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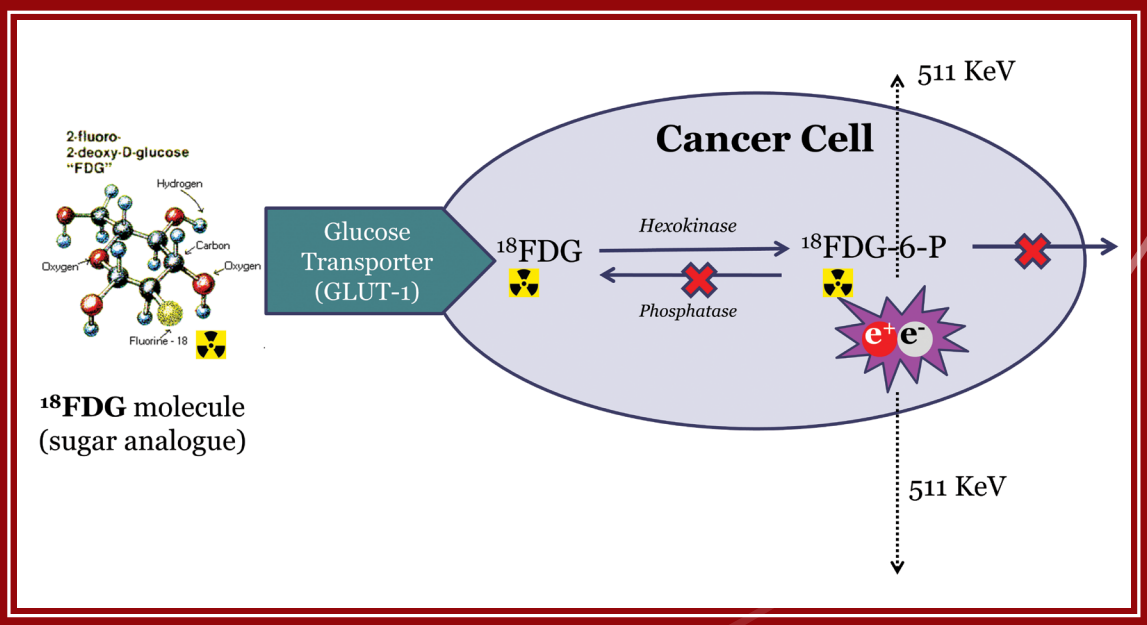

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Quarterly Official Journal of the
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CONTENTS

Dear colleagues,

In the current issue, the editorial by Alexopoulou-Prounia et al. includes an overview of the recently reported guidelines for the care of patients with Chronic Venous Disease (CVD) of the Lower Limbs.

The original article by Argyropoulos et al. investigates the prevalence of dementia and depression in the elderly with chronic diseases in West-Greece and estimates potential risk factors.

Moreover, this issue includes three reviews. The first review, by Zarkavelis et al. presents most recent data on the role of immunotherapy in the treatment of colorectal cancer and demonstrates the results of available clinical trials as well as the relevant future perspectives. The review by Papathanasiou et al. provides an update on the main strengths, limitations, and whole spectrum of clinical applications of Positron Emission Tomography (PET) and Computed Tomography (CT), PET/CT, in the management of oncological patients. The review, by Strimpakos et al. presents data on the role and significance of BRAF mutations and especially the dominant V600E mutation, in colorectal cancer.

Lastly, this issue includes a case report by Chatzi-ioannou et al. presenting an emerging clinical entity, denominated Multisystem Inflammatory Syndrome in Children (MIS-C), in a patient with SARS-CoV-2 infection.

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New European Society for Vascular Surgery (ESVS) 2022 Clinical Practice Guidelines on the Management of Chronic Venous Disease of the Lower Limbs

Loukia Alexopoulou-Prounia[1], Stavros K. Kakkos[1], Marianne G. De Maeseneer[2]

INTRODUCTION

The European Society for Vascular Surgery (ESVS) has developed new guidelines for the care of patients with Chronic Venous Disease (CVD) of the Lower Limbs. [1] The aim of these guidelines was to provide an update to the existing ESVS Guidelines published in 2015 [2] on the diagnosis and management of CVD, related to the pathology of the superficial, perforating, and deep veins of the lower limbs as well as to abdominal and pelvic venous pathology. In contrast to the 2015 CVD Guidelines, the 2022 Guidelines do not include patients suffering from venous or arteriovenous malformations.

The ESVS CVD 2022 Guidelines include 94 recommendations of which 65 are new additions. 34 of them are Class I, 36 are Class IIa, 17 are Class IIb, and 7 are Class III recommendations, based on different levels of evidence (A, B, C). Most chapters include a strategy subsection, illustrated with a clear flowchart. Management strategies are presented in a way they will be useful and applicable in daily clinical practice. There are many new recommendations of interest worth mentioning.

