%; Plt 17.9%; Leu 23.2%) and grade 0 (Hb 5.4%; Plt 66.1%; Leu 44.6%). Interestingly, patients with thrombocytopenia had a significantly shorter survival compared to those with normal Plt levels (21 weeks vs not reached; P<0.003). As expected patients with pre-therapeutic low Hb-level (<10g/dL) had a significantly shorter survival compared to those with Hb-level >10g/dL (28 weeks vs not reached, P<0.004), whereas survival of patients with mildly impaired Hb (>10 but <13.5g/dL) did not differ from patients with normal levels of Hb (X vs. Y, P=...). Also patients with impaired Hb also developed significantly more grade 3 and 4 HT (Hb <10g/dL: 42.9 vs 14.3%, P<0.001; Plt <150G/mL: 25.0% vs 6.3%; P=0.002) and received significantly fewer treatment cycles (Hb<10g/dL: 5.1 vs 5.8, P<0.04; Plt <150G/mL: 3.4 vs 5.6; P<0.001). Neither extent of bone metastases nor previous chemotherapy were associated with survival, number of ²²³Ra cycles and HT. **Conclusions:** Patients with metastatic castration-resistant prostate cancer and impaired hematopoiesis, in particular thrombocytopenia and anemia, before ²²³Ra therapy suffer from significantly more high-grade HT, shorter survival and receive significantly fewer ²²³Ra treatments. Therefore, Hb-levels and platelet counts are essential parameters for adequate patient selection for ²²³Ra therapy.

The authors declare that they have no conflicts of interest.

Individual kidney depth determination is mandatory to assess split renal function in nephrography irrespective of age

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Keywords: Kidney depth -Split renal function -Nephrography

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Abstract

Nephrography having been introduced more than 60 years ago still now is one of the most frequently used and informative procedures in Nuclear Medicine. Although being considered a well standardized method, a worldwide study in 34 centers in 21 countries revealed that determination of split kidney function shows unacceptable high variation, particularly in patients with a relative kidney function below 30%. Furthermore, kidney depth usually is not considered. The calculation of kidney depth by various formulas available, each claiming to be more predictive, is different in races and does not allow individual information, which particularly in patients with a diseased kidney may become unreliable. We investigated 331 patients (167m, 164f) aged 1 to 76 years (84 of them being less than 20 years old), where kidney depth has been estimated by means of sonography as well as by a lateral view gamma camera image obtained immediately after the investigation. At the age of 10 years the kidney depth may vary already by 20%, in some patients increasing to 30% at the age of 20 and showing further increase with increasing age. In adults, >50% show a depth difference between right and left kidney of >1cm. There is an excellent correlation between sonographic and nephrographic kidney depth determination, at mean there was no difference between the kidney depth of the right hand and left hand side. Furthermore, we demonstrate that the incorporation of waist circumference instead of body mass index into the formulas is more precise. These findings indicate that the assessment of split kidney function particularly in patients with kidney disease, transplant donors and atypical localization irrespective of age should be mandatory in clinical routine.

The authors declare that they have no conflicts of interest.

Our solution for fusion of simultaneously acquired whole body scintigrams and optical images, as useful tool in clinical practice in patients with differentiated thyroid carcinomas after radioiodine therapy. A useful tool in clinical practice

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Keywords: Whole bodyscan with optical image -Differentiated thyroid carcinomas -After radioiodine therapy -Clinical practice

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Abstract

Objective: After radioiodine therapy of differentiated thyroid cancer (DTC) patients, whole body scintigraphy (WBS) is standard procedure before releasing the patient from the hospital. A common problem is the precise localization of regions where the iod-avid tissue is located. Sometimes it is practically impossible to perform precise topographic localization of such regions. **Methods:** In order to face this problem, we have developed a low-cost *Vision-Fusion* system for web-camera image acquisition simultaneously with routine scintigraphic whole body acquisition including the algorithm for fusion of images given from both cameras. For image acquisition in the gamma part of the spectra we used e.cam dual head gamma camera (Siemens, Erlangen, Germany) in WBS modality, with matrix size of 256x1024 pixels and bed speed of 6cm/min, equipped with high energy collimator. For optical image acquisition in visible part of spectra we have used web-camera model C905 (Logitech, USA) with Carl Zeiss® optics, native resolution 1600x1200 pixels, 34° field of view, 30g weight, with autofocus option turned "off" and auto white balance turned "on". Web camera is connected to upper head of gamma camera (GC) by a holder of lightweight aluminum rod and a plexiglas adapter. Our own *Vision-Fusion* software for image acquisition and coregistration was developed using NI LabVIEW programming environment 2015 (National Instruments, Texas, USA) and two additional LabVIEW modules: NI Vision Acquisition Software (VAS) and NI Vision Development Module (VDM). Vision acquisition software enables communication and control between laptop computer and web-camera. Vision development module is image processing library used for image preprocessing and fusion. Software starts the web-camera image acquisition before starting image acquisition on GC and stops it when GC completes the acquisition. Web-camera is in continuous acquisition mode with frame rate f depending on speed of patient bed movement v ($f=v/\Delta_{cm}$, where Δ_{cm} is a displacement step that can be changed in *Settings* option of *Vision-Fusion* software; by default, Δ_{cm} is set to 1cm corresponding to $\Delta_p=15$ pixels). All images captured while patient's bed is moving are processed. Movement of patient's bed is checked using cross-correlation of two successive images. After each image capturing, algorithm extracts the central region of interest (ROI) of the image, with the same width as captured image (1600 pixels) and the height that is equal to the Δ_p displacement in pixels. All extracted central ROI are placed next to each other in the overall whole-body image. Stacking of narrow central ROI introduces negligible distortion in the overall whole-body image. The first step for fusion of the scintigram and the optical image was determination of spatial transformation between them. We have made an experiment with two markers (point radioactivity sources of ^{99m}Tc pertechnetate 1MBq) visible in both images (WBS and optical) to find transformation of coordinates between images. The distance between point markers is used for spatial coregistration of the gamma and optical images. At the end of coregistration process, gamma image is rescaled in spatial domain and added to the optical image (green or red channel, amplification changeable from user interface). **Subjects:** We tested our system for 10 patients with DTC who received radioiodine therapy (8 women and two men, with average age of 50.10±12.26 years). Five patients received 5.55GBq, three 3.70GBq and two 1.85GBq. Whole-body scintigraphy and optical image acquisition were performed 72 hours after application of radioiodine therapy. **Conclusion:** Based on our first results during clinical testing of our system, we can conclude that our system can improve diagnostic possibility of whole body scintigraphy to detect thyroid remnant tissue in patients with DTC after radioiodine therapy.

