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Comments from the particpants of the 5th International Medical Olympiad

"I express my heartfelt gratitude to the executive members of the Medical Olympicus Association for successfully organizing the 5th Medical Olympiad and providing local hospitality. It was indeed a memorable experience both at Academic and Recreational fronts".

Professor Devinder Dhawan

I would like to thank you very much for your kind invitation and in particular for the honor you offered me during the conference. In a country where you know that I love to be staying it was more than a pleasure for me.

Professor Helmut Sinzinger

"It was such a great experience. I was so so happy to participate. I cannot wait the next one"!

Dr Agata Pietrzak

"I would like to thank you for the excellent organization of the 5th International Medical Olympiad".

Professor Andreas C Petropoulos

"I want to congratulate the whole organization because the event as always was extremely interesting".

Professor Giuseppe Rubini

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Glucose and lipid abnormalities in patients with adrenal incidentalomas

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Keywords: Adrenal incidentalomas -Metabolic disorders -Glucose -Cholesterol -Insulin.

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Abstract

Adrenal incidentalomas (AI), defined as masses detected during imaging procedures of non-adrenal disorders, have become a common clinical problem that appear to have impairment of glucose and lipid metabolism. **Patients and Methods:** One hundred and ten patients (mean 53.5 age; 24-72), who were diagnosed with functioning and non-functioning AI, were assessed. Patients with hormone-secreting AI underwent biochemical evaluation regarding metabolic disorders. Data about hormone status (cortisol profile and DEX screening test), lipid profile, glycemia, insulinemia were evaluated. **Results:** This prospective study included 41 (37.28%) patients with non-functional and 69 (62.72%) with functional AI. Tumors associated with (sub)clinical Cushing's syndrome (functional AI) are considered to have higher cortisol concentration at 8h ($p=0.027$), 16h ($p=0.025$) and after DEX screening ($p<0.010$), compared to the controls. Patients with cortisol-secreting AI have significantly higher concentrations of cholesterol ($p=0.040$), triglycerides ($p=0.027$) and insulin ($p<0.01$) than controls. The patients with metabolic disorders have a significantly higher total cholesterol, triglyceride and insulin concentration ($p<0.001$) compared to controls. There was significant positive correlation between cortisol concentration after DEX screening and total cholesterol ($r=0.727$, $p=0.007$), triglycerides ($r=0.564$, $p=0.041$) and insulin ($r=0.957$, $p=0.043$) in the group with metabolic disorders. **Conclusion:** The present study demonstrates the patients with functional AI have significantly higher lipid, glucose and insulin concentration than controls. There was a significant positive correlation between metabolic parameters and cortisol concentration.

Introduction

Adrenal incidentaloma (AI) is term for adrenal tumors that has been accidentally detected during radiological visualization procedures, performed for reasons unrelated to adrenal diseases [1]. The incidence of AI has been reported from 4% in radiologic series [2] to 8% in autopsy series [1].

According to clinical manifestations AI are most often (in 60%-80% cases) functionally inactive [1]. Patients usually do not show any physical signs of adrenal hormonal excess. Recent studies, however, have shown that a high percentage of these tumors can be subclinicaly functioning, causing symptoms milder than those encountered in adrenal hypefunctioning syndrome, but still harmful to the patients [3]. According to literature high percentage of patients with AI have disorders of glucose and lipid metabolism (dyslipidemia, glucose intolerance, hyperinsulinemia). Adrenal incidentaloma also has been associated with hypertension, dyslipidemia, glucose intolerance, and obesity that are all parameters closely linked to insulin resistance (IR) [3]. Detailed investigation patients with AI found that 24% patients have subclinical Cushing's syndrome, 92% had hypertension, 50% obesity, 42% DM type 2, 50% abnormal serum lipid concentration, 61%-66% abnormal tolerance glucose [4, 5]. The aim of our study was to determine hormonal profile in patients with AI, as well as the relationship between hormone activity (primarily subclinical Cushing's syndrome) and glucose and lipid abnormalities in patients with AI.

Subjects and Methods

Subjects were drawn from a series of consecutive patients with adrenal mass discovered by abdominal ultrasound or computed tomography (CT) scan performed for the evaluation of adrenal unrelated diseases. Research was conducted as a cross-sectional study which included 110 patients with adrenal incidentaloma evaluated in the Center of Nuclear Medicine in Clinical center Kragujevac during 2017 and 2018. This study was approved by the Ethics Committee of the Clinical center Kragujevac. All study participants signed the informed consent.

Based on the results of laboratory findings patients were divided into two groups: subjects with functional, and subjects with non-functional incidentoma (controls). In the group of functional tumors, two subpopulations have been examined: subjects with disorders of glucose and/or lipid profile and subjects without disorders.

The study not included: patients under the age of 18; patients with an adrenal tumor detected during follow-up of extraadrenal malignancies; patients with associated pancreatic disorders and primary diabetes mellitus; pregnant women; patients with adrenal tumors which are present on some of the hereditary disorder of lipid metabolism.

The patients underwent routine laboratory evaluation, and a glycemia, insulinemia were analised after oral glucose tolerance test (OGTT). At 08 h, after a 10- to 12-h overnight fast and 30min after cannulation of an antecubital vein kept patent by slow infusion of isotonic saline, subjects received a 75-g OGTT. Blood samples were collected at 0, 30, 60, 90, and 120min for the measurement of plasma glucose and insulin concentrations.

All patients also underwent the following endocrine workup aimed to study the hypothalamic-pituitary-adrenal axis: 1) measurement of serum cortisol daily profile: the basal 8 AM serum cortisol level and the cortisol serum level at 16, 20, 24h following overnight dexamethasone suppression test (DEX screening):1 mg of dexamethasone ie, two tablets of 0.5mg) is taken orally at midnight, and a single blood sample is drawn at 8 AM 2)

adrenocorticotropic hormone at 8 AM 2) catecholamines, plasma metanephrine, 3) lipid profile (triglycerides, total cholesterol, HDL, LDL), were analised. The hormonal activity of the adrenal marrow was examined (blood level of adrenaline, noradrenaline and metanephrine).

Elevated values of cortisol was defined when concentration is: cortisol 8h ≥638nmol/L; cortisol 16-20h ≥388nmol/L; cortisol 24h ≥200nmol/L. Absence of cortisol suppression after DEX screening with 1mg dexamethasone (subclinical Cushing syndroma) was defined when cortisol concentration is: >150nmol/L and suppressed ACTH (reference range 7.2-63.3pg/mL).

Elevated catecholamine concentrations in the serum was defined when adrenaline is >27μg/dU, noradrenaline >97μg/dU; methanephrine > 65pg/mL.

Impaired glucoregulation was defined when fasting glycemia >6.1mmol/L, fasting insulinemia >17μIU/mL. Dyslipidemia was defined when: total cholesterol ≥5.2mmol/L, decreased HDL cholesterol (≤1.3mmol/L), elevated LDL cholesterol (≥3.5mmol/L) elevated triglyceride concentration (≥1.7mmol/L).

Assays

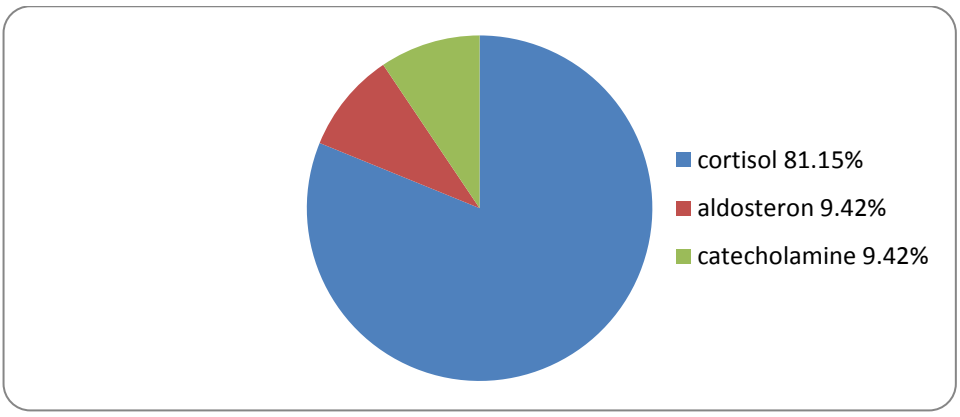
Hormonal variables were measured in the nuclear medicine laboratory by radioimmunoassay (cortisol CORT-CT2 Cisbio Bioassays) or immunoradiometric assay methods (Insulin IRMA CT Cisbio Bioassays), using commercially available kits. Total serum cholesterol, high-density lipoprotein cholesterol, glucose and serum triglycerides were measured by routine clinical biochemistry methods. All samples for an individual subject were determined in a single assay in duplicate.

Statistical analysis

For statistical data processing, the SPSS package, version 17.0, was used. The statistical significance of differences between different sample groups was calculated using Student's t-test and the Mann-Whitney U test. Correlation coefficients were determined using Spearman's rank correlation. The results are presented as the mean±SD. Threshold of statistical significance was defined as $p<0.05$.

Results

In our study there were 110 patients, 68 women (61.82%) the average age of 58.8±12.6 years, and 32 men (38.18%) average age 53.2±9.9 years. The average age of all subjects is 54.8±10.9 years. There was 41 (37.28%) patients with non-functional tumors and 69 (62.72%) with functional tumors. Among functional tumors was 56 (81.15%) with elements (sub)clinical production of cortisol. There was 9.42% catecholamine secreting and 9.42% aldosteron secreting tumors (Graph 1).



Graph 1. Distribution of secretory active subtypes of adrenal tumors

Tumors secreting adrenal androgens were not detected in the study population. As shown in Table 1, there is a statistically significant difference in the concentration of cortisol at 8 AM and 4 PM between patients who have functional and non-functional incidentalomas (cortisol at 8 AM 528.27±186.90 vs. 379.80±132.91nmol/L, p=0.027). Impaired cortisol suppression by dexamethasone (p=0.01) were observed in patients with functional incidentaloma (455.57±234.19 vs. 60.33±38.05nmol/L, p=0.045). We observed hyperinsulinemia, and statistically significant difference in the concentrations of insulin, cholesterol and triglycerides between patients with functional and nonfuctional AI. Evaluated metabolic parameters are shown in Table 2.

Table 1. *Hormone profile of study and control group*

	functional AI[1]	non-functional AI	value p
cortisol 8h (nmol/l)	528.27±186.9	379.80±132.91	p=0.027
cortisol 16h (nmol/l)	349.41±165.42	212.40±47.38	p>0.05
cortisol 20h (nmol/l)	181.73±90.89	161.70±83.48	p>0.05
cortisol 24h (nmol/l)	108.45±73.33	103.96±80.75	p>0.05
DST[2] "low dose" (nmol/l)	455.57±234.19	60.33±38.05	p=0.010
ACTH (pg/ml)	6.40±4.04	5.66±3.81	p>0.05
adrenaline (µg/du)	49.14±54.94	15.81±20.22	p>0.05
noradrenaline (µg/du)	127.29±96.08	54.5±75.57	p=0.038
free metanephrine (pg/ml)	106.84±205.65	56.21±55.89	p>0.05

1-adrenal incidentaloma; 2-dexamethasone suppression test

Table 2. *Evaluated metabolic parameters*

	functional AI[1] n=69	non-functional AI[1] n=41	value p
cholesterol (mmol/L)	5.46±0.95	4.66±0.70	p=0.040
triglycerides (mmol/L)	8.44±10.55	1.22±0.30	p=0.027
HDL cholesterol (mmol/L)	1.18±0.32	1.72±1.79	p>0.05
LDL cholesterol (mmol/L)	3.64±0.71	2.94±0.49	p>0.05
fasting glucose (mmol/L)	4.66±0.84	4.92±0.51	p>0.05
2h glucose	5.98±0.74	5.62±0.49	p>0.05
fasting insulin (µIU/mL)	96.99±54.94	14.65±3.52	p<0.001
2h insulin	176.21±27.14	21.18±0.62	p<0.001

1-adrenal incidentaloma

Without disorder of glycoregulation and/or lipid disorders was 16 cases of functional tumors (23.18% of all patients) and 40 cases of functional tumors (57.73% of all patients) had a glucose and lipid abnormalities. Difference in hormones level between patients with functional adrenal incidentalomas with and without metabolic abnormalities are given in Table 3.

Table 3. *Difference in hormones between patients with functional adrenal incidentalomas*

	with dyslipidemia and insulin resistance	without dyslipidemia and insulin resistance	value p
cortisol 8h (nmol/l)	553.33±204.51	518.87±193.9	p=0.940
cortisol 16h (nmol/l)	341.50±209.57	352.37±162.82	p=0.929
cortisol 20h (nmol/l)	191.53±92.40	196.66±89.67	p=0.879
cortisol 24h (nmol/l)	117.43±73.36	119.15±74.11	p=0.915
DST[1] "low dose" (nmol/l)	478.90±292.92	209.75±111.22	p=0.045*
ACTH (pg/ml)	8.57±9.29	5.78±2.32	p=0.630
adrenaline (µg/du)	50.17±21.80	32.41±17.73	p=0.708
noradrenaline (µg/du)	138.62±63.41	95.24±53.30	p=0.491
free metanephrine (pg/ml)	58.19±61.80	49.25±44.19	p=0.897

1-dexamethasone suppression test

In group patients with functional incidentaloma we observed statistically significant difference in cortisol suppression by dexamethasone between patients with disorder of lipid and glucose metabolism and without disorder of lipid and glucose metabolism (478.90±292.92 vs. 209.75±111.22, p=0.045). There was no statistically significant difference in other evaluated parameters.

There was positive, significant correlation between cortisol concentration after dexametasone suppression test and insulin, cholesterol and triglycerides concentration. Correlation between hormones concentration and evaluated parameters are given in Table 4.

Table 4. *Correlation between hormones concentration and evaluated parameters*

	cholesterol	triglycerides	HDL	LDL	glycemia	insulinemia
Cortisol 8h (nmol/L)	r=0.218, p=0.418	r=0.289, p=0.296	r= - 0.143, p=0.787	r=0.132, p=0.639	r=0.165, p=0.590	r=0.174, p=0.552
Cortisol 16h (nmol/L)	r=0.427, p=0.398	r=0.143, p=0.787	r= -0.886, p=0.019*	r=0.081, p=0.878	r=0.089, p=0.867	r=0.029, p=0.957
Cortisol 20h (nmol/L)	r=0.805, p=0.043*	r=0.400, p=0.505	r= -0.564, p=0.244	r=0.700, p=0.188	r=0.532, p=0.468	r=0.696, p=0.125
Cortisol 24h (nmol/L)	r=0.822, p=0.178	r=0.888, p=0.112	r= -0.437, p=0.563	r=0.200, p=0.800	r=0.791, p=0.419	r=0.410, p=0.210
DST[1] "low dose"(nmol/L)	r=0.727, p=0.007*	r=0.564, p=0.041*	r= -0.281, p=0.375	r=0.245, p=0.467	r=0.283, p=0.428	r=0.957, p=0.043*
ACTH (pg/mL)	r= -0.055, p=0.898	r= -0.180, p=0.670	r=0.566, p=0.143	r=0.072, p=0.866	r= -0.266, p=0.524	r=0.232, p=0.658
Adrenaline (µg/dU)	r=0.619, p=0.102	r=0.107, p=0.819	r= -0.500, p=0.253	r=0.321, p=0.482	r=0.543, p=0.266	r=0.214, p=0.645
Noradrenaline (µg/dU)	r=0.581, p=0.131	r=0.117, p=0.845	r= -0.082, p=0.862	r=0.939, p = 0.002*	r=0.332, p=0.520	r=0.250, p=0.589
Metanephrine serum (pg/mL)	r=0.198, p=0.706	r=0.257, p=0.623	r= -0.368, p=0.473	r=0.429, p=0.397	r=0.429, p=0.396	r=0.029, p=0.957

** -statistically significant difference; 1-dexamethasone suppression test*

Discusion

The main objective of our study was to investigate the effect of adrenal incidentalomas on the occurrence of endocrine-metabolic disorders, i.e. the relationship between appropriate hormonal tumor activity and disorders of glucose and lipid metabolism. We showed that patients with cortisol-secreting AI have significantly higher concentrations of insulin, cholesterol and triglycerides than controls.

The frequent use of visualization procedures increased the frequency detection of adrenal tumors [6]. These incidentally discovered adrenal masses are mostly benign and asymptomatic, however, some patients who do not have clinically active tumors have a certain metabolic disorders. Recent studies suggested, that a certain fraction of incidentalomas produce excessive amounts of cortisol and can suppress the activity of the hypothalamic ±pituitary ±adrenal (HPA) axis at various intensities [7, 8].

Contemporary literature suggests that AI patients have a higher prevalence of glucose homeostasis disorders and dyslipidemia, and that functional AI is an unrecognized manifestation of the metabolic syndrome. The frequency of metabolic disorders depends on the length of exposure of the organism to the altered endocrine status and individual sensitivity of the person, while showing a positive correlation with the size of the tumor mass. However these data are inconsistent [9]. It is not completely clear to what extent the incidentalomas affect the development and expression of metabolic disorders. A group of Korean authors found that functional incidentalomas most commonly autonomously secrete cortisol (giving the subclinical form of Cushing's syndrome), catecholamines (pheochromocytoma), and aldosterone (the occurrence of primary hyperaldosteronism) [10]. Due to the fact that it is mainly a subclinical production of cortisol, impaired glucose metabolism could be due to the effects of hormonal excess on gluconeogenesis, insulin-dependent glucose uptake in peripheral tissues, and inhibition of pancreatic beta-cell secretion. Glucose metabolism disorders can be on different levels, from impaired glucose tolerance and insulin resistance to manifest diabetes [11].

Recent studies found an increased prevalence of glucose tolerance abnormalities and insulin resistance among patients with subclinical form of Cushing's syndrome [12-16]. Moreover, one study found a significant correlation between adrenal lesion size and insulin resistance [14].Our results are consistent with the previously mentioned findings. We observed statistically significant differences in insulin concentrations between patients with functional and nonfunctional AI. There was positive, significant correlation between cortisol concentrations after dexametasone suppression test and insulin concentrations. Similarly, Reincke *et al.* [12] assessed insulin sensitivity in subjects with AI and find that all the studied subjects were insulin resistant.

This *in vivo* result was subsequently confirmed *in vitro*, revealing that insulin promotes proliferation of cells in a time- and dose-dependent manner [12]. The association between AI and glucose metabolism has been also suggested in a retrospective study assessing the prevalence of AI in a large group of type 2 diabetes mellitus patients, reporting an increased prevalence of AI in diabetics compared with controls (4.6% vs. 0.6% to 4.0%) [13]. Although chronic hyperinsulinemia observed in these patients could represent a factor that promotes tumor initiation and/or progression due to the mitogenic effect of insulin [15] or could be a consequence

of insulin resistance caused by cortisol excess [13, 15], it should be considered the key therapeutic target of the treatment of AI.

When it comes the impact of AI on lipid metabolism disorders, some authors have shown that patients with functional incidentalomas have a higher prevalence of lipid disorders [17], while the others have found no effect of tumor hormone production on lipid profile in the absence of impaired glucose metabolism [18]. It seems that the occurrence of dyslipidaemia was associated with the presence of impaired glucose metabolism rather than to the subtle cortisol hypersecretion per se [18-20]. Thus, Kim et al have shown that the values of fasting glucose and total cholesterol are significantly higher in patients with functional versus patients with nonfunctional incidentalomas [10]. Our results are in agreement with those. We consider it can be explained by the fact that existing insulin resistance modulates lipid metabolism by altering lipoprotein lipase activity in peripheral tissues, muscle and fat, resulting in increased fatty acid input into the liver. In our study, the patients with functional AI had significantly higher concentrations of total cholesterol and triglyceride compared to controls. There was a significant positive correlation between cortisol concentration after DEX screening and total cholesterol as well as between cortisol concentration after DEX screening and triglycerides.

In conclusion, the patients with functional AI have significantly higher glucose, insulin and lipid concentrations than controls. There was a significant positive correlation between metabolic parameters and cortisol concentrations. In the other words, this study indicates the importance of functional adrenal incidentaloma in the onset and / or severity of disorders of glucose homeostasis and lipid metabolism. In this regard, AI could be understood as one of the risk factors for the expression of metabolic syndrome parameters. The importance of this study is reflected in the ability to predict and possibly prevent the occurrence of comorbidities in patients with hormone-active incidentalomas.

The authors declare that they have no conflicts of interest.

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Analyzing social cognition and understanding of social inferences in patients with multiple sclerosis. A comparative study

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Abstract

Objective: The aim of the present study was to investigate the process of understanding social inferences and metaphors and the pragmatic use of language through emotion recognition and social inference tests in patients diagnosed with Primary Progressive Multiple Sclerosis (PPMS) mainly characterized by neurodegeneration. Additionally, we tried to identify a cerebrospinal fluid (CSF) biomarker correlated with the degree and rate of cognitive decline in progressive MS patients. **Method:** For the purpose of the present study 25 patients, aged 20 up to 55 years, with PPMS were evaluated. All patients were admitted in the First Department of Neurology, AHEPA Hospital of Thessaloniki. The control group was 30 healthy individuals which participated in the study voluntarily. The groups were matched for age, gender, years of education and intelligence. **Instruments:** Social inference was examined with the Awareness of Social Inference Test (TASIT). The TASIT consists of two different parts. The "Tasit Part I: Emotion Evaluation Task (EET - FORM A). The EET is the first part of a broader audiovisual tool designed for the clinical assessment of social perception that is called "The Awareness of Social Inference Test (TASIT)". The second Part of the test TASIT – Social Inference examines the viewer's ability to determine the speaker's meaning and intentions based upon the dialogue, emotional expression, and paralinguistic cues. **Results:** The findings indicate that patients with PPMS show decline in emotion recognition and social inference abilities, as compared with the control group. More specifically, PPMS patients have problems to understand the affective state of the others

mirroring a specific problem in ToM. **Conclusions:** The level of Theory of Mind in the form of sarcasm understanding decreases significantly in MS patients compared with healthy group potentially mirroring impairment in ToM in general. The results indicate that MS group is not resilient to understand metaphoric speech. More specifically, their pathology seems to be able to affect complex ToM abilities.

Introduction

The percentage of patients with cognitive impairment in MS has been estimated at 40%-70%, depending on the population under study and the different cognitive assessment tests and occurs in all MS subtypes [1-2]. The common pattern includes deficits in information processing speed, attention, working memory and episodic memory processes, whereas impaired spatial processing, verbal fluency and executive functions have been described to a lesser extent [1-3]. According to literature review, it is of vital importance to emphasize the fact that cognitive impairment typically consists of domain-specific deficits rather than global cognitive decline in all MS phenotypes [3]. Moreover, executive functions are recognized as one of the core cognitive domains involved [2-3]. Furthermore, literature review emphasizes on the heterogeneity in the type of cognitive impairments presented by patients with different MS phenotypes [2-4]. It is an axiom to consider that cognitive impairment associated with MS may be found early in the disease course [5], but occurs with increased frequency and severity in progressive MS [6]. Progressive MS is divided into primary progressive (PP) MS (approximately 15% of all patients) and secondary progressive (SP) MS that follows a period of relapsing-remitting (RR) disease course [7]. In both cases, progression starts at a mean age of around 40 years. PPMS causes a distinct type of damage. It tends to cause fewer lesions on the brain and more lesions on the spinal cord, for example, compared with other forms of MS [7-8]. Physical impairments in PPMS include motor, sensory, visual, and autonomic symptoms [8). The Expanded Disability Status Scale remains the typical outcome measure in PPMS trials [8]. In addition, there is a consensus on cognitive profile of PPMS including attention deficits, information processing speed, impairments in verbal episodic memory and phonemic verbal fluency, limited visuospatial functions and impairments in executive functions, as well [8-9]. Finally, although clinical phenotypes may differ in the prevalence or severity of cognitive impairment, main determinants are physical disability as measured by EDSS, and patients' age [8-9].

The presence of oligoclonal bands (OCB) in the cerebrospinal fluid (CSF) of multiple sclerosis patients is a constant finding [9]. The value of OCB has always been considered in the diagnostic criteria of MS [10].The presence of OCB is thought to provide supportive evidence of the immune and inflammatory nature of demyelinating lesions, specifically when imaging criteria fall short or lack specificity or when the clinical presentation is atypical [11]. However, the pathogenetic and prognostic role of the CSF- OCB presence in MS is still unclear [11].

Albeit, research community have highlighted different cognitive deficits with MS patients in different phenotypes, literature review reveals that ecologically valid studies with PPMS patients and social cognition such as emotion perception and Theory of Mind seems to be quite limited and only recently started to receive attention [6-5]. Social cognitive deficits are an

underestimated but important aspect of impairment in MS, reflecting how people process, store, and apply information in social interactions [8-6]. Deficits in these domains have been associated with reduced social and psychological quality of life, even after controlling for severity and duration of the disease, age, and neurocognitive performance [9].

It is of vital importance to add that, impairments in social cognition are found in all disease subtypes and are evident even in the early stages of MS [3] but these social deficits seems to be closely aligned with progressive MS [9-10]. In these conditions, social cognition abilities are crucial to hold relationships and consequently to preserve social support network significant resource for a patient's quality of life [12-13].

Literature review indicates that a plethora of cross-sectional studies evaluating social cognition use photographs or written vignettes, which mirrors an invalid representation of the complex social information that people navigate during interactions with others and limits the generalizability of these tests. As a consequence of the above mention states, it is clear that there is an essential need for assessing social cognition with more ecologically valid tools for the assessment of ToM [17-18].

Aim and hypotheses of the study

Based on the literature research data and a variety of studies concerning Theory of Mind the present study aims at investigating the processes of emotion recognition and understanding social inferences and the pragmatic use of language in patients diagnosed with PPMS, compared to healthy controls. Second aim of the present study was the evaluation of the relationship between emotion recognition and social inference performance and the value of cerebrospinal of fluid oligoclonal bands (CSF-OCB). Third aim was the evaluation of the relationship between the value of physical disability in patients with PPMS, and emotion recognition and social inference performance. In this cross-sectional study, we aimed to investigate the relationship between physical disability in patients with PPMS and social cognition.

Method

Participants and Procedure

For the purpose of the present study 25 patients (13 woman and 12 men), aged 20 up to 55 years (M.O.=43.60, S.D.=5.40) with PPMS were tested. All patients were admitted in the First Department of Neurology, AHEPA Hospital of Thessaloniki. The Expanded Disability Status Scale (EDSS) was used to score physical disability by an experienced neurologist. Inclusion criteria were: (1) to have been diagnosed with PPMS by an experienced neurologist, 2) to have been clinically evaluated, based on the Expanded Disability Status Scale (EDSS), with a disability level ranging from 0 to 5, 3) to have no history of other neurological disorders, 4) to have no dementia and their score in the MoCA to be greater than or equal to 27, 5) to have no history of major psychiatric disorders or psychotic symptoms, 6) to be native speakers of Greek, 7) to have normal or corrected vision and hearing, and, 8) not alcohol abuse or abuse of illegal drugs or steroids.

The control group was consisted of twenty-five healthy participants (13 women and 12 men) aged 20 up to 55 years (M.O.=43.60, S. D=5.40). Participants of the two groups were matched for age, education, gender, mental state, fluid intelligence and on BDI Scale Inventory. The results revealed no statistically significant differences between groups for Verbal Fluency Phonological Test F (1, 48)=.337 p>. 05, for Verbal Fluency Semantic Test F (1, 48)=2.35, p>.05, for Number Series Test F (1, 48)=1.04, p>.05, and finally on BDI Scale F (1, 48)=.30, p>.05.

The Ethical Committee of AHEPA Hospital of Thessaloniki approved the study and all participants gave written informed consent before evaluation. Participants from both groups were residents of Thessaloniki or from different regions of Central Macedonia. Participants were examined at an individual basis, at a quiet room in the Department of Neurology of AHEPA hospital. No time limit was assigned for the completion of the examination and the participants were informed that they were free to withdraw from testing at any time. The authors assert that all procedures contributing to this work comply with the ethical standards on the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All participants participated voluntarily in the study and were informed that all results were confidential.

Neuropsychological Instruments

Screening tests

Four tests were administered in order to evaluate cognitive and emotional profile of both groups. Cognitive ability of both groups was assessed with three different instruments. More specifically, Montreal Cognitive Assessment (MoCA) [19] was used to screen for cognitive impairment. Fluid intelligence- inductive reasoning and abstract thinking was evaluated with the Number Series test (NS) [20]. For the assessment of phonological and semantic fluency, the Greek Verbal Fluency task was used [21]. Finally, in order to exclude cases with depressive symptomatology, Beck Depression Inventory Scale was administered [22]. The Expanded Disability Status Scale was used to score physical disability based on an examination by a neurologist [23]. The Scale ranges from 0 to 10. EDSS value 1.0 to 4.5 refers to people with MS who are able to walk without any aid whereas EDSS value 5.0 to 9.5 are defined by the impairment to walk [23].

Main measures of the study

TASIT – Emotion Evaluation Test (EET)

The EET is the first part of a broader audiovisual tool designed for the clinical assessment of social perception that is called “The Awareness of Social Inference Test (TASIT) [24]. The EET examines a person’s ability to identify six basic emotions, namely happiness, pleasant surprise, sadness, anger, anxiety, disgust and discriminate these from neutral expressions. It consists of 28 alternative forms of a series of short videotaped vignettes of people (actors) interacting in ‘everyday’ situations. The EET is administered with the sound turned off, so as to focus on the ability to read dynamic visual cues.

TASIT – Social Inference (SI-m)

The second part of the TASIT examines the viewer's ability to determine the speaker's meaning and intentions based upon the dialogue, emotional expression, and paralinguistic cues [24]. There are three types of exchanges (in 15 scenes): (1) Sincere, the text and the cues are consistent, (2) Simple sarcastic, the participant needs to read the paralinguistic cues of the sarcastic speaker, and (3) Paradoxical sarcastic, the dialogue does not make sense unless it is understood that one is being sarcastic. In the Sincere exchanges the targeted speaker means what they are saying. In the Sarcastic exchanges one of the speakers means the opposite of what they are saying and intends the recipient to understand the real meaning.

The participants must watch carefully 15 short-duration scenes, (5 for each condition of social exchanges) and then answer four simple questions for every scene. The first question refers to the ability to understand what someone thinks is doing to the other person (Condition "Do"). The second question asks what the viewer thinks someone is trying to say to the other person (Condition "Say"). The third question refers to what the viewer thinks someone is thinking – what is the underlying belief, which may be different from what actors are saying (Condition "Think"). The last question refers to what the viewer thinks someone is feeling – what is the emotion the actors are feeling (Condition "Feel"). The participants have only to respond with saying "*yes*", "*no*" or "*don't know*".

Results

Data analysis was conducted using the SPSS v.21.

Recognition of the emotion of happiness in PPMS patients and healthy controls

According to the factorial structure analysis (CFA) conducted in Greek population for the Emotional Evaluation Test (EET) all the emotions except happiness- were grouped in one factor representing emotion decoding. Recognition of happiness and emotionally neutral condition represented two separate variables included in the model (Variable 1. Happiness, Factor 1. Basic Emotions "sadness", "anger", "anxiety", and "disgust" and "positive surprise" and Variable 2. Emotionally neutral Expressions) [24-25].

The application of one-way ANOVA with group (two levels: PPMS group and healthy controls) as the independent variable and the recognition of emotion Happiness as the dependent variable, showed that the performance on the recognition of this emotion differed statistically significantly between the two groups, F (1,48)=16. 574, p<. 05 η^2=94 (see Figure 1). PPMS patients showed lower ability to recognize happiness compared to healthy controls.

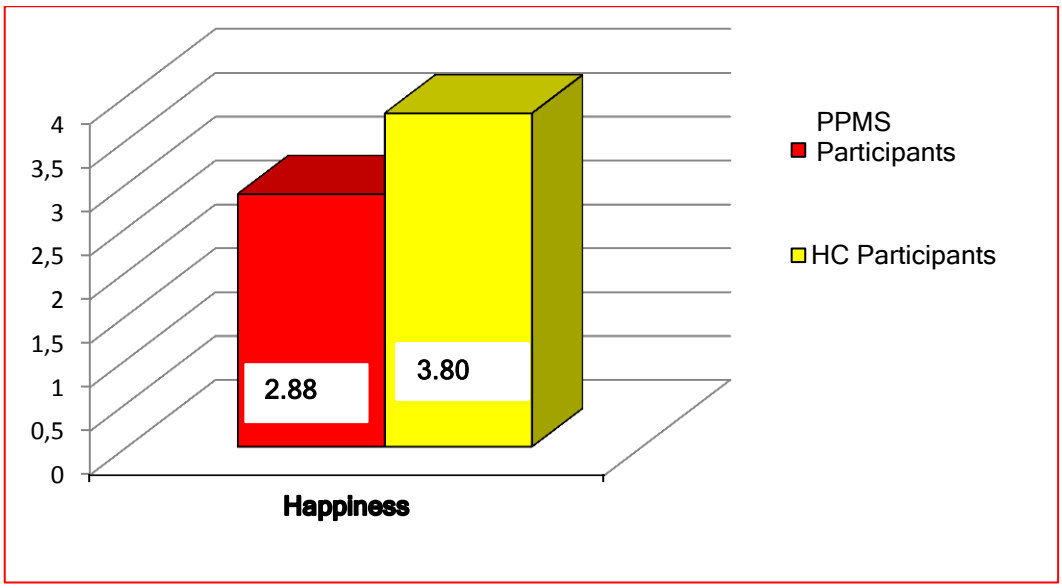

Figure 1. Recognition of Happiness in PPMS patients and healthy controls.

One-way ANOVA with group (two levels: PPMS group and healthy controls) as the independent variable and recognition of basic emotions (the sum of scores as regards recognition of sadness, anger, anxiety, disgust, and positive surprise) as the dependent variable, revealed that the two groups displayed a statistically significant difference in their performance as well, F (1, 48)=16.416, p<.05, n2= .95 (see Figure 2). Finally, for the evaluation of the Neutral Expressions, one-way ANOVA revealed statistically significant difference between the two groups F(1,48)=4.31 p< .05, n2= .69.

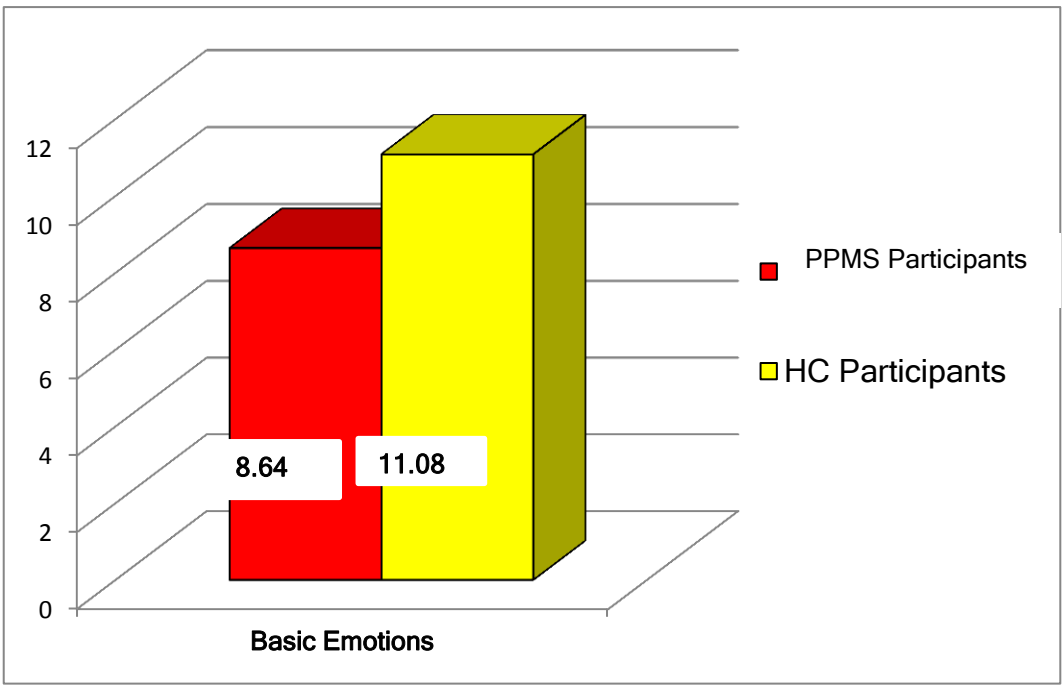

Figure 2. Recognition of Basic emotions in PPMS patients and healthy controls.

Recognition of Sincere, Simple and Paradoxical Sarcasm Conditions

As concerns Social Inference Test, a series of repeated measures analysis showed that the two groups differed in the condition "DO", F (2,96)=89.004, p<. 05, n2= .65 in the condition "SAY", F (2, 96)=111.579 p<. 05, n2=.68, in the condition "THINK", F (2, 96)=97, 953 p<. 05, n2= .69 and in the condition "FEEL" F (2, 96)=146, 163, p<. 05, n2= .75. More specifically, analysis revealed that significant difference was found between two groups in the condition "DO" in Sincere exchanges, F (1, 48)=727.44, p<.05, n2=.93, in Simple Sarcasm, F (1, 48)=954.83, p<.05, n2=.95, and in Paradoxical sarcasm, F (1,48)=656.68, p<. 05 n2=.93. As concerns the condition "SAY" analysis revealed statistical differences in Sincere exchanges, F (1, 48)=593.64, p< .05, n2=.92, in Simple Sarcasm, F (1, 48)=1215.70, p<.05, n2=.96, and in Paradoxical sarcasm, F (1, 48)=430.63, p<. 05, n2=.90. Moreover, in the condition "THINK" statistical differences between the two groups were found in Sincere exchanges, F (1, 48)=954.147, p<.05, n2=.95, in Simple sarcasm F (1, 48)=714.053, p< .05, n2=.93, and in Paradoxical Sarcasm F (1, 48)=8679.89, p<.05, n2=.99. Finally, in the condition "FEEL" statistical differences were found between the two groups in Sincere exchanges, F (1, 48)=4213.50, p<.05, n2=.98, in Simple Sarcasm, F (1, 48)=580.167, p<.05, n2=.92, and in Paradoxical Sarcasm, F (1, 48)=38.628, p<.05, n2=.44.

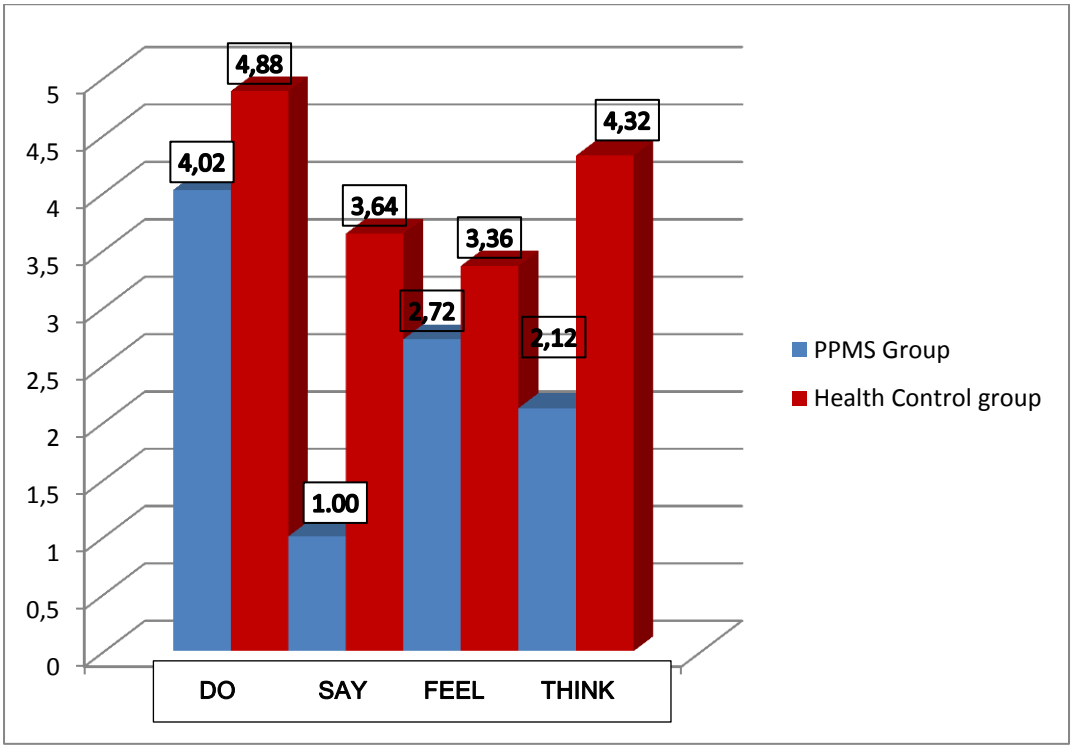

Figure 3. Group differences in PPMS patients and Health control group on understanding Sincere Exchanges.

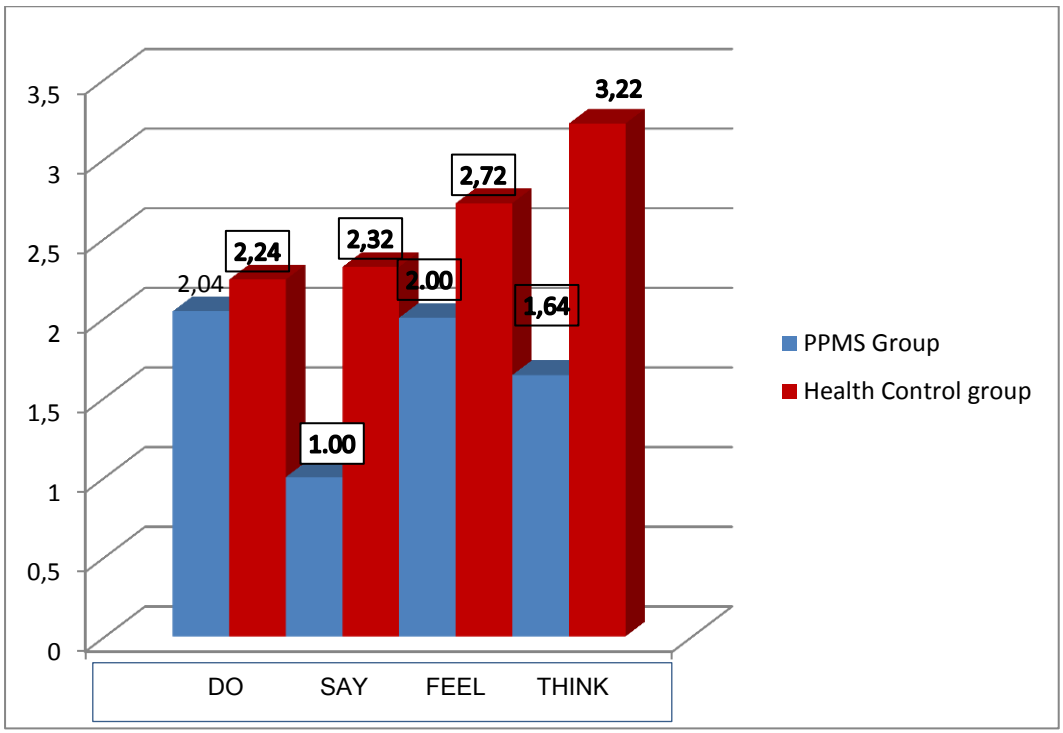

Figure 4. Group differences in PPMS patients and Health control group on understanding Simple Sarcasm.

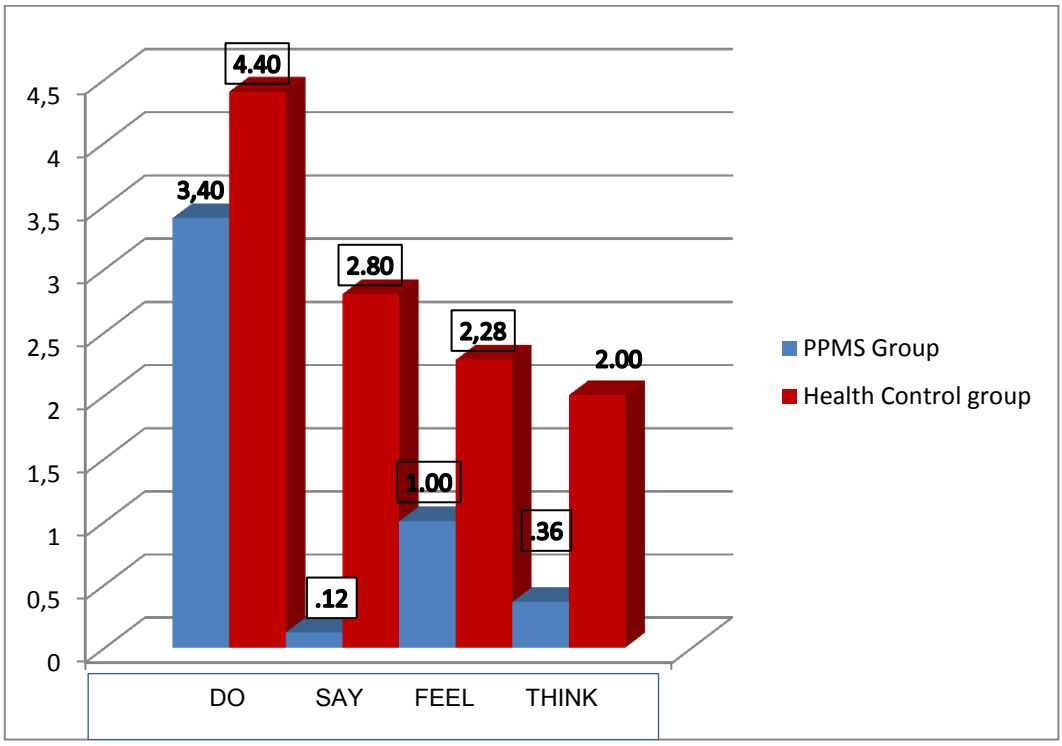

Figure 5. Group differences in PPMS patients and Health control group on understanding Paradoxical Sarcasm.

Results for patients' group

Finally, in order to evaluate the effects of the type of the OCB on recognition of emotions and metaphoric speech understanding, the respective ANOVAs revealed no significant differences between patients with types 2 or 3 OCB's on recognition of Happiness, Basic Emotions and Non-emotional expressions in the Emotion Evaluation Test (EET) and in performance variables on the Social Inference Test.

As concerns physical disability of patients and decline in ToM, negative significant correlations between EDSS and two conditions of Social Inference Test were emerged. More specifically, negative significant correlations were found in Condition "Does" of Sincere Sarcasm of the Social Inference Test , r=- .48 DF=23, p<.01 and in Condition "Does" of Paradoxical Sarcasm of Social Inference Test, r =- .47 DF=23, p<.01.

Discussion

Although exhaustive data interprets cognitive functions in MS, only a little attention has been laid on social cognition [25]. The present study is an attempt to enlighten the social cognition function in PPMS patients when compared with healthy control individuals. It is an axiom to consider that the majority of research on social cognition in MS has focused on relapsing-remitting MS [26]. Present study is an attempt to fill in the literature gap by identifying the pattern of differences in emotion decoding by patients with PPMS. Based on the performance on the Emotional Evaluation Test the ability of PPMS patients to decode Happiness and Basic emotions from dynamic visual cues is decreased when compared with healthy control adults. It appears that emotional recognition difficulties are the basis of impairment in social cognition. These effects of MS on emotion perception might reflect a specific problem in the processing of affective information indicating that emotional skills should be considered when evaluating functioning in MS [26]. Hence, this finding is consistent with previous research studies the results of which revealed impairment in social cognition [25-28]. Efficiently identifying other people's emotions is critical for interpersonal functioning, with difficulties in understanding others' emotions linked to lower social competence [29], communication skills [29] and quality of life [30]. This finding is consistent with previous studies identifying significant impairment in decoding emotional states in MS patients [31]. These effects of MS on emotion perception might reflect a specific problem in the processing of affective information or, instead, more general changes in perceptual processing [31-32].

Furthermore, analysis revealed difficulties in understanding Sincere exchanges, Simple sarcasm and Paradoxical sarcasm for the MS group of patients mirroring a severe impairment in Social Cognition. The decreased sarcasm comprehension seems to be connected with specific regions of the temporal lobe which may be involved in social signal detection and higher-level conceptual processing. Functional imaging studies shows that knowledge of higher-order social concepts is primarily associated with bilateral anterior temporal cortex [26]. It is fundamental to mention that the differences between the two groups in the condition "Believe" and condition "Feel" indicate a specific problem in the affective ToM [26-27].

Thus, PPMS patients seem to have difficulty to understand what one really wants to say to the other person and this might mean that they are not so able anymore to compare paralinguistic cues with the linguistic ones, when the two types of cues have opposite meanings. Moreover, this finding supports the idea that PPMS patients appear unable when compared to healthy control group to understand the affective states of the other, something that could indicate a specific problem in the affective Theory of Mind. This specific impairment could be due to specific brain network damage and dysfunction [26-27] in MS patients. More specifically, the 'social brain' includes brain regions such as the superior temporal gyrus, fusiform gyrus, medial prefrontal cortex, amygdala, precuneus, and temporal pole [31].

As concerns physical disability, EDSS seems to partially relate to the performance on ToM evaluation. The negative relationship indicates that the increasing trend of physical disability mirrors a decreasing performance on social inference, a finding closely aligned with other research findings [28-31].

Thus, PPMS patients may show an essential ToM decline and their examination using this particular ToM task could be able to "capture" the profile of social cognition impairment. Although we did not assess neuroanatomical substrates of ToM in the current study, it is likely that MS related damage to neural networks important for social cognition may underlie the current findings. Connectivity of cortical and subcortical areas and the integrity of white matter tracts, including the corpus callosum, appear to play a crucial role [31]. With widespread white matter pathology and frequent callosal damage, MS can be considered a CNS network disorder [32, 33]. Efficiently identifying other people's emotions is critical for interpersonal functioning, with difficulties in understanding others' emotions linked to lower social competence communication skills [29, 33-35]. Additionally, difficulties with correctly identifying emotions, thoughts and intentions in social situations may result in interpersonal problems and could contribute to the psychosocial burden of MS. It is of vital importance to mention that TASIT can become a screening tool and be related in a prognostic manner to specific lesion patterns in magnetic resonance imaging (MRI). More research is needed in order to shed light on the relationship between the social cognition test and the pathology that enhance the impairment in ToM in PPMS and to confirm these disorders in a larger population with progressive form of MS.

Limitations and future directions

Our research findings are characterized from a series of limitations. A significant limitation was the relatively small sample size of both groups. More investigation is needed in order to support the social cognition deficit in general in MS patients. Furthermore, research and comparison in all MS phenotypes concerning social cognition in order to provide us with a complete and in depth picture about Tom impairment.

The authors declare that they have no conflicts of interest.