An extensive chapter has been entirely dedicated to superficial venous incompetence (SVI). Indications for intervention have been considered for each clinical class of the CEAP Classification. Interventional treatment is recommended for both patients with symptomatic varicose veins (VVs) (CEAP C2s) and patients with skin changes (CEAP C4-C6). When an intervention is required, endovenous thermal ablation (EVTA) is strongly recommended (Class I, Level A). For patients with SVI undergoing intervention, new recommendations about risk assessment for venous thromboembolism (Class I, Level C), individualized thromboprophylaxis strategies (Class IIa, Level B) and duplex ultrasound (US) surveillance (Class IIa, Level C) have been included in the current Guidelines. The duration of post-intervention compression should be decided on an individual basis (Class I, Level A).

When a non-thermal non-tumescent technique is preferred, cyanoacrylate adhesive closure should be considered (Class IIa, Level A), whereas mechanochemical ablation may be considered (Class IIb, Level A) for patients with great saphenous vein (GSV) reflux. Alternatively, if EVTA options are not available for patients with GSV incompetence, high ligation/stripping (Class IIa, Level A) or catheter-directed foam sclerotherapy (Class IIb, Level B) are suggested. The latter technique may mainly be considered for treating saphenous trunks with a diameter less than 6 mm (Class IIb, Level B).

The current Guidelines suggest ambulatory phlebectomy, US guided foam sclerotherapy or a combination of both for the treatment of varicose tributaries (Class

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Key words: *Chronic venous disease; varicose veins; guidelines*

I, Level B). In patients with combined superficial and deep venous incompetence, treatment of incompetent superficial veins should be considered (Class IIa, Level C).

For the management of small saphenous vein incompetence, EVTA is strongly recommended, in preference to surgery or foam sclerotherapy (Class I, Level A); an upgrade compared to previous Guidelines. It is highlighted that for those treated with EVTA, care should be taken to avoid injury to the sural nerve, if cannulation is carried out below midcalf level (Class I, Level B). Endovenous non-thermal non-tumescent ablation methods are weakly recommended for this patient population (Class IIb, Level B). A new subsection on the incompetence of perforating veins suggests endovenous ablation, division or ligation as a Class IIa, Level C recommendation.

For patients presenting with reticular veins and/or telangiectasias, duplex US of lower extremity veins is recommended to look for associated incompetent veins, which should be treated before considering treatment of smaller veins (Class I, Level C). Compared to previous Guidelines, sclerotherapy for reticular veins has been upgraded to Class I, Level A recommendation and first-choice treatment. Transcutaneous laser has also been upgraded as treatment option for telangiectasias (Class IIa, Level B).

For patients with uncomplicated symptomatic VVs (CEAP C2s), phlebectomies with preservation of the saphenous trunk (ASVAL) have been suggested as a weak recommendation (Class IIb, Level C); a downgrade compared to previous Guidelines. For patients with an incompetent GSV with a large truncal diameter (more than 12 mm), EVTA should be considered (Class IIa, Level C). For patients presenting with foot and ankle VVs, phlebectomy, sclerotherapy, and foot perforating vein ligation may be considered during or after ablation of proximal reflux (Class IIb, Level C). For patients with symptomatic recurrent VVs without truncal incompetence, US guided foam sclerotherapy and/or ambulatory phlebectomy is recommended (Class IIa, Level C). If there is residual or recurrent truncal incompetence, EVTA or US guided foam sclerotherapy should be considered (Class IIa, Level B). In general, re-exploration of the groin or popliteal fossa is not recommended in patients with recurrent VVs (Class III, Level B).

Deep venous pathology is discussed in a separate chapter, with an emphasis on the increasing evidence in the field of managing iliofemoral and iliocaval obstruction. For patients with iliac vein outflow obstruction, endovascular treatment remains the first-choice treatment (Class IIa, Level B), while the authors point out that evidence to support endovascular treatment of iliac vein outflow obstruction is still heterogeneous and weak. Management by a multidisciplinary team is highly recommended (Class I, Level C). New recommendations, such as the use of intravascular US to guide treatment (Class IIa, Level C) have been included. US surveillance is also recommended for patients undergoing either endovascular or surgical reconstruction of iliac vein outflow obstruction (Class I, Level C).

Management for acute deep vein thrombosis has been thoroughly covered by a previous ESVS Guidelines document [3]. Principles of treatment for patients who have post-thrombotic syndrome (PTS) are reviewed in the present CVD Guidelines. Surgical or hybrid deep venous reconstruction may be considered in case of a recalcitrant venous leg ulcer (VLU), severe PTS, or disabling venous claudication, when endovascular options alone are not appropriate, as a recommendation Class IIb, Level C, which has been upgraded compared to the 2015 Guidelines.