The authors declare that they have no conflicts of interest.

Characteristics, socioeconomic status and ethnic variations of primary idiopathic macular hole repair in vitreoretinal centers in the United Kingdom

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Keywords: Socioeconomic status - Ethnic variations - Primary idiopathic macular hole - Vitreoretinal centers in U.K

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Abstract

Objective: The purpose of this multicentre retrospective study was to investigate the characteristics and role of ethnicity and socioeconomic status amongst patients with idiopathic macular holes (IMH) and the surgical outcome. **Subjects and Methods:** Consecutive patients undergoing primary IMH surgery at three vitreoretinal units in the UK (King's College Hospital, London, UK, Western Eye Hospital, London, UK, Sunderland Eye Infirmary, Sunderland, UK) between January 2007 and May 2017 were included. The main outcome measure was anatomical closure of IMH. **Results:** Two hundred and thirty three primary IMH surgeries were included. All patients underwent pars plana vitrectomy, internal limiting membrane peeling, and gas tamponade. 69.10% of patients were European Caucasian, 6.44% were Asian, and 24.46% were Afro-Caribbean. The mean base macular hole diameter (BD) was 475.5mcm. Mean BD was 432.2mcm in European Caucasian patients, 481.3mcm in Asians (P=0.005), and 505.61mcm in Afro-Caribbeans (P=0.006). Regression analysis demonstrated that BD and Afro-Caribbean ethnicity were independent significant risk factors for surgical failure. Those who have longer duration of symptoms (Afro-Caribbeans) and live in more deprived places (Afro-Caribbeans) in England were found to have lower success rate on macular hole closure. **Conclusions:** Asian and Afro-Caribbean patients present with larger IMH than European Caucasians. In addition to IMH base diameter, black origin and lower socioeconomic status are independent risk factors for surgical failure. This study presents a large population-based data analysis on ethnic variation in macular holes and may assist in the management and predicting the surgical outcome.

The authors declare that they have no conflicts of interest.

Vitrectomy with fibrovascular membrane delamination for proliferative diabetic retinopathy with or without preoperative Avastin

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Keywords: Vitrectomy -Fibrovascular membrane delamination -Diabetic retinopathy -Preoperative Avastin

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Abstract

Objective: To describe and compare 1. The changes in intraretinal microstructure using serial spectral domain optical coherence tomography (SD-OCT) preceding and following pars plana vitrectomy and delamination of fibrovascular membranes and 2. Intraoperative and postoperative complications in patients with proliferative diabetic retinopathy (PDR) who had preoperative Avastin (group A) or not (group B). **Subjects and Methods:** This retrospective, interventional case series includes 113 eyes. Outcome measures included LogMAR distance best-corrected visual acuity (BCVA), SD-OCT integrity of photoreceptor inner and outer segments junction (IS/OS), and integrity of external limiting membrane (ELM), intraoperative and postoperative complications. **Results:** Pre-operative central macular thickness (CMT) was significantly correlated with the final post-operative LogMAR BCVA in group A. Both groups were also categorised into three sub-groups based on post-operative IS/OS integrity (group 0: IS/OS intact; group 1: IS/OS irregular but not completely disrupted; group 2: IS/OS completely disrupted). Mean BCVA improved significantly and IS/OS integrity and ELM integrity postoperatively, were significantly and positively correlated with final BCVA in group A. Intraoperative complications such as iatrogenic tears and haemorrhage and postoperative such as vitreous haemorrhage and neovascular glaucoma were significantly less in group A compared to group B. **Conclusions:** Pre-operative Avastin reduces the risk of intraoperative and postoperative complications and results in better postoperative anatomic and functional outcomes in fibrovascular delamination surgery for patients with PDR.

The authors declare that they have no conflicts of interest.

The neurophysiological and evolutionary considerations of close combat: A modular approach

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Keywords: Close combat -Modular approach -Neurophysiological view

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Abstract

Close Combat may be identified as a physical confrontation involving armed or unarmed fighting, lethal and/or non-lethal methods, or even simply escape from and/or de-escalation of the confrontation. Our model hypothesizes that distinct areas of the brain are utilized for specific levels of violence, based on evolutionary criteria, and that these levels of violence bring into effect distinct physiological criteria and kinesiology. This model is outlined similar to Paul D. MacLean's triune brain theory, but incorporates distinct processes inherent to the autonomic nervous system (i.e. a "quadrune brain"), and correlates the observed level of violence to a particular response to a specific neural complex associated with very specific reactive kinesiology in the body. Our hypothesis is that the reverse also holds true: specific movements, scenarios and breathing will "activate" corresponding neural centres that in turn correlate to a respective level of violence. Moreover, socio-historic records bear out the premise that specific behavioural violations of social protocols act as "triggers" for assaultive and lethal force involving weapons, and it is very likely that these triggers (and the concomitant decision to engage in assault or lethal force) are processed through neural centres in what McLean has described as his "limbic system." A modular system of close combat is being researched and developed in accord with the above, readily adaptable to the level of violence professional peacekeepers and law enforcement officers may encounter in the course of their duties, but also directly relevant to the self-protection needs of civilians and youth. Distinct modular training regimes have been identified and developed for situations involving escape from a threat, submission of an adversary, and assaultive/lethal force, with the hope of strengthening neural bridges between the four neural complexes postulated in our model, and therefore via these bridges limiting adverse reactions to the psyche from combat stress.

The authors declare that they have no conflicts of interest.

Brachytherapy in soft tissue tumours: an interdisciplinary challenge!