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Think big - Plastic surgical treatment of complicated large sacral ulcerations: A case comparison

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Abstract

Objective: Large pressure ulcers are a well know problem occurring frequently on immobilized patients. They can develop rapidly especially over bony prominences on the elderly, ICU patients and on patients after spinal cord injury. Plastic surgical treatment can be challenging if the defects are large and complications occur like affection of anal region or development of a Marjolin's scar ulcer. Large defects of the sacral region are well known in our university hospital. Common local flaps like gluteal rotation or (double) V-Y advancement flap are often used for the treatment of smaller defects. In special cases these therapies are not sufficient. Rarely we use fillet flap of the lower extremity to cover large sacral defects on patients who were unable to walk before. **Subjects and Methods:** In this case report we demonstrate two relatively young paraplegic patients (49 and 57years old) with large sacral defect wounds. One case occurred in 2017, the other in 2019. After spinal cord injury many years ago both of them developed chronic pressure ulcers of the sacral region. In the case of 2017 a Marjolin's scar ulcer developed as a complication. Both patients had previously lost a leg during the surgical treatment. We used the other remaining leg as a fillet flap in combination with interdisciplinary rectum extirpation for sufficient surgical treatment. **Results:** In both cases adequate coverage of the sacral defect was achieved after interdisciplinary surgical treatment including rectum extirpation. Fillet flaps were safe, even after necessary surgical revisions. In one of the cases a vacuum wound therapy and several debridements were needed. After rehabilitation the patient of the earlier case is able to fully mobilize himself in everyday life and is even able to use public transport. **Conclusion:** Using a fillet flap of the lower extremity to cover large sacral ulcers is often the last possibility of surgical treatment. Though many complications can occur, full rehabilitation and social participation is possible after fillet flap surgery even with loss of both legs. Depending on patient's motivation and availability of orthopedic technology like special electric wheel chairs and other tools full mobility can be achieved.

A critical survey of a dedicated craniofacial surgery outpatient's clinic within a public health service structure

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Keywords: Multidisciplinary team -Care -Craniofacial surgery -Outpatient clinic -Public health

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Abstract

The management of craniofacial conditions, especially in the setting of a large general hospital, is ideally achieved by a focused team in a multidisciplinary clinic. The purpose of this article was to present the design, modus operandi and outcomes of a newly formed dedicated craniofacial multidisciplinary outpatients' clinic at the first year of operation endpoint within a public healthcare structure.

Introduction

Craniofacial surgery is the subspecialty of plastic, reconstructive and aesthetic surgery that corrects congenital and acquired deformities of the head, skull, face, neck, jaws and associated structures. This subspecialty of Plastic Surgery was pioneered by a French plastic surgeon named Dr. Paul Tessier in addressing facial anomalies.

Although the subspecialty of craniofacial surgery was born from Tessier's innovation in skeletal surgery of facial and skull abnormalities, the specialty had previously grown after WWI both in France and in the UK to become the subspecialty of plastic, reconstructive and aesthetic surgery that restores not only facial appearance, but also function and aesthetics by addressing both skeletal and soft tissues. It is nowadays recognized as a distinct entity or subspecialty on its own merit within plastic, reconstructive and aesthetic surgery.

Currently, craniofacial surgeons have expertise in surgery on bone, muscle, nerves, fat and skin, while most patients are seen in a multidisciplinary craniofacial clinics with a variety of presenting pathologies and are treated in corresponding dedicated Units.

To the best of our knowledge this is the first clinic of its kind established in a public healthcare facility in this country to functions on a dedicated Multidisciplinary team (MDT) basis with plastic surgery craniofacial lead.

In terms of geolocation and population, it covers the broader Thessaloniki city and surrounding area with a population of 1.057.000, and Northern Greece with a population of 3.100.000.

Material and Methods

The purpose of this article was to discuss the multidisciplinary team care requirements to treat craniofacial patients in a dedicated environment of a public health structure.

A dedicated multidisciplinary craniofacial clinic was formed and started operating in October 2018 within the scope of specialized outpatients' clinics of the University Department of Plastic Surgery at the Papageorgiou General Hospital of Thessaloniki. It runs strictly on a monthly basis and has a rigid configuration of 6 appointments per session. From its inception it has run at 100% capacity rate except for the month of August 2019 where it underran at 33.33% due to low demand, Table 1. The pathology encountered included the entire spectrum of craniofacial surgery from minor conditions to complex cases. These are all presented together with complete patients' demographics and measurable outcomes, Table 2, Table 3 and Table 4.

Patients were seen via either the hospital's portal (telephone line or internet booking system), through self-referral, general practitioner or inter-specialty referral.

All medical documentation is filed in the Hospital's SAP System with standard computerized entry. In this system any editing is noted and irreversibly traced. Clinical photography was that of standard craniofacial or specific views obtained with a Canon Powershot Digital Camera allowing for date, time and geolocation. All CT – MRI Images were stored on the hospital's central computer and viewed on Impax Xero Viewer.

This dedicated craniofacial clinic operates monthly every third Tuesday of each month. Patients are allocated in 20 minutes slots. A maximum of 6 new (previously) to 9 patients (currently) are examined. The objective setup is to function as a multidisciplinary team (MDT) in order to involve all other relevant specialties as well i.e.: Plastic Surgery (Lead), Maxillofacial Surgery, Orthodontics, Neurosurgery, Ophthalmic Surgery, Speech Therapy, Psychology and Social Workers.

The promoted working concept is that we are a team of professionals dedicated to the treatment of facial deformity and associated disorders in children and adults. We treat all craniofacial surgery conditions (including facial plastic surgery and oral/maxillofacial surgery reconstruction) that is conditions that are either inherited, neoplastic or as a result of trauma. We also offer offsite dental and orthognathic surgical treatments related to facial problems and conditions.

As such, craniofacial conditions that are seen and treated jointly or independently are: clefts (primary and secondary), hypertelorism, skull base and orbital tumours, vascular malformations, craniosynostoses, neurofibromatosis type I and hemifacial microsomia. With regards to facial plastic surgery: nasal reconstruction, skin cancer, facial palsy, facial trauma, oculoplastic surgery, peri-orbital reconstruction, cleft rhinoplasty and post traumatic deformities.

Since the start of its operations in October 2018 this dedicated craniofacial clinic has seen a total of 68 patients resulting in 112 patient episodes. An extended multidisciplinary consultation

was needed in over 17 patients.

Table 1. *Monthly capacity at end of Year 1 (2018-19).*

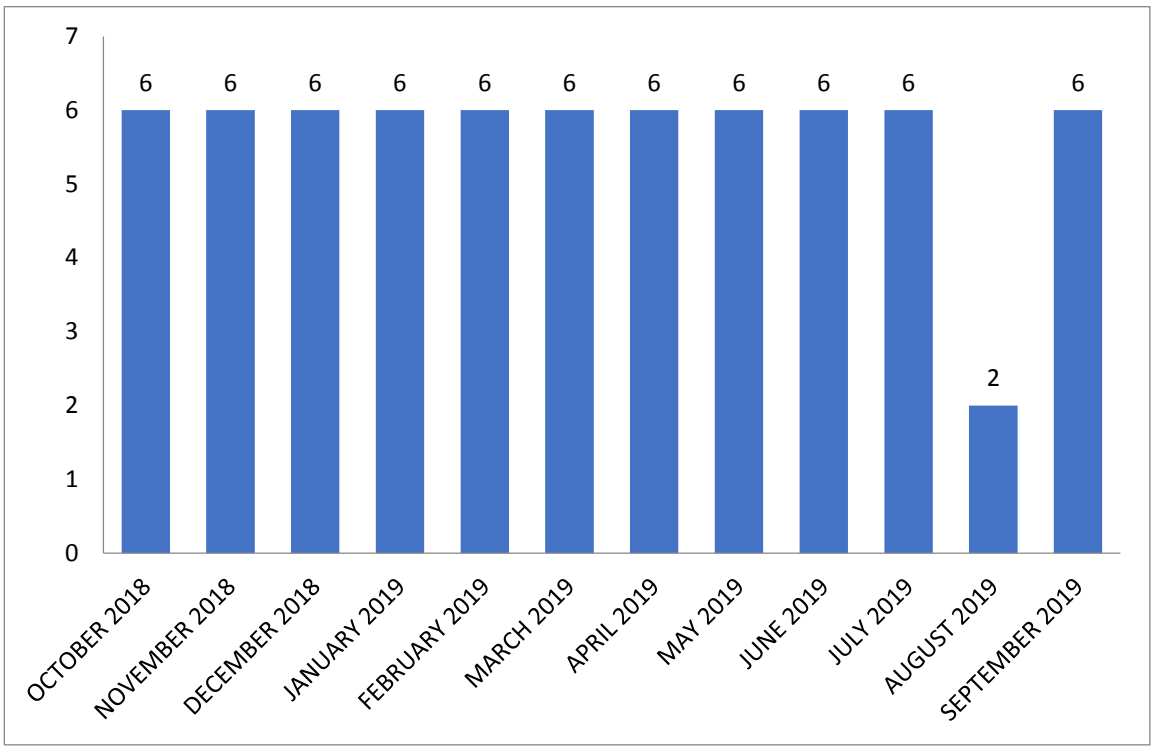


Table 2. *Sex distribution at end of Year 1.*

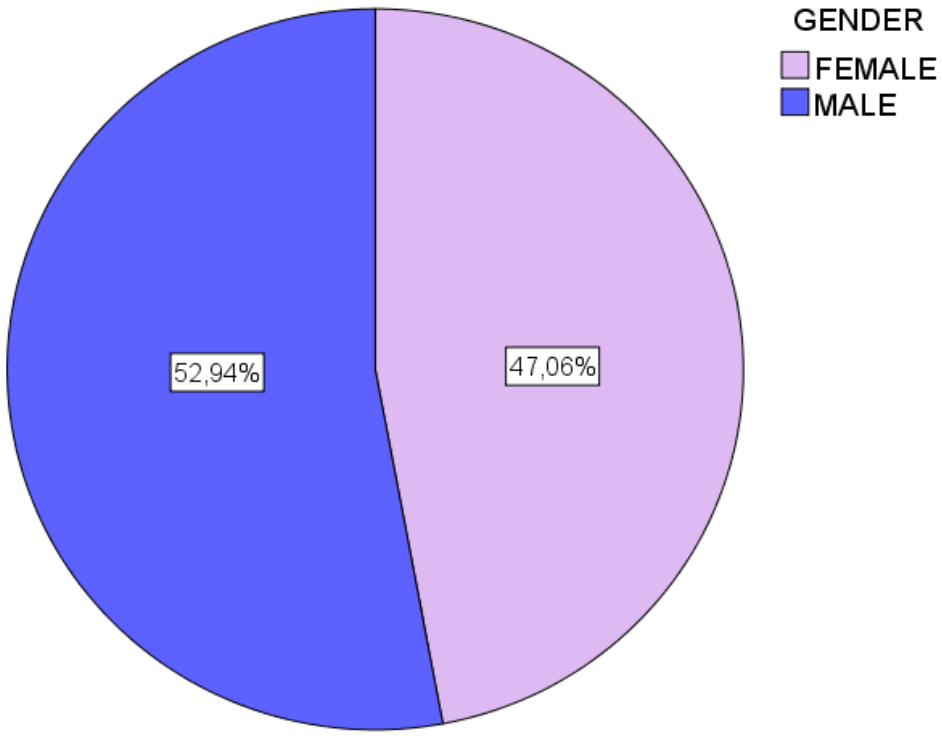
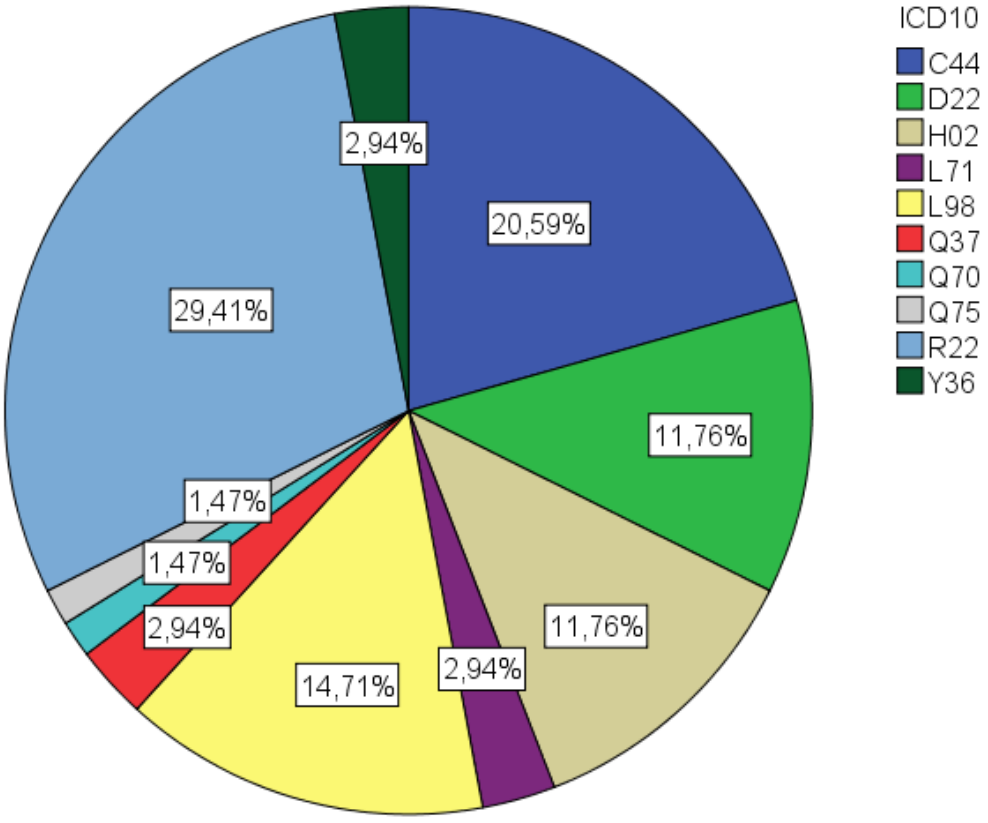


Table 3. *Age distribution at end of Year 1.*

			Statistic	Std. Error
AGE	Mean		48,79	2,254
	95% Confidence Interval for Mean	Lower Bound	44,30	
		Upper Bound	53,29	
	5% Trimmed Mean		49,02	
	Median		44,50	
	Variance		345,479	
	Std. Deviation		18,587	
	Minimum		10	
	Maximum		88	
	Range		78	
	Interquartile Range		32	
	Skewness		-,070	,291
	Kurtosis		-,826	,574

Table 4. *Age distribution at end of Year 1.*

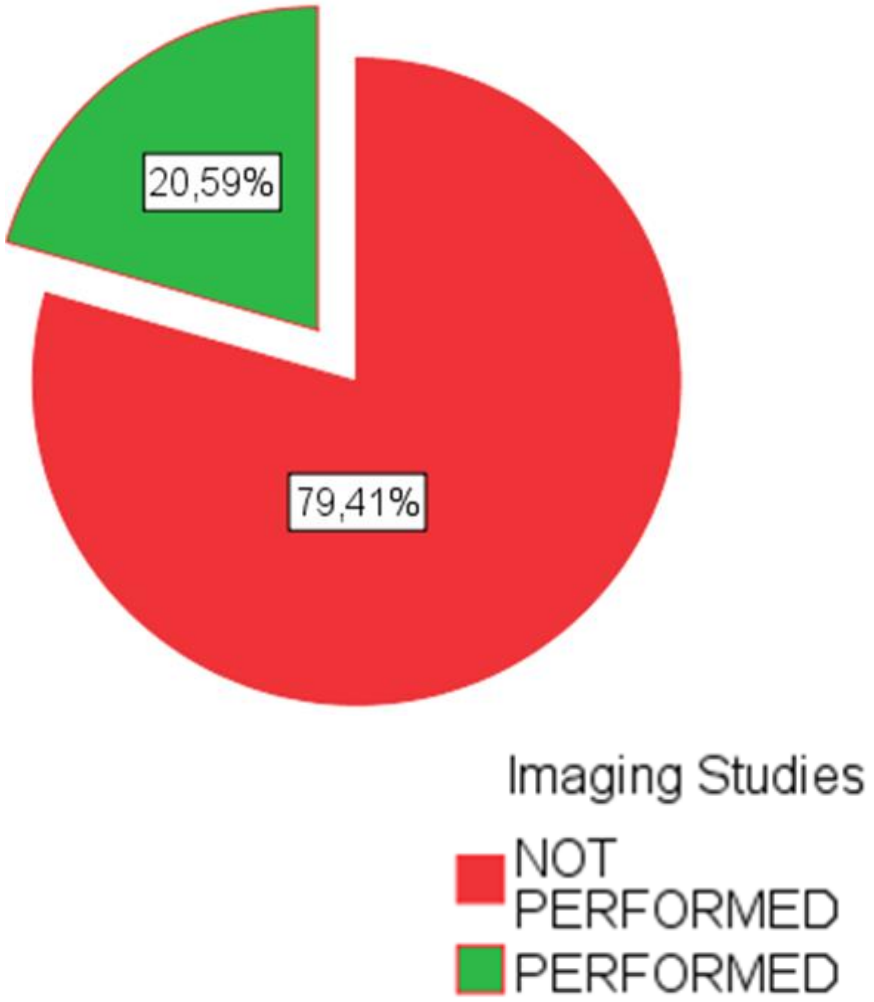


In terms of casemix, in order to maximize the University Plastic Surgery Department's cost benefit balance, each of the patients were assigned to a specific ICD-10 code and according to the designed management comorbidities and demographics, patients were related to a specific diagnosis related group (DRG) for future activity-based funding, Table 5.

As stated above, owing to increased demand, the capacity of the clinic was expanded from 6 new patients to 9 new patients per session in October 2019. Eleven patients were referred for further investigations or evaluation.

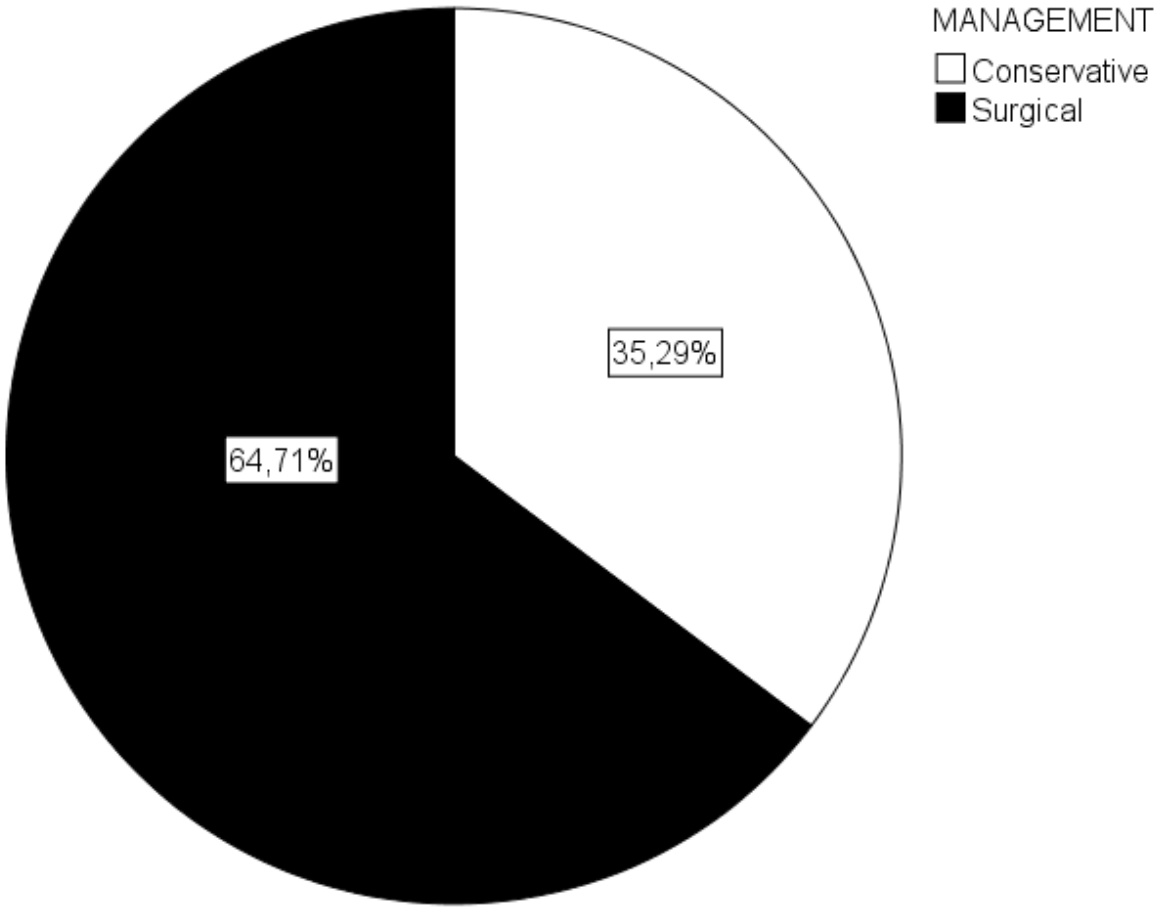
Diagnostic modalities used were: computerized tomography (CT), soft tissue ultrasonography (U/S), magnetic resonance imaging (MRI), plain X-rays and lymphoscintigraphy (SLNB in extended and recurrent SCCs), Table 6.

Table 6. *Diagnostic imaging performed at end of Year 1.*



In terms of management, this was either surgical by means of lesion excision and reconstruction, reconstructive surgery or laser surgery, or conservative, usually with referral to a specialized health-care provider.

Table 7. Management at end of Year 1.



Conclusions

According to unpublished confidential audit data, the innovative institution of a dedicated multidisciplinary quaternary care craniofacial surgery outpatient's' clinic in a general hospital has been initially welcomed by specialists' colleagues, the medical community and the general public. It has positive outcome indicators and although a significant amount of information diffusion and public awareness are required, its establishment offers adequate specialist craniofacial surgery cover at local and regional level. Possibly at national level as well considering WHO guidelines, provided the formation of a singular national craniofacial center is established and secured.

Our appraisal is that it has realistic potential for immediate to medium-term SMART (Specific, Measurable, Attainable, Realistic, and Timely) expansion as a center for excellence and expertise in the field of craniofacial surgery. Given that all specific specialties and disciplines required are present, this would also be an innovation and creative source of public health improvement at local, regional and national level and act as an important advance in assessment, policy development and assurance in this country.

Cardiotoxicity among adult survivors suffered from childhood malignancies

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Keywords: Cardiovascular Disease -Cardiotoxicity -Early detection of CV cardiotoxicity -Survivors from childhood malignancies

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Abstract

Late cardiotoxicity following treatment of malignancy diseases has been long established. Cancer therapeutics-related cardiac dysfunction (CTRCD), acute arrhythmias, pericardial disease, valvopathies and early atherosclerotic Cardiovascular Disease (CVD), are the clinical presentations of cardiotoxicity. Although these clinical modalities can affect adults treated for malignancies, they are more common to present in the pediatric survivors as improvement of prognosis, nowadays exists. Studies have shown that CVD can present earlier than thirty years, post treatment. If adding on this the early and late effect of cardiotoxicity on the developing in childhood cardiovascular system, we are then faced with a new Risk Factor (RF) for CVD. Anthracyclines and its derivatives have served for over fifty years as the road model of studding early, mid and late term cardiotoxicity. Today a vast number of chemical agents are used, many of them with very good results in treating the existing malignancies. Unfortunate, little or even less are known on their potential mechanism of derived cardiotoxicity when used by their own or combined with others and/or radiotherapy (RT). The 2013 existing guidelines by ACC/AHA on surveillance of the cardiovascular health of oncology survivors, are mostly addressing early cardiac adverse effects and CTRCD. Little is mentioned about the development of early CVD, its subclinical diagnosis, prevention and the need of early intervention before clinical events are present. The aim of this paper is to review the exist knowledge and practice on this condition with growing numbers of survivors facing the risk of early atherosclerotic CVD.

Introduction

Cancer, although rare, is the leading cause of death among children over one year old in the United States. In 2014, in USA, 15,780 children and adolescents age up to 19 years had been diagnosed with cancer and 1,960 died of the disease [1]. Cancer occurs more frequently in the age group of 15 to 39 years than in younger children, although incidence in this group is still much

lower than in older adults [2].

The most common types of cancer diagnosed in childhood are: leukemia, central nervous system tumors, lymphomas, rhabdomyosarcoma, neuroblastoma, Wilms tumor, bone cancer, and gonadal germ cell tumors (testicular and ovarian). The number of survivors will continue to increase, given that although the incidence of childhood cancer has been rising slightly in recent decades by 0.6%, the survival rates are also improving [2].

The overall survival rate for children with cancer has improved greatly over the last fifty years. Just over 50% of children diagnosed with cancer before age 20 years survived at least 5 years in the mid 70's. In the 2000's, more than 80% of children diagnosed with cancer before age 20 years survived at least 5 years [3].

These survivors are seven times more likely than the general population to die as a result of CVD. This fact makes CVD the leading cause of noncancer mortality in this population [4]. This increased risk for development of CVD is largely attributed to cancer treatment exposures at a young age, most notably anthracycline (e. g. doxorubicin, daunorubicin, epirubicin, idarubicin) agents, mitoxantrone chemotherapy, and chest directed radiatiotherapy (RT). It is estimated that as many as one in eight survivors of childhood cancer treated with anthracyclines and chest RT will experience a life-threatening cardiovascular event in a 30-year period after treatment of childhood malignancy [5].

Cardiotoxicity secondary to oncology treatment includes cardiomyopathy/heart failure, coronary artery disease, stroke, arrhythmias, valvular diseases, systemic and pulmonary hypertension, pericardial disease and vascular dysfunction, is a major concern for long-term survivors of childhood cancer [6].

The most well studied clinical entities are of those of 1. acute cardiotoxic events, during the period of induction of the oncology therapy and 2. related mostly CTRCD. This last most common condition has been related to the use of anthracyclines historically and now days to the use of trastuzumab; used for breast cancer patients with a positive HER2 receptor [7].

The aim of this short review is to focus mostly on the existing data, clinical approach, diagnosis and prevention of early onset atherosclerotic CVD. A condition with growing numbers affecting survivors suffered from childhood malignancies.

Pathophysiology of Cardiotoxicity leading to early CVD

The process of atherosclerosis is an aging condition that involves both traditional and non, RF's for CVD (Table 1). This is a multifactorial, chronic, and inflammatory process targeting the endothelial of arteries. The first event is an "endocrine dysregulation" of the vascular endothelial. This leads to decrease of the endogenous NO regulating vascular dilatation and increases the levels of angiotensin II, provoking smooth muscle hypertrophy, accumulation of: lipid particles, immune cells, autoantibodies and autoantigens. Their reaction will lead to multiple production of inflammatory cytokines among them tumor necrosis factor-α. Oxidation of low-density lipoprotein cholesterol (ox-LDL) and increased levels of homocysteine will create thickening of the intima media, decreasing elasticity and narrowing the arterial lumen. These will increase the systemic blood pressure, provoking more endothelial damage by sear stress and plaque formation, and thrombophilia. Activated monocytes proliferate the vessel wall and phagocyte ox-LDL molecules lead to formation of the Foam cells that enrichen the atheromatic plaque. Other macrophages

associated with the instability of the plaque are also recruited to the subendothelial space. These lead to the plaque destabilization and the increased risk for rupture [8].

The natural history of the plaque will be either to increase and obstruct the vessel, or rupture and its fragments will create topical or peripheral thromboembolic clinical conditions. These are early-onset Peripheral Vascular Disease, Severe Carotid atherosclerosis, lead to Strokes, Congestive Heart Failure, Coronary Artery Disease, Myocardial Infract, or even Sudden Cardiac Death [9].

Studies have proven that some chemotherapeutic agents as gemcitabine and 5-fluorouracil can increase the risk of atherosclerosis and provoke early Acute Coronary Syndromes, during the second to fifth day of induction treatment. This is not a dose dependent, effect and thought to be a direct result of vasculitis, coronary spasm and /or thrombosis [10]. Bevacizumab and cisplatin have been associated with myocardial ischemia through endothelial dysfunction, hypercoagulability and thrombosis. The frequency of cisplatin-associated Acute Coronary Syndromes is near 2% [11].

The association of CVD with RT is well known. In Hodgkin lymphoma survivors, the cumulative incidence of Coronary Artery Disease in those that have been exposed also to RT is near 20%, 40 years after treatment. Long-term follow-up(F/U) for several years after RT is recommended. CVD and cancer share similar risk factors (Table 2) and pathophysiological pathways. Modification of CVD-RF's has been proven to prevent the development of both Coronary Artery Disease and Cancer [10].

Methods detecting CV cardiotoxicity

In contrast to CTRCT where even in asymptomatic or near asymptomatic patient's regular F/U by Echo-2D can easy and early establish the diagnosis, in cases of accelerated sub-clinical forms of atherosclerotic CVD, this is more limited. As invasive coronary arteriography -gold standard for diagnosis- is far by considered aa a screening test, new techniques have been used to detect sub- clinical CVD, (Table 3). A combination of methods can be used to detect sub-clinical CVD. Among them the most common are cumulation of RF's, measurement of IMT, Coronary Artery Calcium Score and CT coronary angiography [8, 11-13].

Discussion

For the last 4 decades, oncologists have been aware that some chemotherapeutic agents and RT, used in some cancer protocols can result in cardiovascular complications, such as atherosclerotic CVD, CTRCT with clinical or subclinical Heart Failure, Valvular disease and Arrhythmias [4-6]. Treatment options for patients with cancer have evolved with the introduction of new targeted therapies. Never the less a growing appreciation of the cardiac toxicities of cancer therapies has led to a new field of medicine where oncology meets up with cardiology creating "cardio-oncology [5, 10-11]. More, studies have shown that physicians dealing with oncology patients show a sensitivity of 47% and a specificity 68%, in been able to diagnose symptoms and signs related to cardiotoxicity [14].

In contrast with the well-known acute or chronic cardiotoxic events of CTRCD little is known for the adverse effect that oncology treatments can provoke early atherosclerosis. The luck of a diagnostic method, used in outpatient clinics, as Echo-2D is used in early detection of CTRCD, has not permitted regular screening of the surviving population suffered from childhood malignancies [5, 11].

Framingham Risk Score is one of the most common calculators in use for estimating CVD risk. It also guides both preventive and therapeutic interventions aiming to lower the possibility of developing clinical atherosclerotic CVD in the general population [15]. However, does not take in consideration that some chemotherapy agents and RT each or both together used frequently in treating pediatric malignancies are independent RF's for early CVD. Thus, survivors from childhood malignancies treated with those agents are a high-risk population, independent of the presence of other existing RF's for CVD. This has been already recognized and addressed by many specialists in the field as Children's Oncology Group [16]. Further, a task force of the American Academy of Pediatrics sponsored by the National Heart, Lung and Blood Institute and the National Institute of Health have addressed the need of early detection, primordial or primary prevention of common RF's for CVD among the youth [17].

For the above mention facts, a surveillance program addressing the needs of childhood malignancy survivors is of a high importance. Studies have shown that even in survivors under thirty years old and even as earlier as eight years from the end of their treatment evidence of existing accelerating atherosclerosis. Regarding the traditional RF's for CVD dyslipidemia has been found in near 30%, hypertension in 25% and Diabetes Mellitus in 13%. More noble evidence as a high Framingham risk score, the 10-year general CV risk was risen to 7.6% and the mean vascular age of patients older than 30 years was 8 years higher than their chronological age. Additional to that 60% had a pathologic IMT measurement and 27% had diastolic heart dysfunction in their Echo-2D suggesting that the underlying risk for CVD in this cancer survivor's population is elevated [17].

From data arriving from the Children's Oncology Group, the young seem particularly at risk for cardiac toxicity [16]. Increasing the awareness and ability of physicians dealing with this population to detect early symptoms and signs of accelerating atherosclerosis must be our first target. Regular follow-up by cardiologists trained to access early CVD by a combination of methods stated at Table 3 will be our next target. Finally, primordial when possible or primary prevention of the existing RF's for CVD, even by medicine means when alternation of life style is not enough to reduce them [5, 8, 15-19].

Conclusion

With rapidly growing understanding of the cardiac toxicity related to cancer therapies, it is important to have a growing collaboration between cardiology and oncology. Mostly as the two devastated diseases shear common RF's and affected populations [10]. There seem to be related underlying mechanisms between these two diseases [11]. Given the plethora of cardiac toxicity from new emerging targeted therapies, a general awareness of the cardiac risks of these treatments is necessary [5]. Finally, additional research is needed to for the incorporation of both

imaging and blood biomarkers to determine the best practice in early diagnosis of accelerating atherosclerosis among survivors from childhood malignancies.

Table 1. *Most common used Traditional and Non-traditional RF's for CVD [8, 9, 15]*

TRADITIONAL	NON-TRADITIONAL
Total Cholesterol	Lp(a)
Hypertension	Homocystinemia
Age	High Uric Acid
Diabetes Mellitus	High sensitivity CRP
Smoking	High Fibrinogen
Gender	Insulin resistance
High Density Lipoprotein Cholesterol	Obesity
	Family history of CVD
	Birth Weight

Table 2. *Cardiotoxicity RF's prior to initiation of oncology treatment [5, 6, 10]*

Genetic Factors (Syndromes-Familial) predisposing to malignancies
Age of presentation of malignancy: < 15 > 65 years old
Type of chemotherapy agent and cumulative dose
Known Ischemic Heart disease and/or clustering of more than 4 RF's or Diabetes Mellitus (any type)
Female gender
Hypertension
Combined Chemotherapy and Chest RT
History of existing HF
Low normal Ejection Fraction (50-55%), before treatment
Renal Failure
Obesity (BMI >30%)
Time elapsed since end of treatment

Table 3. *Non-invasive techniques and biological markers used to detect early CVD [8, 12-13, 18-19]*

Intima media thickness (IMT) calculated by ultrasonography
Coronary Calcium Score calculated by CT scan
MRI coronary angiography
CT coronary angiography
Endothelial function tests by ultrasonography
Ankle-Brachial index (ABI) blood pressures
Arterial stiffness measured by pulse wave velocity
Scintigraphy Techniques (Positron Emission Tomography), (Single-Photon Emission Computed Tomography
Traditional RF's for CVD
Non-Traditional RF's for CVD
Pericardial adipose tissue, measured by cardiac-MRI or Echo-2D
Waist and Waist to Hip ratio

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The authors declare that they have no conflicts of interest.

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A study about neck pain on active population who visit Primary Health Centers

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Keywords: Neck pain -Assessment -Algometer -Pain questionnaires -Primary health

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Abstract

Background: Cervical pain is very common among population and several methods have been used until now in order to evaluate it. The main aim of this study is to estimate the prevalence of non-specific neck pain and the special features of it through its relation to demographic agents, habits and physiology parameters. Additionally, this study aims to select the most valid and objective methods for neck pain evaluation, which could also be useful in Primary Health Care and could give new prospectives. **Methods:** This is a cross sectional study with a sample of 440 people 20-40 years old who visited the Public Centers in the County of Thessaloniki. The sample was chosen randomly. The subjects were separated in two groups (neck pain and no pain group). A questionnaire was distributed to the sample. The devices were an algometer which was used to estimate pain threshold and an electronic hand watch/smartphone which was used to evaluate pulse and skin humidity. **Results:** The 38.6% of the samples seemed to suffer from neck pain but real neck pain was found only in the 21% of the sample. The disturbance in neck area because of stiffness and fatigue was found in 51% of the subjects and 25.9% of theme felt both pain and fatigue. The factors which were related to neck pain were female sex ($p<0.001$), age after 40 ($p=0.007$), marriage status ($p=0.042$), smocking ($p=0.034$), lack of physical exercise ($p=0.01$) and stress ($p<0.001$). Neck pain was strongly associated to pain pressure thresholds (PPT) ($p<0.001$). The PPT were lower in the group who suffered from neck pain. The agents which were not statistically associated with neck pain were the hardness of work ($p=0,369$), computer use ($p=0.07$), educational level ($p=0.274$), alcohol consumption ($p=0.748$), siesta ($p=0.913$), depression ($p=0.086$), heart rate ($p=0.216$) and skin humidity ($p=0.141$). There was no correlation between NRS and pain thresholds ($p=0.947$). The majority of people seemed to suffer from mild disability caused by neck pain (56.7%). Almost the half of the sample did not asked for professional help for their neck pain (48%) and seemed to be ignorant about the role of general practitioner at the management of their neck pain (44.1%). **Conclusion:** The examination of the patients showed that neck pain is often confused with stiffness and fatigue at the neck area. The NDI and HADS questionnaires could be easily used to evaluate respectively the disability and the psychological status of a person who suffers from neck pain. Algometry seems to enhance the objectiveness at the field of neck pain evaluation. The algometer showed high validity and reliability as a mean of neck pain evaluation under the condition that it is applied by a health professional with experience on its use. Neck pain is a complexed symptom and should be evaluated from an integrated point of view. These tools could probably be used in Primary Health Care as contemporary resources in the medical practice but more research is needed.

Factors influencing corneal predescemetic endothelial keratoplasty (PDEK) graft creation: It's all in a bubble

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Abstract

Aim: To assess the effect of pneumatic (air) and fluidic (transport medium) injection to the type of bubble (I, II or mixed III) and the resultant dissection of corneal endothelial grafts PDEK or DMEK. **Materials and Methods:** All grafts were obtained from Dr Agrawal Hospital's Eye Bank. Air injection was the initial preferred mode of graft harvest. If pneumatic dissection was unsuccessful after 10 tries, fluidic dissection with transport medium was tried. SPSS 23.0 was used to statistically analyse the data. **Results:** 40 consecutive donor corneas with a mean age of 46.5 and a mean endothelial count of 2980 were analysed. Air dissection lead to the harvest of 27 endothelial grafts and fluid dissection led to the creation of 7 endothelial grafts. Statistically significant difference was found the different bubble types and the type of injection (x2 chi square=10.02, 0=0.008). **Conclusion:** In young donors pneumatic (air) graft dissection leads to PDEK in a high proportion. This percentage is reduced when transport medium is tried after unsuccessful air injection. Injection of transport medium increases the percentage of grafts harvested but also increases the ratio of Type II and III DMEK grafts created.

Introduction

Predescemetic Endothelial Keratoplasty (PDEK) was first described by Agarwal et al. in 2014 [1] after the description of the predescemetic layer of the by Dua et al. in 2013 [2]. PDEK is associated with a number of advantages over other types of transplants for endothelial dysfunction which include greater rigidity compared to Descemet Membrane Endothelial Keratoplasty (DMEK) grafts allowing greater manipulation with smaller chance of primary and/or secondary failure and possibility of using the intact anterior lamella for anterior keratoplasty i.e Deep Anterior Lamellar Keratoplasty (DALK) or tectonic as in the case of DMEK grafts. PDEK grafts can be harvested from younger age donors which are usually associated with higher endothelial cells counts, extending in this way the donor age of eye donations. Despite all these

advantages, the harvest of PDEK grafts has been a matter of controversy as the creation of Type I and Type II bubbles is not always predictable leading eye banks and some eye surgeons to be reluctant to introduce PDEK to their practice despite the many advantages that are associated with PDEK. These include greater rigidity compared to DMEK grafts allowing greater manipulation with smaller chance of primary and/or secondary failure and possibility of using the intact anterior lamella for anterior keratoplasty i.e Deep Anterior Lamellar Keratoplasty (DALK) or tectonic as in the case of DMEK grafts. Type I bubbles lead to the creation of PDEK grafts, whereas Type II bubbles lead to the harvest of DMEK grafts. Agarwal et al. [2] originally described the harvest of the PDEK grafts using pneumatic dissection, however it has been postulated that fluid (i.e transport medium that is conveniently red) can increase the harvest of PDEK grafts.

Aim

In order to assess the efficacy of the fluidic dissection in relation to solely a pneumatic harvest we retrospectively reviewed 40 consecutive endothelial graft harvests. We assessed the results obtained after the standard pneumatic and the fluidic dissection that was used once pneumatic dissection was deemed unsuccessful.

Materials and Methods

All grafts were obtained from Dr Agarwal Hospital's Eye Bank. The standard PDEK technique was followed with pneumatic dissection as described by Agrawal et al [1]. The technique can be summarised as follows. A 30-gauge needle attached to a 3.5 ml air filled syringe was inserted from the limbus into the mid-peripheral superficial stroma in the bevel up position and air was injected in the central stroma to facilitate the pneumatic dissection of the type I PDEK graft. In the case of a type II bubble formation the procedure continued as a Descemet's membrane endothelial keratoplasty (DMEK) graft. In case a type 3 (mixed bubble) was created, a DMEK graft was carefully dissected and the procedure was concluded as a DMEK case. In cases where pneumatic dissection could not be achieved after 10 tries, fluid (transport medium) was used to facilitate the creation of grafts.

The statistical package used to analyse the data was SPSS 23.0. Normality of the data was tested by Shapiro-Wilk normality test. P value <0.05 considered statistically significant.

Results

Donor corneas from 40 consecutive donors aged between 8 and 74 years of age were used (mean 46.5, mean endothelial counts 2980). 34 PDEK grafts (Type 1 bubble) 25 with air separation and 9 with transport medium, 4 Type II DMEK grafts (2 with air and 2 with fluidic dissection) and 2 Type III (that were used for DMEK) grafts all with fluidic dissection were created.

All of the mixed bubble were created with transport medium. The results can be summarized in Table 1.

Table 1. *Type of injection found to be significantly different between the bubble types (x^2 chi square=10.02, 0=0.008)*

Bubble type			Type of dissection (Air or Medium)		Total
			Air	Medium	
Bubble Type	Type I Bubble	Count	25	9	34
		%	73.5%	26.5%	100.0%
	Type-2 Bubble	Count	2	2	4
		%	50.0%	50.0%	100.0%
	Type 3 bubble	Count	0	2	2
		%	0.0%	100.0%	100.0%
Total		Count	27	13	40
		%	67.5%	32.5%	100.0%

Discussion

After the description of the first successful keratoplasty by Edward Zirm in Olomouc (then Czechoslovakia) in 1906 [3], few things changed at least in the surgery of corneal endothelial disorders for almost 100 years until the development and description of Descemet Stripping Endothelial Keratoplasty (DSEK) by Melles et al [4] and Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) by Gorovoy et al [5] in 2004 and 2006 respectively. These revolutionized the treatment of corneal endothelial dysfunction as they are almost sutureless relying on air to attach the posterior corneal graft to the host cornea (Figure 1), they induce minimal astigmatism, they lead to rapid visual recovery compared to traditional PK and they maintain the host cornea's architecture due to the small incision used for the introduction of the graft.

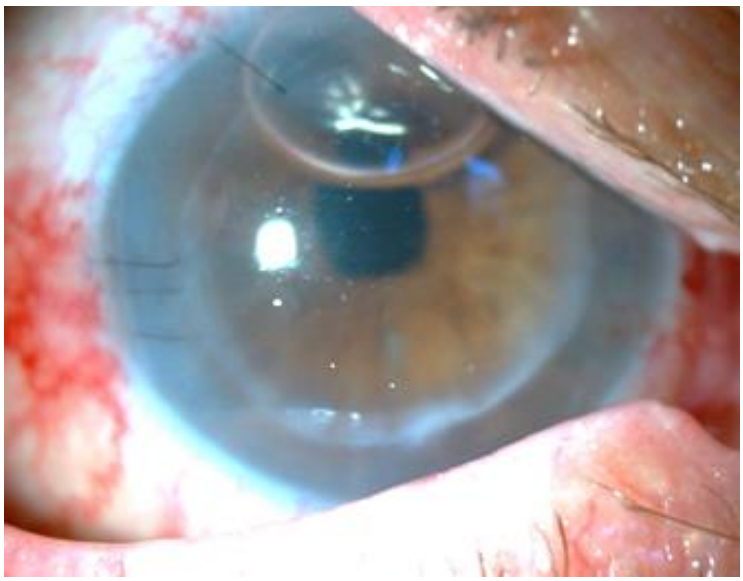

Figure 1. First post-operative day of a DSEK graft with evidence of an air bubble that is used to facilitate attachment of the donor graft to the host posterior stroma.

Since then, more advancements were introduced that led to even more rapid rehabilitation and were associated with more surgical challenges and difficulties. DMEK that was described by Melles et al [6] in 2006 leads to even faster rehabilitation but is associated with a higher failure rates and the need for use of older donors; and PDEK that was described by Agrawal et al [7] in 2014 whic is smaller in size than DMEK but allows internal and external manipulation, leads to almost as fast visual rehabilitation and can be performed with very young even infant donors.
Despite the many advantages associated with PDEK, there are some surgeons and Eye Banks that are reluctant to incorporate PDEK harvest in their practice due to lack of experience with pneumatic (air) or other medium graft dissection.

Type I bubbles used for PDEK harvest are smaller than Type II- DMEK bubbles and always start from the center towards the periphery making the two types of bubbles easily distinguishable.

In our cohort 85% of the graft harvests led to Type I – PDEK bubble with 10% Type II and 5% Type II (mixed). These later ones are used for DMEK graft harvesting.

67.5% of the grafts were pneumatically dissected with the vast majority being harvested as Type I PDEK grafts. The use of transport medium after 10 tries of air dissection led to the harvest of more PDEK grafts. Air dissection led to the creation of PDEK (Type I bubble) grafts in 92% of the cases whereas medium-fluidic dissection led to PDEK (Type I) graft dissection in 69% of the cases. It is characteristic that out of the mixed bubble (Type III) grafts, that are difficult to dissect, all were injected with transport medium. The type of injection was statistically significant ($p=0.008$) between the bubble types suggesting that an easier pneumatic dissection normally leads to a Type I- PDEK bubble. The addition fluidic (medium) injection allows more grafts to be harvested, but also leads to the creation of Type II and Type III (mixed) DMEK grafts.

It is important to note that our cohort consisted of quite young donor corneas with an associated high endothelial count. Although, this is usually the requested type of donor material, most European eye banks supply also older donor corneas (naturally within the requirements and

regulations) with adequate endothelial counts. We postulate that lower quality donor material maybe associated with a higher percentage of Type II and Type III grafts in both air and mainly fluidic dissection, however a larger prospective would better assess the relationship between donor quality and predictability of graft type creation.

Until this is performed, validated and reconfirmed we suggest that surgeons use donor material of the best possible donor quality for all types of endothelial keratoplasties and do not give up their graft dissection even after 10 pneumatic tries. In these cases fluidic dissection should be performed allowing the harvest of more grafts but care should be taken in patient consenting as the percentage of these later fluidically dissected grafts is higher for DMEK transplants.

The authors declare that they have no conflicts of interest.

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Functional and anatomic results of up to 24 months aflibercept treatment for diabetic macular edema in real-life setting

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Abstract

Purpose: Treatment with intravitreal injections of anti-vascular endothelial growth factors, like aflibercept, has revolutionized the management of diabetic macular edema. The purpose of this study is to evaluate the 2-year results of treatment with aflibercept in newly diagnosed, treatment-naive patients with diabetic macular edema in a real-life setting in a tertiary hospital of Southwestern Greece. **Methods:** In this retrospective, real-life, single-center, cohort study the records of diabetic patients were reviewed. In the study we included treatment naive eyes that started treatment with intravitreal injections of aflibercept in the Department of Ophthalmology of the University Hospital of Patras. The scheduled treatment regimen of aflibercept was based on the Summary of Product Characteristics of the product and included a loading dose of 5 monthly aflibercept injections followed by bimonthly treatment until the completion of the first year. During the second year a treat and extend treatment regimen was applied. We recorded data such as age, gender, number of visits and injections, best corrected visual acuity (BCVA) and central macular thickness (CMT) as it was evaluated by a spectral domain optical coherence tomography (SD-OCT). **Results:** Thirty treatment-naive eyes of 22 patients (14 male, 8 female) received treatment with aflibercept for at least 1 year during the period between January 2017 and August 2019. The mean age of the patients was 68.64±7.35 years. Ninety percent of the patients suffered from type-II diabetes and 9% from type-I. The median time between the diagnosis of diabetic macular edema and initiation of treatment with intravitreal injections of aflibercept was 0.5 months (range 0-3 months). Median baseline logMAR BCVA was 0.398 (range 0.046-1.301). The mean CMT at baseline was 388.0±162.94μm. Over a period of 12 months, and after a mean number of 7.5±2.3 visits, patients received a mean number of 7±1.12 intravitreal injections of aflibercept. Eighteen eyes (60%) received an induction phase with 5 monthly injections according to aflibercept SPC. After 12 months the median BCVA (0.324, range 0.0-1.3) was statistically significantly better compared to baseline (p=0.024) and the CMT (295.67±70.99) was significantly lower compared to baseline (p=0.017). Eighteen eyes (60%) completed 2 years of treatment with aflibercept. Over the 2-year period patients made a mean number of 12.7±3.08 visits and received a mean number of 10.2±1.64 intravitreal injections of aflibercept. The median

logMAR BCVA at 2 years (0.301, range 0-0.52) was statistically significantly better compared to baseline (p=0.013) and the CMT (293.53±65.93) was significantly lower compared to baseline (p=0.01). No serious adverse events were recorded during this period.**Conclusion:** Aflibercept resulted in significant functional and anatomic improvement after 12- and 24-month treatment in diabetic macular edema eyes in a real-life setting. The majority of the eyes completed the 2-year treatment regimen of aflibercept.

Introduction

Diabetes mellitus (DM) has become a global pandemic. There were 382 million people suffering from diabetes in 2013, while this figure is anticipated to rise to 592 million by the year 2035 [1]. According to the World Health Organization records, diabetic retinopathy is accountable for 4.8% of the 37 million cases of blindness throughout the world [2]. Diabetic macular edema (DME) is one of the major complications of diabetic retinopathy and one of the leading causes of vision loss in the working age population in industrialized countries [3]. The prevalence of DME is estimated to be 6.81% among diabetic patients [4]. It may develop at any stage of diabetic retinopathy and is the main cause of visual impairment in these patients [5].

Diabetic macular edema is characterized by the presence of retinal edema, vascular leakage and deposition of lipid exudates at the area of the macula [6]. Hyperglycemia induces a number of molecular and biochemical changes that lead to over-expression of chemokines and cytokines and contributes to the establishment of an inflammatory state at the retina. Several mediators, including vascular endothelial factor (VEGF), promote disruption of the retinal blood barrier and result in increased vascular permeability and development of diabetic macular edema [6].

The previously available therapeutic options for DME, like grid or focal laser photocoagulation at the macular area, could stabilize visual acuity but failed to provide significant visual gains [7]. The introduction of anti-VEGF agents has revolutionized the management of DME. Aflibercept is a soluble decoy receptor fusion protein [8] that is comprised of VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 fused to the Fc portion of the human IgG1 immunoglobulin. Aflibercept binds and inhibits all VEGF-A and VEGF-B isoforms as well as the placenta growth factor (PGF), a VEGF homologue binding the VEGF receptor 1[9].

The purpose of this study is to evaluate the 2-year results of treatment with aflibercept in diabetic macular edema in a real-life setting in a tertiary hospital of Southwestern Greece.

Patients and Methods

This was a retrospective, real-life, single-center, cohort study at the Ophthalmology Department, University Hospital of Patras. The study was conducted according to the ethical guidelines of the Declaration of Helsinki and received approval by the Institutional Review Board of the University

Hospital of Patras.

The medical records of treatment-naive eyes treated with intravitreal injections of aflibercept monotherapy due to DME for at least one year were retrospectively reviewed. The treatment regimen for DME in our clinic is based on the Summary of Product Characteristics (SPC) of aflibercept (Eylea 40 mg/ml, Bayer Hellas A.G.) [9] and included 5 monthly consecutive intravitreal injections followed by bimonthly injections until the completion of the first year of treatment. Provided visual and/or anatomic results were stable after the first year, intervals between injections during the second year were extended by 2 weeks up to a maximum of 3 months.

We analyzed the data from the medical records of DME patients treated with aflibercept from January 2017 to August 2019. We excluded eyes that did not complete at least 1 year treatment, eyes with intravitreal hemorrhage, tractional retinal detachment, epiretinal membrane or vitreomacular traction or eyes with any other concurrent severe ocular pathology, eyes that had undergone cataract surgery or any other surgery in the study eye during the study period or eyes that received any additional treatment for DME except aflibercept. Patients with insufficient records were also excluded. Finally, we did not include patients that participated in any interventional clinical trial.