An entirely new chapter has been dedicated to the management of patients with VLUs. For patients with an active VLU, objective arterial assessment is highly recommended (Class I, Level C). Compression, exerting a target pressure of at least 40 mmHg at the ankle, is strongly recommended to improve ulcer healing as a Class I, Level A recommendation; a stronger level of recommendation compared to previous Guidelines. Compression stockings should be considered for small and recent onset ulcers, as well as for healed VLU in order to reduce ulcer recurrence (Class IIa, Level B). For patients with active VLU and SVI there is a very important new recommendation stating that early endovenous ablation is highly recommended to accelerate ulcer healing (Class I, Level B). For healed VLU, treatment of the incompetent veins is strongly recommended to reduce the risk of ulcer recurrence (Class I, Level A). Moreover, for active or healed VLU, treatment of incompetent superficial veins is recommended, even in the presence of deep venous incompetence (Class I, Level A). Ablation of the sub-ulcer venous plexus using US guided foam sclerotherapy should also be considered as part of the treatment strategy (Class IIa, Level C). For patients with active or healed VLU and iliac vein outflow obstruction, venous stenting should be considered (Class IIa, Level B).

A new chapter describes the management of patients with pelvic venous disorders (PeVD). When suspecting PeVD in women, exclusion of other causes of chronic

pelvic pain is highly recommended (Class I, Level C). Abdominal and/or transvaginal US should be considered in these patients (Class IIa, Level B). In case of symptomatic VVs that may be of pelvic origin, specific duplex US assessment of pelvic escape points is highly recommended (Class I, Level C). Local procedures for VVs and related pelvic escape points should be considered as an initial approach in case of VVs of pelvic origin without pelvic symptoms (Class IIa, Level C). Pelvic vein embolization should only be considered if pelvic symptoms appear, in which case embolization may considerably reduce them (Class IIa, Level B).

Considerations about the management of acute complications have been included in the new Guidelines. In case of spontaneous bleeding from superficial veins, referral for urgent assessment and treatment is highly recommended (Class I, Level C). For patients with CVD who have suffered from an episode of acute bleeding of superficial veins or telangiectasias, local foam sclerotherapy should be considered to prevent recurrent bleeding (Class IIa, Level C). In addition, special patient considerations have been included for the treatment of venous disease in specific patient populations, such as pregnant women, elderly, children, obese and patients under anticoagulant therapy. The case of venous aneurysms is also discussed. For patients with a popliteal vein aneurysm with thromboembolic complications or those that are saccular, fusiform exceeding 20 mm, or containing thrombus, surgical repair should be considered (Class IIa, Level C).

In conclusion, in the current document several new key points and innovations have been identified in comparison to previous Guidelines. As expected, the ESVS CVD 2022 Guidelines have provided many novel recommendations. Meticulous evaluation of the published literature made it possible for the authors to develop well justified recommendations providing an evidence-based standard that helps clinicians in selecting the best management strategies to achieve optimal outcomes for the care of patients with Chronic Venous Disease of the Lower Limbs.

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Prevalence and risk factors of dementia and depressive symptoms in the elderly: A cross-sectional study in west -Greece

Konstantinos Argyropoulos, Christos Liatsos

Abstract

Background: The aim of the present study was to estimate the prevalence of cognitive impairment and depressive symptoms in the elderly with chronic diseases in West -Greece.

Methods: A cross-sectional study was conducted among 127 people aged 65 and over, who visited the General hospital of Krestena, Elis, West- Greece. An anonymous questionnaire was developed to collect basic demographic data. The Greek version of the Geriatric Depression Scale (GDS-15) was administered to screen the elderly for depressive symptoms and the Mini Mental State Examination (MMSE) was used to assess cognitive deficits. Statistics was processed with SPSS 24.

Results: According to the GDS-15, 27.6% (21.3% moderate and 6.3% severe type) of the studied population screened positive for depressive symptoms. 24.4% of older people were classified as presenting mild and moderate dementia, based on MMSE. Depressive symptoms were more frequent in participants without a supportive environment ($p<0.001$), in lower-educated ($p=0.002$), in single older adults ($p=0.000$), as well as in the elderly with no children ($p=0.022$) and with the presence of comorbidity($p<0.05$). Cognitive impairment was strongly associated with age ($p<0.001$), rural place of living ($p=0.007$), marital status ($p=0.001$) and comorbidity ($p<0.05$).

Conclusions: Cognitive decline and depressive symptoms are common among the elderly and strongly associated with several demographic and socioeconomic risk factors.