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Keywords: Plastic surgery -Flaps -Brachytherapy -Soft tissue tumours

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Abstract

Objective: Interdisciplinary work including surgery and additive radiotherapy is often needed for the therapy of tumours. Beneath this, brachytherapy is an important part of the radiotherapy. It was first used over 100 years ago and is in regular use after the development of afterload technology in the early 1970s. Today it is often used in different tumour therapies, for example in soft tissue sarcoma [2, 4, 6, 8] or breast tumours [1, 5, 7], in order to decrease the risk of local recurrence. Concerning its benefits, higher doses could be used because of the localized effect with equivalent local control rate and less toxicity of treatment [3]. Moreover, brachytherapy can also shorten the treatment time from 5-7 weeks to some days and is better reconcilable due to its localized effects, thus reducing side effects, as radiation-induced reactions, teleangiectasia and brosis [3]. Precondition for application of brachytherapy is the need of a good soft tissue coverage and wound healing. Therefore, good interdisciplinary cooperation between plastic surgery and radiotherapy is important. After wide surgical resection reconstruction with different kind of flaps are often required, for achieving early wound healing and fast start of radiotherapy. **Patients and Methods:** Between 2011 and 2017 we applied brachytherapy to 13 patients with soft tissue sarcomas and other tumours like merkel-cell-carcinoma, schwannoma, and breast cancer. The treatment consisted of tumour resection, intraoperative insertion of brachytherapy catheters and after that brachytherapy alone or in combination with external beam radiotherapy. In half of the patients a reconstruction with different flaps was required, including pedicled trapezius flap, musculus latissimus dorsi flap and radial forearm flap; in some cases nerve and tendon reconstruction for better function and faster wound healing and so faster start of postoperative brachytherapy was also needed. The mean age of the patients was 55 years (± 19) and we could start brachytherapy after 3-21 days after the operation, with a mean start on day 8 ± 5 postoperatively. Three patients received additional percutaneous radiotherapy. The patients who received only brachytherapy got a dose of 2, 5 or 3Gy twice daily, with a mean total dose of 31 ± 3 Gy. **Conclusion:** Multidisciplinary work, including surgery as the main procedure and radiotherapy additionally, is needed for a successful treatment of soft tissue tumours. Depending on the type and the stadium of tumour plastic and reconstructive surgery provides soft tissue coverage, faster wound healing and the chance for limb salvage; on the other hand, additive brachytherapy contributes to a good tumour control. Therefore, a close collaboration between the two specialties is of particular importance, in order to improve the effectiveness of the therapy and the postoperative quality of life of the patient.

The authors declare that they have no conflicts of interest.

Stem cells therapy: the future in the management of systemic sclerosis? A case report

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Keywords: Stem cells -Scleroderma -Finger amputation

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Abstract

Objective: Systemic sclerosis (SSc) is a connective tissue disorder of unknown etiology, with heterogeneous clinical manifestations and chronic and often progressive course. The diffuse cutaneous form of SSc (dcSSc) is characterized by thickening of the skin (scleroderma) and distinctive involvement of multiple internal organs. Patients with limited cutaneous SSc (lcSSc) generally have long-standing Raynaud's phenomenon before other manifestations of SSc appear. Over the last decade the interest of adipose-derived cell therapy in regenerative medicine has increased continuously. In compare to bone marrow-derived mesenchymal stem cells (MSCs) adipose tissue-derived stem cells (ADSCs) are considered to be ideal for application in regenerative medicine. Zuk et al., introduced a multipotent, undifferentiated, self-renewing progenitor cell population isolated from adipose tissue, called processed lipoaspirate (PLA). However, subcutaneous injections of autologous adipose tissue-derived stromal vascular fraction (ADSVF), which is known to contain mesenchymal stem cells, in hands of Patients with scleroderma for enhancing their impaired hand function is still in an experimental stage, although there are already promising results of the therapy. Commonly available therapeutic options for hands of Patients with systemic sclerosis, vasodilator drugs and physiotherapy, have many restriction and limited effects. **Materials and Methods:** A 62 years old woman with scleroderma presented with progressive digital necrosis, ulceration, gangrene and impaired wound healing, despite of conventional therapy with vasodilator drugs. Water-jet-Assisted Liposuction (Body-jet® evo, human med AG, Schwerin, Germany) of subcutaneous abdominal fat was carried out under general anesthesia by an experienced surgeon. Autologous adipose tissue-derived stromal vascular fraction (ADSVF) was harvested by in a single-use Q-graft® collector (human med AG, Schwerin, Germany). Cells were centrifuged in 400G for 5 minutes and cell pellets were aspirated carefully in a 20mL syringe filled with 0.9% NaCl. A total of ca. 2.72 million cells have been isolated. Meanwhile middle phalangeal amputation of digit 2, 3 and 4 of the left hand were performed, without closing the skin of the amputation stumps. The SVF cell suspension was injected subcutaneous into the area of metacarpophalangeal joints in both hands, as well as into the amputation stump of the left middle finger, and under a skin necrosis in the right hand. **Results:** The therapy was good tolerated by the patient, with absence of adverse reactions. No infection was observed, despite open amputation. Three weeks after the stem cell therapy, no need to further amputation was demonstrated. The patient is still under regular clinical observation, in order to determine the long term effects of the therapy. **Conclusion:** Application of isolated adipose tissue-derived stem cells seems to be a very promising procedure in the treatment of the manifestation of systemic sclerosis. However, more clinical and experimental studies are required, in order to understand the exact mechanisms of action and standardize the therapy.

The authors declare that they have no conflicts of interest.

Right putamen and age are the most discriminant features to diagnose Parkinson's disease by using ^{123}I -FP-CIT brain SPET data by using an artificial neural network classifier, a classification tree (CIT)

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Keywords: Right putamen -Diagnosis Parkinson's disease - ^{123}I -FP-CIT brain SPET data -Artificial neural network classifier