We recorded data such as age, gender, type of diabetes, serum glycosylated hemoglobulin levels (HbA1c) and stage of diabetic retinopathy. We also recorded clinical data like best corrected visual acuity (BCVA) and automated central macular thickness (CMT) as assessed by spectral domain optical coherence tomography (SD-OCT, Heidelberg Engineering, Inc., Heidelberg, Germany) at baseline and after 12 and 24 months of treatment with aflibercept. The number of visits and the number of intravitreal injections administered during the 12 and 24 months of treatment were also documented.

Statistical analysis

Statistical analysis was performed with SPSS 23.0 software (SPSS, Inc., Chicago, IL). All variables were tested for normality with the Kolmogorov-Smirnov test. The Snellen fraction was converted to log MAR for statistical analysis. The Wilcoxon non-parametric test was used for pairwise comparisons between different timepoints and baseline. A level of $p<0.05$ was considered to be statistically significant.

Results

A total of 30 treatment naive eyes (12 right and 18 left eyes) of 22 newly diagnosed DME patients were included in this study (Table 1). The study population comprised of 14 males and 8 females; mean age 68.64±7.35 years. Ninety-one percent of the patients suffered from type 2 and 9% from type 1 diabetes mellitus. The median HbA1c was 6.7 (5.5-9.3). Two eyes had mild non proliferative diabetic retinopathy (NPDR), 10 moderate NPDR, 8 severe NPDR and 4 very severe NPDR wile 6 eyes suffered from proliferative diabetic retinopathy. The median Snellen BCVA at baseline was 0.4 (range 0.05-0.9). The median logMAR equivalent BCVA was 0.398 (range

0.046-1.301). The mean CMT was 388.0±162.94μm.

The median time between DME diagnosis and initiation of treatment was 0.5 months (range 0-3 months). Eighteen eyes (60%) received a loading dose of 5 monthly intravitreal injections of aflibercept.

Over a period of 12 months, and after a mean of 7.5±2.3 visits, patients received a mean number of 7±1.12 intravitreal injections of aflibercept (Table 2). The median Snellen BCVA at 12 months was 0.5 (range 0.05-1).The median logMAR equivalent BCVA was 0.324, (range 0.0-1.3). The BCVA at 12 months was statistically significantly better compared to baseline BCVA (p=0.024) (Table 3). The CMT after 12 months of treatment was 295.67±70.99 and was statistically significantly lower compared to baseline CMT (p=0.017).

Eighteen eyes (60%) completed 2 years of treatment with aflibercept. Over the 2-year period, patients had conducted 12.7±3.08 visits and had been administered a mean number of 10.2±1.64 intravitreal injections of aflibercept. At 24 months, the median Snellen BCVA was 0.5 (range 0.3-1) and the median logMAR equivalent BCVA was 0.301 (range 0-0.52) (Table 3). The BCVA after 2 years of treatment with aflibercept was statistically significantly better compared to baseline BCVA (p=0.013). The CMT at 24 months (293.53±65.93) was significantly lower (p=0.01) compared to baseline.

No serious adverse events occurred or were reported during the time period of the study.

Table 1. *Patient demographics and baseline characteristics.*

Characteristic	
Number of patients	22
Sex (male/female)	14/8
Age in years (mean ± SD)	68.64±7.35
Type of diabetes	
Type 1, n (%)	2 (9)
Type 2, n (%)	20 (91)
HbA1c [median (range)]	6.7 (5.5-9.3)
Number of eyes	30
Study eye (right/left)	12/18
Diabetic retinopathy stage	
Mild NPDR, n (%)	2 (6.7)
Moderate NPDR, n (%)	10 (33.3)
Severe NPDR, n (%)	8 (26.7)
Very severe NPDR, n (%)	4 (13.3)
PDR	6 (20)
Snellen BCVA [median (range)]	0.4 (0.05-0.9)
LogMAR BCVA [median (range)]	0.398 (0.046-1.301)
CMT in μm in OCT examination	
Mean±SD	388.0±162.94
Median (range)	380 (202-821)

HbA1c : glycosylated hemoglobulin, NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, BCVA: best corrected visual acuity, CMT: central macular thickness, OCT: optical coherence tomography

Table 2. *Number of visits and intravitreal injections during the study period.*

	1st year	2nd year	Total
Number of visits			
Mean ± SD	7.5 ± 2.3	5.2 ± 2.24	12.7 ± 3.08
Median (range)	8 (5-13)	6 (2-8)	11 (10 - 20)
Number of intravitreal injections			
Mean ± SD	7 ± 1.12	3.2 ± 1.2	10.2 ± 1.64
Median (range)	7 (5-8)	3 (2-6)	10 (8-13)

Table 3. *Visual and anatomic characteristics of DME eyes at baseline and at 1 and 2 years after treatment with intravitreal injections of aflibercept.*

	Baseline	1st year	2nd year
BCVA (logMAR)			
Median (range)	0.398 (0.046-1.301)	0.324 (0.0-1.3)*	0.301 (0-0.52) #
CMT (μm)			
Median (range)	380 (202-821)	281 (200-500)**	331 (199-366) ##
Mean ± SD	388.0±162.94	295.67±70.99	293.53±65.93

DME: diabetic macular edema, BCVA: best corrected visual acuity, CMT: central macular thickness

** p=0.024 compared to baseline (Wilcoxon test)*
*** p=0.017 compared to baseline (Wilcoxon test)*
 # p=0.013 compared to baseline (Wilcoxon test)
p=0.01 compared to baseline (Wilcoxon test)

Discussion

The efficacy and safety profile of aflibercept for the treatment of DME has been established through the VISTA and VIVID DME studies [10, 11], two large randomized clinical trials. In these studies, aflibercept 2mg administered every 8 weeks following an induction phase of 5 monthly intravitreal injections of aflibercept led to significantly better functional and anatomic outcomes

compared to the control group which was treated with laser photocoagulation. The results were sustained at week 100 and 148 [11, 12]. The mean number of intravitreal injections of aflibercept was estimated between 9 to 12 injections per year. The results of these studies resulted in the regulatory approval of aflibercept for the treatment of DME.

However, the results of randomized controlled clinical trials cannot always be anticipated in the patients treated in a real life setting. The strict inclusion and exclusion criteria of the well-designed randomized clinical trials often result in the exclusion of patients with other concomitant health issues, poor glycemic control, eyes with low BCVA or proliferative diabetic retinopathy, rendering the study population not representative of the unselected population encountered and treated in clinical practice. Furthermore, compliance with scheduled visits is not certain in everyday practice, while possible delays in treatment approval by health insurance funds may result in different treatment regimens compared to the treatment protocols that are administered during a controlled clinical trial. Consequently, real world studies are required in order to evaluate the effectiveness of aflibercept in the treatment of DME in routine clinical practice.

In a real world setting, aflibercept was originally assessed in patients unresponsive to other anti-VEGF agents and the visual and anatomic outcomes were good [13, 14]. Thereafter, a 12-month retrospective real life cohort study conducted at the Moorfields Eye Hospital, London, UK reported significant improvement in BCVA and reduction of CMT after administration of a mean number of 6.92 intravitreal injections of aflibercept, on a pro re nata basis, in 99 treatment naive DME eyes [15]. A smaller real-life study from Spain evaluated aflibercept in 29 treatment naive DME eyes over a 12-month period and showed that aflibercept effectively reduced CME and improved BCVA. Aflibercept treatment regimen was fixed in that study and included five monthly doses followed by every 2 months injections [16]. Another real life study from Japan reported smaller number of intravitreal injections of aflibercept during the first year of treatment (3.8 ± 2.4) that also resulted in significant improvement of BCVA was significant reduction of CMT [17].This study, however, included some patients that had already been treated for DME with other treatment modalities so its conclusions may be interpreted with caution. Finally, a population-based analysis from the United States [18] that included 1379 eyes treated with aflibercept for DME, reported inferior BCVA outcomes (mean gain of 5.5 letters) compared to randomized clinical trials. In that study an average number of 7.5 intravitreal injections of aflibercept were administered over a period of 12 months.

Our retrospective study aimed to evaluate the 1 and 2-year outcomes of aflibercept treatment in eyes with DME in a non-study population treated in real-life practice at a tertiary hospital of Southwestern Greece. We found statistically significant improvement in BCVA and reduction in CMT after a 12-month treatment period. The 12-month results were maintained and at 24 months with a fewer number of intravitreal injections.

We have to recognize that our study has certain limitations. Firstly, we included a small study population, a fact that prevents us from drawing generalized conclusions. The small sample size is also the reason we were unable to assess prognostic factors, correlations between our findings or any possible effect of treatment with aflibercept on the diabetic retinopathy status. Another limitation is the fact that visual acuity was estimated using Snellen optotypes, as is routinely done in clinical practice in Greece, which was then converted to logMAR BCVA for statistical analysis purposes. That deprives us of the chance to accurately compare visual gain or

loss in early treatment diabetic retinopathy study letters as practiced in randomized controlled trials and in the majority of the large real-world studies.

To conclude, in our study aflibercept monotherapy in treatment naive DME eyes was proven safe and effective in a routine clinical practice setting. Significant improvement in BCVA and anatomic restoration in terms of reduction of CMT was observed in comparison to baseline at 12 months and the results were maintained through the 24-month duration of the study. More, larger real-life studies are required in order to evaluate the long-term effectiveness of aflibercept in the treatment of DME on an every-day practice basis.

The authors declare that they have no conflicts of interest.

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Visual and anatomic outcomes of aflibercept treatment in treatment-naive patients with neovascular age-related macular degeneration; real-life data over 24 months.

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Abstract

Purpose: The aim of this study is to evaluate the 2-year visual and anatomic results of treatment with intravitreal injections of aflibercept in newly diagnosed, treatment-naive patients with neovascular age-related macular degeneration in routine clinical practice of a tertiary hospital of Southwestern Greece. **Methods:** In this retrospective, single-center, non-randomized case-series study we analyzed the records of 32 treatment-naive eyes of 28 patients treated with intravitreal injections of aflibercept. Patients received treatment in the Department of Ophthalmology of the University Hospital of Patras from January 2017 to August 2019. The scheduled treatment regimen included a loading dose of 3 consecutive monthly injections of aflibercept and then injections at 8-week intervals for the next 9 months followed by a treat and extend treatment during the second year. Data such as age, gender, best corrected visual acuity (BCVA) and number of injections were recorded. Spectral domain optical coherence tomography (SD-OCT) findings including presence or absence of fluid and automated central macular thickness measurement at baseline, 12 and 24 months were also recorded. **Results:** The mean age of the patients (14 male, 14 female) was 78.5 ± 7.73 years. Over a period of 12 months, and after a median number of 6 visits (range 3-10), patients received a median number of 6 intravitreal injections of aflibercept (range 3-8). Twenty eyes completed 2 years of treatment with aflibercept. Over the 2-year period patients conducted a median of 14 visits (range 9-15) and received a median number of 10 IVAs (range 6-13). The median logMAR BCVA at 12 months was significantly better compared to baseline [0.412 (range 0.046-1.097) versus 0.549 (range 0-1.301) respectively; p=0.003] while median logMAR BCVA at 24 months [0.398 (range 0.222-1.097)] did not differ significantly compared to baseline (p=0.295). The central macular thickness at baseline was 398.75±98.16 μm and decreased statistically significantly at 12 (295.81±80.48μm) and 24 months (289.29±34.25μm) compared to baseline (p=0.0002 and p=0.002, respectively). At baseline SD-OCT examination subretinal fluid (SRF) was present in 26 eyes (81.25%), intraretinal fluid (IRF) was present in 20 eyes (62.5%) while pigment epithelium detachment (PED) was observed in 28 eyes (87.5%) At 12 months SRF was present in

16 eyes (50%), IRF was present in 10 eyes (31.25%) while PED was observed in 23 eyes (71.88%). At 24 months examination SRF was present in 4 eyes (20%), IRF was present in 10 eyes (50%) while PED was observed in 14 eyes (70%). No serious adverse events occurred during this period. **Conclusion:** Treatment with intravitreal injections of aflibercept in a real life setting resulted in a significant improvement in BCVA at 12 months and in a significant anatomic restoration throughout the 24-month follow-up.

Introduction

Age related macular degeneration (AMD) is the leading cause of central vision loss and of irreversible, legal blindness among elderly individuals in the industrialized countries [1]. This entity is a degenerative, aging procedure that involves the macula and, based on the clinical features, is classified as early AMD characterized by the presence of medium-sized drusen, intermediate AMD where larger drusen and pigment abnormalities are present and late-stage AMD where geographic atrophy or choroidal neovascularization are present [2]. In the latter case the term exudative or neovascular AMD is used.

The pathogenesis of neovascular AMD involves the growth of new blood vessels that is promoted by a signal protein called vascular endothelial growth factor A (VEGF-A) [3].The prognosis of neovascular AMD has vastly improved over the last decade due to the available treatments with intravitreal administration of anti-VEGF agents, like aflibercept, bevacizumab and ranibizumab. Aflibercept is a recombinant fusion protein composed of the combinations of portions of human VEGF receptors 1 and 2 extracellular domains that are fused to the Fc fragment of the human IgG1 immunoglobulin. It binds with a high affinity and disables not only VEGF-A but also VEGF-B, as well as placental growth factor that is also involved in the pathogenesis of neovascular AMD [3].

Aflibercept, administered intravitreally, has been approved for the treatment of neovascular AMD [4]. The purpose of this study is to evaluate the 2-year visual and anatomic results of treatment with intravitreal injections of aflibercept in newly diagnosed, treatment-naive patients with neovascular AMD in routine clinical practice of a tertiary hospital of Southwestern Greece.

Patients and Methods

This is retrospective, single-center, non-randomized, observational case-series study. The study met the ethical guidelines of the Declaration of Helsinki and received approval by the Institutional Review Board of the University Hospital of Patras. The records of treatment-naive eyes treated with intravitreal injections of aflibercept monotherapy for at least one year in the Department of Ophthalmology, from January 2017 to August 2019 were retrospectively reviewed. The scheduled treatment regimen included a loading dose of 3 consecutive monthly injections of aflibercept (Eylea 40mg/ml, Bayer Hellas A.G.) followed by administration of aflibercept at 8-week intervals

for the next 9 months. During the second year a treat and extend treatment regimen was applied where, based on the visual and/or anatomic features, the treatment interval could be extended up to 3 months by 2-week increments, provided there were no signs of recurrence of disease activity on the examination with the spectral domain optical coherence tomography (SD-OCT), a newly detected macular haemorrhage in biomicroscopy, or best corrected visual acuity (BCVA) loss of more than 5 letters which could not be explained by other ophthalmic conditions. In case of deterioration, the treatment interval was shortened by 2 weeks to a minimum of 2 months interval. The eyes that were excluded from the analysis were eyes that did not complete a 12-month treatment with aflibercept, those with co-existent severe ocular pathology, eyes that had undergone cataract surgery, YAG laser posterior capsulotomy or other surgery like vitrectomy in the study eye during the study period or eyes that received any additional treatment for AMD except intravitreal injections of aflibercept. Insufficient clinical records were not included in the study. Finally, patients that participated in interventional clinical trials were also excluded.

Patients' medical records were examined and demographic and clinical characteristics were recorded. Data such as age, gender, Snellen BCVA and number of visits and injections over the 12 and 24-month period were recorded. The findings from the examination with SD-OCT device (Heidelberg Engineering, Inc., Heidelberg, Germany) were also recorded. The OCT features that were assessed were automated central macular thickness (CMT), presence or absence of intraretinal fluid (IRF), presence or absence of subretinal fluid (SRF), presence or absence of pigment epithelium detachment (PED). The baseline fluorescence angiography or indocyanine green angiography were evaluated and neovascular AMD was classified as classic, occult or as polypoidal choroidal neovascilarization. The BCVA and OCT findings were recorded at baseline, 12 months and 24 months after the initiation of the treatment.

Statistical analysis

Statistical analysis was performed with SPSS 23.0 software (SPSS, Inc., Chicago, IL). All variables were tested for normality with the Kolmogorov-Smirnov test. Non parametric data are presented as median and range. Parametric data are presented as mean ± SD. The Snellen fraction was converted to logMAR for statistical analysis. The Wilcoxon non-parametric test was used for pair-wise comparisons between different timepoints and baseline. The Chi-square test was applied to compare categorical variables. A level of $p < 0.05$ was considered to be statistically significant.

Results

Thirty-two treatment naive eyes (20 right eyes, 12 left eyes) of 28 patients (14 male, 14 female) treated with intravitreal injections of aflibercept, and who had documented assessments, were included in the analysis. All eyes completed at least a 12-month treatment with intravitreal injections of aflibercept. The demographic and baseline characteristics of the patients are presented in Table 1.The mean age of patients was 78.5±7.73 years.

The median Snellen BCVA at baseline was 0.2 (range 0.05-0.8). The logMAR equivalent

BCVA at baseline was 0.549 (range 0-1.301) (Table 2). Optical coherence tomography was conducted in all patients at baseline. The central macular thickness (CMT) at baseline was 398.75 ± 98.16 μm. Subretinal fluid was present in 26 eyes (81.25%), IRF was detected in 20 eyes (62.5%) while PED was observed in 28 eyes (87.5%) (Table 3). Thirty eyes (93.75%) had undergone baseline examination with fluorescence angiography in order to confirm the diagnosis of exudative AMD while indocyanine green angiography was conducted in 14 eyes (43.75%). According to fluorescence and indocyanine green angiography findings occult CNV was present in 19 eyes (59.38%), classic CNV in 8 eyes (25%) while polypoidal choroidal neovascularization was present in 5 eyes (15.62%) (Table 1).

The median time between the diagnosis of AMD and initiation of treatment with intravitreal injections of aflibercept was 0.75 months (range 0.5-4 months). Twenty-two eyes (68.75%) received an induction phase with 3 monthly intravitreal injections of aflibercept. Over a period of 12 months, and after a median number of 6 visits (range 3-10), patients received 5.81 ± 1.2 intravitreal injections of aflibercept (median 6, range 3-8,) (Table 4). The median Snellen BCVA was 0.4 (range 0.08-0.9). The median logMAR BCVA (0.412, range 0.046-1.097) was statistically significantly better compared to baseline (p=0.003) (Table 2). Compared to baseline, BCVA improved in 29 eyes (90.6%), remained stable in 1 eye (3.1%) and worsened in 2 eyes (6.3%). The CMT (295.81 ± 80.48) was significantly lower compared to baseline (p=0.0002).

At the 12-months, in OCT examination, SRF was present in 16 eyes (50%), IRF was present in 10 eyes (31.25%) while PED was observed in 23 eyes (71.88%) (Table 3). Compared to baseline, statistically significantly fewer eyes had SRF or IRF (p=0.008 and p=0.012 respectively).

Twenty eyes had completed 2 years period of treatment with aflibercept and adhered to the inclusion criteria for the analysis. The reasons for non-completion of the 2-year treatment with aflibercept of the other 12 eyes were lost to follow-up (5 eyes), end of treatment due to disease stabilization (1 eye), cataract surgery during the second year of treatment (3 eyes) and switch to ranibizumab treatment (3 eyes).

Over the 2-year period patients conducted a median of 14 visits (range 9-15) and received a 9.7±1.95 intravitreal injections of aflibercept (median 10, range 6-13) (Table 4). The number of intravitreal injections of aflibercept that were administered during the second year was 3.4±1.39 (median 3, range 1-6). The median Snellen BCVA was 0.4 (range 0.08-0.6). The median logMAR BCVA at 2 years (0.398, range 0.222-1.097) did not differ statistically significantly compared to baseline (p=0.295) (Table 2). Compared to baseline BCVA improved in 10 eyes (50%), remained stable in 4 eyes (20%) and deteriorated in 6 eyes (30%). After two years of treatment the CMT (289.29±34.25) was significantly lower compared to baseline (p=0.002). Subretinal fluid was present in 4 eyes (20%), IRF was present in 10 eyes (50%) whereas PED was observed in 14 eyes (70%). Compared to baseline, statistically significantly fewer eyes had SRF (p< 0.0001) (Table 3).

No serious adverse event occurred during this period.

Table 1. *Patient demographics and baseline characteristics.*

Characteristic	
Number of patients	28
Sex (male/female)	14/14
Age in years (mean ± SD)	78.5±7.73
Number of eyes	32
Study eye (right/left)	20/12
Snellen BCVA [median, (range)]	0.2 (0.05-0.8)
LogMAR BCVA [median, (range)]	0.549 (0-1.301)
OCT examination	
CMT in μm, Mean ± SD	398.75±98.16
Median, (range)	392 (271-554)
Presence of SRF (%)	81.25
Presence of IRF (%)	62.5
Presence of PED (%)	87.5
Fluorescein angiography examination (%)	93.75
Indocyanine green angiography examination (%)	43.75
Type of AMD	
Classic (%)	25
Occult (%)	59.38
PCV (%)	15.62

BCVA: best corrected visual acuity, OCT: optical coherence tomography, CMT: central macular thickness, SRF: subretinal fluid, IRF: intraretinal fluid, PED: pigment epithelium detachment, AMD: age related macular degeneration, PCV: polypoidal choroidal neovascularization

Table 2. *Visual and anatomic characteristics of AMD eyes at baseline and 1 and 2 years after treatment with intravitreal injections 2 aflibercept.*

	Baseline	1st year	2nd year
BCVA (logMAR)			
Median (range)	0.549 (0-1.301)	0.412 (0.046-1.097)*	0.398 (0.222-1.097)
CMT (μm)			
Median (range)	392 (271-554)	292 (145-499)**	278 (249-352)***
Mean ±SD	398.75±98.16	295.81±80.48	289.29±34.25

AMD: age related macular degeneration BCVA: best corrected visual acuity, CMT: central macular thickness

* *p=0.003 compared to baseline (Wilcoxon test)*

** *p=0.0002 compared to baseline (Wilcoxon test)*

*** *p=0.002 compared to baseline (Wilcoxon test)*

Table 3. *Optical coherence tomography findings of AMD eyes at baseline and 1 and 2 years after treatment with intravitreal injections of aflibercept.*

	Baseline (32 eyes)	1st year (32 eyes)	2nd year (20 eyes)
IRF, n (%)	20 (62.5%)	10 (31.25%)*	10 (50%)
SRF, n (%)	26 (81.25%)	16 (50%)**	4 (20%)***
PED, n (%)	28 (87.5%)	23 (71.88%)	14 (70%)

AMD: age related macular degeneration, IRF: intraretinal fluid, SRF: subretinal fluid, PED: pigment epithelium detachment

** p=0.012 compared to baseline (Chi-square test)*
*** p=0.008 compared to baseline (Chi-square test)*
**** p< 0.0001compared to baseline (Chi-square test)*

Table 4. *Number of visits and intravitreal injections.*

	1st year	2nd year	Total
Number of visits			
Mean±SD	6.25±1.95	5.9±1.86	12.1±2.65
Median (range)	6 (3-10)	5 (4-10)	14 (9-15)
Number of intravitreal injections			
Mean±SD	5.81±1.2	3.4±1.39	9.7±1.95
Median (range)	6 (3-8)	3 (1-6)	10 (6-13)

Discussion

Age-related macular degeneration (AMD) is a multifactorial disease that constitutes the leading cause of vision loss in individuals over the age of 60. Its incidence is expected to increase due to the increase in life expectancy and the ageing of population [1]. It is distinguished as exudative or neovascular AMD and non-exudative AMD.

During the last decade, the introduction of anti-VEGF agents such as aflibercept has revolutionized the treatment of neovascular AMD. Randomized, controlled clinical trials have demonstrated the efficacy of intravitreal injections of aflibercept in the treatment of neovascular AMD and reported significant improvement in terms of visual acuity gains and anatomical stability. The VIEW 1 and VIEW 2 clinical trials are two identical, randomized, double-masked, active-controlled, phase 3 studies that were conducted in order to evaluate the safety and efficacy of intravitreal injections of aflibercept in the treatment of neovascular AMD [5]. The VIEW 1 study recruited patients from Canada and the United States while VIEW2 enrolled patients from other countries. The outcomes of these studies, that enrolled more than 2,400 eyes, resulted in the regulatory approval of aflibercept for the treatment of neovascular AMD. The treatment regimen of

aflibercept for neovascular AMD that was approved was the intravitreal administration of 2 mg aflibercept every 8 weeks after 3 initial monthly injections during the first year, since this treatment protocol resulted in similar efficacy and safety outcomes compared to monthly ranibizumab administration. The proposed treatment regimen for the second year is the treat and extend treatment protocol which is based on the functional and/or anatomical response of the eye to the previous injection. The treatment interval is incremented in case of favorite response and shortened when recurrence occurs.

Nevertheless, the results of the standardized, randomized clinical trials cannot be anticipated in a real-life practice where the strict adherence to a fixed treatment protocol is not always possible, even in a highly standardized clinical setting. Based on that premise, several large-scale, real-life studies have been conducted in order to evaluate the "actual" results of neovascular AMD treatment with aflibercept. The PERSEUS observational study evaluated more than 800 eyes treated with aflibercept for neovascular AMD in Germany [6]. The 12-month results highlighted that regular treatment is associated with better BCVA outcomes. The RAINBOW study is an ongoing, observational 4-year study which aims to evaluate the effectiveness of aflibercept, administered in several different protocols, in the treatment of neovascular AMD in actual treatment conditions at 55 centers across France [7]. The 1-year visual and anatomical results of this study are in agreement with the outcomes of the VIEW studies, although only 10% of the patients received the same protocol with the VIEW studies. The National Aflibercept UK User Group retrospectively evaluated the outcomes of 2 years of treatment with aflibercept in 17 UK centers [8]. In this study aflibercept was administered in more than 1,000 eyes according the VIEW studies protocol during the first year while a variable treatment approach was followed during the second year. The study concluded that the BCVA gains from the first year of aflibercept treatment subsided during the second year, whereas higher number of aflibercept injections during the second year of treatment results in better BCVA outcomes.

Our retrospective study aimed to evaluate the 1 and 2-year outcomes of aflibercept treatment in eyes with exudative AMD in a non-study population treated in real-life practice at a tertiary hospital of Southwestern Greece. According to our results a statistically significant improvement in BCVA and a statistically significant decrease in CMT were observed at 12 months compared to baseline, in addition to preservation of visual acuity and decrease of macular thickness at 24 months.

The 1-year results of our study are in agreement with the RAINBOW study, regarding the visual and anatomical outcomes [7]. Compared to the RAINBOW study almost the same number of intravitreal injections of aflibercept were administered over the first year of treatment (5.81±1.2 in our study and 6±2.1 in the RAINBOW study) which were fewer than that in the VIEW studies (average 7.5 injections in 12 months) [5]. Nevertheless, fewer patients in our study received a loading dose of 3 monthly intravitreal injections of aflibercept (68.75% in our study versus 76.9% in the RAINBOW study). The two year results of our study demonstrated the preservation of BCVA and statistically significant decrease of macular thickness compared to baseline. Our 2-year findings are in agreement with the findings of the National Aflibercept UK User Group study [8]. Furthermore, similar number of intravitreal injections of aflibercept was administered during the second year (a mean number of 3.4 in our study compared to 3.7 injections in the National Aflibercept UK User Group study) [8].

However, we have to admit that our study has certain limitations. The retrospective nature of this single-center study and the limited number of eyes are the major limitations. Due to the small sample size we did not evaluate possible prognostic factors for neovascular AMD response to aflibercept treatment. Furthermore, BCVA was measured with Snellen and not with the Early Treatment Diabetic Retinopathy Study (ETDRS) letter charts, a fact that prevented us from making precise measurements of BCVA gain or loss in EDTRS letters or to make direct comparisons in the functional outcomes with other studies which report results in EDTRS letters.

To conclude, intravitreal injections of aflibercept in treatment-naive patients with neovascular AMD were well tolerated in clinical practice and resulted in a significant improvement in BCVA at 12 months and in a significant anatomic restoration throughout the 24-month follow-up in routine clinical practice at a tertiary hospital. Certainly, more studies are required to report the outcomes of AMD treatment with aflibercept in a real-life setting at Greek hospitals.

The authors declare that they have no conflicts of interest.

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New application of membrane blue-dual dye for retinal or iatrogenic break staining in retinal detachment surgery

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Keywords: -Chromovitrectomy -Undetected retinal breaks

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Abstract

Introduction: The principles of surgery for managing primary rhegmatogenous retinal detachment (RRD) are to precisely identify and correctly treat all causative retinal breaks. Estimates regarding unidentified breaks complicating RRDs vary from 2.2%-22.5%. **Purpose:** To evaluate the efficacy of membrane blue-dual heavy dye solution for staining of undetected preoperatively retinal and iatrogenic breaks. **Subjects, material and methods:** This is a prospective interventional study. 23 and 27-gauge vitrectomy surgeries were evaluated for primary repair of 5 patients with rhegmatogenous retinal detachment (RRD). No breaks where identified prior to surgery despite meticulous pre-operative examination using binocular indirect ophthalmoscopy with indentation. 0.1ml of MembraneBlue-Dual™ was applied onto the vitreous cavity, while it was completely filled with fluid, and all excess dye was immediately aspirated with a blunt backflush instrument. In all eyes with RRD, the surgery was completed by gas tamponade (C3F8 or SF6). Follow up was 6 months. **Results:** We compared the number of breaks identified when examined intraoperative with internal peripheral indentation before and after injection of the dye and found that in all cases (100%) at least one more break was found after injection of dye which was subsequently treated with cryotherapy or endolaser. At last follow up six months after surgery the success rate was 100% and none of our cases re-detached. **Conclusions:** The greatest advantage of use of this 'heavy' dye solution membrane blue-dual is improved intraoperative identification of ILM at the edges of retinal breaks and the discrimination of them from surrounding intraocular structures. Due to its increased molecular weight and viscosity properties it eliminates the need for fluid-air exchange, injection of PFCL or subretinal injection.

Introduction

Chromovitrectomy is a novel approach to visualise the vitreous or retinal surface during vitreoretinal surgery and was motivated by the difficulty in visualising several thin and transparent tissues at the vitreoretinal interface such as the internal limiting membrane (ILM), epiretinal membrane (ERM) or vitreous, particularly the posterior hyaloid membrane. In 1932, Lobeck [1] and coworkers were the first to perform intravitreal injection of vital dyes; a pioneer report has been released by Abrams et al. [2] demonstrating the first intraoperative use of vital dye, with

fluorescein as a good adjuvant for vitreous identification. Since 2000 the application of dyes to stain preretinal tissues during vitreoretinal surgery has become a widespread technique among vitreoretinal surgeons.

Numerous vital dyes with high specific affinity for the ILM have been applied in ILM peeling such as indocyanine green (ICG) and trypan blue (TB). Intravitreal injection of ICG facilitated the visualization of ILM [3]. However, the use of indocyanine green (ICG) has been associated with adverse effects such as visual field defects, reduced visual acuity, and persistent staining [4] which may be aggravated by low osmolarity, bright endoillumination, and use of concentrations greater than 0.05 mg/mL. [5] Later, TB was proposed as a helpful tool to identify epiretinal membranes, and intravitreal triamcinolone acetonide (TA) was found to stain the vitreous [6]. A few other dyes including infracyanine green, patent blue, bromophenol blue, brilliant blue (BBG), and sodium fluorescein have been proposed as alternateve dyes during chromovitrectomy [7 -8].

Recently, extensive laboratory studies have yielded two novel commercially available and CE-approved dye solutions, MembraneBlue-Dual™ and ILM-Blue™, that may have a higher efficacy as a result of the synergistic effect through the use of two dyes within the same sample, combined with polyethylene glycol (PEG) to increase the molecular weight and viscosity. MembraneBlue Dual® tissue dye is a sterile, non-inflammatory solution of Trypan Blue and Brilliant Blue G, dissolved in a physiological sodium chloride phosphate buffer with 4% Polyetheylene Glykol. [9-11].

Future studies may explore the use of fluorophore-labeled antibodies directed against specific macular tissue which, if combined with selective illumination and barrier filters, may lead to highly specific fluorescent staining of ocular tissues. [12]

Each ml MembraneBlue Dual® contains (±5%): [9]

0.25mg Brilliant Blue G purified
1.5mg Trypan Blue, Purified
40mg Polyethylene Glycol 3350
1.9mg disodium hydrogen phosphate dihydrate
0.3mg sodium dihydrogen phosphate dihydrate
8.2mg sodium chloride
Balance to 1ml: water for injection

Advantages of Dual Blue dye:

1. Stains both ERM and ILM (double stain as with Brilliant Blue) to facilitate peeling
2. Improves intraoperative visualisation of membranes of interest and helps the discrimination of these membranes from surrounding intraocular structures as it yields enhanced color-staining contrast against the orange-red attached retina or the whitish detached retina
3. Can be injected in a BSS filled vitreous cavity (no need for additional air exchange as with membrane blue)
4. Stains the posterior hyaloid membrane which can then be grasped and elevated with the vitrectomy cutter (Figures 1 and 2)

5. Has minimal toxicity or safety concerns

Properties of Dual Blue:

1. Sinks immediately as a cohesive ball to the posterior pole and provides a viscous and dense solution
2. Only stains the targeted tissue
3. Can be also diffuse throughout the whole globe and stain the peripheral ILM (indented dispersion) [9]

Staining the ILM or epiretinal membranes allows surgeons to work more quickly and precisely, thereby potentially improving surgical safety and anatomical outcomes. There are four main target tissues for staining during chromovitrectomy. Vital dyes such as TB exhibit outstanding affinity for ERMs because of the strong presence of dead glial cells within those membranes. ILM staining with TB is subtler than with ICG, and possibly TB stains the fine ERM overlying the ILM rather than the ILM itself. The usefulness of TA to highlight the vitreous has also been proposed. TA enables the visualisation of both prolapsed vitreous to the anterior chamber or posterior vitreous remaining in the vitreous cavity.

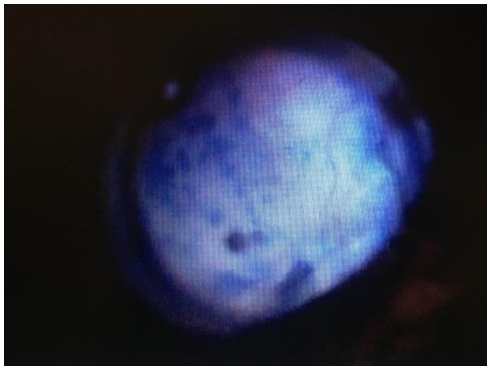

Figure 1. Patchy staining of the posterior hyaloid membrane with Dual Blue.

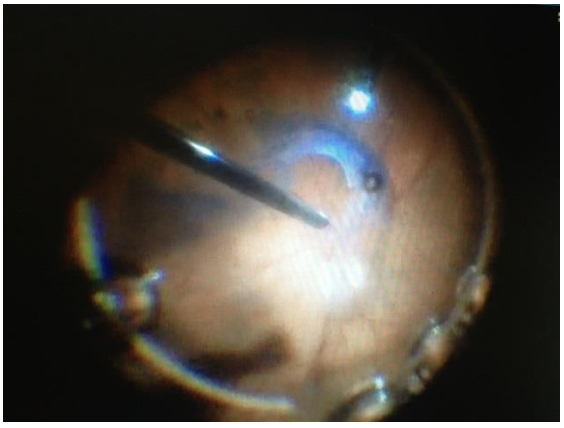

Figure 2. Posterior vitreous detachment with a 23-gauge cutter assisted with Dual Blue staining.

The principles of surgery for managing primary rhegmatogenous retinal detachment (RRD) are to

precisely identify and treat all causative retinal breaks. Breaks responsible for RRDs may not be seen preoperatively owing to opacities in the media, pseudophakia, poor dilation or other causes. Estimates regarding unidentified breaks complicating RRDs vary from 2.2-22.5% [13-15].

Various strategies have been used to identify and manage primary retinal breaks intraoperatively when they have not been found before surgery, including circumferential buckling, broad retinopexy, scleral buckling, and pars plana vitrectomy (PPV) [16-21]. When retinal breaks can be identified intraoperatively, primary reattachment rates are significantly higher than when retinal breaks cannot be identified before or during surgery [16-21].

During recent years, advances in the PPV technique have allowed the surgeon to conduct a detailed intraoperative examination of the peripheral retina and, thereby, identify small retinal breaks. A new application for dyes in chomovitrectomy consists of staining retinal break edges during vitrectomy for rhegmatogenous retinal detachment repair was suggested in 2007. Jackson et al. [22] demonstrated the success of this technique to identify retinal breaks in 4 out of 5 patients and concluded TB-guided retinal break detection to be a very useful surgical technique. In this technique, TB 0.15% is injected transretinally into the subretinal space using a 41-gauge cannula.

In order to evaluate the efficacy of MembraneBlue-Dual heavy dye solution for staining of retinal and iatrogenic breaks we performed a prospective non-randomized interventional study using the high molecular weight dye MembraneBlue-Dual (0.15% trypan blue, 0.025% brilliant blueG, 4% PEG) in 23 gauge vitrectomy surgeries for primary repair of 10 patients with rhegmatogenous retinal detachment (RRD) where no breaks where identified prior to surgery despite meticulous pre-operative examination using binocular indirect ophthalmoscopy with indentation. In all cases MembraneBlue-Dual enhanced staining, thus facilitating the identification of undetectable retinal or iatrogenic breaks. None of the surgeries required the use of perfluorocarbon heavy liquid, fluid-air exchange to assist the dye application, or subretinal injection of dye. Subretinal fluid was stained which enabled surgeons to identify and drain through the break (Figures 3, 4 and 5). A drainage retinotomy was not required in any patient. No retinal adverse effects related to the surgery or use of the dye were observed based on serial autofluorescence images up to 6 months after surgery.

Vitrectomy surgeries were performed during which a posterior vitreous detachment was created. The surgeons performed all scleral depression using a muscle hook which was started at the most probable location of the retinal break and then continued along the entire retinal periphery. Without performing a prior fluid-air exchange 0.1 ml of MembraneBlue-Dual™ was applied into the vitreous cavity, while it was completely filled with fluid, and all excess dye was aspirated with a blunt backflush instrument. In all cases, the intention was to identify peripheral PVD-related retinal or iatrogenic breaks and to treat them with cryotherapy or endolaser. A retinal break was defined as primary when it was judged from the contour of the detachment that this break alone could account for the detachment. A break was defined as secondary when the contour of the detachment could not be accounted for by this break alone. In one case a parafoveal iatrogenic break was identified close to the area where macular ERM peeling was performed. In all eyes with RRD, the surgery was completed by gas tamponade (C2F6, C3F8 or SF6). After absorption of the gas tamponade all retinas remained attached. We compared the number of breaks identified when examined intraoperative with internal peripheral indentation

before and after injection of the dye and found that in all cases (100%) at least one more break was found after injection of dye which was subsequently treated with cryotherapy or endolaser. At last follow up six months after surgery the success rate was 100% and none of them re-detached. (Figure 6 a.b.c.)

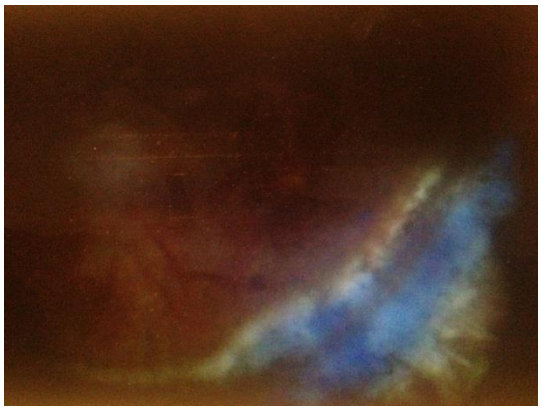

Figure 3. The intravitreal Dual Blue dye penetrated the peripheral detached subretinal space through the retinal break.

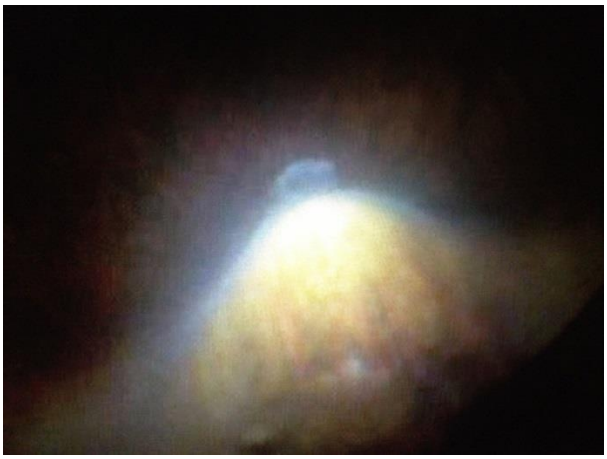

Figure 4. The posterior retinal break was detected with indentation after drainage of the subretinal fluid.

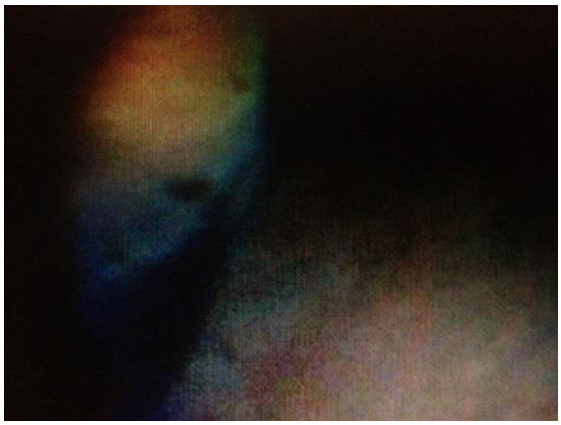

Figure 5. Multiple peripheral breaks identified after Dual Blue dye staining.

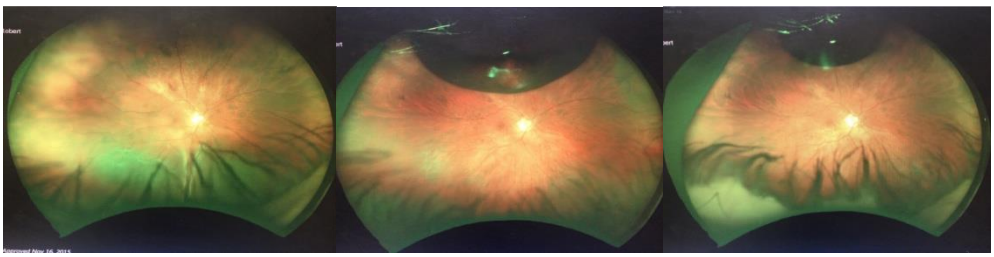

Figure 6.a.b.c. Serial color photos with Optos in a patient with RRD. The retina remained attached after absorption of the gas tamponade.

Discussion

A number of management techniques and strategies are available for treating retinal detachments in which the breaks are undetected pre-operatively. An internal search for retinal breaks using deep kinetic indentation of the sclera with combined endoillumination was first described by McLeod, using PPV and enables identification of retinal breaks in 95% of RRD cases. [23] This has been further elaborated to a dye extrusion technique, [22, 24] involving injection of sub retinal trypan blue, using a 41-gauge needle, into the SRF. Extrusion of the dye is encouraged through the presumed break using perfluorocarbon liquid to fill the eye and systematic rotation of the globe, with the aim of seeing a plume of dye venting out of the break. The use of a dye provides color contrast that aids detection and is an advantage over the use of heavy liquid alone. In some instances, the dye stains the devitalized tissue of the break itself. This was reported in the context of both primary and repeat RD repair. [22, 24] In small size retinal breaks there is small amount of subretinal fluid streaming out through the breaks even after the use of PFO thus, the Schlieren effect is difficult to identify. 18 Both these techniques carry the potential risk of retinal toxicity, especially as some dye may be left in the subretinal space.

Gupta D et al. [25] published a case series where Vision Blue (0.006% version of Trypan Blue) has been injected trans-sclerally into the subretinal fluid under the detached retina to identify clinically undetected (occult) retinal breaks in the setting of retinal re-detachments. This technique offers the advantage that iatrogenic break creation is avoided compared to trans-retinal injection. However, after injection of dye an air exchange is required to encourage the dye posteriorly. Theoretically, possible complications of this technique include hypotony, choroidal haemorrhage, retinal haemorrhage and retinal and vitreous incarceration [26]. Wu et al. [27] suggested the use of scleral buckling that extended over the circumference of the RRD with cryotherapy in the initial treatment of pseudophakic RRD with undetected retinal breaks with 72% anatomic success increased to a higher overall success rate after PPV with long-term tamponade for recurrent RD after primary buckling. Martinez-Castillo V et al. [28] reported 98% and 100% primary and final success rate respectively using pars plana vitrectomy alone with diffuse illumination and extensive vitreous dissection in order to identify and manage retinal breaks undetectable before surgery.

Conclusion

The greatest advantage of use of this dye solution MembraneBlue-Dual is improved intraoperative identification of ILM at the edges of retinal breaks and the discrimination of them from surrounding intraocular structures. Due to its increased molecular weight and viscosity properties it eliminates the need for fluid-air exchange or subretinal injection.The better the ILM can be identified the lower the chance that further surgery due to undetectable retinal or iatrogenic breaks will be needed.

Our results suggest that intravitreal injection of Dual Blue dye is a simple, safe and effective technique that can facilitate the identification of clinically undetectable retinal breaks in patients with primary retinal detachment and can result in high primary reattachment rate at 6 months follow up (in our case series we had 100% success rate). The use of intravitreal Dual Blue dye can be one additional option in vitreoretinal surgeons' armamentarium of surgical techniques dealing with unidentified retinal breaks.

The author declares that they have no conflicts of interest.

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Novel smartphone assisted device for neurotargeting

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Abstract

Introduction: Despite the widespread use of external ventricular drainage, revision rates, and associated complications are reported between 10 and 40%. Current available image-guided techniques using stereotaxy, endoscopy, or ultrasound for catheter placements remain time-consuming techniques. Also, brain targeting procedures in emergency setting are challenging. The development of an easy-to-use, portable, image-guided system could reduce the need for multiple passes and improve the rate of accurate catheter placement and other brain targeting interventions in emergency setting. This study aims to design a novel smartphone assisted device for external ventricular drainage (EVD) placement and neuronavigation. **Technique:** In this study, authors have designed a novel 3D system composed of 3D software based on Android operating system and 3D design of a device in Autodesk 3D max using simple cranial measurements by DICOM PACS software for data input from Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). We plan to utilize this software as a guide and as a replacement for far more advanced neuronavigation systems. We have designed and launched the pilot version of this software and tested and compared it by DICOM PACS and also in an artificial skull for accuracy assessment. Our evaluation confirmed high accuracy performance of this smartphone application compared with DICOM PACS software for initial surgical approach to candidates for EVD placement or other emergency setting procedures which require neurotargeting interventions. Also, our neurotargeting device's accuracy was tested using provided angles by the application, which results in acceptable performance. **Conclusion:** This smartphone application coupled with targeting device can be used in various settings such as EVD placement in hydrocephalus and in other brain targeting candidates in emergency settings. Also, it may be used in any emergency or neurosurgery department centers with no access to advanced neuro-imaging facilities, only using patient's simple cranial measures to achieve acceptable and highly accurate brain targeting compared with conventional, time consuming and costly techniques. We plan to expand this study to clinical trials for further evaluation.

Introduction

Ventriculostomy, is a process of creating an ostomy at surface of skull of a patient to gain access to the ventricular system. The process is done via drilling the skull and dura by application of an external ventricular drainage (EVD) for cerebral spinal fluid (CSF) outflow or intracranial pressure (ICP) monitoring, which are termed as provisional catheterization [1]. On the other hand, permanent catheterization includes cerebral shunts that are named by their end place. Ventriculo-peritoneal shunt which drains CSF to peritoneal cavity, ventriculo-atrial shunt which ends at right atrium, ventriculo-cisternal shunt and ventriculo-subgaleal shunt which drain to cisterna magna and sub galeal space respectively. Each shunt is designed for a specific purpose and thus variable valves are used [2]. A delta valve opens when ICP reaches to a pre-determined value and is designed for prevention of over drainage. Thus, shunted ventricles are larger than non-shunted ventricles. Also, application of a cylindrical valve with medium pressure could result in unequal ventricular drainage. A two-ball valve connected by a screw without pressure adjustment, makes a Spitz and Nulsen valve. Furthermore, as same as delta valves, anti-siphon valves have similar mechanism of action [3]. Albeit, EVD is indicated in patients whom their intracranial hemorrhage disseminated to the ventricles (intra-ventricular hemorrhage), suffered from sub-arachnoid hemorrhage or traumatic brain injury with ventricular system disruption. Also, EVD placement in infection events with involvement of meningeal layers and ventricles (bacterial meningitis and ventriculitis) is advocated for more precise diagnosis, as well as antibiotic introduction [4] Moreover, ICP monitoring in suspected patients to hydrocephalus is the mainstay of management in regards of primary intervention in emergency department. Also, increased ICP could result secondary to intracranial tumors, traumatic brain injury with cisternal effacement, cerebral edema secondary to ischemic stroke with vasigenic edema or hypoxy due to ischemic encephalopathy. Pathologies of venous return system such as venous thrombosis or jugular vein thrombosis could lead to raised ICP [5]. Besides, metabolic disorders such as hyponatremia, uremic or hepatic encephalopathy, choroid plexus tumors, idiopathic intracranial hypertension and pseudotumor cerebri, are among other causes of increased ICP. Ventriculostomy is a common procedure in neurosurgical setting and precise insertion of a ventricular catheter is a major determining variables of longevity of a shunt [6]. Despite large amount of patients suitable for this practice and development of technology, failure rate due to catheter's proximal end occlusion by blind techniques without guidance remains considerably high which mentioned rate were stated in multiple reports as 12, 32, 36, 38 and 45% [7]. Optimal placement of the catheter in ipsilateral anterior horn of lateral ventricle just anterior to the foramen of Monro, depends on accurate placement of catheter's tip in the ventricle far from choroid plexus and ependyma, in a proper trajectory by precise calculation of catheter's length [8]. A retrospective study with 90 patients who underwent catheterization based on anatomical prominences, correct catheter's tip placement in ipsilateral ventricle was achieved only in 56% of cases. Also, 7% were located in contra-lateral ventricle, followed by 6% in 3rd ventricle. Notably, catheter's tip was situated outside relevant ventricular structures in 22% of patients [9]. High failure rate of accurate catheter placement all over the world, ignites investigation for an optimal method with acceptable results. In past 25 years, factors such as guided techniques, precise evaluation of catheter length based on computed tomography (CT) images and utilization of navigation based on stereotactic coordinates

given by anatomical prominences of patients, were invented and introduced [10]. Mentioned techniques continued their development following the emergence of ultrasound transducers, neuronavigation and endoscopic guidance methods. Trans-cortical drilling of ventricles is a well-known simple procedure of neurosurgery which is trained as one of the first procedures by trainees [11]. In cases of lifelong necessity of a patient to a shunt secondary to underlying disease, ventricular catheterization considered as a permanent and important implant and its accurate placement based on proportional relativity between catheter's location and probability of proximal shunt occlusion is considered somehow vital intervention. Some studies reported the inaccuracy of blind EVD placement from 12.5 to 40%. The efficacy of non-guided and blind technique from superficial anatomical landmarks relates to surgeon's expertise [12]. In total, in cases of encounter to complicated cases, surgeons and experts used mentioned above procedures and technologies which in relative to the final purpose, are time consuming and costly. Based on above statements and the importance of the spirit of this procedure and the necessity to prevent treatment-related complications and avoidance of catheter misplacement, authors set out to design a new system for accurate localization of ventricular system for optimal EVD placement.