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Abstract

Objective: The differential diagnosis of Parkinson's disease (PD) and other conditions, such as essential tremor and drug-induced parkinsonian syndrome or normal aging brain, represents a diagnostic challenge. ^{123}I -FP-CIT brain SPET is able to contribute to the differential diagnosis. Semiquantitative analysis of radiopharmaceutical uptake in basal ganglia (caudate nuclei and putamina) is very useful to support the diagnostic process. An artificial neural network classifier using ^{123}I -FP-CIT brain SPET data, a classification tree (CIT), was applied. CIT is an automatic classifier composed of a set of logical rules, organized as a decision tree to produce an optimised threshold based classification of data to provide discriminative cut-off values. We applied a CIT to ^{123}I -FP-CIT brain SPET semiquantitative data, to obtain cut-off values of radiopharmaceutical uptake ratios in caudate nuclei and putamina with the aim to diagnose PD versus other conditions. **Subjects and Methods:** We retrospectively investigated 187 patients undergoing ^{123}I -FP-CIT brain SPET (Millenium VG, G.E.M.S.) with semiquantitative analysis performed with Basal Ganglia (BasGan) V2 software according to EANM guidelines; among them 113 resulted affected by PD (PD group) and 74 (N group) by other non parkinsonian conditions, such as Essential Tremor and drug-induced PD. PD group included 113 subjects (60M and 53F of age: 60-81yrs) having Hoehn and Yahr score (HY): 0.5-1.5; Unified Parkinson Disease Rating Scale (UPDRS) score: 6-38; N group included 74 subjects (36M and 38 F range of age 60-80 yrs). All subjects were clinically followed for at least 6-18 months to confirm the diagnosis. To examine data obtained by using CIT, for each of the 1,000 experiments carried out, 10% of patients were randomly selected as the CIT training set, while the remaining 90% validated the trained CIT, and the percentage of the validation data correctly classified in the two groups of patients was computed. The expected performance of an "average performance CIT" was evaluated. **Results:** For CIT, the probability of correct classification in patients with PD was $84.19 \pm 11.67\%$ (mean \pm SD) and in N patients $93.48 \pm 6.95\%$. For CIT, the first decision rule provided a value for the right putamen of 2.32 ± 0.16 . This means that patients with right putamen values < 2.32 were classified as having PD. Patients with putamen values ≥ 2.32 underwent further analysis. They were classified as N if the right putamen uptake value was ≥ 3.02 or if the value for the right putamen was < 3.02 and the age was ≥ 67.5 years. Otherwise the patients were classified as having PD. Other similar rules on the values of both caudate nuclei and left putamen could be used to refine the classification, but in our data analysis of these data did not significantly contribute to the differential diagnosis. This could be due to an increased number of more severe patients with initial prevalence of left clinical symptoms having a worsening in right putamen uptake distribution. **Conclusion:** These results show that CIT was able to accurately classify PD and non-PD patients by means of ^{123}I -FP-CIT brain SPET data and provided also cut-off values able to differentially diagnose these groups of patients. Right putamen uptake values resulted as the most discriminant to correctly classify our patients, probably due to a certain number of subjects with initial prevalence of left clinical symptoms. Finally, the selective evaluation of the group of subjects having putamen values ≥ 2.32 disclosed that age was a further important feature to classify patients for certain right putamen values.

The impact of lung perfusion scintigraphy in the emergency management of patients with suspected pulmonary embolism

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Keywords: Lung scan -Q scan -Pulmonary embolism -Emergency

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Abstract

Background and Aim: Pulmonary Embolism (PE) is an emergency condition that requires immediate treatment. As the symptoms and the risk factors are nonspecific, PE differential diagnosis is often required. Even if angio-CT is considered the gold standard for PE diagnosis, the frequent allergic condition and/or chronic renal failure of patients make, in most cases, not possible the use of contrast enhancement in emergency with even more increasing use of Lung Perfusion Scintigraphy (LPS), as a simple and fast examination with no preparation/contraindication. The aim of our study is to highlight the role of LPS in the management of patients (pts) with suspected PE admitted to our hospital as an emergency in the "on-call" 24 hours (hrs) service. **Materials and methods:** We retrospectively revised 2166 LPS performed for suspected PE from January 2012 to December 2016, of which 1730 were urgent. LPS was performed according to the EANM guidelines in the 4 standard projections. The relation between symptoms, risk factors, dosage of D-dimers, other imaging diagnostic tools and LPS results were evaluated by contingency tables and Odds Ratio (OR). **Results:** The origin unit of pts was: emergency (56.7%), pneumology (10.8%), neurology (4.8%), internal medicine (6.5%), surgery (5.2%), cardiology (3.3%) and other departments (11.2%). 59.3% of the examinations were performed during the on-call 24 hrs service. Symptoms were chest pain in 39%, dyspnea in 75%, cough in 22%. In 34% were present two symptoms, while 10% were asymptomatic. D-dimer dosage before LPS was increased in 97% (>500 ug/L). 55.5% had only one risk factor, 18.7% had two or more risk factors. 75.5% of pts had previously performed another diagnostic exam (Chest X-ray in 57%, chest CT in 8.4%, both in 10.1%) while 24.5% did not undergo previous diagnostic exam. The Chest X-ray and/or chest CT resulted negative in 25.4%, suspected for PE in 24.4%, non-specific with pleural effusion in 18.8% and non-specific with inflammatory interstitial diseases in 31.4%. LSP resulted positive for PE in 17% and then treated; LPS resulted negative in the remnant 83%. LPS results were associated with those of CT and Rx ($\chi^2=17.5$ P=0.001). LPS resulted positive in 13.8% with negative Chest X-ray and/or CT, in 23.4% with suspected PE, in 15.2% with pleural effusion and in 14.7% with inflammatory interstitial diseases. Furthermore LPS resulted positive in 17.32% without previous diagnostic exam. The increased value of D-Dimers (>500ng/ml) observed in 97% was not predictive of PE (OR=0.598 P=0.152). A similar result was observed for cough (OR=1.146 P=0.395) and chest pain (OR=0.927 P=0.601). Conversely, dyspnea appeared to be a significant symptom of PE (OR=1.596 P=0.003). The presence of risk factors is not predictor of PE detected by LPS (OR=1.297 P=0.089). **Conclusion:** LPS has a key role in the early diagnosis but even more in the exclusion of PE, optimizing the management of pts who do not require admission to intensive care unit with high costs and limited availability. LPS confirms to be a simple, quick and inexpensive examination. It does not require preparation and has no side effect so it can be performed in all types of pts including pregnant women, politraumatized and complicated patients, with great impact on resource optimization for intensive care units. Our multi-year and large-scale experience related to a metropolitan area suggests that, to date, given the great demand and relevance of this examination, Nuclear Medicine Units must necessarily be organized in order to provide LPS as emergency in on-call 24 hrs service.

He was called Fellow of Medical Olympicus Association for his immense contribution to Medicine and to the Association

Curriculum Vitae

Alavi Abass

Professor of Radiology, Director of Research Education



Education/Training

1. University of Tehran, School of Medicine: MD, 1964, Medicine, 2. A. Einstein Medical Center, Philadelphia: Intern, 1966, Rotating Internal Med, 3. VA Medical Center: Resident, 1968, Internal Medicine, 4. Hospital of the University of Pa: Fellow, 1969, Hematology-Oncology, 5. Beth Israel Hospital, Boston: Resident, 1970, Radiology.

Personal Statement

Dr. Alavi has spent the past 4 decades of his career in developing novel techniques that have substantially influenced the evolution of the discipline of molecular imaging. Among his accomplishments, the introduction of ¹⁸F-Fluorodeoxyglucose (FDG)-positron emission tomography (PET) has truly revolutionized the field and has allowed this powerful modality to expand to daily practice of medicine worldwide. The impact of using FDG-PET along with CT (and soon MRI) in managing so many serious diseases and disorders is unparalleled by any other technique in recent history. He has received many awards for his outstanding contributions to the field of molecular imaging. Currently, he is actively involved in conducting research on a full time basis.

Positions and Honors

Positions and Employment

1971-73 Fellow, Nuclear Medicine, Hospital of the University of Pennsylvania, Philadelphia. **1973-74** Instructor in Radiology, Dept. of Radiology, Univ. of Pennsylvania School of Medicine. **1974-77** Assistant Professor Radiology, Dept. of Radiology, Univ. of Pennsylvania School of Medicine. **1977-82** Associate Professor of Radiology, Dept. of Radiology, Univ. of Pennsylvania School of Medicine. **1979-06** Chief, Division of Nuclear Medicine, Hospital of the University of Pennsylvania. **1979-91** Co-Director, Positron Emission Tomography Center, University of Pennsylvania. **1991-** Medical Director, Positron Emission Tomography Center, University of Pennsylvania. **1982-** Professor of Radiology, Department of Radiology, University of Pennsylvania School of Medicine. **1984-** Associate Director, Center for the Study of Aging, University of Pennsylvania. **2006-** Director of Research Education, Department of Radiology, University of Pennsylvania

Awards, Honors and Membership in Honorary Societies

1971-73 Postdoctoral NIH Fellowship in Nuclear Medicine. **1982** Member, Steering and Executive Committees, Prospective Investigation of Pulmonary Embolism Diagnosis, Chairman, Nuclear Medicine Working Group, NIH.-**1984** Who's Who, Frontiers of Science and Technology.-**1985** Fellow of the Center for

the Study of Aging, University of Pennsylvania Fellow, American College of Nuclear Physicians. **1992** Fred Joliot Professorship at Orsay, France.-**1992** Evan-Jone Memorial Lecture, U of MDS, London.-**1994** Taylor Lectureship award - Dept. of Psychiatry, University of Maryland.-**1997-2000** Vice Chairman & Chairman-elect American Cancer Society Study Section.-**1997** Sarabhai Memorial Oration award - Society of Nuclear Medicine of India.-**1999** The 11th Annual Berson-Yalow Award - Greater NY Chapter Society of Nuclear Medicine.-**1999**- Best Doctors in America. 2001 One of two Finalists, "Most influential Radiology Researchers".-**2001** Distinguished Service Award, Indo-American Society of Nuclear Medicine.-**2001** Taplin Pioneer Award.-**2004** Georg Charles de Hevesy Nuclear Pioneer Award, 51st Annual Meeting of the SNM.-**2005** Member, (Ad Hoc Chairman), NIH/CSR/ZRG1 SBIB-Q50, Chairman, NIH/CSR/ZRG1 SBIB-Q13.-**2006** Mentor of the Year Award (2006&2014), American College of Nuclear Medicine Physicians Fellow, World Innovation Foundation, Honorary Doctoral Degree (*Laurea ad honorem*, Medicina e Chirurgia), University of Bologna, Italy, Consultant Editor (ongoing), PET Clinics.-**2007** Honorary PhD Degree in Molecular Biology, University of Shiraz, Member, Honorary Editorial Boards, Multiple Journals.-**2008** Life Member, American Board of Nuclear Medicine, Honorary Doctoral Degree (DSc), University of the Sciences in Philadelphia, The most published and most cited faculty at PENN. **2010** Member, College of CSR Reviewers, National Institute of Health.-**2012** Benedict Cassen Prize for Research in Nuclear Medicine, Annual Meeting of the SNM, Featured Speaker on the occasion for dedicating Brookhaven National Laboratory as Historic Chemical Landmark by the American Chemical Society for introducing FDG to medicine.-**2014** Mentor of the Year, American College of Nuclear Medicine.-**2014** Marquis Who's Who in America 2014 (68th Edition). **2015** Recipient, honorary MD degree from Tabriz University of Medical Sciences, Tabriz, Iran.-**2015** Honorary citizen of City of Tabriz, Iran.-**2015** Alavi International Institute of Aging in Tabriz, Iran, established in his name.-**2016** Recipient, first Gold Medal from Ministry of Health (NIMAD), Tehran, Iran.-**2016** Recipient, honorary Doctor of Medicine degree from the Medical University of Gdansk, Gdansk, Poland.-**2016** Recipient, honorary doctorate degree from the University of Southern Denmark.-**2016** Among top 1% Scientists worldwide.-**2017** Gold Medal Recipient, American College of Nuclear Medicine.-**2017** h-Index of 116 and more than 55,000 citations, Google Scholar.-**2017** RG score 55.41

Contributions to Science

Introduction of Molecular Imaging with PET and SPECT

In 1973, along with my colleagues David Kuhl, and Martin Reivich I introduced the concept of the synthesis of ¹⁸F-Fluorodeoxyglucose (FDG). In 1976, I conducted the first human study with FDG by generating tomographic images of the brain and planar whole body scan. During the past 40 years, we have secured multiple grants from the NIH and other agencies to carry out numerous research projects, starting with brain imaging and expanding to other disciplines including oncology, infectious diseases, and inflammatory disorders. At PENN, we have been either the first or among the first in initiating these research projects. The introduction of FDG has transformed the field of molecular imaging to a vivacious and active specialty and is rapidly changing the practice of medicine in many domains. Our work with PET has not been confined to imaging with FDG and has dealt with many compounds related to receptor imaging, gene therapy, and novel approaches such as the detection of hypoxia and other molecular abnormalities.