Technique

In this study, we have designed a novel 3D system composed of 3D software based on Android operating system and 3D design of a device in Autodesk 3D max using simple cranial measurements by DICOM PACS software for data input from CT and magnetic resonance imaging (MRI). We plan to utilize this software as a guide and as a replacement for far more advanced neuronavigation systems. We have designed and launched the pilot version of this software and tested and compared it by DICOM PACS and also in an artificial skull for accuracy assessment. By conducting this study, we aim to prevent high EVD misplacement rate which reported from 12.5% to 40% in previous section. Also, this system will capable of accurate catheter's tip insertion without time and expenditure consumption that are used via more advanced live imaging or ultrasound techniques. Predicted expenses to be used for development our proposed system is significantly less in relative to current complicated technologies. Upon completion of development of this system and initiation of clinical trials, widespread application of this system to patients with ventricular disorders who require EVD placement or ICP monitoring is expected. Patient's load admitted to emergency departments who require ICP monitoring or EVD placement for CSF drainage is considerably high and prompt management of these group of patients to prevent continued elevated ICP and hydrocephalus with potential of occlusion of posterior cerebral artery occlusion due to trans-tentorial hernia, chronic papilledema as a result of optic disc injury, increased size of third ventricle with optic chiasm's compression, cognitive disorders, ataxia and incontinency is vital. To this date, multiple surveys were conducted to evaluate the efficacy and complications of current widespread blind technique along with novel methods, either from admiration or criticism viewpoints, are available in online data banks which raise the importance of achievement to a rational solution. This novel system is capable of

improvement of precise catheter's tip placement, exact calculation of catheter's length from skull's surface to ventricular cavity, exact calculation of entering angle of catheter's tip from skull's surface aimed at ventricle in axial, coronal and sagittal planes, catheter's entry point and burr hole's location on skull's surface based on initial CT or MRI images, hastening EVD placement's procedure compared to blind method, decrease potential peri-ventricular tissue injury and the possibility of eloquent as well as infection rate secondary to enhanced catheter placement and skull-related deformity compensation in cases of blind EVD placement due to uneven surface via utilization of uploaded primary images of CT or MRI. For enrollment of patients to approve its clinical efficacy, authors assess following variables at initial survey, including age, sex, previous medical history, underlying etiology of current intracranial pathology, presence of any deformity of skull's morphology and multiple diameters of the skull. Our evaluation confirmed high accuracy performance of this smartphone application compared with DICOM PACS software for initial surgical approach to candidates for EVD placement or other emergency setting procedures which require neurotargeting interventions. Also, our neurotargeting device's accuracy was tested using provided angles by the application, which results in acceptable performance.

Conclusion

Multiple conducted studies investigating the risk and failure rate of catheter's tip location, reported on high inaccuracy and misplacement. In the setting of traumatic brain injury, intracranial lesions and narrow cerebral ventricles, altered brain's anatomy exists which leads to complicated catheterization [13]. For instance, a compressed and shifted cerebral ventricle makes cannulation for the surgeon a challenging process. Moreover, in cases where shunt systems composed of ventricular catheter, mentioned high misplacement rate results in increased necessity of re-catheterization and its following morbidity and expended cost [14]. Meanwhile, there are variable anatomic, technical, patient-related and surgeon-related aspects which should be considered simultaneously, with exclusive attention derived to ventricular size and distance from choroid plexus [15]. Pre-operative ventricular size considered a significant factor regarding catheterization's accuracy with greatest impact in pediatric population. Commonly, larger ventricles catheterize more accurately compared to others, thus diminish the possibility of proximal end occlusion and following enhancement of shunt's performance period. Albeit, a recent study by Wilson et al. on 170 patients with mean age of 56 years, demonstrated no significant relationship between ventricle's size in blind technique (assessed by Evans ratio) and misplacement rate. In addition, distance to choroid plexus is regarded as an imperative contributing factors in catheter's tip occlusion that have crucial importance in pediatric population due to relatively small ventricular size and large choroid plexus [16]. The most important factor of shunt failure is related to occlusion events that results from debris of choroid plexus and ventricles with consequent lumen blockage, glial cell growth in lumen and decreased ventricular size secondary to continued drainage. Ferguson et al evaluated 396 patients of adults and pediatric population who underwent ventricular catheterization for the first time and discovered 116 shunt malfunctions and stated possible etiologies of previous phenomenon as proximal catheter

blockage in 38% of patients, followed by infection in 29%. Current evidence suggests the proper selection of catheter's length and accurate placement of its tip just anterior of foramen of Monro as the main preventing factor of mechanical obstruction [17]. In general, hydrocephalus underlying etiology is associated with catheter's survival rate with most threatening hazard is present in ventricles complicated with hemorrhagic events and in patients diagnosed with ventriculitis. Based on a study conducted on pediatric group, factors such as sex, American Society of Anesthesiologist (ASA) status, emergent nature of the situation, prophylactic antibiotic administration and duration of surgery have no significant association with shunt failure [18]. In cases of complicated catheterization due to obvious altered intracranial anatomy and for low-experienced surgeons, other techniques such as frameless neuronavigational stereotactic interventions with robotic image guidance assistance, showed increased ventricular catheter accurate placement in desired location [19]. Along with intracranial neuroendoscopy, catheterization by ultrasound guidance performed in several studies in pediatric population were associated with a 1.5-2cm burr hole drilling, prolonged operation interval and increased cost [20]. This smartphone application coupled with targeting device can be used in various settings such as EVD placement in hydrocephalus and in other brain targeting candidates in emergency settings. Also, it may be used in any emergency or neurosurgery department centers with no access to advanced neuro-imaging facilities, only using patient's simple cranial measures to achieve acceptable and highly accurate brain targeting compared with conventional, time consuming and costly techniques. We plan to expand this study to clinical trials for further evaluation.

The authors declare that they have no conflicts of interest.

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Conscientious objection in health care: A principlism-based compromise position

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Abstract

Background: Conscientious objection (CO) is a complex topic of great clinical and philosophical importance which recently came again under fire. Both the so-called absolutist and pro-choice extreme positions (pro and against CO, respectively) cannot stand up to arguments. Moreover, there is not satisfactory compromise position between the conflicting rights. **Discussion:** The conflicting claims (objectors' and patients') are (almost) equally strong and as such should be accommodated at the same time, when the following conditions are met: objectors' claims are entrenched in society, no anti-democratic values are manifested, and patients' claim is incontestably de lege lata legitimate. The judgment about the de lege lata legitimacy of any given patient's claim should result from the dynamic process of an ongoing dialogue in accordance with the rawlsean "reflective equilibrium" held against a background of shifting sand: people change, medicine changes, society changes. The dialogue should be informed by the ongoing universal dialectic between absolutism and relativism. In accordance with the principle of mutuality, the state and other involved stakeholders (i.e. institutions) have the moral obligation to investigate all the "alternative options and circumstances" under which the conflict can be eliminated, circumvent or a true compromise can be achieved. With this path locked, the conflicting parties should find a fair mutual concession accommodating both the conflicting claims to the greatest extent possible, at the same time. Both the conflicting parties are placed under the obligation to tolerate a 'reasonably' minimal harm. This may be the case with referral obligation. If an objective (not personal) referral obligation would be recognized the right to CO would be limited without, however, losing its core physiognomy, provided that the right to CO is a flimsy subjective right that is structured like a molecular aggregation. Besides, a very low amount of wrongness can be conferred upon the act of referral. Who makes it is an in-the-rear-actor in a 'wrongdoing' which, in addition, is preparatory act of the principal moral wrongdoing. **Conclusion:** On the basis of the bioethical principle of mutuality the paper provides a proposal in two steps for obtaining a normatively reasonable (if not true) compromise position.

Background

Conscientious objection (CO) is a complex topic of great clinical and philosophical importance. In the context of contemporary medical ethics the legitimacy of CO is an increasingly contentious issue. The legal, ethical and political issue of conscientious objection in health care was recently put again under spotlight and fueled heated debates between scholars. The ever-accelerating advance of medical technology result in more and more newly appeared ethical dilemmas. Besides, in many countries of the Western world laws concerning medical areas as euthanasia, abortion or medically assisted reproduction was liberalized.

A widespread assumption has been accommodated for far too long in the field of medical ethics that health professionals must be allowed the right to conscientious objection (CO). This consideration is based on the assumption that health professionals' deeply held values and beliefs which constitute a part of their narrative identity deserve respect in case that a medical procedure goes against these values and beliefs, and hence, against their autonomy and moral integrity.

In this context, it should be noted that the positions against the (in principle) accommodation of CO in the context of health care are called "pro-choice" positions. In other words, some authors have taken a clear stance against the legitimacy of CO in medicine arguing that it goes against the core professional values which a health professional has already accepted when voluntarily chose to join the particular profession [1].

On the contrary, the positions in support of the accommodation of CO are called "absolutist" positions. The pro-choice positions are mainly based on the assumption that patients claim a legitimate right to undergo a treatment that they are entitled to. Indeed, such a right should be respectful in a liberal democracy. Fiala and Arthur stated with regard to the CO to abortions: "individuals should not be allowed to boycott a democratically decided law because of society's deference to religious beliefs and traditional views that assign women to a childbearing role" [2]. Similar considerations may be supported with regard to many other areas of medicine. However, taking a strong stance against CO is likely to increase health professionals' complains that their CO rights related to their professional integrity are dealt a major blow.

At any rate, it is not surprising that neither absolutist nor pro-choice positions are satisfactory. A great number of scholars mentioned below (i.e. Ancell and Sinnott-Armstrong, Card, Wicclair, McLeod, Hughes, Cowley, Lamb) have been in search of a compromise solution between the conflicting parts, namely, between conscientious objectors and patients. Importantly, as yet the literature has not given a mutually acceptable answer on what is blameworthy contribution to a principal moral wrongdoing. Schuklenk puts it best in saying that "the legal literature on the subject is growing due to the impossibility of satisfactory compromises" [3].

Indeed, it is a difficult task to find a balance between the conflicting positions between patients' right to access legal health services and health professionals' right to practise with respect to their own consciences. In principle opposing CO (namely, adopting overly restrictive CO policy) seems to be equally ineffective to excessively inclusive CO policy as a way of solving the conflict between objectors' and patients' rights. Note, however, that this conflict translates into a conflict between the principle of autonomy and the principle of beneficence (or nonmaleficence,

to the extent that refusal of healthcare provision might be viewed as an attitude that prevents letting go of patient sufferings).

Furthermore, it is important to keep in mind that the legitimacy of CO is not equally addressed by all countries. For instance, in secular and genuinely liberal Scandinavian countries as Sweden or Finland already CO is banned. On the other hand, in the USA the (deeply root in society) conviction that CO health professionals must be allowed the right to religion-based conscientious objection, makes authorities to behave in ways that in effect constitute contra legem behaviors. Nelson in his recent article highlights the political dimension of the topic. He argues that bringing charges against those who fail to provide an abortion when a woman's life depends on it would be highly politically charged [4]. Therefore, district attorneys do not prosecute them by making use of their wide discretion in deciding whether or not to do so, thus failing to honor the constitutional duty to provide women the equality before the laws [4].

The (unsatisfactory) compromise positions

Fovargue and Neal state that "lack of clarity about the proper limits of conscientious refusal to participate in particular healthcare practices has given rise to fears that, in the absence of clear parameters, conscience-based exemptions may become increasingly widespread, leading to intolerable burdens on health professionals, patients, and institutions" [5].

Below, I provide a short overview of the relevant literature related to the topic. Wicclair argued that "an appeal to conscience has significant moral weight only if the core ethical values on which it is based correspond to one or more core values in medicine" [6]. In this perspective he argued that even though there is not a universally accepted ranking of values within medicine, we can make "uncontroversial comparative judgments" about those values without uncritically endorse them. More recently, however, he has taken a somehow different stance arguing for a compromise approach that rejects both the incompatibility thesis (arguing that CO is always incompatible with professional obligations) and the compatibility thesis (arguing that CO is not always incompatible with core professional obligations). He argues that the justifiability of conscience-based refusals is context-dependent as well as that core professional obligations to patients such as to promote patient's health/well-being, respect patient dignity and autonomy, do no harm, do not discriminate, disclose options and refer or facilitating transfer justify ethical constraints on the exercise of conscience [7]. However, such a compromise position is difficult to obtain and requires further clarification of its justification.

Chavkin et al. published a study where they examined four countries—England, Italy, Portugal, and Norway and purported to show that it is possible to accommodate CO to legal medical services (i.e. abortion) while assuring that patients (i.e. women seeking abortion) have access to health care services [8]. Nevertheless, Fiala and Arthur strongly criticized this study as being flawed and providing no real support to CO [2].

Ancell and Sinnott-Armstrong (2017) argue that in case of invidious discrimination the costs arising from the refusal should be paid not by the patient but by the health care providers "as a correction to their framework for preserving medical providers' freedom to determine the scope of their practices" [9]. In any other case, patients bear the costs of conscientious objection. Notwithstanding, very recently Card (2019) arguably writes that "a free market argument to determine the proper scope of conscience objections in medicine has poor prospects" [10].

Hughes states that "arguing for robust enforcement of the no-impediment condition, rather than opposing conscientious objection in principle, may be a more effective way of addressing the harms resulting from an over-permissive conscientious objection policy" [11]. However, this solution may further limit the already limited health care resources.

As to whether a CO should be accommodated or not, some scholars confine their focus to the values and beliefs that motivate CO and form the basis of conscientious claims. Savulescu and Schuklenk (2017) are right in saying that conscientious objections, theologically motivated or not, are based on ethical statements related to certain cultures, times, groups, or individuals [1]. Indeed, if all values and beliefs could form a licit base of CO, this would be ethical relativism that practically is ethical nihilism [1]. Nevertheless, this consideration cannot be used as an argument in defense of a complete removing of CO right (as the authors argue). A closer look reveals that not all values and beliefs can form a licit base of CO. A CO may deserve accommodation, though, it must meet certain criteria. It is crucial to bear in mind that even the proponents of the accommodation of CO acknowledge that constrains should be placed on it [12]. For instance, Sulmasy who is a clear proponent of accommodation of CO argues that the broad discretionary space of physicians (that he recognizes) may be constrained when CO would be destructive to individuals and society [13]. Sulmasy argues that "a substantial risk of serious illness, injury or death" would be sufficient grounds to compel conscience, though not simply "inconvenience, psychological distress or mild symptoms" (i.e. a continuing dizziness) [13]. While these criteria are more than questionable, the following criteria appear to be less questionable than the aforementioned ones: a) CO should be based on genuinely and deeply held core values and beliefs closely allied to the objector's narrative identity, and b) CO should be consistent with relevant empirical data [14-16]. In this perspective, Card draws on the rawlsean "public reason" that justify these values and beliefs by the terms "common sense" and "plain truths" [14]. At any rate, however, it is true that values and beliefs are too subjective to admit public justification [15, 16]. Further, some scholars shift their focus from values and beliefs motivating CO to the circumstances the patient would face if the objection were accommodated. In this perspective it has been argued that CO can be accommodated if and only if that it does not cause 'needless or unjustified' [17] or 'unwarranted harm' to patients in the sense that it interferes with 'a patient's timely access to clinically appropriate healthcare services.' [15]. Recently Zolf finds these criteria to be "too nebulous to draw a line between acceptable and unacceptable practices", namely, between legitimate and arbitrary or unjust conscientious claims [12]. He argues that a normative criterion is required for judging individual claims [12]. However, Blackshaw (2019) disagree with Zolf and suggests "a judgment of what degree of harm should be tolerated as the cost of permitting conscientious objection" [18].

The solution of referral

Interestingly, McLeod considers that the referral obligation is justified on the basis that the medical norms are morally justified [19]. Nevertheless, the author herself has expressed some concerns about this argument without, however, to reject it [19]. According to Cowley a patient seeking a service is entitled to ("at the very least") referral information from every physician who is representative of the health service, provided that society has conferred upon certain medical procedures not only immorality, but also "a certain amount of moral legitimacy', and hence, made

these procedures to be 'essentially contestable" [20]. This, according to the Cowley's thought, is due not only to the fact that there are "generations of legal, democratic and professional decisions" (I borrow this statement from Finegan) entrenched within society, but also to the fact that there are many people who are "well-informed, morally serious and open-minded" who do not regard as morally wrong a procedure (i.e. abortion) that conscience objectors see as morally wrong [20]. Objecting physicians should respect the pluralism, namely, the moral views of their non-objecting colleagues and of patients who know better their best interest, as well. The Cowley's position received strong criticism by Finegan. Not surprisingly, Finegan argues that these arguments of Cowley can be used in favor of CO [21]. Besides, Finegan argues that the Cowley's arguments in favor of referral obligation are seriously mistaken since they overlook the "internal, deliberating perspective of those with a CO and the good of moral integrity" [21].

It is true that the compromise solution of referral raises concerns about whether a particular most remote contribution to a principal moral wrongdoing (such as referral, supervision or delegation) constitutes a morally blameworthy contribution or not. Finegan argues that a physician who makes referral provides significant material cooperation with patient plans since he or she aids the accomplishment of these plans in a significant way [21]. The author argues that the chain of practical reasoning of patient 'intersects' with the chain of practical reasoning of physician [21]. He argues that patient's practical agency 'intersects' with physician's practical agency, and, hence, there is not causal independence between these agencies. In other words, the "chain of cooperation" is not broken due to the fact that patient after being referred leaves the physician's space as Cowley argues [21].

Lepora and Goodin have offered a formula to calculate the blameworthiness of a cooperation based on the following criteria: "badness of principal wrongdoing, responsibility for contributory act, extent of contribution, extent of shared purpose with principal wrongdoer" [22]. Oderberg (2017) defends a refinement of the doctrine of double effect in the context of cooperation with a morally wrong act [23]. According to this doctrine an agent can knowingly and freely perform an act intending to obtain a result, but this act may have a secondary effect that the actor has foreseen but not intended. Oderberg argues that a cooperation with a morally wrong act may be morally neutral in light of the doctrine of double effect if it is not sufficiently proximate, indispensable (namely, necessary) or disproportionate [23]. This approach supports the conscience absolutism since these conditions are open to much different interpretations in various contexts and particularities of each case so that even a mostly remote contribution to a principal wrongdoing may be classified as morally wrong. More recently, Oderberg returned to address the matter at the time of the recent discussion on the Conscientious Objection (Medical Activities) Bill in the UK. The author argues that judges will develop what Oderberg calls "civil jurisprudence of co-operation", thereby drawing a demarcating line between activities that "constitute proximate co-operation amounting to assistance" and those do not [24].

In UK the Supreme Court in its judgment (on 17 December 2014) given on Doogan and another case argues (in § 17) that the crucial point is to make clear "what is meant by 'to participate in' the course of treatment in question" [25]. It state that "a broad meaning might cover things done in connection with that treatment after it had begun." "A narrow meaning would restrict it to 'actually taking part', that is actually performing the tasks involved in the course of treatment" [25]. "On any view, it would not cover things done before the course of treatment

began…" The prestigious court did not go into details concerning the distinction between morally relevant and morally irrelevant remote cooperation, namely, in the broadest (over-inclusive) sense cooperation. Thus, the High Court left the door open for recognizing the duty to refer.

The perspective adopted in this study: a principlism-based compromise position

This study shifts the focus from searching a normative justification of the values and beliefs (which in each individual case constitute licit ground of CO), as well as from drawing a sharp distinction between morally relevant and irrelevant remote contribution to a principal wrongdoing in each individual case, towards searching justification of a fair compromise between the two competing social groups: conscientious objectors and patients. The study proposes through the lens of the principle of mutuality and proportionality an objective (not individual) compromise position, thus obtaining a congruence in attaining a fair mutual concession of both the conflicting parts in all the areas of clinical practice. Below, I briefly outline the two-step proposition here advanced.

At a first step, the involved stakeholders (state, institutions, professional associations) are obliged to investigate all the possible and feasible (under the holding circumstances) alternatives for eliminating the conflict. In a second step, if the conflict cannot be otherwise eliminated or circumvent, and hence, a true individual compromise cannot be achieved, a fair compromise position can be achieved by making both the conflicting parties (regarded as members of conflicting social groups) morally and legally bound to make minimal mutual concessions at the same time, under the particular circumstances. A genuine liberal system should provide maximal degree of protection to both "the most deeply and sincerely held ethical or religious beliefs" [23] and patients' right to a legitimate service, if certain requirements are met, namely, if the strengths of both the conflicting rights are almost equal. Referral is an activity which conscience absolutists regard as most remote and material (not formal) blameworthy contribution. This study defends the objective referral obligation applied in any area of medicine and context of health care whilst securing a minimal harm of patients (i.e. simple "inconvenience, psychological distress or mild symptoms" [13]) and objectors, as well. The harm of the objectors in case of referral obligation should be considered minimal for the following reasons: a) The line of distinction between merely preparatory acts and participation in a principal moral wrongdoing should be drawn in analogy to the distinction between merely preparatory acts and participation in crime. b) The right to CO constitutes an aggregation of micro-rights. The right to opt out a referral is not a core but an associate right to CO, the elimination of which does not significantly changes the basic physiognomy of the right to CO.

The proposal advanced in this study aims at securing the minimal harm of the patient being refused service while reflecting on both objectors and patient agential practical reasoning. Below, I go a bit deeper into the proposal.

In case that both patients' and objectors' rights are almost equal a reasonable and fair compromise position should be respectful of the bioethical principle of mutuality. This study offers a fair compromise position that is 'normatively reasonable' while not rejecting the internal perspectives and deliberations of objectors. The provided position is based on the bioethical principles of mutuality and proportionality. In the perspective of the here advanced proposal, the nature of the right to CO and the association between moral and criminal assessment of an

individual's degree of involvement in a (criminally or morally) wrongdoing are of essential importance. In light of the proposal advanced in this study, the state (as well as other involved stakeholders) has a moral (if not legal) obligation to go to great lengths to investigate all the feasible (under the current circumstances) alternatives to eliminate or circumvent the conflict between patients and conscientious objectors rights (for instance through obtaining a true compromise position). With this path blocked, the genuine referral obligation is proposed as fair compromise solution where both the conflicting rights are 'accommodated' at the same time, on the condition that both the conflicting claims are a) deeply entrenched in society, b) do not represent values that go against democracy, and c) the medical norms which constitute licit ground of patients' rights are normatively justified in a clear and incontestable way. Note, however, that this compromise position is not a true individual compromise but a normatively justified compromise between two competing social groups of people (conscientious objectors and patients). In light of the here advanced proposal, health professionals are placed under the obligation to tolerate effective referral obligation as a fair 'normatively reasonable' slight concession of their right to CO. Health professionals who voluntarily chose to join and practice their profession under the actual normative framework should show respect to the internal 'reasonableness' of this (legal and bioethical) normative framework. In consistency with the perspective advanced in this study, normative ethics can set effective limits on conscientious objectors' rights in the context of health care by justifying referral obligation as a sustainable normative 'compromise' between patients' legitimate right to access healthcare services and (equally strong) health professionals' right to opt out this service for genuinely moral reasons.

Discussion

In this section I provide a further clarification of the justification of the here advanced proposal and discuss it in detail.

The ill-defined and flimsy right to CO

The right to CO is a highly controversial right. It can neither overweigh the patient's right to a medical service, nor be overridden by it. This assumption advocates for equal accommodation of objectors' and patients' rights, to the greatest possible extent. Below, I cite two opposite positions over the topic from the recent literature. Fiala and Arthur (2017) argue that "CO in reproductive health care should not be considered a right, but an unethical refusal to treat" that we allow to health professionals on the basis of a relative widespread assumption that has taken hold in the field of medicine, as well as that "supporters of CO have no real defense of their stance" [2]. Ortiz-Millan argues that there is a right to CO based on autonomy and moral integrity [26]. The conscience objector's autonomy, freedom and moral integrity have been regarded as grounds of the right to CO. However, none of them can in itself be an isolated licit ground of this right.

Moral integrity

Some scholars have offered arguments in defense of CO by appealing to the importance of

preserving an agent's moral integrity [27]. Wicclair (2000) writes that the moral importance of a conscientious objection to providing a legal service "can be grounded in the value of moral integrity and self-respect as well as the significant harm associated with self-betrayal and loss of self-respect" [6]. Moral integrity is a complex and abstract term. It represents a virtue that is fundamental to all other virtues. Importantly, coherence and internal consistency are constant and essential elements of moral integrity so that without them it makes no sense [28]. The behavior of one who has moral integrity shows external consistency (between what he professes and what he actually does), as well as internal coherence and consistency (between his various convictions). Lamb (2016) arguably puts it best in writing that moral integrity is a moral unity between personal and professional values and responsibilities [29]. Nevertheless, it is crucial to bear in mind that practically the internal consistency of a health professional's moral integrity is at stake, for the following reason: The one's core values and beliefs (namely, what one professes) are strictly related to him or her as an isolated individual. However, it is arguably suggested that one's professional autonomy (on which is based what one actually does as professional) is relational in nature [30]. As regards the autonomy of an individual (as moral agent) as possible ground of CO, the autonomy of a health professional who voluntarily joined a profession that involves core professional obligations is rather relational in nature than conceived in light of the "in-control agent" model of isolated individualism that highlights the individual's self-sufficiency. Hence, his or her CO may not only be based on his or her core values or beliefs, but also it may be profoundly shaped by external influences in a world of interactions and independences. Moreover, freedom cannot constitute an isolated ground of licit CO given that when one voluntarily joins a profession he or she concedes some of his or her freedom.

In addition, the currently ill-defined concept of conscience is insufficiently explored at both conceptual and empirical level in the context of CO in health care. Lamb arguably states that it is difficult to make sense of conscience in the context of health care and the concept of conscience is "largely absent in definition or in common definition in the discourse surrounding conscientious objection in health care practice" [29]. It is important to bear in mind that the concept of conscience is volatile since personal moral (or meta-moral) judgments and intuitions probably play important roles in defining it [13]. The concept of conscience may mainly be based on meta-moral judgments, probably resulted from conscious-cognition-bypassing (perhaps intuition-based) processes. Fleming et al. (2018) recently cite some authors who "while they agreed that respecting conscience was important, there was failure to agree as to the nature of conscience and when it manifested itself" [31]. It is argued in literature that conscience exists within all human beings referring to an "inner voice" the ignoring of which is to induce guilt, remorse and the "nagging" feeling of shame [32, p:1395, 33, p: 97]. Sepper from a different perspective argues that conscience "may be experienced retrospectively, generating guilt or regret, or prospectively, generating a sense that failure to resolve these conflicting demands will risk one's sense of self" [34, p: 1528]. This perspective appears to be close to the Wicclair's (2000) perspective according to which CO can be grounded not only on objector's moral integrity, but also on his or her self-respect and self-trust [6]. Interestingly, scholars regard conscience as being deduced from natural law [35]. Lamb (2016) considers that exploring the "language necessary to communicate the meaning of conscience and its rights is of great importance" [29]. In addition, she considers necessary to explore the nature of influences surrounding the reasons of CO. The author states

that the status of conscience and perceptions of moral welfare may substantially differ from individual to individual [29].

While reflecting on the controversial issue of the legitimacy of CO, it has been placed considerable emphasis on the value of freedom of conscience [17]. Clarke writes that "some conscientious objections are justified by appeal to all-things-considered moral judgments, and some are justified by appeal to the 'dictates of conscience'" [36]. Notwithstanding, Giubilini is right in saying that he shifts the focus from the freedom of conscience towards a benefit-based approach of the issue of CO [37]. He argues that freedom of conscience of not an absolute right in liberal secular societies and conscience is variously conceived in philosophy [37]. Hence, he argues that it would be problematic the argument shared by scholars that one's professional judgments are necessarily informed by one's conscience [29, 38]. Interestingly, while Giubilini considers that a benefit-based approach does not leave room for CO, in the same forum, Schaefer writes that a benefit-based approach still leaves room for CO [37]. The here advanced proposal shares this compromise position and struggles to accommodate both the conflicting rights, at the same time.

What matters is whether individuals is strictly committed to those values/beliefs so that their participation in an activity that they would be thought of as being incompatible with these values would give rise to CO [7]. However, the motivations of objectors may (quantitatively and qualitatively) vary significantly in the context of CO in health care according to the degree of an objector's commitment to his or her own core set of moral values and beliefs which are closely allied to his or her identity, as well as what the objector views as goal of medicine, concept of the good life and patient's best interest. Certain core values of an objector may become peripheral (to a greater or lesser extent), and vice versa. Note, however, that the line of distinction between the core and peripheral of an objector may be blurry and shifting over time and in various contexts. Furthermore, it is crucial to bear in mind that people should be allowed to change core values and that an individual's narrative identity may change in the course of his or her life. Wicclair (2017) has defended the assumption that the identity conception of moral integrity, is "sound and suitable in the context of responding to health professionals' conscientious objections and requests for accommodation" [39] Although, as McFall argues, there is a sharp distinction between peripheral ethical beliefs that are defeasible commitments and self-defining (identity-conferring) commitments [40], Soeharno arguably considers that it is difficult to make clear which values are identity-conferring, without exploring one's intentional orientation [41].

Moreover, it is crucial to bear in mind that the claim to conscientious objector's status may be shaped by "social, political and economic pressures" that may profoundly influence objectors who do not have any moral or religious objections [42]. For instance, the fear of social stigma towards the physicians who perform abortions may cause a physician to raise CO. In addition, some researchers make a distinction between true CO and 'obstructionist CO', based on the motivations or actions of various objectors [2].

In conclusion, the right to CO is a flimsy, ill-defined and flexible (as below is presented) right, of which the strength varies from case to case and, at any rate, it is too subjective to admit external (objective) assessment. Moreover, the degree of the blameworthiness of a (in objector's opinion) wrongdoing (or contribution to it) is ill-defined as opposed to the patient's right to a medical service where the crucial point is to obtain the provision of the particular (well-defined)

medical service.

The right to CO as a macro-right

Practically, subjective rights are often macro-rights which may represent a concurrence of two or more micro-rights. These macro-rights often are named as the most important of their components, called nuclear or core right [43]. Wellman goes back to Hohfeld's distinction among rights and views each right as having a “defining core” surrounded by “associated elements”. In a particular case the ‘associated elements’ may be present or absent [44, 45]. The subjective rights may be viewed as a “molecular aggregation” [46]. Recognition or rejection of a right to CO may underpin various combinations of elementary positions, as is the case with the right to procreate.

In the case of CO the core right is the right to claim immunity from the obligation to formally (normatively understood) perform or cooperate in certain medical procedures on moral or religious grounds. Associate rights may be context-depended. For instance, the objector’s right to opt out a contribution to a principal wrongdoing that might be viewed not as participation but as preparatory act (by analogy to the provisions and rules laid down in criminal law) would not be a “core” but an “associated” right to CO.

In principle, the objectors’ right to CO in the health care should be accommodated

Savulescu and Schuklenk argue that even the physicians who are objectors should act with “professionalism” [1]. The authors state that physicians who would be providing services “half-heartedly”, “possibly to the detriment of patient care”, “would be acting with a gross lack of professionalism”. In their opinion CO is “simply unprofessional.” However, in my opinion, medicine cannot be thought of as a profession the practice of which can be motivated by a pure commitment to professionalism. As an expert clinician, I do not agree with this assumption. Professional obligations cannot unconditionally be enforced against a physician being within the physician-patient relationship that constitutes the keystone of healthcare. GMP cannot be compelled in violence of conscience. GMP becomes questionable when the physician does not act in good conscience, namely, wholeheartedly. The physician-patient relationship is a sui generis relationship which involves normative and extra-normative elements. The safeguarding of this relationship bound to reciprocal trust is necessary in order to achieve the goals associated with the development of good medical practice. It is a matter of public health to prevent the development of physician-patient relationships that translate into reciprocal discharge (full performance) of the contractual obligations. White and Brody arguably state that conscientious objection is an instrumental means of promoting the integrity and quality of medical care [47]. Pellegrino and Thomasma state: “Medical good is only one of the components of the complex notion of patient good. The key concept is beneficence in trust.” [48]. Pellegrino and Thomasma view medicine as a moral profession that goes well beyond the notion that health is a normative category [49]. They consider that medicine is not only science and art but also a virtue. Indeed, doctors are not mere technicians. Medicine is a “moral enterprise” and medical decisions are guided and informed by its professional values and patient’s interest and health/well-being [6].

Recently, Schuklenk remarks that “defenders of conscientious objection maintain that in a liberal society respect for a professional's conscience is of sufficient importance that conscientious objectors ought to be accommodated. To deny conscientious objectors

accommodation would reduce diversity in the health care professions, it would deny objectors unfairly equality of opportunity, and it would constitute a serious threat to the moral integrity of conscientious objectors" [3]. Pellegrino puts it best in writing that "in the justifiable concern for patient autonomy, we must remember that the physician is a moral agent as well as the patient" [50, p:171-173]. Not surprisingly, this threat to the objectors' moral integrity may be thought of as being a threat to their health / well-being. Lamb argues that health may be defined as a state of moral well-being for all humans, be patients or health professionals [29]. Hence, in case that a health professional is not allowed to object to refraining from participating in practices that go against his or her moral well-being, his or her ability to carry out ethical care for patients may be compromised [29]. Different authors argue that the ignoring of an individual's conscience may generate guilt, regret, remorse, or the "nagging" feeling of shame may risk one's sense of self [31-34]. In my opinion, further research is needed to explore whether work-related stress responses (such as vicarious traumatization, secondary traumatic stress, compassion fatigue, and burnout due to high level of moral distress') may occur among physicians who engage themselves in activities with which they feel profoundly uncomfortable for genuine moral reasons. Sulmasy goes to great lengths to show through his theoretical analysis that "respect for conscience is not a trivial matter" [13]. Sulmasy argues that proscribing CO would entail eliminating physician discretionary space and finally undermining good medicine [13]. Notwithstanding, McConnell arguably writes that "professional ideals will promote good medicine better than Sulmasy's wider discretionary space" [51]. Sulmasy is right when argues that proscribing CO would entail undermining good medical practice [13]. In my opinion, this is not due to the objector's narrow discretionary space. However, it may be due to the fact that the narrow discretionary space may be negatively perceived by objectors and, hence, their health/well-being may be negatively affected. Moreover, against the right to CO it is argued that an implicitly recognized right to CO would simply be a slippery slope to disobedience to laws and authorities in general [26]. However, slippery slope is a flawed argument as it represents a specific type of logical fallacy.

Ultimately, the accommodation of claims to the status of conscientious objector is justified for both deontological and consequentialist reasons.

a) Deontological reasons: 1) The deeply held core values that constitute a part of an individual's identity ("the most deeply and sincerely held ethical or religious beliefs" Oderberg writes) merit respect in an individual-centered Western-type liberal culture. 2) Prescribing CO may entail violating objectors' health / well-being, namely, violating the principle of nonmaleficence.

b) Consequentialist reasons: Practicing medicine in good conscience is effectively promoting of good medical practice. Health providers cannot only wholeheartedly engage in therapeutic relationships while also feeling insecure and threatened by these relationships. A health professional should not be (professionally or legally) obliged to perform a requested service.

In principle, the patients' rights to health care should be accommodated, too

CO is not a question that mainly concerns health professionals and their rights. CO is strongly related to patients and their rights to healthcare. CO may be used as a subtle method for limiting these rights. A health provider who voluntarily joined the profession and apparently its core professional obligations cannot in principle opt out a medical service because of his or her

commitment to his or her core values and beliefs even if these values and beliefs are parts of his or her identity. Nevertheless, conscientious objectors claim to violate the actual core professional obligations despite the fact that they have voluntarily chosen to join the profession. This violation of the principle of beneficence is more important in the context of modern health care ethics where the position of solidarity may have been revised upwards in hierarchy and, hence, the principle of beneficence is enhanced. Besides, CO may violate the principle of nonmaleficence. Sepper arguably writes that "the presence of any refuser risks delays, the traumatizing of patients, and bodily harm" [34, p: 1552]. Note, besides, that CO may be turned into an ideological obstruction i.e. for women to have a voluntary abortion. The health professionals' right to refuse treatment may be hidden under the guise of CO. Giubilini and Savulescu are right in saying very recently in a forum that "patients should not be held hostage to the values of an individual doctor, especially when the doctor has a monopoly power over the patient's health or life" [37]. Note, besides, that in countries that have historically made provision for CO there are voices for CO limited or conditioned by the patients' rights [52].

The physician as healer

From a traditionalist standpoint, physicians are committed to professional core values that are essential of caring professions. Good medical practice is related to "professional values going back to the Hippocratic tradition which long preceded any notion of patient autonomy or rights" [53]. Sommerville put it best in saying that physicians can hardly contravene their intuition-based conviction that they play the role of the healer [53]. This is what the author calls "intuitive medical response". "Intuitive medical response" of most physicians (objectors or not) to patient that request a particular kind of service is to abide by the moral duty to fulfill their primary obligation to provide care to the patient who needs it. Provided that physician's primary obligation is to promote patient's well-being objectors should not prioritize their personal core values over core professional obligations. This assumption has already been supported by empirical research [55]. From a literature review carried out by Morrell and Chavkin resulted that physicians who are well positioned to support CO at the same time honor their obligations towards patients [56].

Note, however, that the above mentioned approach goes beyond the limits of the strictly conceived traditionalist medical ethics. Svenaeus arguably writes that from the beginning in ancient Greece through the entire history of Western medicine medical practice carries in itself a very basic structure of it that has preserved certain features that are expressed differently in different historical epochs [54]. The author believes that this traditional basic structure of medical practice and the modern concepts of health (i.e. holistic, phenomenological, which are products of their own time) are not mutually exclusive [54].

The role of healer is deeply ingrained in the notion physician, by its very nature. The physician's primary moral duty of care is defined in relation to the interests of patients and society and can be eliminated if and only if there are very strong countervailing considerations. Breach of a grave duty of care, for instance in case of (obstetrical) emergencies, may result in an unlawful killing that involves reckless disregard for human life, and hence, deserve, more moral blame than manslaughter (speaking in legal terms of common law). The American College of Obstetricians and Gynecologists (ACOG) [57] and the American Medical Association (AMA) [58] directly address health providers' opt-out on conscience grounds in this perspective.

Patients claim a 'secundum legem' claim

In addition to the aforementioned arguments, it should be highlighted that objector's claim goes against the actual legal framework, namely, it is a contra legem claim. On the contrary, in the vast majority of cases of patients' claim to undergo a treatment that they are entitled to, they claim a clearly defined right. Their claim is a secundum legem claim. The behavior of disobedience to a law on the ground that it is "intrinsically unjust" [59] is in reality a contra legem behavior (negatively understood), albeit hidden and veiled with a specified secundum legem argument. In principle, the right to a secundum legem behavior (treatment provision) ought to not be overridden by (if not prioritized over) the right to such a contra legem behavior of disobedience, when there is no other way to accommodate both the conflicting rights, at the same time.

Conditions that should be met for having to accommodate (almost) equally strong conflicting rights at the same time

According to the in this paper advanced proposal, all the types of conscientious objections have claims which are equally strong with patients' claims when the following conditions are met:

a) Not only the patients' rights (as Cowley argues), but also the objectors' rights are deeply entrenched in our society. The values and beliefs manifested through CO should be entrenched in our society. I go into this requirement. From the standpoint of the "identity account" of moral integrity (an account that indeed has received many objections which, however, seem defeasible) [6] "the selves of persons having moral integrity are constituted by socially-shared moral identity-conferring commitments" (italics added by the author) [40]. In the same vein of thought, I think that he values and beliefs that may acceptably be manifesting through CO should have been entrenched in (a part of) society, in the sense that generations of discussions have been made on this basis. In other words, currently or historically there should be a currency across a non insignificant portion of society which might raise a particular type of CO. That is to say that the population who are strictly committed to these values and beliefs (for religious or moral reasons) are of non-negligible interest within our westernized pluralistic societies, especially in the field of socio-dynamics.

Cowley argues that in our society have been entrenched generations of legal, democratic and professional decisions which confer "a certain amount of moral legitimacy" on abortion [20]. This is also the case with other medical procedures. Finegan criticizing Cowley's arguments writes that one's right to CO also conforms to "legal, democratic and professional judgments concerning the moral controversy at hand" [21]. However, morally relevant is not the fact that CO is in accordance with democratic legality and professionalism but the fact that the moral ground on which a CO is raised may also be deeply entrenched in a part of society (i.e. religious people) that is to be reckoned with in a democratic society. The CO is based on a ground constituted by generations of decisions entrenched within our Western-type societies which are individual-centered pluralistic democracies, and which contemporarily tolerate ideological fragmentation.

As a consequence, idiosyncratic values and beliefs cannot form the basis of acceptable CO without further scrutiny. Importantly, tolerance for bizarre or discriminatory values and beliefs may have as consequence intolerable harmful outcomes [12], even though these values and beliefs do not cause "a serious risk of injury or death." Salmasy's consideration that objections even those based on values and beliefs that are discriminatory or bizarre / idiosyncratic should be

tolerated as long as they are not "destructive of society" in the sense that they do not cause "a serious risk of injury or death" [13], seem implausible. Ancell and Sinnott-Armstrong argue that health professionals' right to CO falls within a range of freedom they possess to determine the scope of their practice, and hence, it would be plausible to create a system which grants accommodation even to discriminatory refusals, while protecting the rights of the patients [9]. Nevertheless, such a compromise position appears to be an utopia based on an absurdum. Very recently Card (2019) puts it best in considering that such a "market view" would be overvalued and causing to thinkers to develop and follow absurdum-based arguments throughout to implausible conclusions [10].

Note, besides, that Wicclair (2000) states that "an appeal to conscience has significant moral weight only if the core ethical values on which it is based correspond to one or more core values in medicine" [6]. Notwithstanding, in the position advanced in this paper, the values on which a CO is based may correspond to any professional value or belief which, however, is entrenched in society, as it is anticipated above.

b) The values and beliefs which form a basis of an acceptable CO should not go against the fundamental values of democracy. As a consequence, a CO cannot be motivated by discriminatory values and beliefs.

c) Patients claim should de lege lata be incontestably legitimate. As regards the medical norms, the actual legal landscape should be clarified to the greatest extent possible, with regard to patients' rights, health professionals' obligations and what actually the scope of medicine encompasses. This is a difficult task to be fulfilled by professional associations or the state. Only a patient's claim that is incontestably de lege lata legitimate can incontestably be characterized as secundum legem claim. In the here advanced position 'incontestable de lege lata legitimacy' of a patient's claim to a medical treatment means that the actual normative framework does not admit reliable interpretations that are prohibitive of this claim, at least from the standpoint of an objective interpreter. Otherwise, health professionals cannot be viewed as obliged to provide the requested service or at least to make a referral. As normative framework is meant the actual framework that encompasses all the rules falling onto a continuum that ranges from the formal legal framework to the normative rules issued by professional associations which may involve interpretation of the actual legal framework or not. However, it is to be highlighted that the clarification of the normative framework is not always an easy task. It may not be feasible at all due to the fact that it is not a matter of principle but a context-dependent matter. For instance, withholding and withdrawing of life-sustaining treatment are widely regarded as ethically equivalent in medical guidelines and ethics literature when all other factors are equal. This is the so-called Equivalent Thesis (ET). Wilkinson et al. argue that "preference for withholding over withdrawal could represent a form of cognitive bias" ("withdrawal aversion") [60]. Notwithstanding, Ursin argues that withholding and withdrawing are not in general ethically equivalent on the basis that "health care personnel widely perceive moral differences between withholding and withdrawing" [61]. The author puts it best in writing that "whether the ethical justification for withholding treatment is the same as or different from the ethical justification for withdrawing treatment is not a matter of principle, but a matter of substantial discussion in different contexts" [61]. In addition the author writes: "For guidelines simply to state that ET holds as a general moral principle in medical ethics is not to offer clarification, but to argue for a substantial and controversial ethical position" [61].

Not surprisingly, the legitimacy of some medical norms is reasonably contestable under the applicable normative framework (i.e. the moral equivalence between withholding and withdrawing of life-sustaining treatment or the moral relevance of the simple act of prescribing lethal medication for a terminal patient aiming to commit a medically assisted suicide, at least under legal provisions such as the Greek article 301 of Greek Criminal Code). Another exemplary case: Under the Greek law in the process of giving birth it is not clear the accurate turning point between fetus and person (completely protected human life). Hence, the legitimacy of a particular patient's claim may be a matter of interpretation of the current legislation (where it is not entirely clear) and hence, moral values and judgments may play a crucial role in determining the normative message that arises from the legal text.

The flexibility of professional values

Note, however, that the legitimacy of a patient's claim can be professionally disputed, not personally contested on the basis of personal moral judgments [62]. Note, however, that in a certain society the lines of distinction between morally accepted and rejected medical practices are not stable over time. These lines are blurry and shifting, especially in modern Western-type open, individual-centered and fragmented societies. Note, besides, that medicine is changing rapidly. Society and its prevailing values are changing rapidly, too. Of great importance is to identify the values that are appreciated as 'absolute' in a certain society as well as those being viewed as carrying a deep sense of human values. Such values are necessary for any society keep its integrity i.e. the value of social peace is commonly accepted by all the co-existing ideologies. There is a dynamic process of interaction between these changes. The scope of medicine expands, the core professional obligations become peripheral and vice versa, and the wrongness of some procedures is not universally accepted. Indeed, what is now considered core professional obligation in medicine may tomorrow become peripheral professional obligation (to a greater or lesser extent), and vice versa. Medicine is increasingly conceptualized as holistic medicine. The scope of medicine is rapidly expanding because of the ever-accelerating progress in medicine along with the increasing transformation of social structures. It is extremely difficult to draw a line of distinction between contentious issues and established standards within the currently prevailing scope of medicine. Health is viewed as a wide-ranging concept covering not only the well-being but also the one's capability of being capable to achieve one's goals, at least those characterized as vital goals. In this light, not only the core professional obligations, but also the effective medical norms are constantly being revised. More and more aspects of our everyday life are increasingly medicalized. Newly appeared entities and values are arising from these changes (i.e. artificial gametes may give rise to new types of parenthood). As a) the ever-accelerating advance of medical technology results in more and more newly appeared ethical dilemmas being raised, b) laws that regulate the delivery of medical services in areas as euthanasia, abortion or medically assisted reproduction may be liberalized in many countries of the Western world, thereby raising CO to these services, especially as pertains to religious freedom in healthcare, and c) as societal structures and people's beliefs and attitudes change (i.e. the traditional heterosexual nuclear family is being expanded) and the concept of health expands (as the holistic accounts of it are gradually gaining ground so that the line of distinction between health and well-being becomes increasingly blurry), some professional obligations that were in

the past to a greater or lesser extent peripheral may become core professional obligations, and vice versa. The degree of moral wrongness (if any) of certain behaviors is not universally the same. For instance, there are people who consider that active killing (doing harm) is much more serious moral wrong than giving up in a fight against illness (withholding a benefit), even though in modern bioethics prevails (though there is not a clear legal framework related to the topic) the view that these are morally equal behaviors. CO may be directly related to (based on) values that a country appreciates as being integral to humanity [29]. Nevertheless, it is not easy to draw a line of distinction between a sense of values that is human and one that is not.

Determining the current professional values: A dynamic process

The medical services which are professionally disputed should be defined not by isolated individuals (namely, subjectively) but inter-subjectively. The professional justification of a particular medical procedure should be the product of a honest, substantial, balanced and respectful dialogue between all the members of a professional society. As Stahl and Emanuel state, it can be defined through processes aiming at the rawlsian dynamic process of "reflective equilibrium" to self-correct, namely, a dynamic process regarding the professional prevailing values [62]. Through this process it should be defined not only which medical practices are professionally disputed, but also which professional activities might effectively carry the weight of "execution" (even in the broad sense of the term) of a particular professionally disputed practice. The "reflective equilibrium" should be keeping informed by the results of the ongoing universal dialectic between moral absolutism and moral relativism, which are gradually coming closer to each other. Nowadays, the practitioners of the religion-based absolutism often reveal themselves to be less obstinate and intransigent than in the past whereas the practitioners of relativism increasingly realize that a degree of commitment to certain absolute values (i.e. the value social peace) is necessary for a society with various ideologies to keep its integrity.

The bioethical principle of mutuality

In modern bioethics principlism alone has proved inadequate for providing a coherent bioethical solution to some hard-to-solve or inextricable bioethical dilemmas. Whenever there is a conflict of principles in a hard-to-solve dilemma, this is solved by eliminating bioethical principles for the sake of a priori decided solutions, thus resulting in half-hearted solutions of the conflict. According to the "mutuality principle" (devised by DeMarco), such drawbacks of principlism should be addressed by creating "alternative options and circumstances" [63] under which a true compromise can be achieved or the conflict can be eliminated, circumvent or solved coherently by enhancing all the principles that get into conflict with each other (e.g. autonomy of conscience vis-a-vis woman's reproductive autonomy). We are morally obliged to attempt to achieve a fair compromise between competing principles according to the "mutuality principle". For instance, in case of equally strong conflicting rights of objectors and patients, health professionals would be permitted to practice without compromising their moral integrity, and, at the same time, patients would be permitted to particular services that they are legally entitled to. In light of the principle of mutuality this conundrum can be addressed in two steps. At a first step, all the involved stakeholders (the state, professional associations, institutions etc.) have the obligation to go to great lengths to investigate under the holding circumstances all the "alternative options and

circumstances" under which the conflict can be eliminated, circumvent or a true compromise can be achieved. With this path locked, in a second step these stakeholders should find a fair mutual concession of all the conflicting claims (corresponding to ethical principles) in order for the conflict between two (almost) equally strong claims to be solved coherently by enhancing all the principles that get into conflict with each other, at the same time. That is to say that both the conflicting rights should be accommodated to the greatest extent possible, at the same time. All the involved stakeholders (state, institutions etc) have the moral obligation to go to great lengths to secure the accommodation of both the patients and objectors rights at the same time.

More precisely, in light of the here advanced proposal this obligation translates into the following consideration: a minimal harm of patients claiming a right to access legitimate healthcare services should be appreciated as weighing heavily against health professionals' right to CO (expanding to include objection to referral), on the condition that the moral harm of the objectors can be considered 'reasonably' minimal, too. Both the conflicting parties are placed under the obligation to tolerate a slight harm. In my opinion this is the case with referral obligation. Note, however, that reasonableness is a volatile and fishy notion [37]. Notwithstanding, some scholars in the debate on CO appealed to the reasonability view [17, 64, 65]. In the here advanced proposal, the 'reasonableness' is conceived as internal reasonableness of the actual normative framework, thereby limiting the volatility of the concept.

A mutuality-based solution for fair accommodation of both the conflicting rights

Below, I outline in detail each step of a mutuality-based solution. In case that the requirements for securing that both the conflicting rights are (almost) equally strong are met, all the involved stakeholders are obliged to go to the aforementioned efforts. In this paper it is provided the required normative justification for explaining not what constitutes necessary harm but what normative conditions should be hold for securing almost equal strengths of both the competing rights. Therefore, this paper provides the following proposal summarized below and articulated in the following two steps.

A) First step

The first step moves toward the goal of obtaining alternative options and circumstances that could eliminate minimize, or circumvent the conflict between health professionals' and patients' claims. The state has the obligation to go to great lengths to achieve that goal, namely, to make resolutions that are achievable under the current economic and social circumstances, after having put under rigorous scrutiny all the possible alternatives. For instance, there might be resolutions in addition to those proposed by Savulescu and Schuklenk: "selecting candidates into relevant medical specialties or general practice who do not have objections" and "demonopolizing" the provision of certain services "away from the medical profession" [1]. Minor modifications to the organizational context of the public health care would probably be needed. It has been suggested that physician objectors should be required to defend their claim before a tribunal [66]. This proposal is troublesome. CO in health care is profoundly different from CO in military service [67]. Besides, it would be offending for physicians and ineffective solution for ensuring patients safety in health care.

However, society cannot afford to spend limited resources on expensive programs

needed to accommodate CO instead of trying to figure out ways to provide mildly expensive treatments for elderly patients most at risk of dying. This path of addressing the conflict is often blocked or does not suffice. For instance, the "robust enforcement of the no-impediment condition" proposed by Hughes [11] may be disproportionally expensive. Securing a fair access to health care services in a context of over-permissive CO policy may further limit the already limited health care resources. The health service in all likelihood should expend a great disproportion of resources to protect conscientious objection while ensuring universal access.