Additionally, I have been involved with SPECT imaging from the early days of my career in imaging and have introduced new techniques with this modality for over 45 years. Particularly, my team and I have been pioneers in introducing novel SPECT-based radiopharmaceuticals by synthesizing dopaminergic and other tracers labeled with I-123 and other radionuclides in collaboration with investigators at the Wistar

Institute and other organizations. I also have been heavily involved in developing radiolabeled antibodies for both diagnostic and therapeutic purposes. Particularly, I was among the first to use radiolabeled mononuclear antibodies to detect clots and cancer. I was also among the first investigators to use therapeutic radiolabeled antibodies for treatment of lymphomas and solid tumors.

Related references are available.

Molecular Imaging of the Brain

I have extensively studied the effects of aging on brain function with FDG and other novel agents for the past 35 years. Also, I was among the first to study patients with Alzheimer's disease and other CNS disorders with FDG. Similarly, along with colleagues at the NIH, we were the first to examine patients with brain tumors which was the first human use of this compound in oncology. During the 1980s, our group was the first to use FDG to determine the deleterious effects of head injury and its recovery. We have heavily investigated the role of PET in psychiatric disorders including schizophrenia, depression and bipolar disorders. We have used SPECT-based tracers which has been unique to PENN. In addition, our group was among the first to explore the possibility of amyloid imaging in Alzheimer's Disease with both SPECT and PET.

Related references are available.

FDG-PET applications in Cancer

Our interest in oncology began with examination of brain tumors. Soon after the introduction of body imaging, we have investigated applications of FDG in various cancers including those of breast, lung, thyroid, GI, skin, hematologic system, and pediatric malignancies, and sarcomas. We have been interested in early diagnosis and optimal staging of cancer, assessing response to treatment and detection of recurrence with novel techniques. We have worked with almost every group at the Hospital of the University of Pennsylvania (HUP) and Children's Hospital of Philadelphia (CHOP), including Radiation Oncology, in exploring the role of PET in various organs during the past three decades. Our innovative research in oncology has transformed the practice of medical and radiation oncology.

Related references are available.

FDG-PET applications in infection/inflammation including atherosclerosis

For the past 20 years, we have explored the role of FDG in assessing infection and inflammation. Particularly, we have been interested in orthopedic applications. This includes detection of osteomyelitis as well as infected prostheses in the hips and knees. Our work in inflammation has dealt with detecting and quantifying disease activity in autoimmune diseases such as sarcoidosis, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, and atherosclerosis. We have demonstrated the critical role of FDG imaging in accurate diagnosis of both infection and inflammation. This is becoming valuable in testing the efficacy of modern biological agents. Since 2000, our research in the cardiovascular system has focused on detecting plaques in the major arteries. We were the first to describe this phenomenon that has now been confirmed in other laboratories. Since FDG is taken up by macrophages in the plaques, these lesions are readily visualized in major arteries. We have conducted multiple studies in atherosclerosis. We have established that inflammation outside the cardiovascular system is associated with increased risk of atherosclerosis. This includes patients with psoriasis, HIV/AIDS and rheumatoid arthritis. Furthermore, we were the first to describe the role of ¹⁸F-Sodium Fluoride (NaF) PET imaging in detecting calcification in the heart and major vessels. This appears to be a novel approach for early detection of calcification and

may replace CT-based diagnosis.

Related references are available.

Novel Quantitative Techniques with PET Imaging

I have also been a pioneer in the quantification of PET data including partial volume correction, global disease assessment, dynamic or multiple time point imaging with FDG. It is apparent that correcting for partial volume effect is a must for accurate quantification of uptake of various radiotracers including FDG in malignant and benign lesions. Similarly, measuring global disease activity is essential for assessing response to therapeutic interventions. The use of dynamic imaging with FDG and other tracers introduced by our group allows investigators to determine tumor biology and heterogeneity which is the major factor for lack of response to treatment of cancer.

Related references are available.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/1tm-FMkBWk9QS/cv/174899/>

Research Support-Ongoing Research Support: -Evaluating the impact of cocaine use and HIV infection in arterial wall inflammation by FDG-PET/CT imaging. - A Trial to Determine the Effect of Psoriasis Treatment on Cardiometabolic Disease. - Optimized Arterial Spin Labeling MRI for Cognitive Decline

Completed Research Support: - Assessment of Cardiovascular and Central Nervous System Complications of HIV/AIDS by PET/CT Imaging. - Intellectual and Developmental Disabilities Research Center-Neuroimaging Core. - Phase 2, Open-Label, Imaging Trial of I-124-CLR1404 in Patients with Newly Diagnosed or Recurrent Glioblastoma. - Sensitivity and Specificity of [124I]FIAU PET-CT Scanning in Detecting Prosthetic Joint Infection in Patients Presenting with Pain in a Prosthetic Knee or Hip Joint (BVD002). - Exploration of the use of [124I]FIAU Positron Emission Tomography-Computed Tomography Scanning in Diagnosing Osteomyelitis in Patients with Diabetic Foot Infection (BVD003). - Obesity & OSA: Understanding the importance of tongue fat and metabolic function

He was awarded the merit of honor for his immense contribution to Medicine and Medical Technology

Curriculum Vitae

Anastasios Johannes Tousimis

Professional and Academic Biographic



Place of birth: Agra-Edessa, Macedonia, Greece. United States of American citizen.

Education: Agra-Edessa Elementary and High School. Greece-Aristotelis University Medical School. Thessaloniki, Macedonia-University of Pittsburg, PA. (Chemistry and Biophysics), Max A. Lauffer (Biophysics) and David Holiday (Physics)-Armed Forces Institute of Pathology (AFIP). Washington, D.C (Biophysics), Ernst Goodpasture, Gustav Klinck and Richard Follis-National Institutes of Health (NIH). Bethesda, MD, David B. Scott and Ralph W.G Wyckoff (X-ray Physics)-George Washington University Graduate School. Washington, D.C (Biophysics), Benjamin D. vanEvera.

Positions Held

1954-1956 Section Chief, Walter Reed Army Institute of Research (WRAIR), Washington, D.C

General Dr. Joseph Smadel. Viral and Rickettsial Diseases

1956-1961 Branch Chief, AFIP Cell Pathology and Biophysics.