In any case, the state has the moral obligation to put a great deal of effort to investigate all the attainable alternatives under the current circumstances, taking into account the prevailing values, the principles of social and distributive justice, its priorities and the cost-effectiveness of an alternative. McLeod arguably states that genuine compromise is not the one out of respect or epistemic humility [19]. The state should encourage genuine (true) forms of compromise by providing health professionals with the core values and obligations that are actually prevailing in community and profession, resulting from the ongoing dialogue in society and in professional associations (anticipated above in this paper).

Encouraging for dialogue

By engaging people with different views in this dialogue the state fulfills its obligation to be not only impartial, but also respectful towards the principle of mutuality. The state should prompt robust and respectful dialogue between patients and health professionals with regard to the topic of CO. Fleming et al. write that "while the arguments in the literature emphasize the need for provision of conscientious objection, a balanced debate is necessary in this field, which includes all relevant health professionals" [31]. The state is obliged to foster the above mentioned discussion between the members of a professional society (aiming at the rawlsean "reflective equilibrium") to make clear the currently holding professional values (and scope) of medicine so as to clarify the landscape with regard to moral justification of medical norms. In my opinion, these discussions should be informed by the ongoing universal dialectic between moral absolutism and moral relativism. In many cases, reliable moral justification of medical norms is necessary for providing strong interpretation of the actual normative framework, thus ruling under enough certainty on the de lege lata legitimacy of a patient claim to healthcare services. The aforementioned discussions are necessary not only to minimize the likelihood of making mistakes when providing moral justification of professional norms, (provided that professional societies can make mistakes "as with all humans and human institutions" [62]), but also to prevent professional societies from ending up with moral (if not normative) positivism. Moral (as well as normative) positivism is dangerous not only for liberalism but also for democracy. What results from democratic procedure is not necessarily always morally right. The Christ trial is illustrating of this assumption. Modern professional societies should facilitate the dynamic process of "reflective equilibrium" while keeping open the communication channels with the ever-ongoing global dialectical relationship between intransigent absolutism of values (religion-based or not) and abyssal relativism. Professional societies should continuously be informed by this universal dialectic. The state should encourage these processes while being impartial, namely, holding the role of a 'neutral operator'. Ford et al. state that "with advancements in technology, changing healthcare policies and increasing scope of practice, both reflection and dialogue on

conscientious objection are critical for the continuing moral development of nurses in Canada" [68]. This is also the case with all relevant health professionals in any country. The state must work to thoroughly foster an ongoing public debate on core professional values in medicine and biology as well as to ensure broad public participation and open exchange with experts and professionals. In addition, the state must work to secure the provision of frequently-updated reliable information with regard to the core professional values as they are currently result from the dynamic process of an ongoing dialogue which, however, is held against a background of shifting sand. Moreover, the state must encourage ongoing education of health professionals, thus keeping them adequately informed for both the justification of medical norms and the prevailing values in various fields in order to present them with possibilities to reach a true compromise, namely, a compromise that is not reached out of respect or epistemic humility [19].

Reducing the number of objectors and objections

Furthermore, government healthcare administrators should strive to reduce the number of objectors in health care as much as possible. The (minimally) modificated healthcare system should be flexible to accommodate newly appeared objectors. As the advance of medical technology is ever-accelerating, more and more health professionals may find themselves troubled by questions of conscience over newly appeared ethical issues and dilemmas. Besides, the ethical issues need to be explored on a continuing basis as the technology is evolving so rapidly. Moreover, it is to be stressed that in light of "mutuality principle" the state has a beneficence-based moral obligation to reduce the number of cases that might cause raised CO. For instance, if all patients had access to high quality palliative care there would be much fewer cases for assisted suicide. If all women had access to high quality family planning services there would be much fewer cases for abortion (particularly third trimester abortion) and in-vitro fertilization in women of advanced age.

"Alternative options and circumstances" may be created through developing comprehensive strategies, such as cross-agency actions, demonopolizing, creating lists with the names of the conscientious objectors that are opposed to serving in certain parts of medical practice, ensuring that non-objecting providers are reasonably accessible, or making (minor) modifications to the organizational context of both the public healthcare and particular settings so that neither objectors will participate in activities with which their deeply held beliefs may conflict, nor patients will be deprived from the full use of their right to legitimate medical treatment practices.

Encouraging further (empirical) research

Prior to making the aforementioned minor modifications to the organizational context of the public health care, the state has to encourage further research into social and epidemiological aspects of medicine with regard to practices that usually raise pressing normative questions about the legitimacy of eventual CO of health professionals to opt-out of these practices. CO is understudied especially with respect to sexual and reproductive healthcare [56]. There is a lack of empirical research on the topic [69]. It is most likely that the way the issue of CO is approached by analytical thinking does not reflect reality [70]. In the USA researchers launched consortium called "The Research Consortium on Religious Healthcare Institutions" to investigate and better

understand the growing impact of religiously affiliated healthcare institutions on women's reproductive health [71]. Cross-agency co-operation between health care providers and settings seem necessary.

As these practices most often regard issues that in society are taboo or highly controversial (i.e. abortion or euthanasia), we need courageous politicians to fulfill the above mentioned tasks. In this perspective, empirical studies carried out with confidentiality are needed.

B) Second step

With the first step moving in a path that is blocked, the second step should move toward the goal of obtaining the accommodation of both physicians' and patients' claims at the same time. Patients being refused service should be obliged to tolerate a slight harm (i.e. simple "inconvenience, psychological distress or mild symptoms" [13]) and conscientious objectors should be mutually obliged to tolerate a 'reasonably' slight harm of their moral well being, too.

In terms of bioethical principles that enter in conflict, the goal is to obtain the lesser possible limitation of each of these. While developing my arguments it is essential to present the following: In a genuine liberal democratic society the individuals-members of this society have autonomy and freedom to the maximum possible degree. However, in order for these individuals to peacefully co-exist in a community that keeps its integrity, they should be subject to a mutually proportional limitation of their autonomy and freedom, provided that all the members of the community have equally strong right to autonomy and freedom. Since the conflict is between qualitative similar rights (autonomy to autonomy or freedom to freedom) the mutually proportional limitation is based on the principle of proportionality. Much of the same holds for the case of conflict between bioethical principles of autonomy, nonmaleficence and beneficence (in terms of conflict between equally strong rights: patient right to health care and physician right to object) in a genuine liberal democracy that shows respect for medical ethics, autonomy and patient safety. Since the conflict is between qualitative different principles (principle of autonomy, beneficence or nonmaleficence) the mutually proportional limitation is based not only on the principle of proportionality, but also on the principle of mutuality. Below, I provide a two-fold explanation why referral obligation might be considered a *mutually minimal* concession of both the conflicting parties, which is *'normatively reasonable*'.

The solution of referral: A novel approach

In case of referral, patients are practically not deprived of the rights to healthcare they are entitled to, while health professionals are being given enough moral space in which they can practice without compromising their moral integrity to greater than *insignificant* extent. In short, in case of referral obligation, patient's harm is *normally insignificant* while objector being harmed to a *normatively reasonable* extent. Therefore, both sides of the conflict are obliged to engage in the process of making mutual concessions. I elaborate on this assumption.

a) In case of referral, the harm of the patients is normally insignificant. The patients may experience only some slight inconvenience, a very mild psychological distress or very mild symptoms. In contrary, opting out referral may be considered a health hazard since it may constitute a cause of adverse health effects on patients potentially. These adverse health effects may be harmful to health (traditionally understood) i.e. impairment or not improvement of the

patient's health status due to delay or mobility. These adverse health impacts may cause conditions ranging from very mild to potentially fatal. Nelson arguably remarks that 'conscientious refusals by clinicians to end a pregnancy can constitute murder or reckless homicide under American law if a woman dies as a result of such a refusal. Such refusals are not immunized from criminal liability' [4]. Moreover, provided that in accordance with the modern holistic conceptualization of health where the line of distinction between health and well-being is blurry, opting out referral may cause more adverse health-related effects (i.e. undue psychological distress, anxiety, or negative emotional responses). In case of opting out the referral, the harm of patient may be unperceivable by the objector due to the fact that the latter can only be in possession of those data concerning the patient's health/well-being which are visible from the outside. The degree of such a risk cannot be assessed by the objector in the very short frame time of an encounter between objector and patient. In the limited time of an objector's encounter with a patient, the objector who opts out a referral cannot be in possession of all the relevant health-related data of the patient in order to assess the degree of the harm with which the patient who is denied the service might be faced. In contrary, when the referral requirement is respected the adverse health effects that may occur on patients potentially are insignificant. Besides, as McLeod states referral requirement is in accordance with medical ethics which calls for: a) non abandoning patients, b) respecting patients' autonomy, c) honorising patient's trust and) being beneficent towards patients (acting in their best interest) [19].

b) In case of referral the moral well-being of the objectors may be harmed to a normatively reasonable extent. This is due to the two reasons presented below:

1) First reason. In case of referral obligation the core physiognomy of the right to CO remains intact.

The limited right to CO is viewed as a truncated right of which, however, the core physiognomy remains the same. In other words, the right to CO is not unfairly limited and, hence, its very core is not undermined. Below, I go into details about this consideration.

Interestingly, although Cowley has in the past criticized the referral obligation, in his more recent article argues that referral (i.e. providing of "reliable information" to patients about abortion services) is not formal cooperation in the practice and states that it is not a "necessary or indispensable link in the chain of actions leading to the abortion, since, after receiving the information, the patient leaves the NHS space and becomes a free agent ready to make her…own decisions" [20]. As it is anticipated above, Cowley's position has received strong criticism by Finegan. However, even if Finegan is morally right, he is not also right from a pure legal (criminal) standpoint. According to the here advanced position, while theoretical analysis of moral wrongdoing (conducted to serve the purposes of the existing normative framework) and legal crime theory are not identical, they share a common internal reasonableness.

As is anticipated above, the objector's right to opt out a contribution to a principal wrongdoing that might be viewed not as participation but as preparatory act, would not be a 'core' but an 'associated' right to CO. Given the truth of this assumption and in consistency with the principle of mutuality, it can be accommodated at the same time an almost unimpaired (insignificantly impaired due to referral-based inconvenience) patients' claim to health care, and the objectors' right, which, even if one of its component (associate) rights has been eliminated it stays at its core unchangeable, namely, its core remains unimpaired. This is the only way to

address the relative conflict between the principles of autonomy, beneficence and nonmaleficence in accordance with the principle of mutuality in case that this conflict cannot be circumvented by other means.

The fact that the objectors' harm is also only slight is due not only to the fact that even if the associate right to opt out a preparatory act will be eliminated, the core right to opt out the principal wrongdoing or the participation in it would remain intact, but also to the fact that -as is presented in the next section 'second reason'- the degree of blameworthiness of an objector who was obliged to refer is of rather insignificant importance because he or she is only a participant in preparatory act. In conclusion, both a minimal harm of patients being refused service (namely, their almost intact right to be provided with service) and the objectors' core right to object can in principle be accommodated at the same time.

2) Second reason. Below, I outline the major points of my assumption that the moral wrongdoing of the objector who makes a referral remains insignificant from a normative standpoint. Below, I elaborate on this assumption. This paper shifts the focus from patients' harm towards objectors' harm. In case of referral obligation what matters when judging the degree of objectors' harm is not only the subjective judgment about a particular moral wrongdoing but also the degree to which that wrongness is relevant to the actual normative framework, part of which is the legal rule concerning the legitimacy of CO. Therefore, objectors' harm can admit subjective interpretations which, however, are not completely unconditioned but in consistency with the internal reasonableness of the existing normative framework ('normative reasonableness'). I go into. In my opinion, from the perspective of the actual normative framework the degree of involvement of an individual in a moral wrongdoing should be judged in a way that is similar (or at least in analogy) to the way in which criminal law makes judgments about an individual's degree of involvement in a criminal activity. This is due to the fact that the degree of involvement in an activity is a matter of description of facts and as such it is consistent with an internal normative reasonableness that is the same, whether it concerns contributions in criminal or the moral wrongdoings. Who makes a referral is not an actor of a moral wrongdoing but an aider who has assisted the commitment of a moral wrongdoing, of which the blameworthiness (if any) is (according to the common moral/criminal internal reasonableness) too remote from the principal wrongdoing that is rejected by a conscientious objector. Indeed, who makes a referral committed a preparatory act of the very moral 'crime' (wrongdoing). Note, however, that in criminal law the preparatory acts are normally and traditionally not punishable with rare exemptions appeared in the modern criminal law (i.e. in case of terrorism-related crimes). Below, I provide further explanation of the points raised above.

According to the assumption accepted in light of the position advanced in this paper, the (normatively relevant) degree of blameworthiness of an act appreciated as being contribution to a principal wrongdoing might be judged on the basis of its remoteness from the most remote contribution to the particular wrongdoing which might implicates criminal liability of the actor. The greater the distance of a morally relevant contribution (which is criminally irrelevant) from the most remote criminally relevant contribution to the particular principle wrongdoing, the lower is the amount of its moral blameworthiness, at least the blameworthiness 'to be reckoned with' in the context of the actual normative framework. Indeed, provided that Criminal Law is that branch of law, which deals with public wrongs of offences, it 'knows' better than any other branch of law to

judge the blameworthiness of the most remote contribution to a particular principal legal (criminal) wrongdoing. Of course, some moral wrongs are punishable by criminal law whereas other moral wrongs have nothing to do with criminal law. Nevertheless, when the actual normative framework has to take into account moral wrongdoings (i.e. in the case of CO), by and large, it must use the basic structure of the tools of criminal law to judge the degree of moral wrongness of a contribution to a principal moral wrongdoing, which may be identical to, completely different from or overlapping with the eventual criminal wrongness of the particular contribution. When considering an individual's degree of contribution in a principal moral wrongdoing from a normative standpoint it is not reasonable to develop an absolutely subjective approach that is completely disconnected and far-removed from any legal approach (in effect across our westernized legal systems) to the problem of the particular individual's degree of criminal contribution in the same principal wrongdoing in case that it would be not only moral but also criminal wrongdoing. The degree of an individual's involvement in an activity of moral and criminal wrongdoing should be assessed in an analogical way or, at least, the degree of an individual's involvement in activity of moral wrongdoing should be assessed in analogy to the degree of an individual's involvement in criminal activity, provided that the degree of involvement in an activity is a matter of description of facts.

In the case of a (genuinely effective) referral, the moral blameworthiness of the objector who was obliged to make it should be considered too weak to justify denying patients' requests for legitimate medical services, for two concurrent reasons: a) Referral is in effect a part of preparatory activity which carries a low amount of wrongness (which, in addition, is qualitatively different from the wrongness of the principal act). b) The patient has the hegemony over the act whereas the objector is a participant (aider) in this preparatory activity ('preparatory wrongdoing'). Below, I provide further details.

The act of referral might be loosely defined as an act which is involved in developing a care planning that is the run-up to initiation of the intended principal act (that is classified as moral wrongdoing from the objectors' perspective). In other words, the 'act of developing a care planning' might be viewed as a preparatory act for committing the principal intended moral wrongdoing. Therefore, much lesser immorality there is in the self-standing wrongdoing of developing a care planning in comparison with the principal intended moral wrongdoing of providing a particular healthcare service. Traditionally, from the prospective of criminal law, in the various jurisprudences preparatory acts didn't amount to punishable attempt as a rule with the recently appeared in the criminal law exemption of very particular crimes such as those of the organized crime. Of course, what is not punishable or criminally indifferent is not necessarily morally accepted. However, the moral wrongness of the preparatory acts should be regarded as much lesser than the moral wrongness of the principal intended wrongdoing. As emerged within a recently published research, people are particularly sensitive to violating rules that are in place for preventing acts that can cause harm directly [72]. The preparatory act of developing a care planning itself cannot cause harm directly. Who supports the opposite is based on an arbitrary assumption which, however, is not well-established in society. If the amount of blameworthiness is low for the actor of a preparatory wrongdoing (of developing a care planning), a much lesser amount of blameworthiness would be recognized for the person who simply participates in this wrongdoing (as an aider), namely, for the health worker who makes a referral. Considering an

extension into ethics of the hegemony-over-the-act theory (Tatherrschaftslehre) which has gained considerable support in the German criminal literature and jurisprudence, a healthcare worker who makes a referral is not an actor-up-front but an actor-in-the-rear [73]. Indeed, in case of referral the patient has the hegemony (sovereignty to exert power, to do otherwise, since he or she has a robust agency). The physician is the in-the-rear actor of this moral 'crime'. In light of this theory, the patient is the actor-up-front who maintains control over the execution of the (preparatory) wrongdoing since he maintains his or her agency, regardless of whether the patient after the referral left the health worker's space or not. Even if is given the truth of the Finegan's assumption that in case of reference patient's practical agency "intersects" with physician's practical agency the patient's agency is not eroded to the extent that it may be invalidated. In our everyday life there are several influences (argumentative or non-argumentative) on our (robust) agencies without necessarily invalidating them. However, in case that agency is lost (i.e. in case of patient's unconsciousness), the hegemony over the act passes on to other persons (i.e. physician). It is crucial to bear in mind that one must assume the viewpoint of an objective bystander, namely, one who is in possession of all relevant facts, not only those which are visible from his or her standpoint, in order to judge whether a particular activity constitutes participation or preparation.

The aforementioned arguments regard referral obligation. However, much of the same holds for the case of transferring. Cavanaugh states that "transferring medical records or returning a prescription does not, however, so deeply implicate one in the objectionable act. To transfer a medical record, to return a prescription, or to disclose legal options that other professionals offer is not, thereby, to violate a well-formed conscience" [74].

In conclusion, the blameworthiness of a person who makes the referral is almost insignificant. As such it can be normatively justified (under the principles of mutuality and proportionality) for the sake of patient's right to a medical service, which should be allowed an insignificant only limitation. In consistency with the perspective advanced in this paper, normative ethics can set effective limits with conscientious objectors in the context of health care by justifying referral obligation as a sustainable normative 'compromise' between patients' legitimate right to access healthcare services and (equally strong) health professionals' right to opt out this service for genuinely moral reasons. Not surprisingly, referral requirement in not undeniably accepted by health professionals as a satisfactory solution even in jurisdictions where referral requirement has been introduced in the actual legal framework. For instance, in a very recent publication is stated that in Victoria, Australia, some doctors are not complying with the referral obligation clause introduced in 2008 in the law regulating abortion "with adverse effects on access to care for some women" [75].

Conclusion

In light of the proposal advanced in this paper, the state (as well as other involved stakeholders) has a moral (if not legal) obligation to go to great lengths to investigate all the feasible (under the current circumstances) alternatives to eliminate or circumvent the conflict between patients and conscientious objectors rights (for instance through obtaining a true compromise position). With this path blocked, the genuine referral obligation is proposed as fair compromise solution where both the conflicting rights are 'accommodated' at the same time, on the condition that both the conflicting claims are deeply entrenched in society, do not represent values that go against democracy and the medical norms which constitute licit ground of patients' rights are normatively justified in a clear and incontestable way. In consistency with the perspective advanced in this paper, normative ethics can set effective limits with conscientious objectors in the context of health care by justifying referral obligation as a sustainable normative 'compromise' between patients' legitimate right to access healthcare services and (equally strong) health professionals' right to opt out this service for genuinely moral reasons.

The authors declare that they have no conflicts of interest.

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The importance of postoperative analgesia after major thoracic surgery interventions, protocols, applications, and the following evaluation of the patient's quality of life

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Abstract

Introduction: Major thoracic surgery procedures constitute a standard method of diagnosis and/or therapy against lung, mediastinal cancer and other non-malignant manifestations of the respiratory system. Such patients' recovery and rehabilitation depend directly from the applied postoperative analgesia, with purpose to determine an optimum and long-term quality of life. **Background:** Our database consists of 300 individuals, submitted to major thoracic surgery procedure during a 2 - year period of time (between December 2016 and December 2018) at the "Thoracic Surgery Department" of "Theageneio" Cancer Hospital, Thessaloniki. **Methods:** Every operative method is accompanied with three different types of postoperative analgesia, depending on the demands of the surgical approach: Each patient's postoperative management and evaluation is performed via usage of three "Quality of Life" (QoL) Questionnaires and the "VAS - Visual Analog Scale" for pain, leading to the determination of the "QoL Index". **Results:** Each patient answers the Questionnaires in 4 specific time intervals. The differentiation in their answers is the key point to extract important information about their postoperative health evolution. A detailed questionnaire evaluation follows, both individually and in groups, according to the subgroup of each patient's pain treatment, a combined study which is applied in this form for the first time. **Conclusions:** The measure of a "QoL" index is widely taken into account as one of the most accurate indicators of a patient's health evolution. The results supply us with significant information which is added to the initial management strategy, mainly regarding pain symptomatology and eventual complications and discomforts, while they indicate us towards a thorough realization of each patient's "follow up" individually and the achievement of an optimal Quality of Life level.

Introduction

According to latest data and statistics, lung cancer has managed to climb its way to the top of the list as the commonest manifestation of malignancy in both men and women, reaching a percentage of 11.6% of all diagnosed cancer patients and 18.4% of all cancer-related deaths globally in 2018 [1]. Tobacco smoking remains the major risk factor, indicating a 90% correlation with the final diagnosis [2].

The appropriate surgical procedures against all kinds of pulmonary lesions, malignant or not, such as open thoracotomies and VATS (Video Assisted Thoracic Surgery) are still considered the "gold standard" against this multi – level therapeutic and/or diagnostic task. The evolution of thoracic surgical operations has been established from the standard posterolateral thoracotomies, sternotomies, continuing to less invasive muscle sparing thoracotomies, multiport VATS ("2-Hole & 3-Hole Techniques"), the quite popular Uniportal VATS procedures and finally the innovative Robotic Thoracic Surgery interventions which have broadened the overall operational management during the last decade. Each procedure may be performed for every indicative demand, from a single lung or mediastinal lymph node biopsy, wedge resection, segmentectomy, to a much wider approach such as a lobectomy, bilobectomy or a pneumonectomy. Other operating variations include VATS procedures in "prone position", AVATS Procedures (Awake Video Assisted Thoracic Surgery) applying a combination of local anesthesia and mild sedation, indicative against patients with poor respiratory reserves, etc. [3].

Background

Our patients' database is consisted of 300 individuals, submitted to major thoracic surgery procedure during a 2 - year period of time (between December 2016 and December 2018) at the "Thoracic Surgery Department" of "Theageneio" Cancer Hospital, Thessaloniki.

Figure 1. "Theageneio" Cancer Hospital, Thessaloniki, Greece.

Every patient has been thoroughly studied pre-operatively, concerning his overall health status, current chronic diseases which may affect his postoperative evolution, previous major operations,

eventual oncological status and most important a detailed pulmonary evaluation, including blood gas registration and a spirometry control. The collected data determines the optimal procedural selection for each individual according to the initial purposes and demands.

The main criteria for the selected patients who participated are, the potentiality for performing a "Ro" Tumor Resection Procedure, thus its complete removal within proper surgical margins, in addition with a current personal existing – or not - oncological status that provides promising perspective regarding their following quality of life level [4].

Every procedure is followed by an average of 24 – hour stay in our Intensive Care Department, after which the patient returns in our clinical department's Increased Care Unit Section, where remains under monitoring control for an additional average interval of 24 hours. The above-mentioned postoperative steps are quite essential for achieving optimal regulation of every individual's immediate needs, such as pain control, hydration, eventual required blood transfusion, mobilization, initial respiratory physiotherapy, etc. Uncomplicated "Fast- Track" interventions usually require a 3 to 5 – day interval of immediate postoperative hospitalization, VATS and Mini – VATS interventions a 5 to 7 – day interval, while open thoracotomies an 8 to 10 - day interval until the patient's discharge.

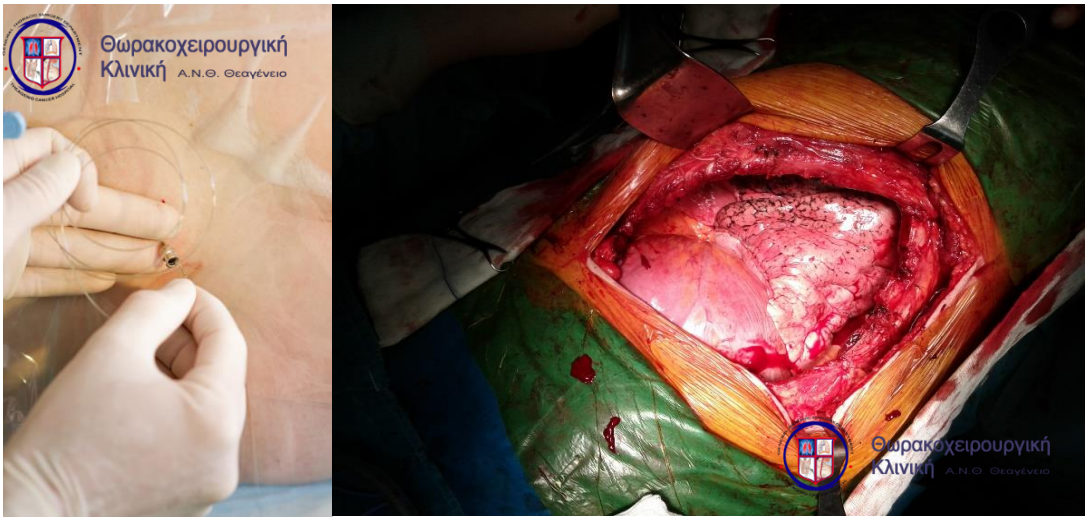
Figures 2 – 3: "Intensive Care Unit" and the "Increased Care Section" in the Thoracic Surgery Clinical Department, "Theageneio" Cancer Hospital, Thessaloniki, Greece.

Methods

Thoracic surgery interventions, especially thoracotomies, are considered to be some of the highest risk and most painful procedures, including surgical handlings, such as skin incision, intraoperative tissue retraction and dissection, etc. which may lead to moderate or/and severe postoperative pain. These facts indicate that successfully applied postoperative analgesia is required and defines every individual's outcome, rehabilitation and their following optimal level of quality of life [5]. The main goals of the conducted pain management are improvement of the postoperative pulmonary function and reduction of any eventual postoperative complications, such as nausea, vomiting, hypotension, shivering, bradycardia, motor block and general respiratory depression.

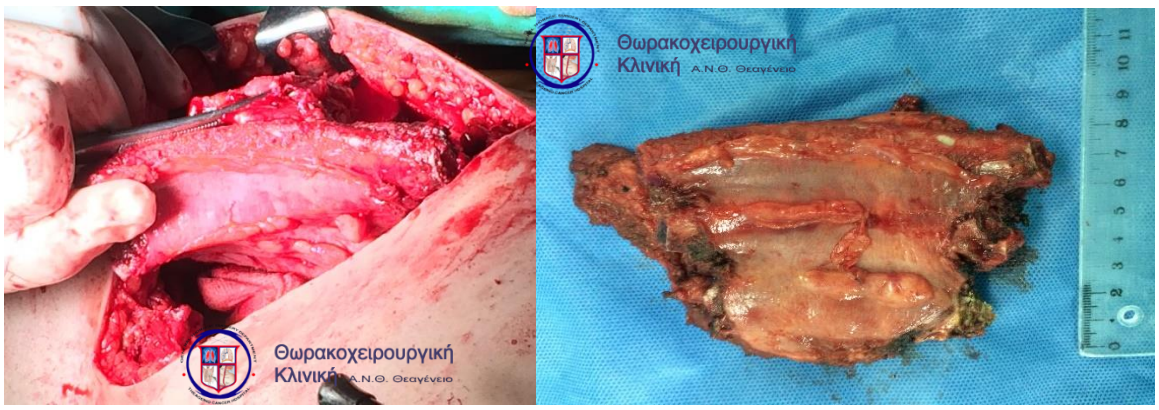
Each operative approach is usually supported by one of three selected pharmaceutical schemes of postoperative analgesia, according to the intervention's type [6]. These schemes are:

1) Thoracic epidural analgesia between the T_3-T_8 Vertebrae level (usually between T_1-T_2 or T_2-T_3 interspace), through intravenous injection of opioids, most commonly Morphine. An epidural catheter is inserted under local anesthesia in a sitting position 3–4 cm into the epidural space, the epidural line is secured with antibacterial filter and the catheter is finally flushed carefully with 3 ml of physiologic saline solution. All patients had their catheters inserted immediately before the procedure. The Cerebrospinal Fluid concentration of Morphine injected in the epidural space far exceeds, in the second order, that in plasma [7]. The catheter is removed after the 2nd postoperative day. Epidural Morphine's pharmaceutical action is characterized with hydrophilicity and a tendency to spread rostrally via the Cerebrospinal Fluid, where may be detected as long as 24 hours later. This scheme is usually applied when a classic open thoracotomy is performed.



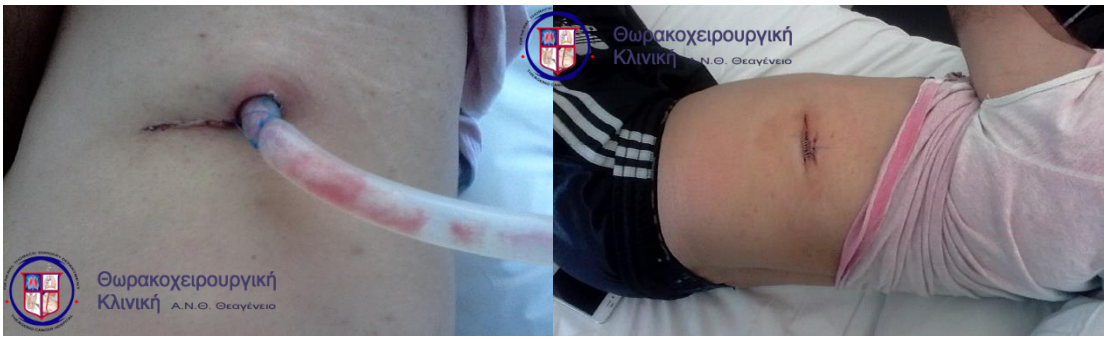
Figures 4 - 5: Open posterolateral left thoracotomy under thoracic epidural analgesia.

2) Thoracic epidural analgesia between the T_3-T_8 Vertebrae level (usually between T_1-T_2 or T_2-T_3 interspace), through combined intravenous injection of opioids, also Morphine, and local intramuscular injection of Ropivacaine [8]. Ropivacaine is a long-acting amide local anesthetic which reversibly inhibits sodium ion influx in nerve fibers. It also is less lipophilic than other local anesthetics and is less likely to penetrate large myelinated motor fibers through its selective act on the nociceptive A, B, and C fibers over the AB (motor) fibers, with significantly less cardiotoxicity and neurotoxicity. This combined scheme is quite effective against more demanding procedures, which may include "En-Bloc" Tumor Resection with infiltrated thoracic wall parts, extensive pleural decortication, etc.



Figures 6 - 7: "En – Bloc" removal of a Germ – Cell Tumor (GCT) along with parts of the 5th, 6th and 7th left rib.

3) Combined intravenous injection of Tramadol and Non-Steroidal anti-inflammatory drugs (NSAID's). Tramadol is an opioid pain killer, used to treat moderate to moderately severe pain with two mechanisms of action. Initially, it binds to the μ-opioid receptor. Secondary, it inhibits the reuptake of Serotonin and Norepinephrine. Nonsteroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents with diverse structural and pharmacodynamics profiles, but have similar mode of action, while they have proven effective in inflammatory conditions such as acute trauma, and pain associated with inflammation [9]. The commonest NSAIDs groups which are usually applied postoperatively are: 2 - Arylpropionic Acids or Profens (Ibuprofen, Flurbiprofen, Ketoprofen, and Naproxen), Oxicams (Piroxicam, Meloxicam), and COX-2 Inhibitors (Parecoxib). The third scheme is applied almost exclusively against minimally invasive procedures, such as VATS, Video – Assisted Mini Thoracotomies, etc.



Figures 8 - 9: Uniportal Left VATS, before and after the removal of the Chest Drainage Tube

Every patient's follow-up and determination of the "QoL Index" is concluded via usage of the "Quality of Life" ("QoL") Protocols and the following "QoL" Questionnaires:

i) The Core quality of life Questionnaire for clinical practice "EORTC QLQ-C30",
ii) The 36-Item Short Form Health Survey "SF-36",
iii) The Health state description and evaluation "EQ-5D" Questionnaire, and additionally,
iv) The "VAS – Visual Analog Scale", exclusively indicative for pain symptoms.

The above-mentioned questionnaires are designed for self-completion. They aim to capture information directly from the respondent and generate direct data from every patient's point of view, which provide significant additional information to every personalized strategic postoperative plan for optimal pain management [10]. The required time intervals necessary for each patient's adequate observation are divided to:

a) The immediate postoperative period (2^{nd} – 3^{rd} postoperative day),
b) The day of discharge,
c) After a 1-month period, following the day of discharge, and
d) After a 4-month period, following the 3^{rd} time interval.

The extracted differentiated information from the answered questionnaires during the overall 6 month - period is evaluated in detail, both separately and in groups, according to each type's subgroup of pain treatment and the person's postoperative health evolution.

Results

The received data, combined with every current individual clinical status, provides significant aid against improving and maintaining the highest possible level of quality of life [11]. The extracted results supply us with important additional information, regarding eventual discomfort, suspicious symptoms and minor/major complications, such as postoperative pain, kinetic or respiratory discomfort, etc., indicating us towards a thorough realization of each individual's optimal and efficient "Follow Up" [12]. The differentiated information from the answered questionnaires during the overall 5-6 month - period is evaluated in detail, both separately and in groups, according to each type's subgroup of pain treatment and the person's postoperative health evolution [13].

According to our data results, 166 male (55,3%) and 134 female patients (44,7%) who underwent a major thoracic surgery procedure, participated in our study.

- The 1^{st} postoperative analgesic scheme was applied to 96 of them (32% overall) ➔ 67 male (69,8%, 22,3% overall) and 29 female patients (30,2%, 9,7% overall),
- The 2^{nd} to 87 patients (29% overall) ➔ 47 male (54%, 15,7% overall) and 40 female patients (46%, 13,3% overall) and,
- The 3^{rd} to 117 (39% overall) ➔ 52 male (44,5%, 17,3% overall) and 65 female patients (55,5%, 21,7 overall).

There is an obvious tendency towards the performance of even more minimally invasive thoracic surgery operations, as long as the experience increases and is accompanied by the latest modern technology in the field of surgical instruments and infrastructures. This kind of medical evolution provides even more promising conditions against a further optimal Quality of

Life level for every patient that undergoes such operational interventions.

The overall 6 – month observation and evaluation presented progressive relief and further comfort concerning pain symptomatology. There were no significant differences between male and female patients according their pain evolution during their immediate and following postoperative period, according the results of the "QoL Index". Additionally, there were no prominent problems during the patient's overall postoperative period and clinical status. A small localized superficial hematoma around the epidural catheter's entry point has been identified with a 1% frequency - 1/100 patients, with no consequent complications.

The patients indicated that they were able to return gradually to their everyday life and habits, especially those that did not require additional management. The rest of them who were submitted to complementary chemo- and/or radiotherapy expressed that they adapted much easier to their new clinical status and way of life, mainly due to the absence of persistent pain symptoms.

This combined study is applied in this form for the first time. The study was conducted in accordance was approved by the Ethics Committee of "Theageneio" Cancer Hospital, with additional consent forms, obtained from all patients. Complete statistical data will be furthermore published and reviewed.

Conclusion

A high level of surveillance and efficiency is necessary to maintain during every patient's postoperative, recovery and rehabilitation period [14].

A "QoL" index, as well as its evaluation, is widely considered as one of the most accurate indicators of a patient's postoperative health evolution [15]. Every individual's final "QoL Index" comes to complete his/her overall "Follow-Up", along with the basic initial strategic plan, the selected surgical intervention and further management.

The patients become active members during their postoperative period, expressing their personal evaluation concerning their progress, based on their "point of view" and perception.

The 6 – month compared data provide significant aid towards successful postoperative analgesia and preservation of the patient's optimal clinical status, especially when malignancy diagnosis is certified and other kinds of forthcoming treatment, like chemotherapy and/or radiotherapy, are imminent to follow.

The authors declare that they have no conflicts of interest.

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Glial and neuroaxonal biomarkers in a multiple sclerosis (MS) cohort

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Abstract

Background: The desire to treat patients with MS early and to target the correct therapy to patients is hampered by a lack of useful prognostic biomarkers that can predict disease progression, severity, and responses to treatment. In addition, clinical trials of new drugs require biomarkers that can predict response to therapy over the course of a few years trial. These biomarkers need to be rapid, relatively inexpensive, and have the potential to be uniformly administered across multiple centres and clinicians. To date, there have been many studies into potential biomarkers for MS. Only serial Magnetic Resonance Imaging (MRI) have emerged as having clinical utility in longitudinal or prospective studies. Small cohort, cross-sectional studies and moderate cohort longitudinal studies have provided a panel of possible biomarkers for MS. Neurofilament light (NfL-l) protein is the light subunit of a structural component in the neuronal axons and increased levels in the cerebrospinal fluid (CSF) reflect axonal degeneration [1]. Tau protein is a microtubule binding protein that contributes to the stability of microtubules. The binding of tau to microtubules is reduced by increases in the phosphorylation state of tau. Hyperphosphorylation of tau disrupts microtubules and leads to degeneration of neurons [2]. Detection of NfL-l protein, tau and phospho tau protein in the CSF in considered to reflect the degree of axonal damage in the central nervous system. Glial fibrillary acidic protein (GFAP) is secreted from astrocytic and is a well-established marker of reactive astrogliosis. Extensive astrocytosis leads to the formation of the astroglial scar, that plays a role in the progression of disease [3]. **Aim:** The aim of the study is to test neuronal (as NfL-l, tau and phospho tau) and astrocytic activation biomarkers (GFAP) and correlate with clinical characteristics of multiple sclerosis patients. **Methods:** This observational case-control study included patients with MS and controls with other neurological conditions. The MS patients were separated in two groups: Relapsing patients included patients with Relapsing Remitting MS and CIS (Clinically Isolated Syndrome) while the progressive group included primary (PPMS) and secondary progressive patients (SPMS) [4, 5, 6, 7]. CSF was collected according to international standards of collection and storage for biomarkers [8]. CSF levels of b-amyloid, tau, phospho tau, NfL and GFAP were determined using enzyme-linked immunosorbent assay. EDSS (Expanded Disability Status Scale) [9] was performed by a neurostatus trained and certified neurologist. The clinical data (sex, age, duration of disease, type of disease, EDSS, previous disease modifying therapy, previous use of steroids and previous use of statins) of the patients were correlated with the measured proteins. There are available studies that showed reduced levels in CSF NfL-l after natalizumab use and serum NfL-l after fingolimod use, hence patients of high efficacy medication as monoclonal antibody natalizumab were excluded from the study and fingolimod patients were kept to a minimum [10, 11]. Follow up EDSS was measured 6-12 months after sampling. We present normally distributed variables using means and standard deviations and non-normally distributed variables using medians along with their ranges. Spearman's correlation coefficients were used to analyse correlations between the CSF's biomarker concentrations, and both unadjusted and Bonferroni adjusted p-values are reported. Orthogonal projection to latent structure

discriminant analysis (OPLS-DA) was also used to find differences in terms of CSF metabolites between the relapsing and remitting patients [CIS, RR, PR versus PP, SP patients]. The OPLS-DA algorithm finds the projection direction, score vector, that gives the largest covariance between the variables and the pre-defined classes (i.e. relapsing and remitting phases) and that maximizes the separation between the classes. The variables that are found to have an influence on the projection (VIP: Variable importance on the projection plots) and that contribute to discriminate between the classes are summarized in a VIP bar-plot. The higher the VIP bar, the more influential is the variable on the model [12]. We used linear regression to examine the association of the biomarkers with EDSS-change, adjusting for age [13]. A global validation test was used in each model to examine if the assumptions are met. All statistical analyses were performed in R [14, 15, 16]. **Results:** 87 MS patients (aged 41.1±11.96) enrolled in the study and 21 controls (aged 44.17±12.8). The female/male ratio was 8 females/12 males in the controls and 64 females/20 males in the patients' group. From the patients' cohort 86% (75 patients) were relapsing forms and 14% (12 patients) were progressive forms of the disease. From the total sample 60 patients had disease duration more than a year and 48 less or equal to 1 year. 31 (28.9%) patients had received prior corticosteroid course and 15 patients (13.9%) had prior statin use. In the MS cohort 31 patients (28.7%) had received previous first line disease modifying treatment. The EDSS that showed mild disability without effect of mobility on grades below 4, affected the majority of the sample with EDSS 1-3 in 70% of the patients. 27% of the patients had EDSS scale score 3.5-6.

General study characteristics are depicted in Table 1 and Table 2.

Table 1. *General study characteristics (categorical values).*

Variable	n	pct (%)
Gender		
Female	74	68.5
Male	34	31.5
Age groups		
18 - 30	18	16.8
30 - 40	36	33.6
41+	53	49.5
Disease status		
no MS	21	19.4
CIS	19	17.6
RR	56	51.9
PR	0	0.0
PP	4	3.7
SP	8	7.4
Disease duration		
Disease duration (>1 year)	60	55.6
Disease duration (<1 year)	48	44.4
Corticoid use (prior year)		
Use	31	28.7
No prior use	77	71.3

Statin use		
Use	15	13.9
No prior use	93	86.1
prior DMT use	31	28.7
no prior DMT	77	71.3
1-3	77	71.3
3.5-6	29	26.9
6.5-9	2	1.9
IgG bands		
Positive	59	62.8
Negative	19	20.2
Inconclusive- oligoclonal bands in CSF and blood	11	11.7
Endothecal IgG without distarbance of the bloodbrain barrier	5	5.3

Table 2. *General study charasteristics (continuous variables).*

Variable	Mean	SD	Median	Min	Max	Q1	Q3
Age at LP (yrs)	41.66	12.42	39.50	16.00	72.00	33.00	49.00
Age, No patients (years)	44.17	12.85	40.00	16.00	72.00	35.00	53.25
Age patients (years)	41.10	11.96	40.00	18.00	68.00	33.00	48.50
Duration (months)	86.52	105.92	33.00	0.50	468.00	2.00	144.00
Duration RR (months)	116.36	109.68	102.00	1.00	468.00	18.00	180.00
EDSS	2.56	1.74	2.00	0.00	7.50	1.38	3.50
EDSS RR	2.50	1.45	2.00	0.00	6.00	1.50	3.50
EDSS (at follow up)	2.73	2.12	2.50	0.00	8.00	1.25	3.50
CIS to RRMS conversion (months)	2.05	4.85	0.00	0.00	18.00	0.00	0.50
AMYLOID (pg/ml)	448.33	171.91	426.00	131.00	883.00	345.00	536.75
hTAU (pg/ml)	86.54	63.75	76.00	17.00	501.00	57.00	93.50
pTAU181	45.94	79.75	36.00	22.00	811.90	29.00	38.95
pTAU181 (serum)	103.52	165.05	40.50	37.00	610.60	39.60	42.20
NFL (pg/ml)	1596.30	2766.46	678.00	96.00	16015.00	457.00	1301.00
GFAP	2.48	3.34	1.66	1.45	26.85	1.58	1.86
GFAP (serum)	3.09	0.54	3.14	2.17	3.95	2.65	3.49

A significant difference was evident in the levels of phospho tau181 in the CSF of patients with relapsing form of MS in comparison to the progressive forms (p=0.03) with phospho tau median 34.5 (INQ 22-89) in the relapsing groups and phospho tau median 40.5 (INQ 26-267) in the progressive one.

EDSS did not correlate with the levels of the biomarkers CSF nor serum, but in a linear regression model a potential association was revealed between GFAP serum and EDSS change from baseline, with a negative association between GFAP serum levels and ED Which means that when GFAP serum levels were elevated in sampling, EDSS in follow-up 6 months after sampling was likely to decrease.

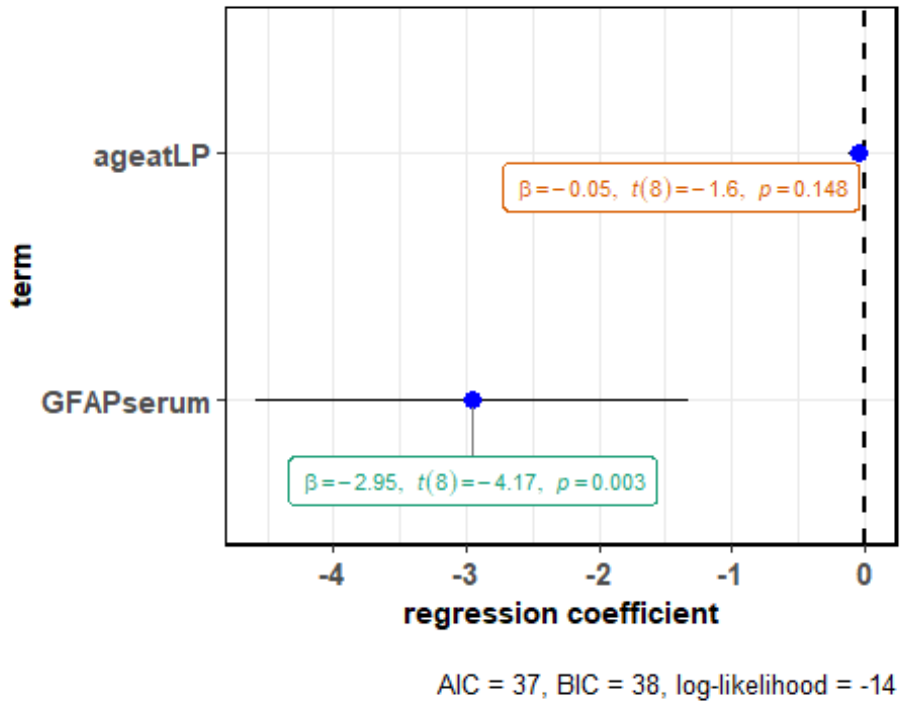

Figure 1. Association between study GFAPserum and EDSS change from baseline. The β coefficients from the regression analysis along with the 95%CI and the p-values are presented.

MS patients that had previous disease modifying treatment showed decreased levels in the phospho tau levels in the CSF, while previous steroid treatment within the last year or previous treatment with statins did not influence the levels of the proteins.

After pairwise spearman correlations of continuous variables, EDSS score showed correlation with age (ρ=0.26, p=0.005) meaning that older patients had higher scores in disability. Also, older patients in our sample had longer disease duration (p=0.00003, ρ=0.38) and disability scores based on EDSS correlated with the duration of symptoms (p=0.011, ρ=0.24). As far as the inter biomarkers correlations, soluble beta- amyloid concentration was proportional with phospho Tau 181 in the CSF (p=0.000009, ρ=0.62) and that phospho Tau 181 in the CSF correlate with the NfL-l concentration (p=0.05, ρ=0.19). Lastly, phospho Tau 181 in the CSF correlate with increased GFAP in the CSF (p=0.0000039, ρ=0.45) (Figure 2).

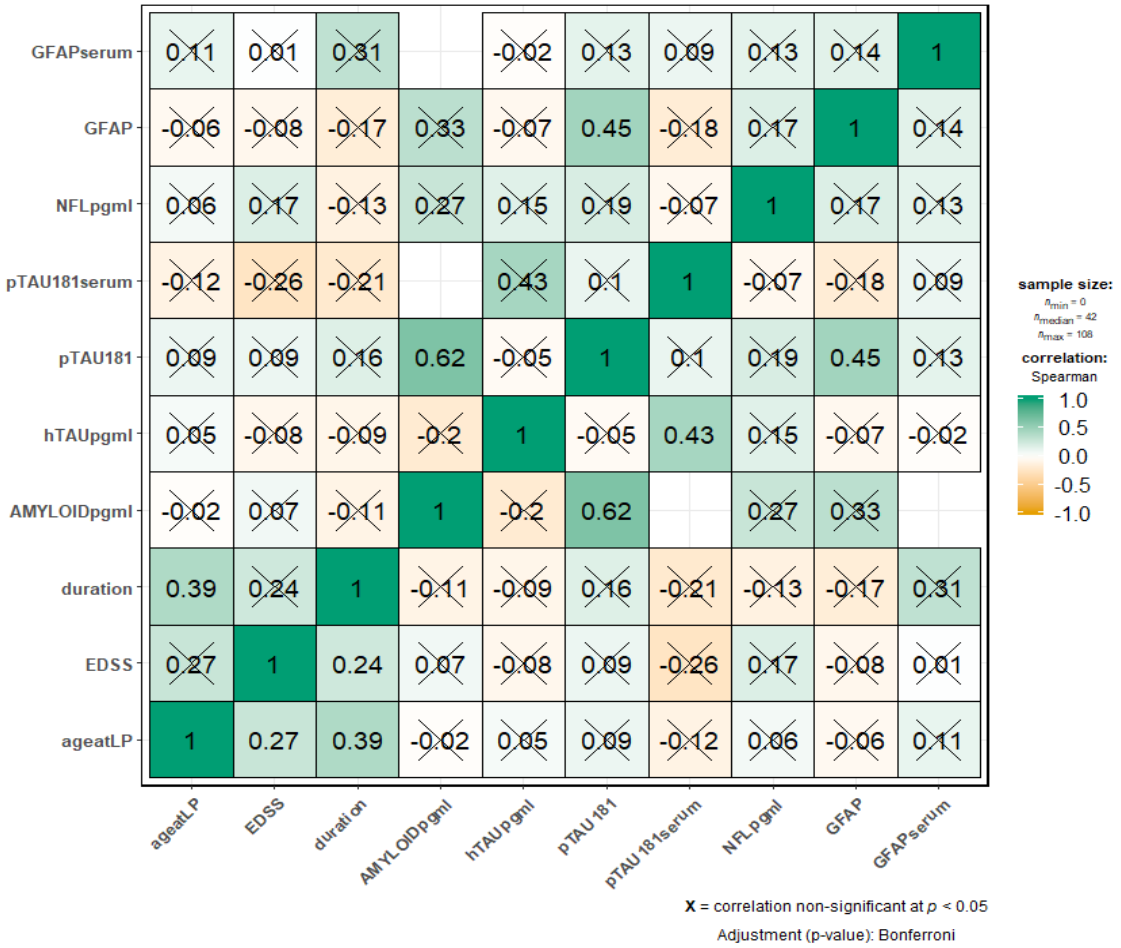

Figure 2. Correlation matrix representing correlations between the study variables. The p-values that correspond to the upper triangle correlations are adjusted for multiple comparisons, while the lower triangle represent the unadjusted estimates. X-crossed are the non-significant correlations.

When orthogonal projection to latent structure discriminant analysis- OPLS-DA was implemented for the discrimination of the most important biomarker to discriminate between relapsing and progressive forms on multiple sclerosis patients, the relative contribution of phospho tau proved higher that the other biomarkers. Hence, phospho tau proved the most important biomarker to discriminate between relapsing and progressive forms on multiple sclerosis patients (Figure 3).