1961 Associate Adjunct Professor and Professor, George Washington University Graduate and Medical School

1961-1993 Consultant and Lecturer in Cell Pathology and Biophysics to Research Laboratories and Universities in the USA and Europe:

AFIP (1961-1991) and Woods Hole Marine Biological Laboratories, Massachusetts

University of Paris, Orsay, France with Professor Raymond Castaign and Professors Galle and Slodjian (1964-1980) and University of Cambridge, England

Awarded numerous research grants from NIH and the U.S. Geographical Surveys to pursue basic research as well as hydrocarbon formation in mud deposits in Florida, USA.

1963 Appointed by President John F. Kennedy as a member of the Scientific Planning Committee for NASA's Apollo Program. Present with Von Braun at the testing of Saturn rockets that took the first man to the moon. Von Braun was an admirer of Aristotle.

1965-1999 Director of Tousimis Biodynamics Laboratory and President and CEO of Tousimis Research Corporation.

1999-present Emeritus Professor, Biodynamics Laboratory.

Highlights of Research

1951-1952 Freeze drying bacteria and biological cells for transmission electron microscopy (TEM) for the preservation of the 3-D structure materials.

1952-1954 Participated in the preparation of the polio vaccine at the University of Pittsburg for clinical trials.

1954-1968 Biophysical characterization and identification of five new viruses: Adenovirus types 4&7 (Summer 1954), Feline Rhinotracheitis Virus (1958), Bovine Rhinotracheitis Virus (1959) and Mouse Leukemia Virus (1968), among others, using his own biophysical methods.

1957 First studies of electron probe microanalysis in medicine and biology.

Additional Research Highlights *in his own words*

Ultrastructural and electron probe x-ray microanalysis studies on numerous human and animal tissues, both normal and pathologic. Organization of the first conference on electron probe x-ray microanalysis and chairman of the Committee, Cosmos Club, Washington, D.C, to establish the Microbeam Analysis society (MAS) in 1966.

Developed numerous instruments starting with the first electron probe x-ray microanalyzer to be used in biologic and medical studies (his PhD) in addition to accessory instrumentation such as: critical point dryers (since 1955), shadow casters (1951) and sputtering devices (1954-used in the preparation of samples for scanning electron microscopy. The critical point dryers are used for MEMS (micro mechanical systems) and NANO devices. Used worldwide today in academia, production and commercial Laboratories. Developed the supercritical point dryers for the preparation of MEMS and NANO devices.

The iPad, iPhone and image stabilization use our Tousimis instruments in preparing the chips for them (MEMS).

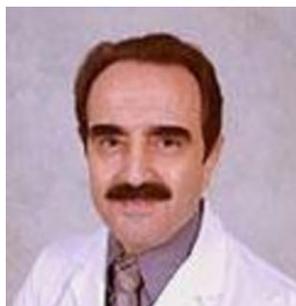
The Tousimis-Lauffer Distinguished Annual Lecture Series at the University of Pittsburg was established in 2006.

A complete list of Dr. A. J. Tousimis' publications in refereed articles, journals and books is available.

He was awarded the merit of honor for his immense contribution to Medicine

Curriculum Vitae

**George N. Sfakianakis,
MD, PhD, MS**



George N. Sfakianakis, MD, PhD, MS is an Emeritus Professor in the Department of Radiology at the University of Miami Miller School of Medicine and an Honorary Professor at the School of Medicine of the Aristotle University of Thessaloniki, Greece. He is a Nuclear Medicine specialist with training in Internal Medicine/Endocrinology and Nuclear Medicine and over 40 years of experience in the practice, administration, research and teaching of Nuclear Medicine.

George N. Sfakianakis was born in Athens, Greece in 1938 and obtained his M.D. degree in 1962 from the Aristotle University of Thessaloniki School of Medicine as a Cadet in the Military Academy. He served in the Greek Army honorably for more than 18 years both in Greece and in Cyprus. After graduation from medical school, he completed his training in Internal Medicine at the same institution and obtained board certification. While in training, he also obtained a doctorate degree. Subsequently, he was awarded a three year scholarship by the Greek State Scholarships Foundation (IKY) to study abroad, and was able to receive a three year fellowship in Nuclear Medicine and Endocrinology at Ohio State University (OSU) in Columbus, Ohio, USA. He began his studies there in 1970. While a fellow, George N. Sfakianakis also enrolled in a graduate program at OSU and obtained a Master's of Science degree in Physiology. After the completion of his studies, he successfully passed the examination and received board certification in Nuclear Medicine. He then returned to Greece in 1973.

Due to the political circumstances under a military dictatorship in Greece at that time, Dr. Sfakianakis went back to the USA in early 1974 and became involved with Greek and American groups opposing the dictatorship. His activities cost him his position in the military. Years later, after the fall of the junta, he was exonerated and reinstated in the Greek Army and was honorably discharged in 1983.

While in the United States, he began a productive and most successful career. Initially, he was appointed an Assistant Professor of Nuclear Medicine at OSU and Columbus Children's Hospital and got involved with all aspects of his specialty. He became very active in his field and began to get recognition at the national level. In 1979 he was offered and accepted an appointment as an Associate Professor at the University of Miami School of Medicine in the Department of Radiology. Throughout his years there Dr. Sfakianakis dedicated his time and efforts to clinical practice (incorporating every new development), administration, research and teaching of all aspects of Nuclear Medicine. He rose quickly through the ranks, was promoted to professor of Nuclear Medicine and Pediatrics and became the Director of the Division of Nuclear Medicine in the Department of Radiology, a position he maintained until his retirement in June of 2016.

Dr. Sfakianakis received national and international recognition for his pioneering research activities--including funded and non-funded projects-- and excellence in clinical work in Nuclear Medicine as well as his innovative approach to training physicians in Nuclear Medicine. To that end and in addition to the regular trainees in his program, he became a mentor and supporter of Greek Nuclear Medicine doctors

by facilitating their placement in his program for advanced training in the newest modalities in the specialty. Since 1990, twelve such physicians came to Miami from Greece for training and then returned to their country where they are now leaders in the field.