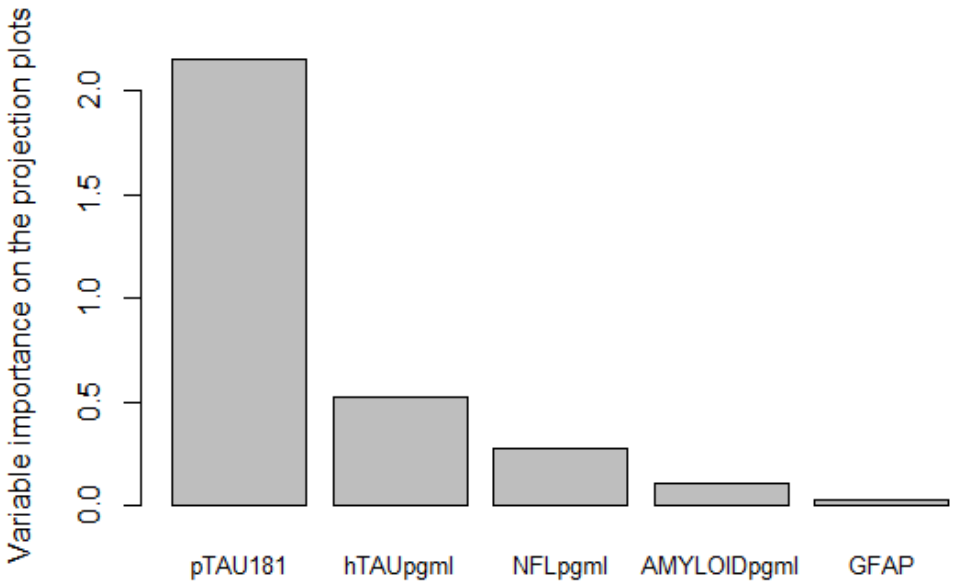

Figure 3. Relative contribution of the CSF biomarkers to discriminate between CIS, RR,PR and PP, SP MS patients, as shown by VIP (Variable Importance in the Projection)

Discussion

In our study the findings that disability progression is associated with age and years form disease onset. Many natural history studies as from Scalfari et al. in 2011 and Confavreux et Vukusic in 2006 [17, 18] showed that in MS many aspects of the clinical course, as clinical symptoms, occurrence of relapses and EDSS scores are associated with age and years from disease onset. Specifically, age has been shown to offer more precise assessment of relative severity of disability on MS through determination of the ARMSS score that incorporates age than disease duration [19]. This is in agreement with reports on the effect of age reaching disability milestones [20] and is well supported and visualised by the clinical course and the functional reserve, as described in the topographical model in MS [21].

Phospho tau is supposed to be a more precise marker than total tau of neurodegeneration [22]. Rostacy et al. in 2005 as Terzi M et al. in 2007 and Tumani et al., in 2009 found increased CSF tau levels in MS as compared to controls [23, 24, 25]. Martinez-Yelamos and co-workers in 2004 described a group of early relapsing-remitting MS patients in which increased CSF tau was predictive of poor short-term outcome [26]. Mitosek-Szewcryk et al., in 2011 used antibodies for phosphorylated and unphosphorylated tau protein epitopes and found increased immunoreactivity of phosphorylation epitopes compared to controls [27]. These data suggest that the recognition of increased phospho tau in the absence of increased tau in the CSF in the progressive forms of the disease may be an early marker of axonal damage as tau protein concentration in the CSF may be normal in unselected MS cases. Recent studies have identified

proline kinase overactivity as a potential new treatment target and our findings are in keeping that a potential kinase autoregulation defect is contributing to disease progression [28].

Previous studies have suggested that GFAP is related to disease progression [29]. In our study this finding was not reproduced but to interpret the results, one must keep in mind the protective role of glial cells following neuronal injury. Microglial and astrocytic activation were reported upon axonal degeneration and contributes to tissue repair and limitation of the inflammatory activity. Reactive astrocytes in active lesions in MS may have both proinflammatory, by secreting proinflammatory cytokines, cytotoxic factors and recruiting cell subtypes, and an anti-inflammatory role supporting differentiation of regulatory T-helper cells and promoting remyelination [30]. As such, elevated levels of GFAP in MS relapse may be followed by clinical improvement and an improvement in EDSS scale.

The inter-correlation between soluble beta- amyloid concentration with phospho Tau 181 in the CSF and NfL-l concentration as well as GFAP, may emphasise the role of astrogliosis in response to neuroaxonal damage in all stages of the disease. All studied proteins are probably closely inter-related in a cascade of neuronal structural integrity change [31].

There was a trend that suggested increased GFAP in serum sampling relates with improved outcomes in follow up 6-12 months after sampling. GFAP expression occurs mainly in the foot processes of astrocytes, which also participate in the formation of blood-brain barrier [32-33]. MS lesions typically are found in perivascular spaces and central vascular sign has been recently suggested as a reliable imaging sign of MS related activity [34, 35]. On the basis of the above, it may be that GFAP serum concentrations reflect GFAP secretion better than CSF concentration as it may partially drain directly into the blood and not into the CSF. Still, though due to limited number of serum samples, further studies are warranted to this direction to enable the verification of results.

The study supports that biomarkers may help in evaluation of neuronal damage in active MS and also reflect secondary pathogenetic mechanisms of repair or progression. Phospho tau may have a role in the discrimination between relapsing remitting and progressive phases of the disease. These findings will need to be further validated in larger cohorts to permit widespread use of biomarkers in clinical practise.

The authors declare that they have no conflicts of interest.

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Elders and mental capacity: Using a qualitative approach to examine views on independence and protection across the Balkans

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Abstract

Objective: How do people in contemporary Balkans think about elders and mental capacity, a particular medical and legal concept? **Subjects and Methods:** Different interpretations and applications regarding elders' mental capacity are explored through in-depth semi-structured interviews and field notes of 28 Greeks, 27 Bulgarians and 10 Romanians of varying ages, all living in Northern Greece. This study attempts to shed some light on the perceptions of ageing, mental disease, civil capacities, family and state involvement across three nearby nations. Interpretative phenomenological analysis (IPA), an approach to psychological qualitative research that aims to offer insights into how a given person, in a given context, makes sense of a given phenomenon was used for the first time applied to this topic. **Results:** Four areas for focus were formed, each relating to the elders and the aging process and presenting the associated themes drawn from the participants' accounts: 1) Discovering the altered-self of the elder, family and society, 2) General experience with old age and capacities in everyday life, 3) Ways of thinking and acting towards old age and capacity issues, and 4) Feelings and comparison thoughts towards old age and capacity issues. Education, occupation, life experience, and especially religious beliefs were all found to be involved in the ways that people from three cultural groups understand the concept of mental capacity and incapacity of elders in their everyday life. A main finding is that the more educated Bulgarians and Romanians tend to speak more easily and to be more positive towards the social construct of aging, while Greeks regardless of their gender, education, religious beliefs, and financial status, tend to consider in their narratives old age as equal to loss of mental capacity, which equals to loss of autonomy and total dependency on others. The process of old age for the group of Greeks begins with retirement which is perceived to reflect withdrawal from social life. This is primarily related to behaviors from individuals and society that result to deprivation of freedom. **Conclusion:** The similarities and differences among these three ethnic groups are discussed, which according to the interviewees discourse reveal peculiar cultural understandings about subordinate themes such as power and its relationship to the self and superordinate themes on emotional control, choice, and individualism.

Introduction

The examination of the effects of the environmental change on human physiology and behavior is an interesting field for research in anthropology, as was the case with the investigation of seasonal change on human behavior in an isolated Inuit community [1]. The above mentioned paper focused on the effects of the economic environment, and more specifically of the effects of the recent financial and social change upon human cognition and behavior in a culturally unique area of the south-eastern Balkans. Originally separated from the rest of Europe by culture, politics, and economics, the Balkans have slowly been integrating into Western Europe since the nineteenth century [2]. Of course, nations and countries in the Balkan Peninsula did communicate and share a lot of things through bilateral interconnections but at the same time they kept their distinct cultural characteristics. Here, we try to explore the relationship between human cognition/behavior and cultural/social/financial variations in people from three neighboring countries all living in Greece: the northern part of Greece, the southern part of Bulgaria and Romania. In an overview of the historical development and contemporary situation of the Northern Greek community, covering social organization, economic adaptations, and recent changes in population structure, we can reach the understanding from state statistics and mass media that the contemporary Greek society is much different from the past: the 'western pattern' of relationships between and among individuals and groups the last 30 years has influenced greatly the way that people think and act, the financial crisis following the global one seems to be one of the most disastrous events in the national history of the country and the demographics of the population is for the first time demonstrating a large proportion of people belonging to different ethnicities. The same is largely true for Bulgaria and Romania. Some rapid social, material, and political changes which are taking place the last few years and right now, and the impact of these changes on the behaviors and attitudes of people regarding elders and ageing are discussed here for the first time.

Recently, growing numbers of researchers worldwide have embraced the challenge of developing new neuropsychological tools to assess different forms of capacities. In the present article, the question that is explored is how people in modern Greece, Bulgaria and Romania interweave diverse ideas concerning elders and mental capacity. Specifically, how rural and urban citizens in a Northern Greek community think about this particular legal and clinical concept in their everyday life and how their fellow neighbors from Bulgaria and Romania living at the same place as immigrants understand and express the same concepts. In investigating mental capacity two main questions are asked: First, how do people understand the mental capacity concept in general and more specifically regarding elders? And second, how are these folk understandings related to their culture and history?

Legal-contractual capacity and mental capacity are terms that are of interest for both medicine and law, but at the same time they appear as a critical term for societies since they concern issues of self and personal power and (in)dependence.

Today, it is well established that the world' s population is growing older, the main reason being the increase of life expectancy, especially over the past few decades and, as a consequence, there is an increasing number of older individuals worldwide. As a result, many

social changes are already occurring and more will be expected in the foreseeable future which, in turn, will pose many challenges to societies and to social and medical sciences. It already appears that one of the most common problems for a large proportion of the older part of the population is the 'normal or abnormal' decrease of cognitive or 'mental' capacities such as failing memory, language deficits, attentional lapses, deficient executive functioning e.t.c. In technical terms such malfunctioning is covered under the diagnostic umbrella of various types of dementias.

When we are referring to capacity, we generally mean the ability to do something or to understand something. If a person lacks capacity with regard to a certain area of life, this may have an effect on other areas of life and even certain legal rights [3]. Legal capacity in all western legal texts is considered to be acquired only when an individual reaches a certain level of intellectual maturity. Ethical issues arising from the practice of law and medicine focus mainly on adult patients with diminished capacity, that is (partial) inability to make independent rational decisions. Mental capacity, in this context, is considered as an abstract concept consisting of a number of different, not always dependent on each other constructs in the legal, medical and psychological fields. In the relevant literature there is a fundamental theoretical distinction between competence and capacity, the former having a legal, not medical connotation in regard to the degree of mental soundness necessary to make appropriate decisions on specific issues or carry out specific actions. Accordingly, older adults are presumed to be competent decision makers, unless otherwise adjudicated by the court. On the other hand, incompetence is related to one's functional deficits due for example to mental illness or mental retardation or any such malfunctioning judged to be sufficiently obvious that the person cannot satisfy the demands for the specific decision-making situation weighted in the light of its potential implications [4]. Only the court can make a determination of incompetence. In contrast, capacity is defined as the individual's ability to make an informed decision, according to mental health expert assessments. In this context, any licensed mental health expert may make a decision in regard to the capacity level of an individual. Whether an individual's capacity to make an informed rational decision or give consent is in doubt, it might be necessary to be referred to a competency hearing or may need the appointment of a guardian. Competence of course is related mainly to a specific situation and is not a global black or white issue, which implies that the question should be expressed in the form of ''Competent for what?''. Thus, it is apparent that the capacity to make a competent informed decision on some issue requires a careful investigation. We add here that other aspects of the individual mental level or diagnosis are not as relevant as the ability to comprehend and make mindful informed decisions regarding the problem at hand.

A relevant issue is that failing mental capacity may not be easy to distinguish from a normal change occurring in the aging process. This increases the possibility for experts such as lawyers and judges to make incorrect decisions about mental incapacity, which raises another common legal issue in regard to competence for the older age group (and especially for those suffering Mild Cognitive Impairment). Moreover, an adult who is presumed to possess legal competence, may lack the capacity to make specific decisions. Psychiatrists, neurologists, neuropsychologists, lawyers-judges and society as a whole, therefore should consider this legal aspect in a serious manner.

The evaluation of competence can take a number of different forms, such as assessment

of specific civil capacities concerning the capacity for medical consent, capacity for sexual consent, financial capacity, testamentary capacity, driving capacity and capacity for independent living [5]. In England and other European countries there is a strong interest in clarifying issues of civil capacities as the Mental Capacity Act 2005 (MCA) implies. In Greece, no clear standards and valid tests exist or, for that matter, any widely accepted kind of examination in the form of a standardized protocol in assessing capacity for decision making for acts that have legal is nonexistent. As a consequence, experts use unstructured clinical interviews for clarifying an individual's decision making process, which lead to legally doubtful judgments and professional malpractice. This chaotic situation has been mainly due to a loose legal framework, which is based on the unsatisfactory definition and explanation by the Greek Civil Code (articles 127-152). Briefly, this states that generally minors, individuals with mental disorders and individuals under the influence of drugs or alcohol have no right to enter into any legal contracts. However, clear criteria on the evaluation are nonexistent. In addition, the Greek Civil Code as the Bulgarian and Romanian and any other formal medical text in the three countries are not offering guidelines for the assessment and there is an absence of national policies on this important issue, which causes further problems of unnecessary court hearings for living elders or repetition of legal procedures on questioning the (in)capacity of deceased elders in these countries.

Dementia studies were and still are dominated by biomedical and psychological models that focus on the individual without taking into consideration the socio-cultural context. There is now a growing scientific interest in how socio-cultural factors may have an impact on the experience of, and response to dementias [6]. This growing interest in the socio-cultural context of dementia with reference to earlier developments in disability studies and ageing research in general is of great importance for social sciences [7, 8].

The relationship between cultural values or culturally mediated experiences and the development or expression mainly of mental illness (psychopathology) is taken today for granted, but at the same time varying attitudes toward mental illness in different cultures is a reality. A 'patient's' culture seems to affect the experience and expression of symptoms [9]. So, thought and experience are related to social and cultural processes. But what about thoughts and experiences about normal elders, elders suffering different forms of dementias and their civil capacities status in the Balkan area?

Adults who lack the capacity to make particular decisions for themselves in paternalistic societies would be considered as individuals to be protected. So, a general hypothesis is made that Greeks due to their different history and culture would be different from the other 'western' European people in the way they think about the capacity issues and would express more paternalistic views for the role of the state due to historical and cultural differences. A widespread idea is that Greece belongs neither to West nor to East, but constitutes a special case, which is unique in history in terms of natural beauty and cultural achievements. Despite their non-static and non-isolated existence, Greeks during their history as a nation always belonged to smaller clans, tribes and states under the rule of a general central state-power. All the above groups offered protection to their members. Activities on political level demonstrated that from its birth in 1830 the modern Greek state in no way sought nor indeed had the skills or capacity to penetrate the geographical area and local communities, but left their government to be mediated by local family elites, with whom the state tried to negotiate [10]. In addition to that the idea of protecting

yourself and other family members from the 'bad' state was a common feeling during Ottoman-Turkish rule [11]. Some contemporary studies indicate that Greece and Bulgaria share several similar cultural values. Greece is traditionally regarded as a collectivist society, but today the country is undergoing an evident process of individualization that increases personal self-evaluation, interestingly without changing the role of the collective self in well-being [12]. Bulgaria is also moving toward individualism, but the collectivistic values seem to strengthen only with the increase of age and the strongest individualism is found in the age group of 15-18 years old [13]. In addition, both Greeks and Bulgarians value providing support for their children and grandchildren during their whole life. For Greek people, there is a positive relation between parents' life satisfaction and providing support for their children and grandchildren in contrast to other cultural contexts [14].

But what about elders? Elder is a role played in an organized community that is most common in subsistence cultures. The elder person in some of these cultures is considered as the Big Man, that is a highly influential individual in a tribe. But what about an elder who has some kind of health problem'? Does this respected image change? Greece and the "post" crisis setting is characterized by a climate of insecurity that gives individuals the impression that they must find ways by themselves to deal with their own family problems with the minimum government or state intervention.

'Western' categories of elders' behavior (normal and abnormal) may not translate across cultures in the same way. More specifically, individuals coming from different societies may have different cognitive and emotional reactions to issues regarding mental capacity and elder members of their society. People think with the aid of schemas that is units of culturally shared knowledge, which shape their understandings of normality and pathology. Another issue is that of stigma for elders and their families. Under the law in Greece (as in the rest of the world) a person is presumed to have mental capacity unless evidence exists to the contrary. The sociocultural context in which dementia or any other mental disorder occurs and the meaning of the disorder to those involved (as sufferers and caregivers) are often missing dimensions. In particular, there is little knowledge about how the disorders of old age in non-Western settings are experienced and understood [15] and these interrelationships among psychological, social and cultural phenomena should be further investigated.

Recent Balkan history is not just the history of the Balkan Peninsula (Southeastern Europe), but a common and at the same time diverse history of numerous struggles for independence and wars for maintaining that independence. Balkan nations tried to control and protect other members of culturally and ethnically different groups using all sorts of methods. This patronizing behavior is apparent not only for the 'others' who belong in different groups (religious, ethnic, linguistic etc.), but also for members of the same group who seem to differentiate from the average. Also, there is a fear of the influence of foreign powers on private matters and a wish to get rid of their rule. This eternal conflict with the 'others' and the 'bad state' shaped the way that individuals and their communities handled their affairs, hiding the truth in order to protect themselves and their families and at the same time trying to find ways by themselves to solve their personal or family problems [11]. One more clue adding to this peculiar way of thinking and acting toward others is the strong Orthodox Christian theological background that shapes every aspect of the everyday life of individuals and their family lives. The majority of the participants in

this study shared these ideas, so it was necessary to investigate two major points: 1) if the fact that they claimed to follow the New Testament Commands and 2) as a consequence of the previous, if the fact of the sense of responsibility for protecting the 'unprotected' would influence the way they arrange their personal familial affairs as they believe that this will give them and their relatives the maximum of well-being when they can no longer make decisions for themselves, all the above in an effort to try to find ways to prevent or diminish incidences of elder abuse, even when there are questions about the legal mental capacity of the other person-family member.

So, how are messages about mental capacity and health communicated in Greece by people with different Balkan ethnicities? How do these ideas change in a given context, for example the Greek society under financial pressure? How do religious beliefs affect ideas on elders and capacity? How do people come to internalize stigmatizing messages on elders and declining capacities? What are the effects of changing cultural norms regarding capacities? These are some of the questions to be answered.

Material and Methods

Purposive sampling was used. This is effective for Interpretive Phenomenological Analysis (IPA). Semi-structured interviews were ideal for obtaining the relevant, rich verbal accounts which IPA requires for thorough analysis and meaningful discussion. This form of interview, as opposed to inflexibly structured interviews or single monologue narratives, gave participants the space to answer questions as fully as they wished, explaining their experiences on and in their own terms. The use of an interview schedule allowed careful development of relevant and meaningful questions prior to the interview, while also enabling movement around the subject area, and flexibility in response to any new concepts which arose during interviews. Simultaneously, this approach allowed the refocusing of interviewees when they departed from the subject. Open questions enabled participants to give information-rich, full answers. Questions reflected the objectives of the research. A brief reminder of the research subject, rationale and aim were given, before asking 3-5 main questions per objective with follow-up questions available where necessary. Finally, to reduce the impact of limitations caused by the researcher's own understanding of the subject area, the participants were asked to add anything they felt was relevant.

This fieldwork was conducted in Thessaloniki, the second-largest city of Northern Greece and in two nearby villages, which are part of the Central Macedonia region. This is a geographical and historical region of Greece in the Balkans, southeastern Europe. From the other side of the geographical and country borders of the Rhodope mountains, participants came from two villages in and near Plovdiv, the second-largest city in Bulgaria after the capital Sofia, who at the time of the interview were temporary residents in Greece. These two places are similar in terms of contemporary population and share a common ancient Greek, Roman and Byzantine history (Plovdiv is the ancient city called Philippoupolis). Some of the Greek participants visited Bulgaria in the past and the majority of the Bulgarians lived and worked for at least 5 consecutive years in

Greece during the past decade, and were settled in Greece at the time of the interview. In addition to the 55 participants, 10 more Romanians living for at least 5 years in Greece were interviewed. Bulgarians and Romanians had close relationships with their family members at their countries of origin and had a good knowledge of the current state of their homeland. Semi-structured interviews were conducted in Greek, which was the native language for the majority of the participants and the second foreign language for the Bulgarians and Romanians. Nearly over half of the interviewees (regardless of ethnicity) had elder alive or deceased relatives with some form of formal psychiatric or neurological diagnosis.

Table 1. *Demographic subject data.*

Total interviews = 65			
Nationality	28 Greeks	27 Bulgarians	10 Romanians
Religion			
	23 Orthodox Christians	24 Orthodox Christians	7 Orthodox Christians
	5 Atheists	3 Atheists	3 Atheists
Residence	28 Thessaloniki	27 Thessaloniki	10 Thessaloniki
Place of origin			
	15 Thessaloniki urban	12 Plovdiv urban	10 Bucharest urban
	13 Thessaloniki rural	15 Plovdiv rural	
Sex			
(%) male	42.85	37.03	50
(%) female	57.14	62.96	50
Occupation			
	12 Farming	18 Farming	6 Farming
	16 Clerk	9 Clerk	4 Clerk
Education			
terminated before high school (age 13)	4	6	3
terminated at age 18	8	5	4
received a university degree	16	16	3
Age			
over 65 years	6	5	2
35–65 years	7	4	5
under 35 years	15	18	3
Financial status (self-characterization)	15 poor	13 poor	9 poor
	13 average income	14 average income	1 average income
Health-status (self-perceived)	13 good	22 good	5 good
	14 bad	5 bad	5 bad

The data collected came from transcribed interviews and field notes. Each interview began with general questions about demographic characteristics such as occupation, education, and family, and then at the second half of the interview the conversation was guided to a more detailed discussion of the issue of elders and mental capacity, discussing everyday experiences on this concept. Questions like “In what kind of situations do you think about mental capacity? Where and when did you officially and unofficially learn about it?” were used. During the interviews, the interviewer also collected personal stories of experiences mainly form family life events in which the idea of elders and (in)capacity was made salient. At the end of the interview, participants were asked to propose suggestions or ideas on how the issue could be investigated better. The aim of

the interviewer was to know not only how the person to whom she was speaking to thought about elders' mental capacity, but how other people might be thinking about it and some of these suggestions were incorporated into later interviews. For example, a point that frequently came up in the very first interviews, was one that had not anticipated: that people often think of capacity in relationship to their small or large scale financial transactions. This domain was incorporated into the first set of questions in further interviews by asking the following: "Which forms of acts with legal implications do you consider to be the most important in your country? or What form of legal capacity does the Greek (or Bulgarian or Romanian) society consider to be the most important?" In this open-ended question the vast majority answered that they believe that the most important acts which involve mental capacity are sales, purchases, loans, leases, donations and testaments. All of the above are acts of financial nature, which was proved to be a rich subject for uncovering thoughts on elders' (in)capacity. Finally, because of the exploratory nature of the study, and as in all qualitative approaches, there was not a strictly fixed hypothesis/es predicting the ways that people would relate to the idea of mental/civil/contractual capacity.

Analysis

For each participant's transcript, resultant super-ordinate themes were considered, in parallel, to identify patterns. Those concepts shared across participants and those which were unique, as idiosyncrasies, were noted. A Master Table of Themes, was then created for all the participants, displaying supportive statements with an assigned participant pseudonym and line number to locate statements in the original transcriptions. This enabled the production of a detailed analytical account for discussion [See Table 2].

Table 2.

Smith's Six Step Model	
Steps	**Actions**
1st Step	Repeatedly Reading
2nd Step	Initial Noting
3rd Step	Coding of Emerged Themes
4th Step	Identification of Recurrent Themes
5th Step	Continue with next Transcript
6th Step	Identification of Patterns Across Interviews- Development of Fixed Superordinate Themes

Results

Here we attempted an understanding of persons, their lives and experiences, based on the knowledge of society and culture, of biology and psychology. We tried to answer the question of how do citizens from three nearby countries think and feel about the medico-legal issue of elders and capacity. We offered a tentative answer to this question, on the basis of three interview-driven

research projects for Greeks, Bulgarians and Romanians. From this in-depth analysis, we raised indicative issues relating to the measures that European Union should take in order to ameliorate the lives of elders and we offered suggestions for future research on this issue in other European nations.

Reflected in the headings and content of 4 ostensible areas for focus, each relating to the elders and the aging process and presenting the associated themes drawn from the participants' accounts:

1) Discovering the altered-self of the elder, family and society
2) General experience with old age and capacities in everyday life
3) Ways of thinking and acting towards old age and capacity issues
4) Feelings and comparison thoughts towards old age and capacity issues

In general the more educated Bulgarian and Romanian people (those that terminated school at age 18 and those who had a university degree, 75 percent of the interviewees) and those who came from the urban areas (59 percent) speak with more ease about this issue and they are more sure about their thoughts. The more educated also tend to be more positive toward elders. Of course, this is definitely not true for the vast majority of Greek origin interviewees (regardless of sex, education and financial status characteristics), who understand ageing as an inevitable route to deprivation of freedom and independence and act accordingly (by taking all responsibility for the actions of the elders according to their statements during the interviews) and by claiming or adopting behaviors that are limiting the elders independence. For example, in a discussion with a young Greek PhD educated man:

Capacity is expressed in our everyday life in different forms. Being able to make your own decisions on finances, testaments, consent issues, voting…. Am I not right? It is very important to be able to decide. To be capable means to be able to identify the decision to be made, gather relevant information, identify alternatives, weigh evidence, choose among alternatives, take action and review decision and consequences…To do these on your own or to know how and whom to ask for help… As I see it…well the most important according to my opinion are cases of sales, purchases, loans, donations and testaments, these are the most important acts with legal implications that I would definitely take care even for my own mother, who at some time when she gets older will definitely become incapable even though she seems to be right now healthy. So, I may say that these are the areas which you should try to assess in some way…. scientific way of course…neuropsychological assessment would be needed, but I doubt if the current tests will give the specific information on this….financial.. contractual capacity…… I believe that most elders have a problem with that or will have in their life. And you know for the state the elders may seem to be ok, but to be sure for yourself and your property just don't let them do things. Of course, it is a burden to take care of the old man, especially for the young. It may sound like a life in prison, but that is what most of us do here. [NS, Field notes, January 6, 2013]

Greeks (96 percent) give as a time limit for the beginning of old age and for being considered as incapable the first years after retirement (around 67 years old). An explanation they give (93 percent) for the loss of capacity at this age is that most even healthy individuals usually withdraw from social life and may experience (most of the times) some sort of cognitive, psychological and physical collapse. According to the above the Greeks find it convenient to put

and treat elders as incapable in order to protect the older people and themselves. Less educated Greek people discussed capacity in a similar way. ES who terminated school at age 18, living today in the urban area of Thessaloniki with her two children, said:

Civil capacity is about every act of yours that has legal implications… And as you know legal implications are linked to money. So, any act that has to do with money and property is an act that shows if someone is capable or not…Elders and financial capacity just don't go together. Look around and you will see that I am right, they can't decide... If their family does not take care of them, then…disaster… The state will arrange for them. Their children should take care of them. This is what we had to do when our grand-mother lost her mind... and believe me all elders lose their mind even if you don't want to call this Alzheimer, it is more or less like that even if you call it with another name…and they are more vulnerable than younger people…You should not end up as a guinea pig in front of a judge or doctor. That's humiliating, isn't it? I do try to put myself in their place. It would be humiliating for your whole course of life..That's why our family should check if you do not have financial capacity and take care of you before anyone notices. [ES, Field notes, January 8, 2013]

Cultural norms do shape psychology and perception. Some norms are healthy and some are not. In the Greek case some contribute to the betterment of individuals, families, and communities; others do precisely the opposite by putting elders in the social margin and forcing them to exclusion. Greeks seem to focus on the negative side of old age and link this period of life with a total incapacitation and some sort of mental disease. Declaring older people in general and more specifically patients with dementia as completely legally incompetent sounds as an unjustified overgeneralization that does not take into account the personality of each individual, but it may also be a sign that they do not really care about elders and that they may notice their difficulties when the seriousness of the health problems have significantly progressed and are visible to others. Any person must be assumed to have capacity unless it is established that he/she lacks capacity. This may contradict with the Greek way of thinking, which overemphasizes the positive side of life, a life without deficiencies. In addition to the ageism against the older group of people always in the fear of not letting the 'others' that is the state to get involved in the clan, strangely for the Greek interviewees a laymans' personal belief in someone's capacity seems to be more important than a formal assessment and legal decision, as only 4 Greek interviewees (14 percent) attributed knowledge on capacity issues to mental health or legal experts.

A similar example comes from an old Greek woman villager with a basic education, who after telling me that she has no idea about psychiatric or legal things and that what I ask is a very abstract concept, she finally said:

Go ask doctors, lawyers and judges……and they may not know what they should tell you…Capacity means to handle your own affairs. I think that you can see if someone is capable by seeing how he manages his financial affairs. You know from everyday supermarket shopping to more complex things like selling…. People of my age…. let's say if I can think about the process of selling this farm. Of course, in cities they would have to buy or sell houses, rent apartments and do a God knows what more with their money in the banks….I think that there you will find out if someone has that civil capacities you are asking me…..And I think that it is important to manage your financial affairs…At least that's what I have learned by my parents and friends half a century

ago…. [DS, Field notes, January 14, 2013]

As the example of DS illustrates, although people were acknowledging the discourse of authority on the subject of capacity, they have their own ideas about family control. Education is not the only and surely not the most important factor contributing to the ideas on civil capacities. Empirical evidence indicating that education could affect the Greek construction of mental capacity/incapacity come mainly from the majority of the recorded interviews of the 13 older Greeks during which they mentioned that they learned this way of behaving at school or at home. Ethnicity of course seems to play a more important role. Bulgarian interviewees as AF a university educated middle-aged man, shows a completely different way of approaching the idea of elders and capacity:

Not all elders have all forms of capacities. Some may be capable to think rationally and decide on some things, let's say on finances, but they may have problems to give their consent for a treatment. There are so many sorts of capacity, for example to make a will, to marry or to make a binding contract. If you asked me from my experience what form of civil capacity I see as the most important I would say …….getting married. But I say again it is not a black or white issue. Greeks as I know, would say that if you lack one, you lack all……and they would overemphasize financial capacity…. and if an elder had some sort of problem with one of these they would treat him like a sick person. They would put elders aside for no real reason….just for having a difficulty…This is my experience. They will talk only for money and elders. Am I not right? Do you think that this will change? [AF, Field notes, January 17, 2013]

Cultural norms often are so strongly ingrained in an individual's daily life that the individual may be unaware of certain behaviors. But this is not the case for the Bulgarian and Romanian immigrants who live in Greece. They recognize the everyday behaviors of Greeks regarding elders in the context of a different culture with different values and beliefs as strange for them and they have difficulty understanding them or may be trying to change them. The majority of Bulgarians and Romanians (93 percent) claim that a minority of elders experience the above total loss of capacity and that the withdrawal is actually caused by members of their families. For them the supposed loss is artificial, in other words the total incapacity issue is the result of a very strict and narrow interpretation of elders' possible problems in some aspects of their everyday life that they can overcome with little or no help. As AA a 40 years old university educated Romanian man said:

From the very first moment I came here I remember an old man sitting at the back of his home and his son shouting at him that he should no more do things by himself, because he was old. He may be a dementia patient, but as I know his family never claimed something like this and never took his guardianship in a legal way. They just not let him handle his money…..And that's all… The same thing is true and for other families I know here….. I can't identify with that. I don't say that Romanians would do something different or better, but I think that they would trust the state and especially the court to declare the old man incapable if and only if that was justifiably necessary…and they would not try to hide it as if he were a leper…[AA, Field notes, January 22, 2013]

Greeks seem to have as their first priority to look strong even though they may not be. This power is about possessing sufficient mental ability to understand an issue, problem, or situation, to make a reasonable decision concerning it, and to understand and appreciate the

potential consequences of that decision. This is an issue that they tend to think on a continuous basis, because when asked in which case and how often they were thinking of the capacity issue and elders, they (71 percent of Greeks) told me that they think of it continuously. For the Bulgarian and Romanian interviewees there is a similar continuous thought of the capacity issue (60 percent), but they do not tend to give the same emphasis on the issue. This is confirmed by what KF, a 63 year old Greek woman told me:

If someone manages financial matters independently (budgets, pays rent, bills goes to bank), collects and keeps track of income, then he is showing that he has the power to live independently. To be himself. To be strong…. To be part of the society. You may not be as strong as you want to be, but it is a matter of not letting others know about being weak. You have to think of it all the time. It's part of our life. Or if you can not hide it, then your family should do it at any cost. [KF, Field notes, January 7, 2013]

As a result an adult is supposed to make all kinds of autonomous choices. But the fear of the public eye and implications of a possible problem with the previous focuses mainly on social relations and is augmented and given huge emphasis in cases of specific financial transactions. Greeks in a case of an individuals' problem, which is linked to the sciences of psychiatry and psychology, find it easy to separate themselves and their families from the state and law of their country in order not to be characterized as mentally ill. But mental illness is more negatively seen when examined in the light of diminished legal capacities and not by its mere clinical and functional deficits. For the majority of the participants fear for the loss of face especially in the public eye is an important issue. Appearance is of course secondary for Bulgarians and Romanians immigrants, but the liberty deprivation is still an important topic for all groups of interviewees and for all ages (28 Greeks, 15 Bulgarians and 9 Romanians) since they kept specifically mentioned freedom and law issues. As EP, a Bulgarian 64 years old woman, said:

To be incapacitated for the state means to lose your liberty. I would not say that you lose your good name in the society. But it doesn't matter anyway what others are saying about you, if the state must take care of you or give your family the right to be your guardian and decide for all or some of your personal affairs. The others would understand that you need help, and what really matters is to get that help. I may lose the ability to carry out some tasks alone, but with more or less help I think that I will manage. Of course, it must be done in a legal way, I mean by the state with an assessment by a doctor and a judge. This would make not only the old person, but any person feel some kind of relief for any liberty loss that he may feel, because it will be done according to the state law, in the right way for the good of everyone. And this liberty loss will be done in a controlled way by the state. So, it is not so tragic.[EP, Field notes, January 3, 2013]

Slight differences between those that belong to the so-called middle-class and working class is evident for the Bulgarian and Romanian interviewees. Adults belonging to different social classes relate to their children and older parents with expectations shaped both by present circumstances and by future envisioned adult roles in society [16]. This may suggest that cultural models play an important role in understanding inter-generational conflict in everyday life. In addition, the information on how people put such models into practice in the context of social interaction is of interest. Of course, for Greeks it seems to be more of a power struggle between the two parts of the relationship. A widely referred conflict management technique is the 'muting' of the elder in the family. Greeks also chose to focus not only on the negative side of capacity, but

also only on its financial implications-dimensions. Unexpectedly, self-perceived personal health and financial status seem not to have an effect on the ideas that people express about health and the socially normal and abnormal. When the interviewees were asked if they believe that their way of understanding capacity issues has changed the last few years due to the ongoing financial crisis, all of the participants denied such a possibility. As KG Greek young woman said:

My views on elders and capacity were and still are the same. I think that it has to do with our culture. I don't think that the current financial crisis changed me or others on this issue. On other issues, maybe yes….. I don't think that it is an expression of neoliberal views. Living abroad for some time did not really change my views…The state the last decade is not just good enough in taking care. Local communities are indifferent… So, you have to do it. If you are an elder you have to trust your family, and if you are someone who has an old person in the family you have to take care of him. [KG, Field notes, January 5, 2013]

According to that the persistence on financial capacity issues and elders is not explained by the change of the country's economic status. In addition to that the Greek interviewees views do not reflect disgust for the old age, but pity, love and protection according to their Orthodox Christian beliefs. 23 Greek interviewees claiming to be Christians explained that what they think and do in their lives for elders is based apart from their own financial protection or profit, on the principles of their faith and that they strive to store up as many good works as possible. In contrast to that the five Greek atheists explained their views on unjustified capacity restrictions based solely on their belief that they should protect first of all their (financial) self-interests. As MP an old Greek woman with basic education said the following:

This is what all Orthodox Christians should do for their salvation. It is a duty to take care of those that are not able to live on their own. This everyday violence is a fact of life…. Some children may be overprotective to their old parents…People my age….I would like to be more independent and do what I like, without having my own children as 'parents' at this age, but….I do what I want, I take part in some things and decisions, I just have to comply to what my family wants…. and my old husband, too…..we do what our children want, mainly on financial issues. Their decisions count more than mine. I have my own wishes, but it is their time to shape the world anyway....It's like having a part of me that wants to live and decide for my life independently and the other one that has to do what the family wants. In this way you will definitely have a better life. [MP, Field notes, January 12, 2013]

Here the aged speak for themselves. Through their words, we find that the aging process is not merely a period of sensory, functional, economic, and social decline. Social identity as expressed by elders themselves (6 Greeks, 5 Bulgarians and 2 Romanians) is negatively colored, but old people (non-Greeks) want and actively try to continue to participate in society, and-more important-continue to interpret more positively their participation in the social world, regardless of the oppression that they may experience from their family members. Through themes constructed from these stories, we can see how the old not only cope with losses and the justified or unjustified manipulation of their families, but how they create new meaning as they reformulate and build viable selves. Creating identity in America, as Kaufman stresses, is a lifelong process [17], but this process and the experience of old age may differ for different ethnical groups. The motivation of Greek elders to conform to the role of the incapable-helpless old guy or 'the incompetent self' is shaped by the cultural beliefs that emphasize on the active role of the young

members of the society and not elders, for whom there is respect and protection, unfortunately without autonomy.

In analyzing the interview data, a number of themes emerge in addition to the capacity issue. What it means to grow old? It seems that Greek elders (from their 6 in-depth interviews) accept the role of the silent figure, of course not without feeling anxiety for becoming incapacitated and anxiety that medical experts/courts/law/state/the authorities can not find ways to provide quality of life for them. The state can not provide a 'refuge', and Greek citizens of all ages and socioeconomic status (rural and urban) during the pre-crisis era [18] and today question the state and its administration of human rights, social welfare, and education for severely mentally ill people and for other groups (e.g. elders), but at the same time immigrants in the country don't seem to share the same rejection.

By claiming in their everyday lives all elders as entirely lacking legal capacity, and not as partially lacking capacity or enjoying full legal capacity, Greeks shift the moral responsibility on themselves and try to do what the state should do, 'to protect the unprotected', of course in a way that is characterized in other cultures as non-scientific and socially unacceptable. Clearly, decision-making capability is proposed as a core concept for a new paradigm of personal autonomy and the right to legal capacity. A person's capability to make personal life/care, health care and financial decisions about their lives can be enhanced sufficiently for making those decisions, but financial viability is recognized as the only field to intervene as the intergenerational dynamics reveal. Greek children or other family members act as caregivers, who explicitly prioritize not necessarily newly introduced individualistic ideas (how they will secure their financial interests), but they do not devalue old values such as ideas coming from Orthodox Christian tradition on helping others. They explicitly express the wish for a legally independent decision making status, even if that is not true in this degree in the everyday life and practice. In addition, the social pressure about elders and the idea of maximum abilities for individuals, shape the cognition on issues of understanding where the cognitive problem is in the lives of elders and trying to find relevant solutions for them.

Discussion

In Western countries people tend to think of capacity-ies as one of the most basic concepts of their everyday life without thinking about its' meaning for them and others. So how do people coming from three Balkan countries think about elders and legal capacity? This study challenges for the first time that the notion of capacity is universally understood by people in the same way as a cross-cultural dichotomy is found to exist despite the common strong Orthodox Christian religious beliefs in all three groups of Balkan interviewees. Social and historical differences might play a more important role in the way that the three groups of interviewees understand capacity, classify elders and express their emotions about them. On the whole, ageing is not a positive concept or experience for the majority of the Greek respondents regardless of age, health status, financial status, religion and education. For the Greek interviewees old age equals to loss of mental capacity, which equals to loss of autonomy and total dependency on others. Interestingly, personal wealth does not affect respondents' experiences and perceptions of mental capacity and

of the process of growing older, although Greeks seem to give a fundamental role to the financial capacity and the legal implications of relevant acts. They believe that there is a black or white value (capable or incapable). If a person has a problem deciding on financial issues this could be seen as a basic criterion for the diagnosis of some form of mental disease, and that they as relatives or friends have to hide this, because otherwise these individuals will be characterized as legally incapacitated, a term that has a so negative connotation that equals to social death. This is a clear fear not only for the stigma of a possible mental illness which for the Greeks is expressed only in relation to a specific capacity, but more importantly for the stigma of being incapacitated due to some form of mental illness. For Greeks there appears to exist an 'obsession' or recurring theme of 'capacity - health and disease', which is strongly linked to the concept of independence and life itself. As historically the motto of the 1821 Greek war of independence states 'Eleftheria i thanatos' (freedom or death), if someone can not handle his affairs and is not free to decide as he wishes by himself/herself, then this leads to the other end that is symbolical or even physical death. In addition to that the strong fear that the Greeks feel that the state will again disappoint them by creating more problems to their loved elders is forcing them to take responsibility, that is to take care of their family-elders by themselves without asking formally for assistance on the custody issue by the state.

On the other hand, Bulgarians and Romanians seem to believe that loss of capacity clinically and legally does not connect primarily and only to financial capacity, which of course may be an important area for the evaluation of elders, but not the one in the inner core of the general capacity concept. In addition to the previous, being incapable or partially incapable is not as disastrous or linked to 'personal death' as Greeks believe. They do not seem to understand issues of capacity as an all or nothing characteristic for individuals, and probably due to an absence of a strong 'small clan ideology', they do not face difficulties in trusting the state for helping or deciding formally on capacity issues. So, the development or expression of mental illness for elders is not associated with financial capacity. Another interesting finding for the Bulgarian and Romanian interviewees living in Greece is that they do not conform to the 'pressure' from the Greek society/modern Greek culture about the issue of elders and capacity and they criticize it strongly. A possible explanation for this lack of conformity could be that individuals who grow up in specific cultures do not necessarily internalize the way people around them think and behave, if they come as adults in the new culture.

In general, more educated interviewees from all countries talk about capacity in a much more confident way than laypeople, possibly because they know more on the issue regardless of their everyday experiences. Greek participants present a uniformly negative view on elders and capacity, which they explain partially based on their religion commands for protection, but at the same time they deny an active participation in life for elders, because of the negative connotation that the state has for them, in contrast to other Balkanians.

Finally, only two of the Bulgarian and Romanian interviewees who had previous experience with elders with some form of serious mental disorder were more negative in their views than participants that did not have such an experience. Greeks on the whole regardless of their previous experiences had a negative stance.

This diversity in the way people see the concept of capacity, even for immediate Balkan neighbors could be explained by cultural differences. I do not by any means claim that there exists

a “correct” understanding of capacity, because my views and interpretations are couched like those of my subjects in a web of relevance particular to my phenomenological-social self, but I do claim that in a globalized world that there must be a framework for the study of capacity issues relating to elders.

This paper reaffirms that different ethnic groups may have different experiences of mental health, but at the same time it seems that there is a ‘human nature’ underneath all cultural differences. Human cognition, emotion, perception, motivation, and mental health may be shaped by the specific cultural groups’ history, language, practices, and conceptual categories. People think with the aid of schemas, units of culturally shared knowledge, which remain to be further explored.

But Greeks don’t lack empathy, as the majority (93 percent) of them during their interviews recognized the emotions that are being experienced by elders. Mental capacity is still a universal and local taboo issue. People who cannot make decisions for themselves are seen as stigmatized. Cultural responses to most illnesses differ and dementia is no exception. For example, in cases from India on dementia patients involving anger and hot temper may be more easily recognized than those involving forgetfulness [19, 20]. In poor agricultural societies, few demands may be made on the elderly, and dementia may not be noticed so quickly, nor be so serious in its consequences, as in faster-moving and more complex cultures. In Greece maybe this could explain the obsession with the capacity issue, because even a slight deviation from the ‘normal’ could have immense and easily detected consequences.

Different types of dementia may be perceived to in different ways. For example, in China medical help may be sought after a stroke, but not in response to failing powers of memory [21]. It is widely held that symptoms of dementia such as deteriorating memory, if present without signs of physical deterioration, may be dismissed simply as the inevitable consequence of old age and this has been reported not only from relatively poor countries [22], but also among the working class Londoners [23], and may be true for other groups of people in the developed world. This is not the case for Greece. The fact that an elder manifests some sort of symptomatology is overlooked if and only if it does not intervene to financial capacity. Also, patterns of support provided by the most obvious source, the immediate family, are not extremely varied, due to cultural influences as all efforts have one aim; to decide for the older individual, not necessarily according or near to his wishes, but according to the best interest of the family as a whole [24].

People seem to think about mental capacity in relation to situations in their lives, rather than as an abstract term [25]. Greeks tend to remember only the negative experiences they had or had heard. On the other hand, Bulgarians and Romanians tend to shape their personal identity of self and others (especially of elders) in a completely different way. The role of cultural difference in the shaping of individual conceptualization of an abstract legal and medical concept of mental capacity in this cross-cultural comparison reveals that globalization is not the case for the Balkans.

How do people with different Balkan ethnicities (in our case Bulgarians and Romanians) think, feel, remember, and interpret the behavior of elders? And more specifically how do the immigrants (our Bulgarian and Romanian interviewees) think about it? How do they understand themselves and others and their place in the world? How do they understand madness-mental incapacity and normality?

So, what can we learn about human variety by considering these questions from an anthropological perspective? There is definitely a difference between neighboring populations, even for those that lived some period of time in another country. The old age incapacity stigma seems not to be a foreign idea to people that have strong religious Orthodox Christian experiences. They seem to accept others and take care of them, but at the same time they do not deny the control of them for their 'own good'. This inquiry gives a new perspective as it suggests that exploring ethnically diverse individuals' views about capacity of the elderly may appear to be a fruitful avenue for continued work in psychological anthropology focusing on how ideas and ideals about abstract concepts may reveal barriers to current or future attempts to create a common legislation in Europe, because of cultural practices which end up in undermining the goals and implementation of a common legislative system [26].

Limitations

Despite the relevance and importance of this study, a number of limitations and should be acknowledged in advance. First of all, the aim of this study was an investigation attempt on the perceptions of a rather small sample of participants on the subject and it can only be seen as such. This means that the findings of this research were not tested for their statistical significance, thus, they cannot be extended to the wider population, something that mostly concerns the quantitative analysis [27, 28, 29, 30]. Moreover, this study was applied to participants living exclusively in Northern Greece [31], and coming from different ethnical groups with the aim of studying in depth the construct of mental capacity in old age [32, 33].

The authors declare that they have no conflicts of interest.

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Impact of reconstruction parameters on quantitative bone SPECT imaging: A novel thoracic spine phantom study

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Abstract

Introduction: The aim of this study was to validate the effect of reconstruction parameters on quantitative accuracy of bone single-photon emission computed tomography (SPECT) images using a novel thoracic spine phantom. **Methods:** We used a novel thoracic spine phantom for bone SPECT image evaluation. This phantom consisted of a body, a vertebral body, a spinous process, and a tumor part (spheres, diameter: 13, 17, 22, 28mm), which were filled with ^{99m}Tc and bone-equivalent solution. The SPECT/CT images were acquired with a dual-head SPECT/CT camera (Infinia8 Hawkeye 4, GE) equipped with low-energy high resolution collimator. The bone phantom images were reconstructed using the ordered subset expectation maximization (OSEM) algorithm with resolution recovery and CT-based attenuation correction and scatter correction. The number of iterations was varied from 1 to 20 and the number of subsets was fixed at 10. The maximum, peak, and mean standardized uptake values (SUVmax, SUVpeak, SUVmean) were calculated for the body and the tumor part, and the relative measurement error was assessed. **Result:** All quantitative values increased as the number of iterations increased and reached plateaus at 50-100 update numbers. The SUVmax of tumor part was 59.2 when the number of iterations was 2 and 55.7 when the number of iterations was 20. The maximum relative measurement error on the tumor part (reference value: 50) was obtained using the number of iteration 1 and over 10. **Conclusion:** Our results indicated that the quantitative accuracy of bone SPECT images was affected by reconstruction parameters. It is important to optimize the reconstruction parameters, and improve quantitative accuracy in bone SPECT imaging.

Basic study of random sampling for compressed sensing using MRI simulator

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Abstract

Introduction: Magnetic resonance imaging (MRI) is a tomography technology that enables the depiction of anatomical structures with information about various features. Compressed sensing (CS) technology has recently been used for magnetic resonance image reconstruction from sparse information. Random sampling methods based on the various probability density function (PDF) are being developed to allow the efficient application of CS technology. Accurate numerical simulation is obviously important for the evaluation of the sampling method that are developed. In this study, the simulation method with MRI simulator and actual MRI scanner was carried out. Moreover, the difference between the result acquired from our simulation and basic one was revealed. **Method**: We first examined a basic method using a 2D Shepp-Logan phantom. This method was only conducted with k-space data obtained from the 2D Fourier transform of the original image. Our method of numerical simulation was applied with the MRI simulator (Bloch Solver, MRI simulations Inc.), an actual MRI system (Vantage Titan 3T, Canon Medical Systems) and a phantom (CAGN-3.0T phantom, Kato Medience). The real and imaginary part of the k-space were acquired with the MRI simulator using a phase map that was imaged by the actual MRI scanner. Random sampling was performed with two types of PDF and image reconstruction was processed by projection onto convex sets (POCS). Hermitian symmetry is a point-symmetry respect to origin and each point located on the opposite side maintains a relation of complex conjugate. Thus, there is no need to acquire data that formed in point-symmetry with the data that had already been acquired. We used the gaussian random sampling method (GA) and a method that considered Hermitian symmetry (GH). The image quality was evaluated using the normalized root mean squared error (NRMSE). **Results and Discussion**: In the basic simulation, the average and standard deviation of NRMSE from GH was better than that from GA because consideration of the Hermitian symmetry enables the efficient acquisition of data. However, in our method of numerical simulation, the average and standard deviation of the NRMSE from GH was worse than that from GA. In this simulation method, the phase error was included in the real and imaginary part of the k-space; thus, the Hermitian symmetry cannot hold and the calculation error of reconstruction images from GH stood out. **Conclusion**: The method of numerical simulation with the MRI simulator using a phase map was close to the actual conditions and was considered to be useful for the validation of new sampling methods. The random sampling method using GH is expected to be useful for the highly efficient acquisition of data under ideal conditions; however, more accurate phase correction is necessary to apply the actual measurement data.

Use of amyloid PET/CT with ^{18}F-Florbetaben in the management of patients with Alzheimer's disease

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Abstract

Introduction and Aim: Amyloid PET/CT is an "in vivo" imaging that may radically change management of Alzheimer's disease (AD) thanks to its ability to identify AD at the earliest stage. A diagnosis of dementia is currently made in terms of probability and is based on clinical evaluation (neuropsycological tests) as well as on the results of morphological imaging investigations (MRI) that can be supported by biohumoral (CSF analysis), and functional imaging only in the case of uncertain diagnosis of disease. The present study aimed to evaluate the role of amyloid PET/CT in the management of patients with suspicion of AD, through comparison with instrumental and clinical evaluation. **Methods:** 38 consecutive patients with suspicion of AD (23 female, 15 male; median age 63 years old, range 46-72), who performed ^{18}F-florbetaben PET/CT, were retrospectively reviewed. All of them performed a previous instrumental evaluation. A subgroup of patients (24/38) were evaluated with Mini Mental State Examination (MMSE). Cohen's K test was used as a measure of agreement between previous instrumental examinations/clinical evaluation and beta-amyloid PET results. **Results:** Twenty-five/38 (65.8%) amyloid PET/CT scans resulted positive for amyloid deposition. Among the four target regions, precuneus was the most frequently involved. Previous instrumental evaluation was: MRI in 26/38 patients (24/26 positive for atrophy), CT in 9/38 (8/9 positive for atrophy), perfusion SPECT in 12/38 (8/12 areas of hypo-perfusion), ^{18}F-FDG PET/CT in 2/38 (1/2 hypometabolism in frontal cortex). The agreement between previous instrumental examinations and beta-amyloid PET results was low (K= 0.084). In the subgroup of 24/38 patients, MMSE was scored positive (MMSE<24) in 14/24 (58.4%) and negative (MMSE>24) in 10/24 (41.6%). The agreement between clinical evaluation (MMSE) and beta-amyloid PET results was fair (K= 0.217). **Conclusion:** The low agreement between amyloid PET/CT and previous clinical and instrumental assessments that we found in our study suggests that the amyloid PET/CT provides additional and early information. To perform an early and differential diagnosis of AD could have a great impact on the patient's management and cost of care in order to perform the correct therapeutic interventions

and to allow family members to manage adequately the patient's demanding care.

Introduction

Alzheimer's Disease (AD) is a progressive, degenerative central nervous system (CNS) disorder characterized by memory impairment and cognitive deterioration plus gradual decline in the so called ABC key symptom domains: Activities of daily living (ADL), Behavior and personality and Cognition.

AD is the most common form of dementia in people age 65 years and over. Two thirds of the cases of dementia are AD, but several other pathological conditions can cause dementia such as vascular dementia to Lewy body and Parkinson's dementia, with an overlap with AD both in terms of pathological characteristics and symptoms [1-4]. For these reasons make correct differential diagnosis and direct the patient to the most appropriate treatment as soon as possible is very difficult.

The social impact of AD is very impressive. It is estimated that currently there are about 50 million of people with dementia and that is expected to increase worldwide with exponential growth in the next 30 years, reflecting also a great impact on the cost of care [5]. In fact, the natural history of the disease is a progressive decline over an average of 8 years, requiring increasing degrees of care: patients with early disease are generally cared at home with or without regular non-professional caregivers; however, as the disease progresses to moderate and then severe stages, the requirement for long-term-care nursing or assisted-living facilities increases dramatically.

The trigger point of AD pathogenesis is the abnormal accumulation of incorrect protein aggregates outside and inside the neurons: Aβ plaques and neurofibrillary tangles (NFTs) respectively. What we know nowadays is that the amyloid plaques begin to build up years, even decades, before people get to stage of dementia, with symptoms appearing only at the end of the cascade when the amount of neuronal loss exceeds brain reserve [6, 7].

Moreover, while a definitive diagnosis of AD was possible only after death, the real breakthrough in this field over the past decade is the possibility to identify AD signs in the brain during life ("in vivo") using different biomarkers (Table 1) [8-10].

Development of ^{18}F-labeled amyloid PET tracers, such as ^{18}F-florbetapir, ^{18}F-florbetaben, ^{18}F-flutemetamol (FDA and EMA approved) has contributed to a widespread use of amyloid PET imaging because they binds specifically to b-amyloid plaques, that are present in the cortical gray matter in all cases of AD [9, 11-12].

The approved indication is for PET imaging of beta-amyloid neuritic plaque density in the brains of adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment [8].

Table 1: *in vivo biomarkers of Alzheimer's Disease.*

MARKERS OF NEURODEGENERATION PROCESSES		
	Specificity	**Sensibility**
• MRI (atrophy)	86-92% [18]	86-88%[18]
• ^{18}F-FDG PET/CT (hypometabolism)	70-80%[18]	63-90%[18]
• Tau protein CSF (increase)	77%[19]	72%[19]
MARKERS OF AMYLOID STORAGE		
• Amyloid-PET (accumulation)	95-100%[19]	92%[19]
• Aβ 42 CSF (reduction)	70%[19]	81%[19]

MRI: Magnetic Resonance Imaging; ^{18}F-FDG PET/CT: 18fluorodeossiglucose positron emission tomography/computed tomography; CSF: cerebrospinalfluid.

A diagnosis of dementia is currently made in terms of probability and is based on clinical evaluation (neuropsycological tests such as MMSE, ADL, IADL) as well as on the results of morphological imaging investigations (MRI) that can be supported by biohumoral (CSF analysis), and functional imaging only in the case of uncertain diagnosis of disease [10, 13-16].

The present study aimed to evaluate the role of amyloid PET/CT in the management of patients with suspicion of AD, through comparison with instrumental and clinical evaluation.

Methods

Subjects

Thirty-eight consecutive patients with suspicion of AD (23 female, 15 male; median age 63 years old, range 46-72), who referred to our Nuclear Medicine Unit in Policlinic of Bari (Italy) between February 2018 and April 2019, were retrospectively reviewed.

Patients who met the following criterions were included in this study: at least one clinical symptom and previous instrumental exams among morphological imaging (MRI/CT) and functional ones (SPET/CT-^{18}F-FDG PET/CT).

Patients' characteristics are displayed in Table 2. All subjects had signed an informed consent form for the data analysis.

Table 2: Patients' features.

Patient characteristics	
Patients	38
M:F	15:23
<65: ≥65	24:14
Familiarity for neurodegenerative diseases	19 yes 19 no
Symptoms:	
Memory impairment	38/38 (100%)
Space-time disorientation	13/38 (34.2%)
Cognitive disorders	12/38 (31.6%)
Depression	12/38 (31.6%)
Disorders of the speech	4/38 (10.5%)
Repetitiveness	3/38 (7.9%)
Behavioral disorders	2/38 (5.3%)

Beta-Amyloid PET technique and images interpretation

^{18}F-florbetaben (NEUROCEQ ®) were intravenous administered at the recommended radioactive exposure of 300 ± 20% MBq per injection through a slow bolus intravenous administration.

A 20-minute PET image was acquired starting 90 minutes after IV injection, with a combined modality PET/CT Discovery 710 (GE Healthcare, Waukesha, Wisconsin, USA) that integrates detectors PET LYSO with 64-slice CT scanner (OPTIMA 660 SE).

The scan of the head was performed with patient supine, positioning head to center and including the cerebellum in the PET scanner field of view. Head movement was reduced by using tape or other flexible head restraints.

Two trained nuclear medicine physicians reviewed images in consensus at a dedicated Xeleris^{TM} Workstation (GE Healthcare, Waukesha, Wisconsin, USA).

Images were displayed in the transaxial orientation using gray scale or inverse gray scale according to the recommended reading methodology interpretation [6]. Sagittal and coronal planes were used for additional orientation purposes while CT images were helpful as anatomic reference.

^{18}F-florbetaben typical transverse PET slices were analyzed to visually compare the activity in cortical gray matter with activity in adjacent white matter at different brain levels in a systematic manner, starting with the cerebellum, as a reference region, and scrolling up through the evaluation of the 4 main target regions: lateral temporal and frontal lobes, the posterior cingulate cortex/precuneus, and the parietal lobes.

Then, for each patient, the PET image assessment was categorized as either beta-amyloid-positive or beta-amyloid-negative as followed: Beta-amyloid negative if tracer uptake (ie, signal intensity) in gray matter was lower than in white matter in all 4 brain regions (no beta-amyloid deposition); Beta-amyloid positive for area(s) of tracer uptake equal to or higher than that present in white matter extending beyond the white matter rim to the outer cortical margin involving the majority of the slices within at least 1 of the 4 brain regions (moderate to pronounced beta-amyloid deposition).

In addition, each region involvement was scored by using the 3 point Regional Cortical Tracer Uptake (RCTU) score; then, a global evaluation of ^{18}F-Florbetaben uptake, called Brain Amyloid Plaques Load (BAPL) score, was assigned to PET/CT considering the sum of RCTU scores in each region, as detailed in Table 3.

Table 3: *regional involvement score methods for ^{18}Florbetaben-PET.*

RCTU Score	
1. No tracer uptake	Tracer uptake in grey matter is lower than that in white matter
2. Moderate tracer uptake	Tracer uptake in a small area is greater or equal to that present in white matter: over most of the slices within each region, extending beyond the white matter rim to the outer boundary of the cerebral cortex
3. Severe tracer uptake	Tracer uptake in a large area is greater than or equal to that present in white matter: whole slices within each region, extending beyond the while matter rim to the outer boundary of the cerebral cortex
BAPL score	
1. Negative	RCTU score of 1 in each brain region
2. Moderate	RCTU score of 2 in any brain region and no score 3
3. Severe	RCTU score of 3 in any of the 4 brain regions (lateral temporal lobes, frontal lobes, posterior cingulate/precuneus, parietal lobes)

RCTU: Regional Cortical Tracer Uptake; BAPL: Brain Amyloid Plaques Load.

Statistical analysis

The concordance between previous instrumental examinations and beta-amyloid PET results was estimated with Cohen's K in the whole population.

Moreover, a second evaluation was performed in a subgroup of patients who were evaluated with Mini Mental State Examination (MMSE) and scored as positive (MMSE <24) or negative (MMSE >24). Cohen's K test was also used as a measure of agreement between MMSE and beta-amyloid PET results.

Results

Twenty-five/38 (65.8%) amyloid PET/CT scans resulted positive for amyloid deposition: 23/25 (92%) had a BAPL=3 while 2/25 (8%) had a BAPL=2. The remnant 13/38 (34.2%) amyloid PET/CT scans resulted negative (BAPL=1).

By considering the evaluation of the four specific regions through RCTU score, in patients with positive scan, 20/25 (80%) had a RCTU=3 in every region, 2/25 (8%) had RCTU=2 in every region, 3/25 (12%) had both RCTU=2 and RCTU=3. Nobody showed a RCTU=1 in at least one region.

Among the four target regions, precuneus was the most frequently involved with a

RCTU=3 in 22/25 (88%) patients (Table 4).

Table 4: *distribution on per region involvement.*

Regional Analysis	RCTU 2	%	RCTU 3	%
Precuneus	3/25	12	22/25	88
Temporal lobes	4/25	16	21/25	84
Frontal lobes	4/25	16	21/25	84
Parietal lobes	5/25	20	20/25	80

Previous instrumental evaluation was: MRI in 26/38 patients, CT in 9/38, perfusion SPECT in 12/38, ^{18}F-FDG PET/CT in 2/38; 9/38 had both MRI and brain perfusion SPECT, 2/38 had both CT and brain perfusion SPECT.

Twenty-four/26 (92.3%) had MRI with morphological signs of localized or diffused atrophy; 8/9 CT (88.9%) were positive for brain atrophy; 8/12 (66.7%) had SPECT with areas of hypo-perfusion (especially to temporal lobes); 1/2 (50%) had FDG hypometabolism in frontal cortex at PET scan.

Concordant results between examinations and beta-amyloid PET were found in 23/38 (60.5%) patients (Positive Concordant in 19/23; Negative Concordant in 4/23); Discordant results between examinations and beta-amyloid PET were found in the remnant 15/38 (39.5%) patients (exams positive and PET negative in 10/15; exams negative and PET positive in 5/15).

The agreement between previous instrumental examinations and beta-amyloid PET results was low (K= 0.084), as reported in Table 5.

Table 5. *Choen's K results*

	Amyloid PET +	Amyloid PET -	Choen's K
Instrumental Examination+	19	10	K=0.084
Instrumental Examination-	5	4	
MMSE +	10	4	K=0.217
MMSE -	5	5	

MMSE: Mini Mental State Examination

A subgroup of 24/38 patients were clinically evaluated with MMSE and scored positive (MMSE <24) in 14/24 (58.4%) and negative (MMSE >24) in 10/24 (41.6%).

Concordant results between clinical evaluation (MMSE) and beta-amyloid PET were found in 15/24 (62.5%) patients (Positive Concordant in 10/15; Negative Concordant in 5/15); Discordant results between MMSE and beta-amyloid PET were found in the remnant 9/24 (37.5%) patients (MMSE positive and PET negative in 4/9; MMSE negative and PET positive in 5/9).

The agreement between clinical evaluation (MMSE) and beta-amyloid PET results was fair (K= 0.217), as reported in Table 5.

In addition, 21/38 patients who had both previous instrumental examinations and clinical evaluation were observationally analyzed. Of those, in 7/21 (33.3%) patients the 3 evaluations

(previous instrumental examinations, MMSE and amyloid PET) were positive concordant; conversely only in 1/21 (4.7%) patient the 3 evaluations were negative concordant.

In Figures 1, 2 and 3 are reported three examples of negative concordant, positive concordant and discordant result of MRI and amyloid PET respectively.

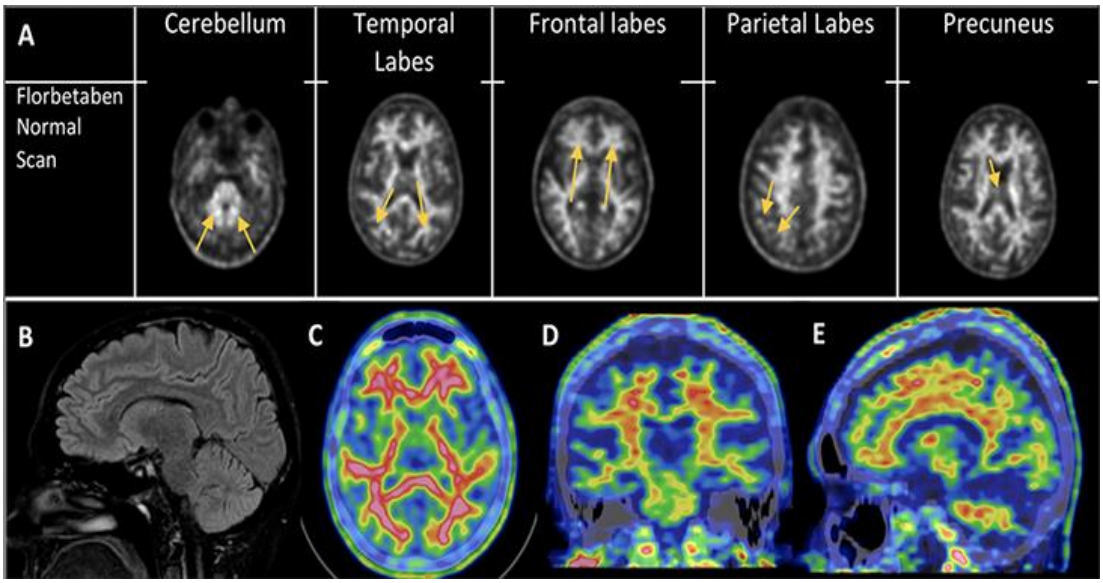

Figure 1. Woman, 52 years old, with neurodegenerative diseases familiarity . From few months appearance of memory deficit and cognitive impairment. MMSE resulted negative (25/30). (A) 18Florbetaben PET axial slices of cerebellum (reference region) and the 4 target regions: no signs of accumulation in none of the 4 target regions, with well-evidenced contrast between gray and white matter (RCTU=1 in all regions). BAPL=1. (B) Sagittal T1-weighted MRI image: non-dilated ventricular system, no atrophy, no areas of altered brain parenchyma signal. (C-E) 18Florbetaben fused PET/CT axial, coronal and sagittal slices.

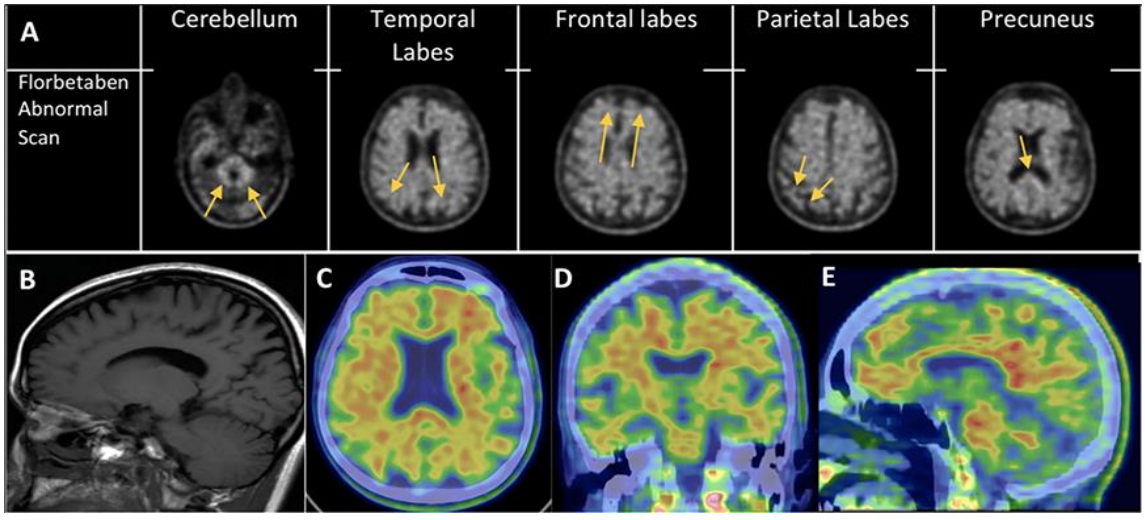

Figure 2. Woman, 62 years old, with neurodegenerative diseases familiarity . From about 1 year appearance of memory deficit for recent events, repetitiveness, confabulation. MMSE resulted positive (12/30). (A) 18Florbetaben PET axial slices of cerebellum (reference region) and the 4 target regions: widespread accumulation of amyloid especially in left temporal (RTCU 3) and frontal lobes (RCTU 3). BAPL=3. (B) Sagittal T1-weighted MRI image: cortical atrophy of the

hippocampus and parietal lobe bilaterally. (C-E) 18Florbetaben fused PET/CT axial, coronal and sagittal slices.

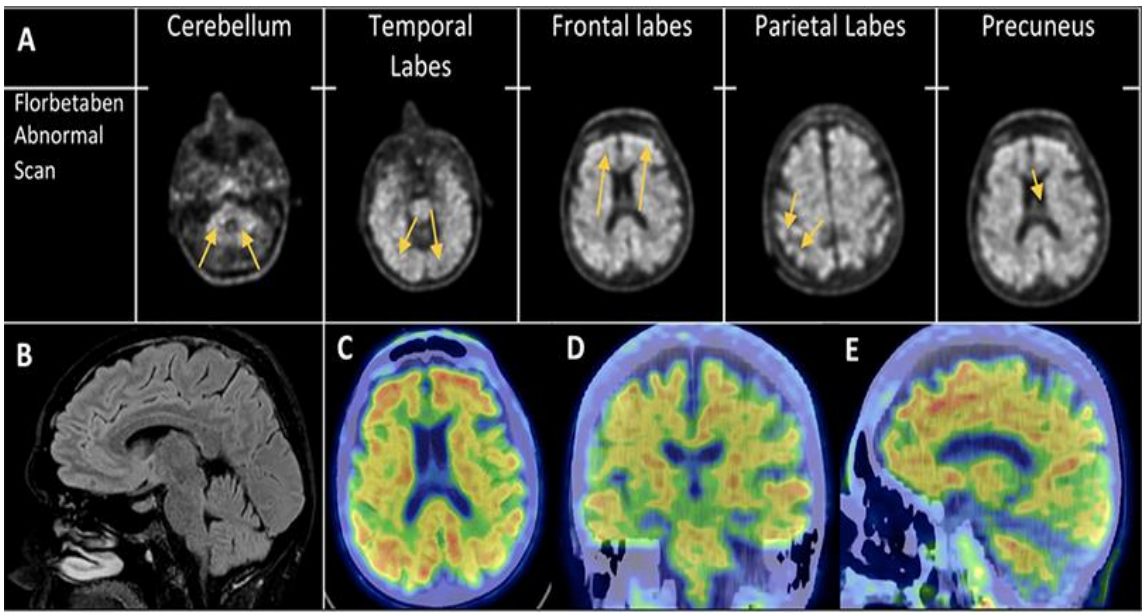

Figure 3. Woman, 60 years old, with no familiarity for neurodegenerative diseases. From about 1 year appearance of memory deficit. The relatives report mood deflection. MMSE resulted slight positive (23/30).

(A) ^{18}Florbetaben PET axial slices of cerebellum (reference region) and the 4 target regions: widespread accumulation of amyloid especially in temporal (RTCU 3), frontal (RTCU 3) lobes and in precuneus (RTCU 3). BAPL=3. (B) Sagittal T1-weighted MRI image: mild cortical atrophy, non-dilated ventricular system. (C-E) ^{18}Florbetaben fused PET/CT axial, coronal and sagittal slices. Amyloid PET has helped in the early prediction the conversion of MCI to AD.

Discussion

By 2050 it is projected that 115 million people worldwide will be affected Alzheimer's Disease (AD). Recent attempts have been made to redefine the diagnostic criteria of AD to include both markers of neurodegeneration and markers of amyloid accumulation [5].

The current diagnosis of AD is made by clinical, neuropsychological, and neuroimaging assessments. Multiple factors, namely amyloid, tau, inflammation, metabolic, and perfusion changes, contribute to the cascade of neurodegeneration and functional decline occurring in AD. These molecular and cellular processes and related functional and morphological changes can be visualized in vivo by imaging modalities that are now part of the diagnostic algorithm and of particular interest for patient stratification and targeted drug development [10-11, 17].

However, literature reports different values of specificity and sensitivity for each diagnostic technique, so which is the biomarker of AD to be used effectively is still debated [18,

19].

A crucial element of biomarker-based staging of AD is the notion of temporal ordering of different biomarkers.

Clifford R et al. [20] hypotized a model, which relates pathological stage to AD biomarkers, by showing a correlation between pathological changes during AD progression, from pre-symptomatic to severe AD, and specific biomarker sensitivity in each stage [21, 22]. In this model, biomarkers of Aβ deposition become abnormal early, before neurodegeneration and clinical symptoms occur. Biomarkers of neuronal injury, dysfunction, and neurodegeneration become abnormal later in the disease. Cognitive symptoms are directly related to biomarkers of neurodegeneration rather than biomarkers of Aβ deposition.

In this setting, if routine structural neuroimaging evaluation, based on nonspecific features such as atrophy, is a late feature in the disease progression, available evidences suggest that functional changes might precede MRI changes [20, 23-25].

This statement could explain the low concordance between instrumental evaluation and PET results in our sample.

Moreover, to distinguish clinically AD from other forms of dementia is an ongoing challenge because of the great overlap in terms of symptoms, but if cognitive symptoms are directly related to biomarkers of neurodegeneration rather than biomarkers of Aβ deposition, amyloid PET can result negative also in presence of symptoms and vice-versa [6, 9, 11].
This could be the reason why we found a low concordance also between clinical evaluation and PET results in our sample.

More interestingly, amyloid PET/CT may radically change management of AD thanks to its ability to identify AD at the earliest stages [26].

Although mild cognitive impairment (MCI) is recognized as a risk factor for dementia, it remains a challenge to predict on an individual level who will convert to become demented. Amyloid β (Aβ) is one of the crucial pathological findings in AD and amyloid-PET may offer the unique possibility for measuring fibrillar Aβ load in the living brain. It is well known, in fact, that b-amyloid plaques are present in the cortical gray matter in all cases of AD, even in earliest stage [27].

So, if amyloid-PET has a very high negative predictive value, because b-amyloid plaques are present also in 50–70% of patients with DLB and in normal elderly people in a percentage that increases with age, a positive amyloid PET does not establish a diagnosis of AD, but it can help to support or exclude the presence of AD in the context of a more comprehensive evaluation of the patient [11].

In addition, amyloid PET images' analysis performed with topographic criteria using the RCTU score, may find a relation with different clinical symptoms, assess the evolution of brain involvement, identify patients who would benefit from therapy and facilitate efficacy evaluation of the new agent.

Therefore, in the near future Aβ-amyloid PET may become an important tool for in vivo amyloid imaging contributing to early (differential) diagnosis, in prediction the conversion of MCI to AD, as well as evaluation of treatment response in AD.

Conclusion

Our study suggests that the amyloid PET/CT provides additional information to primary instrumental evaluations (MRI, CT, SPECT/CT), which are often non-specific, and to clinical examination (symptoms, MMSE), that especially in early stage of the disease can be inconclusive, mostly in patients with MCI in whom is important to predict the conversion to AD.

To perform an early and differential diagnosis of AD could have a great impact on the patient's management and cost of care in order to perform the correct therapeutic interventions and to allow family members to manage adequately the patient's demanding care.

The authors declare that they have no conflicts of interest.

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Impact of pre-treatment variables on the completion of 223radium-dichloride therapy in mCRPC patients with bone metastases

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Abstract

Introduction: Radium-223 dichloride (Ra223) is an alpha-particle-emitter radiopharmaceutical, approved for metastatic castration-resistant prostate cancer (mCRPC) patients with symptomatic bone metastases and no visceral involvement. Its administration is based on a schedule of intravenous injection (55kBq/kg) every four weeks for up to six cycles. Because the biological effectiveness of Ra223-therapy is dose-dependent, the main goal is to complete the entire treatment to achieve a better patient outcome. This study aims to identify potential pre-treatment variables that could impact on Ra223-treatment completion and then be used to improve the clinical and supportive management of mCRPC patients. **Materials and Methods:** 30 consecutive mCRPC patients (mean age 77 years old), who were admitted for Ra223-therapy at our Department from February 2016 to October 2018, were enrolled for the analysis. The population was grouped as patients who completed Ra223-therapy (group Ra223-C) and patients who do not (group Ra223-U). For each group, we analyzed the effects of potential pre-treatment variables (age, Gleason Score, tumor burden, "Time From Diagnosis To Ra-223 therapy", type and number of previous treatments, hemoglobin level, Alkaline Phosphatase, Prostate Specific Antigen and pain) on the Ra223-therapy completion. Statistical analysis was performed to evaluate the association between the completion of Ra-223 therapy and the variables examined. **Results:** 16/30 (53%) patients were Ra223-C, conversely 14/30 (47%) patients were Ra223-U because of an early interrupted treatment. A statistically significant association was found only with tumor burden: 68.7% of patients who completed Ra223-therapy had less than 20 bone metastases ($\chi 2=4.821$, $p=0.028$). **Conclusion:** Our preliminary analysis demonstrates that the high tumor burden represents the most important pre-treatment factor that could affect treatment completion and that needs to be considered before starting Ra223-therapy to achieve a better outcome in mCRPC patients.

Introduction

Prostate cancer is the second most commonly diagnosed cancer and the fifth cause of cancer-related death in men, accounting about 15% of all cancer diagnosed, with an increasing incidence especially in older men (median age 68 years old) [1].

After radical surgery and radiant treatment for localized prostate cancer, a recurrence could develop within 10 years of diagnoses [2]. Positron Emission Tomography/Computed Tomography (PET/CT) has gained an important role to detect primary and recurrent prostate cancer, thanks to the availability of well-established tracers as ^{18}F-Fluorocholine (^{18}F-FCH) and to the new promising radiopharmaceutical as Trans-1-amino-3-18F-fluorocyclobutanecarboxylic acid (anti-[^{18}F]-FACBC) with a favorable biodistribution [3-4]. The most active treatment for advanced prostate cancer is androgen suppression; however, when the disease progresses to Castration-Resistant Prostate Cancer (CRPC), chemotherapy (docetaxel and cabazitaxel) and second-generation hormone therapy (abiraterone and enzalutamide) represent valid therapeutic options. These treatments are associated with improved overall survival (OS) and quality of life, but also with side effects and poor tolerability [5].

Eighty% of CRPC patients develop bone metastases, causing severe pain, pathological fractures, worsened quality of life, increased costs of treatment and a dramatic reduction to 3% of 5-year survival. In this setting of patients, Nuclear Medicine proposes a radiometabolic treatment represented by Radium-223 dichloride (Ra223).

Ra223 is a bone-seeking radioisotope that, accumulating in bone metastases, emits high energy alpha-particles short-range and induces nonrepairable double-strand DNA breaks, with low myelotoxicity and localized cytotoxic effect, ensuring a favorable safety and tolerability profile [6-8].

Based on Alpharadin in Symptomatic Prostate Cancer Patients (ALSYMPCA) study results [9], Ra223 was approved in 2013 by US Food and Drug Administration (FDA) and European Medicine Agency (EMA) for the treatment of mCRPC patients with symptomatic bone metastases, without visceral involvement and lymph nodes metastases (>3cm) [10, 11].

In July 2018, based on ERA223trial data [12], the EMA revised Ra223 previous indications and recommended its use only in mCRPC patients in progression after two previous lines of systemic therapy or when other treatments cannot be performed; in addition, it is not recommended in association with other systemic cancer therapies, except for hormone therapy, and in patients with a low tumor burden (number of osteoblastic bone metastases <6).

Ra223-therapy is based on a schedule of intravenous injection (55kBq/kg) every four weeks for up to six cycles. Literature reports longer OS and better quality of life in patients who received at least 5 doses and a lower probability of completing Ra223-therapy in patients with more advanced disease [13, 14].

Since the evidence that the biological effectiveness of Ra223 is dose-dependent, the main goal is to complete the full therapeutic doses to achieve a better patient outcome. Unfortunately, it happens that Ra223-therapy is early interrupted before completion of 6 doses due to several causes.

This study aims to identify potential pre-treatment variables that could impact on Ra223-treatment completion and then be used to predict outcome and improve clinical and supportive management of mCRPC patients.

Materials and Methods

Subjects

Thirty consecutive mCRPC patients (mean age 77 years old, range 59-90), with at least 2 symptomatic bone metastases and no visceral involvement, who were admitted for Ra223-therapy at our Nuclear Medicine Department from February 2016 to October 2018, were enrolled for the analysis.

Baseline Bone Scan (BS) and ^{18}F-FCH PET/CT were performed to assess tumor burden (TB) disease and to exclude visceral involvement before starting treatment. All patients received the standard dose of Ra223: 55kBq/kg every 4 weeks up to a total of 6 doses. Complete blood count, Alkaline Phosphatase (ALP) and Prostate Specific Antigen (PSA) levels were measured before each cycle of Ra223. The use of luteinizing hormone-releasing hormone (LHRH) analogs during Ra223-therapy was allowed.

Patients' characteristics are displayed in Table 1. All subjects had signed an informed consent form for the data analysis.

Table 1.

Patients' characteristics	
Patients	30
Mean Age	77 years old (range: 59-90)
TB <20:≥20	15:15
Mean TDRT	78.2 months (range: 14-240)
GS <8:≥8	12:18
Type of previous treatments: *Only chemotherapy* *Only second-generation hormone therapy* *Both* *None*	 2/30 (7%) 9/30 (30%) 15/30 (50%) 4/30 (13%)
Previous chemotherapy *<3: ≥3 chemotherapy lines*	13 No 17 Yes 13:4
Previous second-generation hormone therapy *Only Abiraterone* *Only Enzalutamide* *Both*	6 No 24 Yes 13/24 (54%) 4/24 (17%) 7/24 (29%)
Hemoglobin <11:≥11 g/dL	6:24
ALP <115:≥115 U/L	13:17
PSA <500: ≥500 ng/mL	26:4

Pain intensity (BPI-SF scale, 0-10):	
slight (0-4)	9/30 (30%)
moderate (5-7)	11/30 (36.7%)
intense (8-10)	10/30 (33.3%)
TB, Tumor Burden; TDRT, Time From Diagnosis To Ra-223 therapy; GS, Gleason Score; ALP, Alkaline Phosphatase; PSA, Prostate Specific Antigen; BPI-SF, Brief Pain Inventory-Short Form	

Statistical analysis

The population was grouped as patients who completed Ra223-therapy (group Ra223-C) and patients who do not (group Ra223-U). For each group we analyzed the effects of pre-treatment variables on the 223Ra-therapy completion: age, Gleason Score (GS), TB evaluated by metabolic imaging methods, "Time From Diagnosis To Ra-223 therapy" (TDRT), type and number of previous treatments, with particular reference to chemotherapy agents and second-generation hormone therapy, hemoglobin, ALP and PSA levels and pain, assessed by Brief Pain Inventory-Short Form (BPI-SF), as reported in Table 1.

The association between the completion of Ra223-therapy and GS, TB, type and number of previous treatments, ALP and PSA levels and pain intensity was investigated using contingency tables and Chi-Square test; Median test was used to assess the association between the completion of Ra223-therapy with age and TDRT and Fisher Exact Test to evaluate the association with hemoglobin level. $P<0.05$ was considered statistically significant.

Results

As shown in Table 2, 16/30 (53%) patients completed all scheduled Ra223 doses. Considering previous treatments, in Ra223-C group, 2/16 (12.5%) received only chemotherapy, 5/16 (31%) only second-generation hormone therapy, 7/16 (44%) both types of therapy and 2/16 (12.5%) none. Among 9/16 (56%) patients who underwent chemotherapy, 7/9 (78%) received less than 3 chemotherapeutic lines, while 2/9 (22%) equal or more than 3 chemotherapeutic lines. Among 12/16 (75%) patients who received second-generation hormone agents, 4/12 (33%) underwent only abiraterone, 3/12 (25%) only enzalutamide and 5/12 (42%) both agents. In addition, we observed that ALP (<115U/L, 56%) and PSA (<500ng/mL, 75%) values were lower, hemoglobin level was higher (≥11g/dL, 94%) and pain ranged from slight to moderate (BPI-SF 0-7, 75%) in Ra223-C group compared to Ra223-U group.

Conversely, 14/30 (47%) patients early interrupted treatment, due to disease progression (5/14, 36%), hematological toxicity (4/14, 29%), death (3/14, 21%) and other causes (2/14, 14%). In Ra223-U group linked to progression disease, pre-treatment tumor burden was higher (≥20 lesions, 80%) and the pain was intense (BPI-SF 8-10, 60%) compared to Ra223-U group due to other causes. Considering previous treatments, in Ra223-U group, no patient received only chemotherapy, 4/14 (29%) only second-generation hormone therapy, 8/14 (57%) both types of

therapy and 2/14 (14%) none. Among 8/14 (57%) patients who received chemotherapy, 5/8 (62%) underwent less than 3 chemotherapeutic lines, while 3/8 (38%) equal or more than 3 chemotherapeutic lines. Among 12/14 (86%) patients who received second-generation hormone agents, 9/12 (75%) underwent only abiraterone, 1/12 (8%) only enzalutamide and 2/12 (17%) both agents.

A statistically significant association was found between the Ra223-therapy completion and tumor burden: 11/16 (68.7%) of Ra223-C patients had less than 20 bone metastases (χ^2=4.821, p=0.028).

Conversely, there was no statistically significant association between the completion of 223Ra-therapy and neither type of previous treatments nor the number of chemotherapeutic lines or second-generation hormone agents administered. In addition, no statistically significant differences were found between two groups regarding age, TDRT, GS, pre-treatment ALP, PSA, hemoglobin level and pain, as detailed in Table 2.

Table 2. *Comparison between pre-treatment variables in Ra223-Completed and Ra223-U group.*

	Ra223-C group	**Ra223-U group**	**Agreement**
Patients	16/30 (53%)	14/30 (47%)	
Mean Age	78 years old (range: 67-88)	76 years old (range: 59-90)	Median=76.5 vs 78y, p=1.000
TB <20: ≥20	11:5	4:1	χ^2=4.821, p=0.028
TDRT	Mean TDRT 82 months (range: 14-240)	Mean TDRT 74 months (range: 20-209)	Median=54.5 vs 62, p=0.730
GS <8: ≥8	6:10	4:10	χ^2=3.147, p=0.677
Types of previous treatments:			
Only chemotherapy	2/16 (12.5%)	None	
Only second-generation hormone therapy	5/16 (31%)	4/14 (29%)	
Both	7/16 (44%)	8/14 (57%)	χ^2=2.054, p=0.561
None	2/16 (12.5%)	2/14 (14%)	
Previous chemotherapy	7/16 (44%) No	6/14(43%) No	
	9/16 (56%) Yes	8/14 (57%) Yes	
<3: ≥3 lines lines	7:2	5:3	χ^2=6.807, p=0.235
Previous second-generation hormone therapy	4/16 (25%) No	2/14 (14%) No	
	12/16 (75%) Yes	12/14 (86%) Yes	
Only Abiraterone	4/12 (33%)	9/12 (75%)	
Only Enzalutamide	3/12 (25%)	1/12 (8%)	χ^2=2.359, p=0.307
Both	5/12 (42%)	2/12 (17%)	

Hemoglobin <11:≥11 g/dL	1:15	5:9	Fisher Exact Test: p=0.059
ALP <115:≥115 U/L	9:7	4:10	$\chi 2=2.89$, p=0.089
PSA <500: ≥500 ng/mL	13:3	13:1	$\chi 2=0.87$, p=0.351
Pain intensity (BPI-SF scale, 0-10): *slight (0-4)* *moderate (5-7)* *intense (8-10)*	 7/16 (44%) 6/16 (37%) 3/16 (19%)	 2/14 (14%) 5/14 (36%) 7/14 (50%)	$\chi 2=4.58$, p=0.205
Cause of interruption:			
disease progression *hematological toxicity* *death* *other causes*		5/14 (36%) 4/14 (29%) 3/14 (21%) 2/14 (14%)	
TB, Tumor Burden; TDRT, Time From Diagnosis To Ra-223 therapy; GS, Gleason Score; ALP, Alkaline Phosphatase; PSA, Prostate Specific Antigen; BPI-SF, Brief Pain Inventory-Short Form.			

Discussion

Ra223 is a well-tolerated radiometabolic treatment indicated for mCRPC patients with bone involvement to improve painful symptom and prolong OS and time to the first skeletal event [9].

The biological effectiveness of Ra223 is dose-dependent and a better prognosis is achieved for patients who receive all 6 doses; then, the completion of the full scheduled doses is the main purpose at the beginning of therapy to achieve a better patient outcome [13, 14].

Even if several studies explored pre-treatment variables associated with Ra223-therapy completion, currently baseline standard variables useful to guide clinical practice and to optimize patient selection are still not available [15].

Literature data highlighted that Ra223-therapy completion is more likely in patients with normal hematological function, with lower tumor burden, less aggressive disease, less pain (none to mild), lower PSA and ALP level and that disease progression is the major cause for early treatment discontinuation, as it was also observed in our analysis (Figure 1) [7, 13, 16-17].

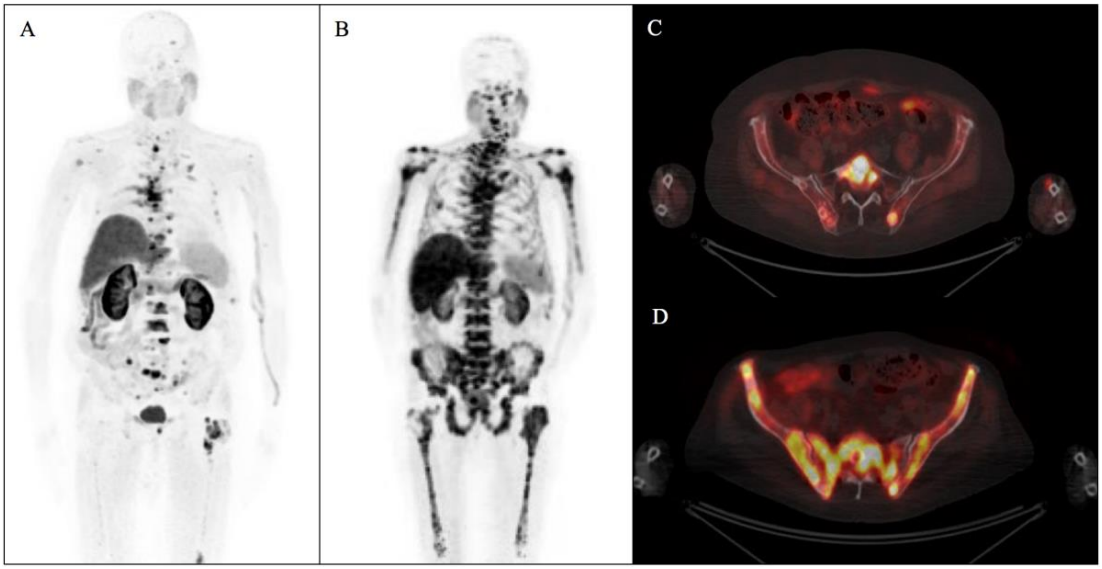

Figure 1. ^{18}F-FCH PET/CT (A) MIP and (C) axial fused pre-treatment images and (B) MIP and (D) axial fused post-IV dose images performed in an 85 years old patient affected by CRPC, GS 8. Ra223-tehrapy has been preceded by chemotherapy and abiraterone therapy. (A, C) Pre-treatment ^{18}F-FCH PET/CT showed high tumor burden (≥20 bone lesions). He started Ra223 therapy, but after the IV dose (B, D) ^{18}F-FCH PET/CT showed disease progression, leading to stop radiometabolic therapy.

According to Costa et al. [18], hematological toxicity, especially anemia, represented the main reason for early Ra223-therapy interruption and was observed frequently in mCRPC patients with greater disease burden and previously treated with docetaxel. This could suggest the needing to anticipate Ra223-therapy in the earliest stages, rather than procrastinate after chemotherapy, to ensure a successful treatment. We experienced mCRPC patients with hematological function compromised by too advanced disease and several previous treatments in our study; in those patients, Ra223-treatment completion was achieved thanks to the prompt supportive management by physicians (Figure 2).

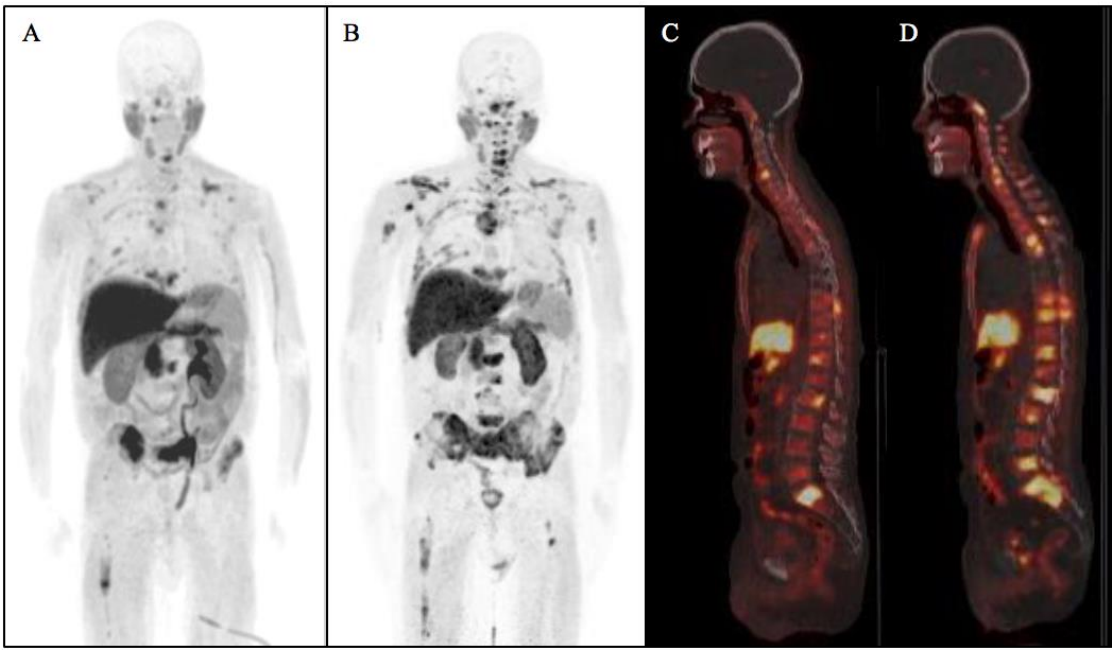

Figure 2. ^{18}F-FCH PET/CT (A) MIP and (C) sagittal fused pre-treatment images and (B) MIP and (D) sagittal fused post-treatment images performed in a 73 years old patient affected by CRPC, GS 9. Ra223 therapy has been preceded by several lines of systemic therapy (docetaxel, cabazitaxel, abiraterone and enzalutamide). (A, C) Pre-treatment tumor burden was more than 20 bone lesions, but his hematological function needs to be supported by blood transfusion and erythropoietin therapy. Only after increasing hemoglobin level, he started and completed Ra223-therapy. (B, D) Post-treatment ^{18}F-FCH PET/CT showed disease progression with an increased number of bone metastases. However, he had an improvement in clinical symptoms (slight vs moderate pain).

After the new EMA guidelines, Ra223-therapy was restricted to the advanced stage of disease, being its use limited in mCRPC patients with more than 6 symptomatic bone lesions and after at least 2 previous treatments, with particular reference to chemotherapy and second-generation hormone agents. Our preliminary analysis, started before the introduction of the new EMA guidelines, has included patients with low tumor burden and without at least 2 previous treatments: those patients completed successfully Ra223-therapy with clinical and survival benefit (Figure 3).

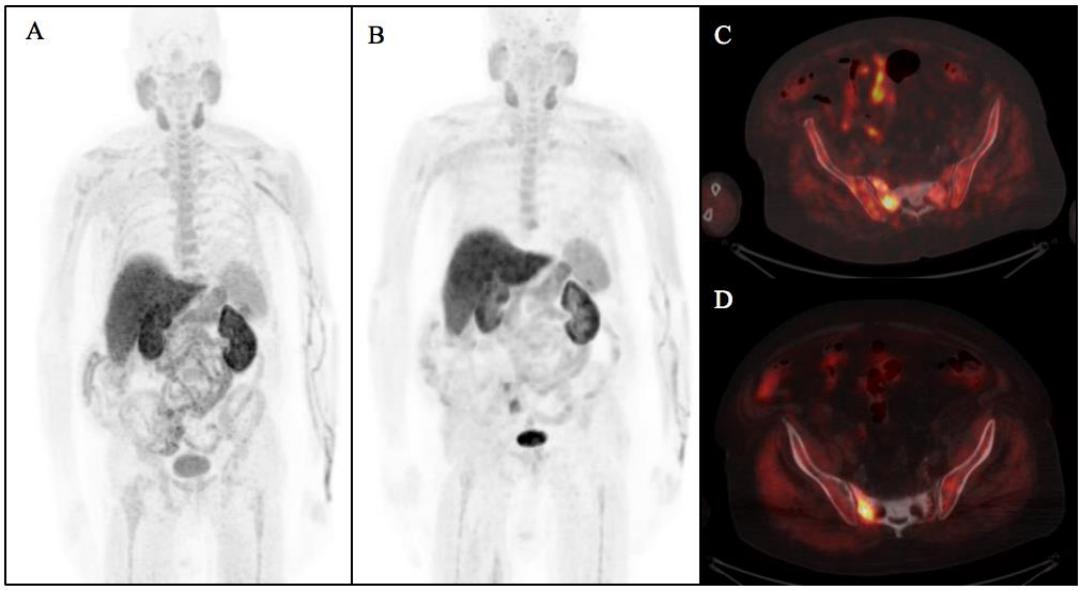

Figure 3. ^{18}F-FCH PET/CT (A) MIP and (C) axial fused images pre-treatment and (B) MIP and (D) axial fused image post-treatment performed in an 83 years old patient affected by CRPC, GS 8. Ra223 therapy has been preceded by only abiraterone, but because he was evaluated before the new EMA guidelines, he was enrolled for this kind of therapy. (A, C) Pre-treatment evaluation showed low tumor burden (<20 bone lesions). He started and completed Ra223-therapy. (B-D) Post-treatment ^{18}F-FCH PET/CT showed stable disease and he had an improvement in clinical symptom (slight vs moderate pain).

In this study, 53% of patients completed Ra223-therapy and the high tumor burden resulted the most important pre-treatment factor statistically associated with Ra223-treatment completion, useful to predict a better patient outcome. Conversely, there was no evidence of statistical association with neither type of previous treatments nor the number of chemotherapeutic lines and second-generation hormone agents. Similarly, Saad et al. [13] in a 135 patients study cohort did not find a significative correlation between the completion of 223Ra-therapy and the number of previous therapies.

Moreover, even if a statistical association was not found in this study probably due to the small sample size, we observed in our clinical practice that pre-treatment pain intensity, hemoglobin, PSA and ALP level deserve to be carefully evaluated to optimize mCRPC patients selection and to eventually manage their adequate supportive care.

Nowadays, because clear recommendations of therapeutic sequence in mCRPC patients still lack, the multidisciplinary team represents a useful opportunity to establish the most appropriate patient's tailored timing and therapeutic choice [19, 20]. The prompt collaboration with oncologists, urologists, radiologists and radiotherapists and a careful and close monitoring of patients, turned out to be fundamental tools to achieve successful treatment and to prevent its premature interruption.

Conclusion

Our preliminary analysis, started before the introduction of new EMA guidelines limitations, demonstrates that a high tumor burden represents the most important pre-treatment factor that could affect treatment completion and that it needs to be considered as pre-treatment variable to predict a better outcome in mCRPC patients.

However, other variables, as pain, hemoglobin, PSA and ALP level, deserve to be carefully evaluated in a multidisciplinary team to optimize patients' selection, to choose the timing of therapeutic sequence and to plan prompt supportive care in collaboration with physicians to improve their quality of life.

The authors declare that they have no conflicts of interest.

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The paleoradiology importance in the study of relics: the unique densitometric analysis of a bone relic of Saint Nicholas.

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Abstract

Introduction: DXA have greatly contributed to the development of paleoradiology, a branch of diagnostic imaging that allows to obtain information about human remains in contexts of archaeological and/or forensic interest. In this manuscript we report the unique experience of DXA performed on the relic of a Saint; in particular we analyzed a skeletal fragment of St. Nicholas, kept in the Basilica of Bari (Italy) since 1087. **Material and Methods:** The bone to be examined consisted of the posterior arch of the ninth left rib that was 12cm long, 1.2cm maximum width and 1.7cm thick at the body. The data acquired from the densitometric study were performed using the anthropometric measures reported in historical records of St. Nicholas' life: sex (male), age (75 years), weight (70kg), height (167cm), and ethnicity (Caucasian). In addition to the examination of the relic, a comparison assessment was made with the rib of a healthy 60 years old man (height of 170cm, without known skeletal pathologies). This sample had a length of 19cm, maximum width at the head 1cm, and 0.7cm thick at the body. The analysis of bone fragments is different from the analysis of bones in the context of the human body (where soft tissues are placed around the skeleton); for this reason, one of the most critical issues was to create a support that would allow the analysis of bone fragments. We simulated conditions similar to those occurring in patients: a density scale was established, using a specific plexiglass phantom on which the bone fragments to be examined were placed. From the analysis it was calculated the parameter bone mineral density (BMD), express in g/cm2, that indicates the relation between mass of bone mineral content and area of examined bone segment. BMD data was compared to a range normalized by age, sex and ethnicity (BMD-N). **Results:** The results of the scannnig of St. Nicholas' rib showed a BMD of 0.97g/cm2 with a BMD-N between 0.77 and 1.08g/cm2. Simultaneous measurements of the relic compared with a reference rib showed highlighted BMD of 0.84g/cm2 for the relic and 0.50g/cm2 for

the reference rib. The St. Nicholas data are 168% higher than reference bone. All our measurements of the relic indicated a high bone mineral density, most likely due to the presence of a high concentration of calcium salts. A relatively higher mineral density of the relic was seen compared to the healthy subject's rib. From the history of St Nicholas' life, we know of the long imprisonment at the age of 51 in damp and unhealthy environment. The results of this study suggest that a good bone mineral density was maintained by the Saint even in old age. An additional element that can influence bone mineral density is diet, certainly different during the time of St. Nicholas. The good bone densitometry indicates that the Saint maintained a proper diet, with a generally fair state of health. **Conclusion:** For this first DXA analysis of the rib relic of Saint Nicholas was necessary a long and complex experimental work to modify standard technique procedure to particular and unusual sample and Create specific supports and complementary instruments. Perform DXA analysis on relics permit to obtain additional information to living conditions, economical situation, behaviours, diet, diseaes, conservations conditions of remains, change of life style in different age. Our experimental work, the first of its kind, creates the way to analyze precious relics that often include only few bone fragments and data obtained by our work can be useful for a better management and movement of fragile relics. We ourselves are working on a new challenge for the analysis of bone finds from shipwrecks found at the bottom of the sea.