George N. Sfakianakis was invited over one hundred times to present his work as a visiting professor and a speaker at national Nuclear Medicine conventions in the USA and also abroad. His body of scientific work includes over one hundred articles published in refereed medical journals, numerous published abstracts and proceedings of symposia, several chapters in nuclear medicine books and numerous scientific presentations and exhibits. In addition, he's had editorial responsibilities with major juried publications in his field. He has enjoyed and appreciates very much the tremendous changes and progress that occurred in Nuclear Medicine over the years and is thankful to have been a part of that transformation.

Dr. Sfakianakis met his wife Eleni Damoulaki-Sfakianaki, MD, MSPH, a Pediatrician-Neonatologist and Public Health Professor, while both were attending medical school- -and they have been together ever since. Together they raised three wonderful daughters one of whom became a lawyer while the other two became doctors-one of them a Nuclear Medicine specialist following in her father's footsteps. George and Eleni have two grandchildren.

An avid reader, and ballet, theater, opera and classical music lover, Dr. Sfakianakis also enjoys gardening, long walks and travel. And he is dedicated to promoting Greek culture and history. In 1981, he and his wife were two of the founding members of the Greek School "SOCRATES" and he served as the first president of the board. The school continues to be in operation to this date.

In Memoriam

Franz Gerstenbrand (1924-2017)-The world ambassador of Neurosciences

"I believe that an excellent man is more valuable than ten thousand others". Heracletus (fr. B 49a).

The Neurological sciences are deprived of the eminent professor of Neurology and honorary doctor of many universities Franz Gerstenbrand, who passed away peacefully on the 30th of June, 2017.

The late Professor was a prophetic authority in Neurosciences, who like the Pre-Socratic philosophers was characterized by a right and acute foresight in Neurology, having also the capacity to harmonize the precious deep knowledge of the Clinical Neurology with the rapid flow of the new data of the clinical and experimental investigation.

Franz Gerstenbrand, was born on 6th of September 1924 in Northern Moravia (Nordmähren). His birthplace was sanctified by the mission of the Greek brothers Saint Cyril and Methodius, the Thessalonian missionaries and scholars, who went in the Great Moravia in 862 AD, for evangelizing the Slavic people, devising also their alphabet, "Cyrillic" alphabet.

Franz Gerstenbrand graduated from Vienna University, "Cum Laude", in 1950, and immediately, he started postgraduate training in Neurology and Psychiatry at the same University, under the directorship of Prof. Hans Hoff. In 1976, he was elected Professor of Neurology and appointed head of the University Department of Neurology at Innsbruck University, where he stayed till his retirement in 1994. Under his directorship the department of Neurology became one of the best organized and functioned university departments in Europe.

Among the numerous awards and honorable distinctions he received Honorary Doctorate of the Charles University in Prague, in June 1997 and his Honorary Doctorate of the Medical School of Aristotelian University of Thessaloniki, on 27th of June 2003.

Franz was also the recipient of the Silver Medal for Achievements for the Republic of Austria, of the Honorary Cross for Science and Arts of the Austrian Republic, of the Valeriy-Gagarin-Medal of the Russian Space Organization, of the Honorary Medals of the Country of Tyrol and the Medical University of Innsbruck, of the award of the Southern Moravia for the Culture. In 2014, Franz was awarded the Gold Medal of the city of Graz. He was also the recipient of Lifetime Achievement Award by the WFN.

Franz Gerstenbrand was elected Honorary Member of the European Academy of Neurology. In addition, he was elected honorary member of the Senate of the University of Krems in June 2003. In November 2001, Franz was elected honorary president of the Austrian Society for Parkinson's disease, honorary president of the Austrian Neurological and Psychiatric Society, and honorary president of many European medical and neurological Societies.

Franz Gerstenbrand's main noble ambitious vision was the unification of the European Neurology. He was endeavoring to increase a peaceful, friendly, respectful and sincere collaboration among the European neurologists.

In 1962, Franz founded the Danube International Neurological Group, which later became a Society and started organizing the Danube Symposia, with the participation of neuroscientists coming from western and eastern countries of the Danube region. Franz Gerstenbrand, founded in Prague in 1989, the Pan European Society of Neurology, which was evolved to be the European Federation of Neurological societies (EFNS). EFNS was later a strong background for the foundation of the European Academy of Neurology in 2014, which unified all the European Neurological Societies in a common Forum.

In addition Franz succeeded in creating a Scientific Society for Neurorehabilitation in Austria and abroad, resulting in the foundation of World Federation of Neurorehabilitation.

Franz Gerstenbrand collaborated constantly with the Hellenic Society for the amelioration of the quality of life of chronic neurological patients, which was founded in Thessaloniki, in 2000. Franz Gerstenbrand was the honorary president of the ten international congresses, which were organized by the society and were held in Constantinople, Vienna, Alexandria, Odessa, Catania, Marseille, Thessaloniki, Delphi and Athens.

Franz Gerstenbrand's intellectual horizons were extended far beyond Neurosciences including philosophy, religion, politics, history, archeology, music, painting, eastern philosophy and culture, African studies and linguistics. He studied extensively Greek philosophy, history and civilization, he delved deeply in Aristotle and Stoics, he admired the classic Greek literature and art, the lyric poets, the ancient drama, the Greek science, and he studied profoundly the scrolls of the Hippocratic collection and the treatises of Galen.

He was gratefully honored and awarded by the Republic of the Union of Myanmar and he was elected President of the Austrian Myanmar Society.

Franz Gerstenbrand, as author gained a unique international recognition. He published 780 papers and 12 Textbooks and Monographs. To 1967 Franz described the traumatic apallic syndrome, in his habilitation treatise, entitled "Das traumatische apallische Syndrom, Klinik, Morphologie, Pathophysiologie und Behandlung". In addition his contribution in the treatment of Parkinson's disease was also of substantial importance, since he was among the first neurologists, after George Kozias, who introduced levodopa therapy in Parkinsonian patients.

He was characterized by profound respect to human dignity, devotion to righteous principles and genuine love and compassion to the patients. Franz was the invaluable mentor of many neurologists and researchers and the pure scientific and moral physician's prototype for young neurologists in Europe.

For Franz Gerstenbrand the following Heraclitus' axiom has an evident validity.

"Among all mortal men, the best are chosen to have an immortal glory". Heraclitus Fragm. DK B29.

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