Introduction

Since the discovery of X-rays, diagnostic imaging techniques have been used to study living subjects, but sometimes also a deceased person or body fragments [1].

Among the subsequent imaging techniques, computed axial tomography (CAT) densitometry evaluates the mineral content in the skeleton, especially calcium and phosphorus that, combined with oxygen, form hydroxyapatite crystals. It is based on the measurement of X-ray attenuation when crossing a body district in which skeletal components are present.

It can be performed with various techniques, but the most widespread is dual-energy X-ray absorptiometry (DXA). The X-rays emitted by an X-ray tube are filtered in order to produce two radiation beams with specific energies that are adequately collimated so as to exclude diffuse radiation. The result of DXA is an image on a two-dimensional plane of the attenuation of X-rays when crossing a bone volume. This representation integrates the data of the compact bone present on the surface with the internally located cancellous bone, when both are present in the bone volume examined [2].

Densitometry evaluates the mineral content in the skeleton, especially calcium and phosphorus that combined with oxygen, form hydroxyapatite crystals. It can be performed with various techniques, but the most widespread is dual-energy X-ray absorptiometry (DXA).

Since the discovery of X-rays, diagnostic imaging techniques have been used to study living subjects, such as menopausal women and in patients with skeletal diseases to evaluate the risk of bone fracture; but sometimes also a deceased person or body fragments.

DXA as well as other morphologic diagnostic imaging techniques have been also applied for the study of relics, greatly contributing to the development of paleoradiology [3].

Paleoradiology is a branch of diagnostic imaging that allows to obtain information about human remains in contexts of archaeological and/or forensic interest. In both areas it is therefore possible to reconstruct biological and behavioral aspects such as the biological profile (age, sex,

height, ethnic group, state of health, reconstruction of the face, etc.); the state of health and diseases of ancient populations; the age, the cause and the context of death [4].

DXA, analyzing the bone mineral density of the skeletal remains of populations lived in past centuries, provides important information on the geographical origin of the populations, their genetic heritage and lifestyles [5].

Saint Nicholas is one of the most famous religious figures of the Christian religion, and his cult is widespread throughout the world, not just in the West.

A deeper bon has been established between the Saint and the city of Bari (Apulia, Italy) since his relics have been moved in the city in the year 1087 a.C. and preserved in the main church of the City dedicated to him ("Basilica di San Nicola").

In the world there are numerous relics attributed to Saint Nicholas, but those present in the Basilica of Bari are the most numerous and those that have been attributed to the saint with greater certainty [6].

In the night between 5 and 6 May 1953 the canonical reconnaissance of the skeletal remains enclosed in the Tomb of St. Nicholas was performed. This survey was an absolutely exceptional event, given that for as many as 866 years nobody could touch or see the Bones of the Saint.

The relics were repositioned in the new restored crypt in 1957; during this period the anatomical analysis of each single fragment was performed allowing to attribute with certainty the relics to the saint.

All the bony pieces were immersed in a clear liquid, similar to rock water, occupying the bottom of the niche at a height of about 2 cm; the parts of the bones that stood above the surface of the water were all moist, while all the marrow spaces of the chipped spongy bones were full of water and abundantly dripping when the skeletal pieces were raised [6].

In this report we present the unique experience of DXA performed on the relic of Saint Nicholas; in particular, we analyzed the skeletal fragment withdrawn from the sepulcher of the Saint on 19th June 2016 for its transport to Russia in honor of the Metting between Pope Francis and Russian Patriarch Kirill.

Material and Methods

Thus, on June 19, 2016, a team of doctors from the Polyclinic of Bari removed the posterior arch of the 9th left rib of the Saint from under the altar of the crypt in the Basilica of St. Nicholas in Bari. In order to minimize damage, they used a light probe and a fiberscope to access the grave through a circular entrance with a diameter of only 7cm.

An X-ray densitometric apparatus [Unigamma Plus/P Compact, I'Acn s.r.l., Italy] was used to quantify bone mineral density. This instrument produces two distinct beams at different energies (40 and 70kV) with a scan range of 15x20cm.

The analysis of bone fragments is different from the analysis of bones in the context of the alive human body (where soft tissues are placed around the skeleton); for this reason, one of the most critical issues was to create a specific system that would allow the analysis of bone

fragments.

While densitometry data analysis of living patients recognizes the different density of the bone, the soft tissues placed around the skeleton and the air present between patient and instrument, the bone fragment analyzed in this study was obviously void of other tissues.

Thus, in order to perform the analysis, we simulated conditions similar to those occurring in patients. A density scale was established, using a specific plexiglass phantom on which the bone fragments to be examined were placed. This phantom reproduced the differences in density between air/phantom and phantom/bone that are necessary for this type of analysis, without creating interference. Prior to each densitometric measurement, the equipment was subjected to routine controls to certify its correct functioning.

DXA studies performed on relics reported in the literature were performed by immersing the bone fragments in a liquid of known density, to simulate the bone tissues and soft tissues. Furthermore DXA analyzes were performed on fragments such as vertebrae and femurs [7].

The analysis on the relic of St. Nicholas presented exceptional feauters: first of all, being a relic of high historical and religious value it was not possible to immerse it in liquids and manage it in dangerous ways.

Furthermore it consisted of a fragment of the ninth left rib that was 12cm long, 1.2cm maximum width and 1.7cm thick at the body (Figure 1); for this type of bone fragment there are no reference parameters in literature.

Figure 1. *Image of the fragment of Saint Nicholas' left ninth rib (posterior arch).*

These feauters made it necessary to overcome three critical issues in order to carry out a reliable analysis.

The first critical point to overcome was to analyze the relic without immerging it in liquids; the second critical point was to recreate the three different densities of the human body (air, soft tissue, bone); the third critical point was to establish the correct distance of the relic from the origin of the x-ray beam.

After experiments with plexiglass plates supplied for the quality control of the equipment we established that plexiglass was the best choice.

Furthermore experiments were performed in order to establish the correct distance of the relic from the origin of the x-ray beam: the optimal thikness of support that permit an appropriate distance of bone to x-ray source was total thickness of 7.5cm. Three plexiglass sheets of 22x22x2.5cm were specifically created.

These phantom and conditions of analysis reproduced the differences in density between air/phantom and phantom/bone that are necessary for this type of analysis, without creating interference (Figure 2).

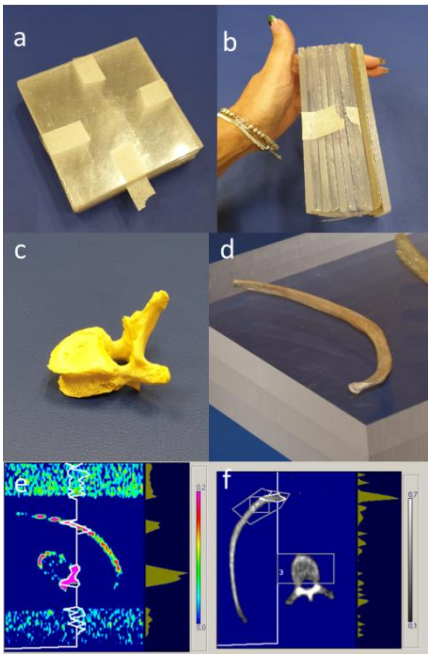

Figure 2. Image of plexiglass sheets used for the experiments (a, b), human vertebra and rib used for experimens (c, d) and images resulted during the experiments (e) and after the final DXA experiment with the optimal setting of support material, its thickness and distance from X-ray beam (f).

Considering the lack of reference parameter for rib relics the analysis was firstly performed on the relic of the rib of a healthy 60 years old man (height of 170-175cm, without known skeletal pathologies) provided by the Section of Legal Medicine of "Aldo Moro" University of Bari. This sample had a length of 19cm, maximum width at the head 1cm, and 0.7cm thick at the body.

The measurement of the relic of St. Nicholas was performed with an X-ray beam parallel to the internal and external surfaces of the rib. The data acquired from the densitometric study were performed using the following anthropometric measures: sex (male), age (75 years), weight (70 kg), height (167 cm), and ethnicity (Caucasian); these parameters were reported in historical records of St. Nicholas' life and by Prof. Luigi Martino who studied the relic in 195 [6].

The first measurement of the relic of St. Nicholas was performed with an X-ray beam parallel to the internal and external surfaces of the rib (Figure 3).

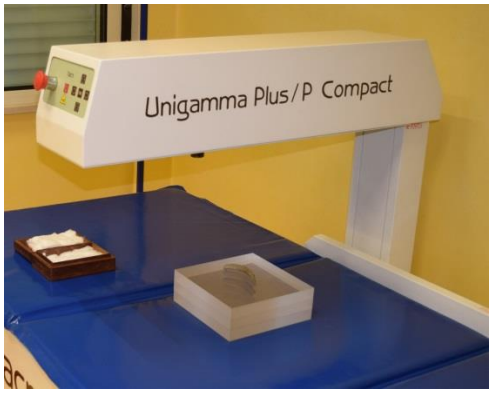

Figure 3. Image of the relic positioned in the densitometer.

Subsequently, a comparative scan between the skeletal relic and a reference rib was performed. In this scan, the ribs were again positioned in such a way that the incident beam was parallel to the internal and external surface of the ribs (Figure 4).

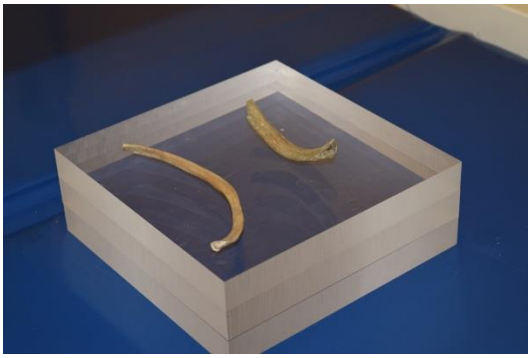

Figure 4. Saint Nicholas skeletal relic towards a reference rib, positioned on a dummy in the densitometer

From the analysis it was calculated the parameter bone mineral density (BMD), express in g/cm2, that indicates the relation between mass of bone mineral content and area of examined bone segment. BMD data was compared to a range normalized by age, sex and ethnicity (BMD-N). [8,9]

Considering the conditions in which the relic was found in the sepulcher that was immersed in a colorless liquid, defined as rock water, a chemical analysis of this liquid was also performed, which proved to be devoid of solutes that could cause the deposition of calcium salts on the relic.

Results

The results of the scannnig of St. Nicholas' rib showed a BMD of 0.97 g/cm2 with a BMD-N between 0.77 and 1.08 g/cm2. (Table 1; Figure 5)

Table 1. *Measurements from the scan of Saint Nicholas' rib.*

BMD g/cm2	BMD-N g/cm2
0.97 g/cm^2	0.77-1.08 g/cm^2

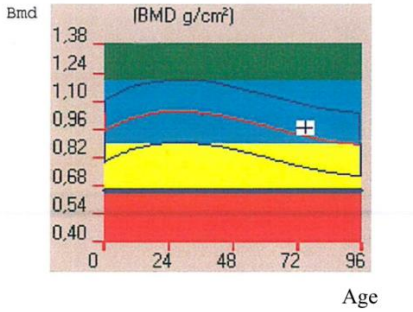

Figure 5. Graphic representation of the relic density (BMD) with respect to age. The bone density falls within the range of values considered normal for the age of the saint (blue band). The curves represent the average density (red line) and the normal range (blue lines) in relation to age.

Simultaneous measurements of the relic compared with a reference rib showed highlighted BMD of 0.84 g/cm2 for the relic and 0.50 g/cm2 for the reference rib. (Table 2)

Table 2. *Simultaneous measurements of the relic compared with the reference rib.*

	BMD g/cm2
Saint Nicholas rib	0.84 g/cm^2
Healthy man rib	0.50 g/cm^2

The St. Nicholas data are 168% higher than reference bone.

Therefore, the densitometry value of the relic was higher than the value of an healthy subject's rib used by comparison.

Discussion

The analysis of very ancient relics always poses the problem of how to manage them as because handling bone fragments stored for hundreds of years is a great challenge, considering their high fragility.

The fragility of the relics of Saint Nicholas was documented by the fine dust and minute fragments produced even by delicate handling of the relics and because they were found immersed in a colorless liquid without solutes.

In the bone tissue of a living subject, the simultaneous presence of both the collagen fibers that function as a robust elastic scaffold, and of phosphorus and calcium crystals, documented by a good mineral content, provide strength and make the skeleton very resistant. The lack of collagen fibers that has occurred after St. Nicholas' death several centuries ago has

instead made the bone relics very fragile, as documented by the fine dust and minute fragments produced even by delicate handling of the relics. In spite of the high bone density linked to a greater quantity of calcium salts, the lack of fibrous support elements, such as collagen fibers present in the bone tissue in vivo but lost after death and with the passing of the centuries, predisposes the bones to a greater possibility of fragmentation that can even occur as a result of small stresses.

Furthermore our sample was a relic of high historical and religious value so the degree of responsibility and the limits to its analysis were elevated.

Bone densitometry by DXA is normally evaluated in vivo, on a specific bone segment surrounded by other tissues and organs with different density each other and from the surrounding air.

The analysis of bone fragments is different from the analysis of bones in the context of the human body; for this reason, one of the most critical issue is to use support that can simulate the in vivo conditions. DXA studies performed on relics reported in literature were performed by immersing the bone fragments in a liquid of known density, to simulate the densities differences among bone tissue, soft tissue and air [7].

For our analysis on the relic of St. Nicholas, being a relic of high historical and religious value it was not possible to immerse it in liquids.

For this reason we have performed numerous serial experiments in order to establish the support material that could replace the liquid and the dimensions of the support and in particular its thickness to be able to recreate the different density of tissues and air present in vivo. Furthermore was also fundamental establish the correct distance of the relic from the X-ray beam in order to obtain also high quality and resolution images.

The other major challenge was to examine a type of bone that is not one of the segments routinely analyzed with the DXA such as vertebrae and femurs. Studies reported in literature on archaeological findings concern femurs and vertebrae, performed mostly on finds of common human bones, which could then be handled more easily than a Saint relic [7].

Our relic consisted of the posterior arch of the ninth left rib that was 12cm long, 1.2cm maximum width and 1.7cm thick at the body; for this type of bone fragment there are no reference parameters in literature.

Our DXA analysis of the relic of a rib is the first reported in the literature, for our knowledge. A rib can be assimilated to a ribbon-like structure, in which the surface predominates compared with the thickness; these features have further complicated the experiments described above, due to the difficulty of positioning of the "isolated" relic with respect to the X-ray source.

The anatomical analysis carried out during the 1953 reconnaisance of Saint Nicholas Relics revealed that they belonged to a man over the age of 70, and the historical chronicles report that Saint Nicholas died around the age of 75, on the December 6th during the year 350 after Christ [6].

Also considering that DXA measurements are related to the patient's age and define an index of bone fragility we analyzed also the fragment of the rib of an old man in good health. [10, 11, 12]

All our measurements on Saint Nicholas relic indicated a high bone mineral density, most likely due to the presence of a high concentration of calcium salts in the relic. A relatively higher

mineral density of the relic was seen compared to the healthy subject's rib.

In spite of the high bone density we found the bone relics very fragile as documented by the fine dust and minute fragments produced even by delicate handling of the relics.

From the history of St Nicolas' life and the study of his relics, we know of the long imprisonment at the age of 51 in damp and unhealthy environment. This was reported to have caused an ankylosing spondylitis as well as widespread hyperostosis of the internal surface of the skull in a plain film radiographic study performed in 1953. The results of this study are in agreement with these findings and suggest that a good bone mineral density was maintained by the Saint even in old age. His bone density was even higher than that of subjects of our modern age who have a more comfortable and consequently more sedentary life-style, and lack stimuli (physical activity, sustained walking) that maintain bone mass [6].

An additional element that can influence bone mineral density is diet, certainly different during the time of St. Nicholas. The good bone densitometry indicates that the Saint maintained a proper diet and good living conditions, with a generally fair state of health. The greater bone density of the rib under examination compared to the reference rib can also be explained by the Saint's known skeletal diseases. Hyperostosis is a condition characterized by overproduction of collagen fibers, which are lost over time after death, and subsequent deposition of mineral crystals that remain unchanged over time [6].

Two possible shortcomings of this study should be emphasized. First, the normal densitometric values of the rib fragment examined cannot be taken as an indication that equally normal densitometric measurements might be found on other regions of the skeleton. Second, it should be considered that the skeleton has remained immobile for centuries in a humid environment that facilitated the deposition of calcium salts coming from the stone of the sarcophagus and conveyed by the condensation of particles of water on the skeleton, and this may have contributed to the higher mineral densitometry.

Conclusion

For this first DXA analysis of the rib relic of Saint Nicholas was necessary a long and complex experimental work to modify standard technique procedure to particular and unusual sample and create specific supports and complementary instruments.

Perform DXA analysis on relics permit to obtain additional information to living conditions, economical situation, behaviours, diet, diseaes, conservations conditions of remains, change of life style in different age; furthermore our results were in line with storical data about the relics belongings to Saint Nicholas.

Our experimental work, the first of its kind, creates the way to analyze precious relics that often include only few bone fragments and data obtained by our work can be useful for a better management and movement of fragile relics.

We ourselves are working on a new challenge for the analysis of bone finds from shipwrecks found at the bottom of the sea.

The authors declare that they have no conflicts of interest.

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Will Rogers phenomenon in the oesophageal cancer patients staging - CT versus ^{18}F-FDG PET/CT: retrospective study

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Abstract

Objective: The oesophageal cancer is one of the most common and aggressive malignancies, especially in elder man. The method of choice in diagnosis of the oesophageal cancer patients are the contrast-enhanced computed tomography (CT) and the 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) examinations. **The aim** of this study was to evaluate and compare the contrast-enhanced CT and the ^{18}F-FDG PET/CT methods of imaging in terms of the oesophageal cancer staging and restaging using the eighth edition of the tumor-node-metastasis (TNM) classification. **The studied group** consisted of 25 retrospectively analyzed patients (23 men, 2 women; mean age±SD: 60±11 years, range: 33-78 years, median: 62 years, p=0.09) who underwent the contrast-enhanced CT and the ^{18}F-FDG PET/CT scanning within one to eight weeks. All mentioned lesions were histopathologically examined. Among these patients, 12 did not receive any treatment and 13 subjects have been treated with the chemotherapy and the external beam radiotherapy using comparable therapeutic protocols. **According to our results**, in 13 subjects PET/CT method occurred as more sensitive in terms of pre- and posttreatment staging than CT and in 10 from 13 cases, involving the ^{18}F-FDG PET/CT imaging into diagnostic management affected the therapeutic protocol. In 11 cases both

methods showed comaparable or similar stage of the disease and in 1 patient both methods showed no pathology. **In this material**, the ^{18}F-FDG PET/CT seems to be more accurate in terms of staging in case of the oesophageal cancer TNM classification.

Introduction

The oesophageal cancer (esophageal cancer, EC) is one of the most common cancers and six cancer-related cause of death worldwide [1]. The two major types of EC are the oesophageal squamous cell cancer (OSCC) and the oesophageal adenocarcinoma (OAC). The method of choice in the oesophageal cancer detection, staging, restaging and recurrence assessment are the contrast-enhanced computed tomography imaging and the 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) examination [1-3]. Both methods are considered useful, however the ^{18}F-FDG PET/CT might be superior in terms of primary and recurrent staging of the disease.

The aim of this study was to evaluate the usefulness of the contrast-enhanced CT and the ^{18}F-FDG PET/CT methods in terms of the oesophageal cancer patients staging and restaging in comparison with the clinical and histopathologic diagnosis, using the eighth edition of the tumor-node-metastases (TNM) classification.

Subjects and Methods

The study was performed upon received of the patients' written informed consent and approved by the Poznan University of Medical Sciences Bioethical Committee (chair: Pawel Checinski, Prof.) as the retrospective analysis based on standardly performed examinations from March 2013 to January 2017.

33 retrospectively analyzed consecutive oesophageal cancer patients underwent the ^{18}F-FDG PET/CT imaging. Among this group, 25 patients (23 men, 2 women; mean age: 60±11 years, range: 33-78 years, median: 62 years) have been examined both with the contrast-enhanced CT and the ^{18}F-FDG PET/CT within one to eight weeks between the studies for the staging and restaging purposes before, during or after the treatment.

Only patients with studies performed at the same step of the oncological management were included into analysis. In this study, 12 from 25 of examined patients did not receive any treatment and 13 subjects have been treated with the chemotherapy and the external beam radiotherapy using comparable therapeutic protocols. According to the histopathologic examination, 8 patients have been diagnosed with the OAC and 17 subjects - with the OSCC.

The ^{18}F-FDG PET/CT study has been performed using the Philips Gemini TF16 (Ohio, Cleveland, United States of America) hybrid scanner due to standard acquisition protocol, using

the radiopharmaceutical's activity of 3.7MBq/kg. Patients laid supine on the PET/CT table with arms above head. The scanning included the area from half-thigh to scull vertex. Patients were scanned using the following CT parameters: 150-200mAs and 120kVp, Pitch of 0.8, x-ray tube rotation of 0.5s. The PET acquisition scans were reconstructed using the spatial resolution of 5mm and evaluated with the Philips-recommended software – the Fusion Viewer. The contrast-enhanced CT was performed with comparable technical parameters.

The necessary statistics have been performed using the STATISTICA, StatSoft, Poland software.

Results

The CT and PET/CT scanning results were compared and confirmed with the final clinical and histopathologic assessment as the golden standard in lesions' distinction. Considering $p<0.05$ as the statistical significance level, the difference between number of women and men in examined group was significantly different with $p<0.01$ and the age distribution in examined group was Gaussian ($p=0.09$). The difference between the sample-size of patients diagnosed with OAC and OSCC was significant with $p=0.02$ (8 OAC and 17 OSCC studied subjects).

In 13 subjects PET/CT method occurred as more sensitive in terms of pre- and posttreatment staging of the disease than CT. In 10 from 13 cases, introducing the ^{18}F-FDG PET/CT imaging into diagnostic management shifted the therapeutic protocol from radical to palliative approach. In 11 cases both methods showed comaparable or similar stage of the disease. In our study, both methods showed no pathology in 1 patient (no tumor recurrence observed, Table 1, Table 2).

Table 1 and Table 2 show the TNM characteristics evaluated with the contrast-enhanced CT and ^{18}F-FDG PET/CT imaging in comparison with the confirmed tumors' grade in each patient and the histologic diagnosis. Tables include all available data regarding the histologic type of the tumors.

Table 1. CT vs PET/CT: staging.

8th edition of the TNM classification - OSCC and OAC staging

In	Gender	Age	CT	PET/CT	Clinical staging	Histopathology	Superior method
1	M	60	TxNxM1	TxN2M1	TxNxM1	OAC G1	similar*
2	M	61	**T2N1M0**	**T2N1M1**	**T2N0M0**	OAC	PET/CT
3	M	62	T+N0M1	T+N0M1	T3N1M1	OAC G2	similar*
4	M	33	T4bN2Mx	TxN2M1b	T4N2Mx	OSCC keratotic G2	similar*
5	M	47	**TxN0M0**	**TxN2M0**	**T4aN2M0**	OSCC G3	**PET/CT**
6	M	65	T4N1M1	T4N3M1	T4N3M1	OSCC	PET/CT
7	M	66	**T3NxM0**	**T3N2M0**	**T3N2M0**	OSCC	**PET/CT**
8	M	66	**T3N1M0**	**T3N2M1**	**T3N2M0**	OSCC akeratotic G2	**PET/CT**
9	F	66	T3NxM0	T3N1M0	T3N1M0	OSCC G2	comp.**
10	M	68	T0NxM0	TxNxM0	T1N3M0	OSCC	PET/CT
11	M	68	**TxN1M1**	**TxN3M0**	**T1N3M0**	**OSCC**	**PET/CT**
12	M	73	T3N3M0	T3N3M0	T3N2M0	OSCC	similar*

similar - same lesions of none, comp.differences in number of detected metastatic lymph nodes '+ no specified, bold font: clinically significant stage migration*

Table 2. CT vs PET/CT: restaging.

8th edition of the TNM classification - OSCC and OAC restaging

In	Gender	Age	CT	PET/CT	Clinical staging	Histopathology	Superior method
1	M	40	T3N0M0	T4N0M0	T4N0M1	OAC G1	PET/CT
2	M	46	TxN3M1	TxNxM1	T4N3M1	OAC G1	comp.**
3	M	48	**no lesions**	**T0N1M0**	**T0N1M0**	OAC	**PET/CT**
4	M	49	**no lesions**	**T0N1M0**	**T0N1M1**	OAC G3	**PET/CT**
5	M	78	**T2N1M0**	**T2N2M1**	**T2N2M0**	OAC G2	**PET/CT**
6	M	55	**TxN1M0**	**TxN2M1**	**T3N2M0**	OSCC akeratotic G2	**PET/CT**
7	M	60	TxN0M0	TxN0M0	T2/3N0M0	OSCC G2	similar*
8	M	60	TxN2M1	TxN3M1	T1bN2M1	OSCC G3	comp.**
9	F	61	T0N3M0	T0N3M0	T0N3M0	OSCC akeratotic G3	similar*
10	M	62	T3N1M1	T+N0M1	T3N1M0	OSCC akeratotic G3	comp.**
11	F	64	T0N2M0	T0N2M0	T1aN1M0	OSCC p. kerat.G2	similar*
12	M	69	no lesions	no lesions	no lesions	OSCC (rec.susp.)	similar*
13	M	70	**T3N0M1**	**T3N3M1**	**T3N3M1**	OSCC akeratotic G3	PET/CT

similar - same lesions of none, comp.differences in number of detected metastatic lymph nodes '+ no specified, bold font: clinically signifcant stage migration, p. kerat. - partially keratotic, rec.susp. - recurrence suspicion*

Discussion

The oesophageal cancer staging and restaging assessment is crucial in the oncological patients' therapeutic management. According to the National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO) and Polish Oncology Union (pl, Polska Unia Onkologii, PUO), the method of choice in the oesophageal cancer treatment is the radical chemoradiation combined with the tumor's surgical resection, however the possibility to introduce radical approach into patient's therapy depends on the stage of the disease. The distant metastasis occurrence and significant lymph nodes involvement shifts the patient from radical therapy to palliative management. The tumor's resectability might be performed when the disease is limited to the *in situ* status or when the metastatic lymph nodes resection can be also performed (located near the area of the primary tumor). Therefore, therapeutic indications depend on appropriate diagnosis in which, accurate imaging seems to be crucial.

The ability to detect all metastatic lesions is limited and depends on the used method of imaging, however combined diagnostic management seems to be of value. Patients suspected of OSCC or OAC undergo usually the contrast-enhanced CT first and the ^{18}F-FDG PET/CT afterwards, most often before the treatment. In some cases, the ^{18}F-FDG PET/CT might be deffered and used for the restaging purpose (i.e. treatment effectiveness assessment) in palliative patients.

According to the eighth edition of the TNM classification, the T characteristic includes four main grades (T1-T4), N – four grades (0 – no lymph nodes involved, 1 – one or two, 2 – three to six, 3 – seven or more regional lymph nodes involved) and the M – two grades (0 – no distant metastases, 1 – distant metastases presence) [1,3]. The main limitation of the PET/CT method is the T feature evaluation. The most relevant method in the locoregional oesophageal cancer staging is the endoscopic ultrasonography (EUS). The PET/CT technique is more sensitive and specific in terms of lymph nodes involvement and distant metastatic lesions detection when compared to CT alone. Therefore, the ^{18}F-FDG PET/CT method can significantly affects the disease's TNM staging which has been defined as the stage migration (commonly recognized as the Will Rogers phenomenon) and occurred in this study in 9 patients [4-6].

According to literature [2, 6-8], the PET/CT examination seems to be of value considering detection, staging and restaging of the disease as well as CT technique. However, in our study, the ^{18}F-FDG PET/CT method occurred as more sensitive and specific than CT scanning in terms of recurrence assessment and distant metastasis detection in 12 cases. In some of studied subjects, the differences between the TNM grade obtained with the CT and PET/CT were significant and the results collected with the ^{18}F-FDG PET/CT scanning were comparable or similar when compared to clinical and histologic staging. More of that, the ^{18}F-FDG PET/CT occurred as useful despite the type of observed lesions (primary tumor, metastatic lymph nodes, bone lesions, OSCC, OAC). Nevertheless, considering the examined sample-size and its heterogeneity, further studies are needed.

In conclusion, in this study the ^{18}F-FDG PET/CT seems to be more adequate in terms of staging in case of the oesophageal cancer TNM classification, however further study and detailed analysis are needed.

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The authors declare that they have no conflicts of interest.

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Administration of the extra virgin olive oil (EVOO) in mild cognitive impairment (MCI) patients as a therapy for preventing the progress to AD

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Abstract

Background: Although Mediterranean diet is connected with longevity and lower rate of many disorders including Alzheimer's disease (AD), the effect of olive oil, which is the principal component of the Mediterranean diet, on fibrinolytic system related to AD and especially on plasminogen activator inhibitor-1 (PAI-1) and a2-antiplasmin in aged participants are not yet examined. This study was performed on 108 aged participants allocated into 5 groups: Mild Cognitive Impairment (MCI) (36) patients subjected to 1-year therapy with extra virgin olive oil (EVOO), MCI without therapy patients (26), MCI without therapy 1-year later patients (11), AD patients (30) and healthy individuals (16). **Hypothesis/Purpose**: To examine the effect of EVOO therapy on the fibrinolytic factors PAI-1 and a2-antiplasmin, on hallmarks of AD, tau and Aβ amyloid fragments and on an oxidative stress biomarker, MDA in the serum of MCI patients aiming to be exploited as a future preventive therapy. **Results**: Using ELISA method, the levels of both fibrinolytic factors PAI-1 and a2- antiplasmin in the serum of MCI patients were reduced notably in the EVOO treated patients versus the control group and were lower than those of all other groups. For better determination of AD from other pathological conditions the ratio Aβ1-42/Aβ1-40 was measured in serum of all participants. The more lessened the ratio is, the more cognitive impairment is observed in patients. The MCI group with one-year EVOO therapy displayed a ratio similar to this of healthy individuals. Moreover, patients with EVOO therapy showed decreased tau protein levels in comparison with all the other groups. The levels of the oxidative stress's biomarker, malondialdehyde (MDA) showed a significant decrease in MCI patients subjected to EVOO therapy revealing the involvement of the beneficial antioxidative properties of EVOO in the progression of AD. **Conclusion**: We demonstrated that EVOO therapy may prevent the risk of patients with MCI to progress to AD via decreasing fibrinolytic factors PAI-1 and a2 antiplasmin that reflecting in the diminution of the hallmarks proteins of AD, tau and Aβ amyloid as well and in a biomarker of oxidative stress, MDA.

Predicting oligonucleotide therapeutic efficacy at the population level

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Abstract

Background: DNA-directed RNA interfering (RNAi) mediators that follow the classic Watson-Crick base pairing to bind to their molecular targets and exert their silencing capacities have been identified to be relatively insensitive to single nucleotide polymorphisms (SNPs). The experimental evaluation of a few putative genomic SNPs in a quasi-species population is the only approach scientists have been employing so far for the experimental validation of the efficacy of oligonucleotide drugs on a given population. These studies are inherently constrained by the number of SNPs that can be experimentally supported in the context of an identified molecular target. **Material and Methods:** To address this sampling limitation, we have developed a method to report the relative sensitivity of all known and unknown polymorphisms to a prospective therapeutic drug. The power of ultra-deep next generation sequencing (NGS) allows us to test drug effect in vitro on all possible SNPs of a molecular target, in a patient-free manner. We are presenting the technical details to our approach that is empowering unbiased pharmacodynamic studies at the population level for sequence-specific oligonucleotide drugs and genome editing tools.

Benchtop systems for in vivo molecular screening of labeled compounds, as a tool to speed up drug research

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Abstract

Background: For every new drug, >10,000 candidate molecules are tested for ~15 years. This is the daily mission of thousands research teams worldwide. It is well proven that small animal imaging speeds up this work, increases accuracy and decreases costs. However, commercial imaging systems have high purchase cost, require high service contracts, special facilities and trained staff. Thus, they are affordable to only few large research centres and not to the majority of small and medium research teams internationally. There are two main reasons that urge the addressing of this problem at large scale now: Firstly, small animal imaging started in 2000 and quickly research community and pharma industry understood its value, which opened preclinical imaging market (>2.5 Bil $). Continuous evolution in medicine and biology clearly shows the need to speed up research using new tools. Asian countries rapidly invest funds in drug research, enlarging existing market. Secondly, until recently such systems were based on complicated electronics and expensive components. Evolution in detector technology, electronics, software and 3D printing, made feasible the development of benchtop imaging systems, with attractive end user price. **Materials and Methods:** Being an active partner of numerous international and national projects, we tried to identify the main requirements that an imaging system should have, in order to become a screening tool for daily use. Thus, we recently developed a new generation of affordable, but high-performance imaging systems, which can fulfil the daily needs of all research labs activated in preclinical research. Our technology covers the field of SPECT (Single Photon Emission Computed Tomography) and PET (Positron Emission Tomography) imaging, while an optical and x-ray imaging system is under development. The systems are based on well tested technology, including pixeliated scintillators, Position Sensitive Photomultipliers, programmable ADCs (Analog to Digital Converters) and FPGAs (Field Programmable Gate Arrays) and are connected with a standard laptop through USB and Ethernet connection. The systems are named “eyes-series” and have been already tested for fast screening of small animals injected with labeled compounds including peptides, antibodies and nanoparticles. Besides their performance, they are offered at a fraction of the cost of the commercial ones, comparable to standard lab equipment such as HPLC, gamma counter etc, opening new prospects in preclinical research. The first system is called «γ-eye^{TM}» and it is a dedicated system for imaging photons (γ-rays) which are emitted from radiolabelled biomolecules (2D-SPECT). The second system is called «β-eye^{TM}» and detects positrons (β-rays) from similar biomolecules (2D-PET). They both have dimensions which are 35x35x30cm and weight which is less that 30kgr. The spatial resolution of both systems is <2mm and their energy resolution <20%. Their sensitivity allows real time imaging for the first second post injection, while images are shown in real time during acquisition. They allow recording of fast frames, down to 1min, thus it is possible to perform fast kinetic studies. Finally, they are both provided along with a laptop that has preinstalled the required software, named «VISUAL-eyes». **Results:** The technical specifications and performance evaluation of our technology will be presented. Different applications including oncology, regenerative medicine, nanomedicine and lung imaging will be given. Finally, the results of the comparison against high performance systems and a typical workflow for optimizing throughput will be demonstrated.

Correlation between cervical carcinogenesis and tobacco use by sexual partners

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Keywords: Cervical carcinogenesis -Tobacco -Smoking -Human Papillomavirus (HPV)

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Abstract

Purpose: Investigating the effects of active smoking, passive smoking and semen of tobacco smoking sexual partners on the carcinogenesis of uterine cervix. Introduction: It is now well-established that persistence of Human Papillomavirus (HPV) infection is the strongest epidemiologic factor associated with intraepithelial neoplasia and cancer of cervix, as well as in other related locations such as in the vagina, vulva, anus, oral cavity, etc. A 1999 study indicates that the worldwide HPV prevalence in cervical carcinomas is 99•7 per cent. Multiple factors seem to intervene on cervical carcinogenesis, many of them related to tobacco, especially by direct local carcinogenic effect and local immunosuppression. Many studies have also shown that active or passive smoking in women (family-work environment, meeting places, etc.) greatly affects the occurrence, progression and degree of malignancy of carcinogenesis. Furthermore, particularly increased levels of nicotine and cotinine in the cervical mucus as well as prostate sperm fluids and urinary cotinine:creatinine ratios in smokers and passive smokers indicate that tobacco constituents do indeed reach the uterine cervix and lead to increased modification of DNA in cervical epithelium, suggesting biochemical evidence consistent with smoking as a cause of cervical cancer. The research presented today, though it took place over 30 decades ago (1975-1986 at the University Gynecological and Obstetric Clinic of Homburg ad Saar), we hope will serve as a reminder and contributing factor for further examination of the increased risk of cervical cancer in non-smoking women living with smoking partners. **Study**: The study analyzed a total of one thousand five-hundred and forty (1,540) medical history sheets (krankenblätter) of women aged from eighteen to seventy-four (18-74) years old that were admitted, treated, examined (PAP TEST) or referred for further tests by their family physicians to the Homburg ad Saar Clinic between 1975-1986. The study evaluated the general medical history of the 1,540 women with a special focus on gynecological and obstetric related data and gathered additional information from patients through written questionnaires completed via phone, mail or personal interviews. Among a range of factors and data studied during the research, our current presentation and discussion will focus on the development of cervical neoplasms in women, examining results from three different study groups: smokers, passive-smokers and women with smoking sexual partners. **Results**: Five hundred and forty-four cases (544) out of the overall study sample of one thousand five-hundred and forty (1,540) women, were identified as cases with pathological cell abnormalities (35.32%). Following diagnosis and treatment of transient lesions due to various inflammations (vaginitis, cervicitis etc.) one hundred and twelve (112) cases (20.59%) showed varying degrees of

mild/reversible up to CIN 1-3 intraepithelial lesions. From the above sample of one hundred and twelve 112 cases, nineteen cases (19) were smoke free women who never smoked themselves, were not exposed to passive smoking and had non-smoker partners (16.96%). Forty-four (44) cases (39.29%) were female smokers, twenty-two (22) cases (19.64%) were women exposed to regular passive smoking (family-work environment) with a smoke-free partner and twenty-seven (27) cases (24.11%) were women non-smokers with a smoker partner. From the above findings, intraepithelial lesions were found to be higher (and with a progressive malign ratio) on the study groups that were associated with tobacco use either active or passive and therefore, the synergistic harmful effect of smoking, progressively from passive smokers to active smokers, is clearly evident on the occurrence and progression of cervical malignancies. As already mentioned above, the presence of HPV has been widely proven to be almost exclusively the cause of different degrees of neoplasia in the cervix for more than 99.7% of cervical carcinogenesis. However, the harmful effects of a) active smoking, b) passive smoking and c) the exposure to tobacco constituents through an active smoker partner, in women, should be sought and possibly attributed to the catalytic reduction of cervical self-defense and overall cervical immunity disruption which results to the exposure of cervix to elevated levels of nicotine-cotinine and cancer-causing chemicals related to smoking, may work together with certain types of HPV limiting the natural ability of the cervix to defend against carcinogenesis and therefore increase the likelihood of developing cancer. **Conclusion**: Since the almost exclusively cause of cervical neoplasms is due to the presence and carcinogenic activity of HPV, the harmful/synergistic effect of smoking, passive smoking and partner smoking cannot be attributed to the direct carcinogenic effect of nicotine but to the overall damage of the immune system as we as the reduction of cervical self-defense making it more vulnerable to the carcinogenic nature of HPV, in particular the increased pathogenic types 16 and 18. Lastly, another potential correlation that could be further examined is the potential effects of tobacco constituents in cervical fluids on the self-defense system of the male reproductive system.

This research was conduced more than 3 decades ago as part of a much broader Doctorate thesis at the University of Homburg a.d. Saar by Dr Siokos, that even though was fully concluded successfully and included groundbreaking results and conclusions for that time and was supervised by the highly esteemed Direktor Prof. Dr. Med. Herr Gerhard Basttert, however the research was not publicly published until today. Today, with updated academic references and under the review and guidance of Dr. Tzafetas of the University of Aristotle University of Thessaloniki, we have the opportunity to present it and for this allow me to thank the committee of the conference.

Today we will be examining only the component dedicated to the correlation between cervical carcinogenesis and tobacco use.

The reason we are presenting this part of the research today is mainly because the study, even if conducted in 1986, remains very much relevant and timely and we hope the presentation will stimulate, reinforce and encourage further research on this direction and especially on the increased risk of cervical cancer in non-smoking women living with smoking partners.

Purpose

Investigating the effects of active smoking, passive smoking and semen of tobacco smoking sexual partners on the carcinogenesis of uterine cervix.

Introduction

It is now well-established that persistence of Human Papillomavirus (HPV) infection is the strongest epidemiologic factor associated with intraepithelial neoplasia and cancer of cervix [1-2] as well as in other related locations such as in the vagina, vulva, anus, oral cavity, etc. (Figure 1, 2). A 1999 study indicates that the worldwide HPV prevalence in cervical carcinomas is 99·7 per cent. [3]

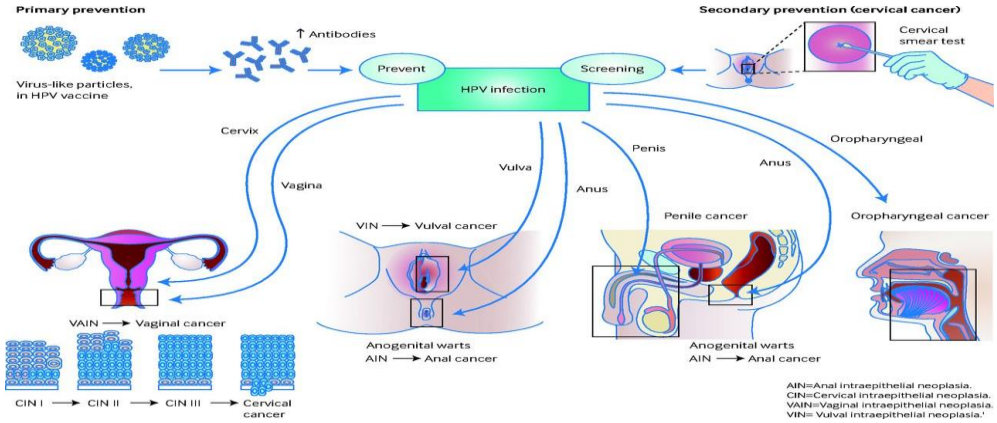


Figure 1

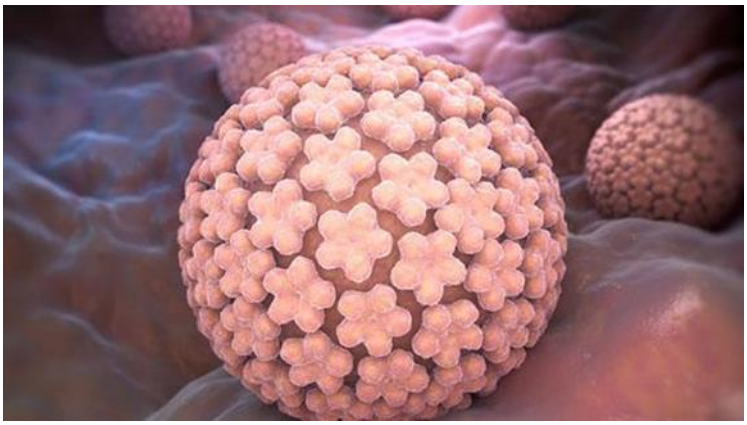


Figure 2

Multiple factors seem to intervene on cervical carcinogenesis, many of them related to tobacco, especially by direct local carcinogenic effect and local immunosuppression [4].

Many studies have also shown that active or passive smoking in women (family-work environment, meeting places, etc.) greatly affects the occurrence, progression and degree of malignancy of carcinogenesis [5-7].

Furthermore, particularly increased levels of nicotine and cotinine in the cervical mucus and prostate sperm fluids as well as the urinary cotinine:creatinine ratios in smokers and passive smokers [8] indicate that tobacco constituents do indeed reach the uterine cervix and lead to

increased modification of DNA in the cervical epithelium, suggesting biochemical evidence consistent with smoking as a cause of cervical cancer [9].

Table 1. *Samples of nicotine-cotinine levels in urine, blood and cervical mucus for women smokers and non-smokers*

Tab. 5: Gehalt an Nikotin und Cotinin in Urin, Serum und Zervikalschleim

	Nikotin	Cotinin
Raucherinnen (10–20 Zig./die)		
Urin (ng/mg Kreat.)	90–3190	664–4120
Serum (ng/ml)	0,1– 39	115– 397
Zervikalschleim (ng/ml)	66–2620	161– 363
Nichtraucherinnen		
Urin (ng/mg Kreat.)	2– 78	2– 30
Serum (ng/ml)	nicht nachweisb.	nicht nachweisb.
Zervixschleim (ng/ml)	5– 31	1– 4

Nach: Sasson et al., New Engl. J. Med. 312 (1985), 315

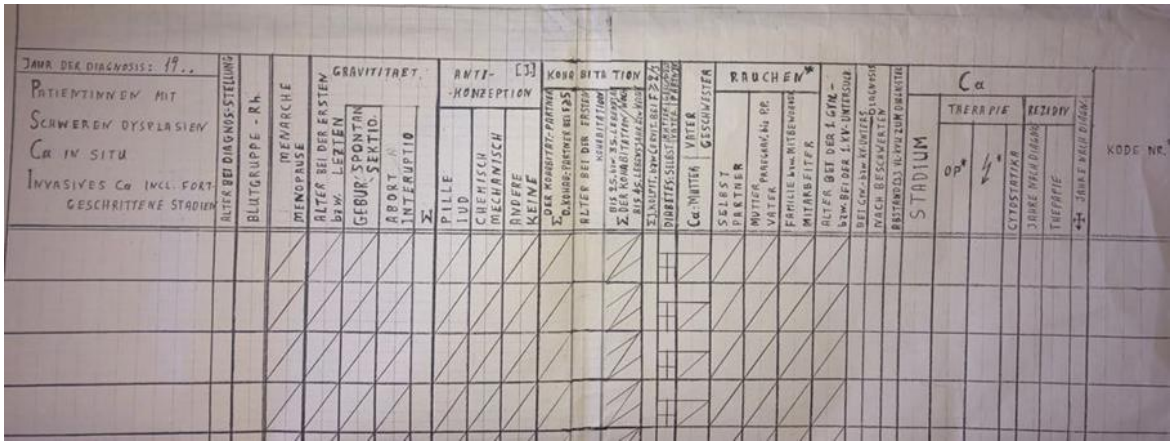
The research presented today, though it took place over 30 decades ago (1985-1986 at the University Gynecological and Obstetric Clinic of Homburg ad Saar), we hope will serve as a reminder and contributing factor for further examination of the increased risk of cervical cancer in non-smoking women living with smoking partners [10].

Study

The study analyzed a total of one thousand five-hundred and forty (1,540) medical history sheets (krankenblätter) of women aged from eighteen to seventy-four (18-74) years old that were admitted, treated, examined (PAP TEST) or referred for further tests by their family physicians to the Homburg ad Saar Clinic between 1975-1986.

The study evaluated the general medical history of the 1,540 women with a special focus on gynecological and obstetric related data and gathered additional information from patients through written questionnaires [11] completed via phone, mail or personal interviews.

Table 2. *Original study questionnaire sample.*



As you can see there was a wide range of indicators and data studied during the research, however, our current presentation and discussion will focus on the development of cervical neoplasms in women, examining results from three different study groups: smokers, passive-smokers and women with smoking sexual partners.

Results

Five hundred and forty-four cases (544) out of the overall study sample of one thousand five-hundred and forty (1,540) women, were identified as cases with pathological cell abnormalities (35.32%).

Following diagnosis and treatment of transient lesions due to various inflammations (vaginitis, cervicitis etc.) one hundred and twelve (112) cases (20.59%) showed varying degrees of mild/reversible up to CIN 1-3 intraepithelial lesions.

From the above sample of one hundred and twelve 112 cases, nineteen cases (19) were smoke free women who never smoked themselves, were not exposed to passive smoking and had non-smoker partners (16.96%). Forty-four (44) cases (39.29%) were female smokers, twenty-two (22) cases (19.64%) were women exposed to regular passive smoking (family-work environment) with a smoke-free partner and twenty-seven (27) cases (24.11%) were women non-smokers with a smoker partner.

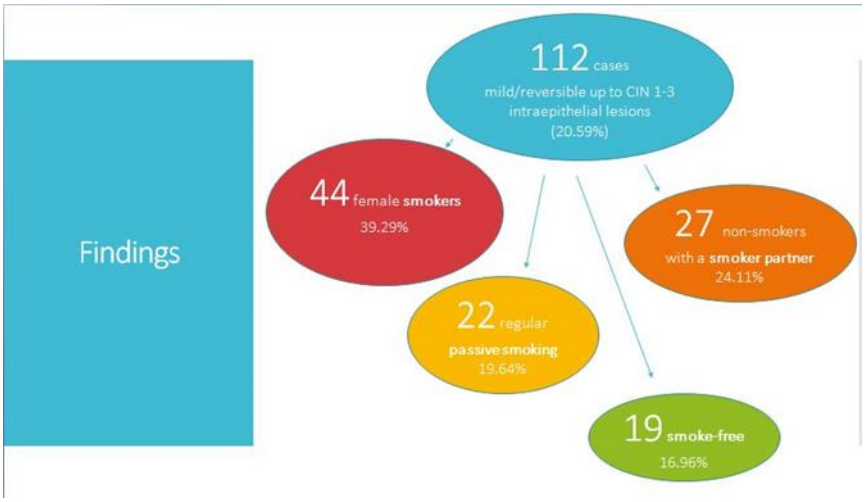


Figure 3

From the above findings, intraepithelial lesions were found to be higher (and with a progressive malign ratio) on the study groups that were associated with tobacco use either active or passive and therefore, the synergistic harmful effect of smoking, progressively from passive smokers to active smokers, is clearly evident on the occurrence and progression of cervical malignancies.

As already mentioned above, the presence of HPV has been widely proven to be almost exclusively the cause of different degrees of neoplasia in the cervix for more than 99,7% of cervical carcinogenesis [12]. However, the harmful effects of a) active smoking, b) passive smoking and c) the exposure to tobacco constituents through an active smoker partner, in

women, should be sought and possibly attributed to the catalytic reduction of cervical self-defense and overall cervical immunity disruption which eventually results to the exposure of cervix to elevated levels of nicotine-cotinine and cancer-causing chemicals related to smoking. Therefore these factors may work together with certain types of HPV limiting the natural ability of the cervix to defend against carcinogenesis and therefore increase the likelihood of developing cancer [13].

Conclusion

Since the almost exclusively cause of cervical neoplasms is due to the presence and carcinogenic activity of HPV, the harmful/synergistic effect of smoking, passive smoking and partner smoking cannot be attributed to the direct carcinogenic effect of nicotine but to the overall damage of the immune system as well as the reduction of cervical self-defense making it more vulnerable to the carcinogenic nature of HPV, in particular the increased pathogenic types 16 and 18.

Lastly, another potential correlation that could be further examined is the potential effects of tobacco constituents in cervical fluids of women smokers on the self-defense system of the male reproductive system of non-smoker male partners.

Figure 4, 5

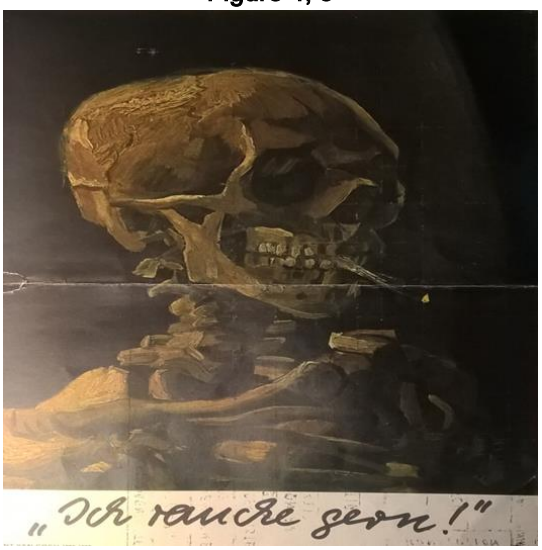


Figure 6

The authors would like to thank the Committee of the 5th International Mecical Olympiad.

The authors declare that they have no conflicts of interest.

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