Key words: *GDS-15; MMSE; dementia; depression; prevalence*

INTRODUCTION

The rapid increase in the population of older people worldwide renders a focus on mental disorders such as depression and dementia, and aging both timely and imperative. Dementia is a syndrome characterized by difficulties in memory and other cognitive skills, affecting 1 in every 14 of the population aged 65 years or older [1]. In 2019, the number of people suffering from dementia worldwide was 50 million, whereas this number is estimated to reach 152 million in 2050 [2]. Between 2000 and 2013, deaths from prostate cancer, heart disease and stroke decreased by 11%, 14% and 23%, respectively, whereas deaths from dementia increased by 71% [2]. Dementia is a complex condition with many influencing factors and it is often difficult to pin down an exact cause. Several demographic and socioeconomic characteristics have been associated with an increased prevalence of cognitive decline including female gender, low educational level, rural place of living and the coexistence of other medical conditions such as cardiovascular comorbidity [3].

Late life depression is estimated to affect one out of seven older people above 65 years according to the World Health Organization [4]. Despite the lower overall percentage in comparison to younger counterparts, the

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consequences of untreated or partially treated depressive symptoms later in life results in higher mortality rates both due to suicide and medical illness [5]. The clinical features of depression observed in the elderly may be different than those seen in early ages, such as memory loss, sleeplessness, loss of appetite and somatic symptoms, mainly constipation and pain [6].

The findings of the studies performed indicate that depression in the elderly is the result of a complex multidirectional interaction of biologic (vascular depression), psychological (including personality based), and social factors. Sociodemographic parameters that have been associated with depressive symptoms later in life are the advancing of age, being a female, low educational and financial level, and the presence of comorbidities especially diseases of the cardiovascular system [7,8].

Mild cognitive impairment (MCI) is an early stage of memory loss or other cognitive ability loss (such as language or visual/spatial perception) and a systematic review in 2012 reported a prevalence of MCI ranging from 0.5 to 42% in different countries [9]. MCI is characterized as an intermediate phase between normal cognitive ageing and overt dementia and is subcategorized into Amnestic MCI that primarily affects memory and Nonamnestic MCI that affects thinking skills other than memory [9].

The purpose of the present study was to estimate the prevalence of cognitive impairment and depressive symptoms in the elderly with chronic diseases who visited the General Hospital of the rural city of Krestena of the municipality of Elis, West Greece, and to estimate possible risk factors.

MATERIALS AND METHODS

A cross-sectional study was conducted among patients over 65 years old who visited the General Hospital of Krestena, Elis, West-Greece, Peloponnese, from January to February 2019. During the study period, the specialist physicians enrolled a total of 127 elderly, excluding patients who had been previously diagnosed either with dementia or depression.

The psychometric measure for patients' assessment, was a structured anonymous questionnaire designed and supplied by the researchers and filled by the treating physician. The questionnaire contained items that assessed information regarding sociodemographic characteristics (age, gender, educational level, marital status, supportive environment: friends and social life), comorbid conditions (hypertension, history of myocardial infraction and stroke) and place of living (urban or rural; rural is defined as the population of those municipalities and communes in which the inhabitants of the largest population center is less than 10.000).

The evaluation of cognitive decline was made by the treating physicians on the basis of objective cognitive test. The Mini Mental State Examination (MMSE), was used to assert cognitive status of the elderly. The MMSE is a widely used 30- point screening test of cognitive function among the elderly; it includes questions of orientation, attention, memory, language, visual-spatial skills, registration, recall, calculation, language and ability to draw a complex polygon [10].

MMSE was first published in 1975 by M. F. Folstein et al, and the translation and validation in the Greek language was made by Fountoulakis et al, [10,11]. The presence of dementia is determined by the total score. Traditionally, a 23/24 cut-off has been used to select patients with suspected dementia [12]. According to Fountoulakis et al, MMSE appeared to be valid during test and at the score level of 23/24, sensitivity is 90.80 and specificity 90.62. The severity of cognitive impairment was assessed as following: Scores 0-10 indicate severe dementia, 10-20 moderate, 21-24 mild dementia, 25-27 mild cognitive impairment (MCI) and 28-30 are considered normal [11].

The Greek validated version [13] of the Geriatric Depression Scale-15 (GDS-15) was administered to all participants to screen for depressive symptoms. The GDS-15 was first developed by Yesavage et al, [14], and has been tested and used extensively in many countries to assess depression in elderly. It is a brief questionnaire, in which participants are asked to respond to 15 yes or no questions, in reference to how they felt on the day of administration. The GDS-15 has been standardized and adapted in a Greek elderly population and was found to have 92% sensitivity and 95% specificity. The severity of depressive symptoms was assessed according to Fountoulakis et al. Scores 0-5 are considered normal, 6-10 indicate moderate depression, and 11-15 indicate severe depression [13].

Informed consent explaining the objectives and procedures was obtained from all participants before the study and they were guaranteed anonymity and confidentiality. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the General Hospital of Krestena, Elis, in West-Greece and every effort was made to adhere to recommended best practice principles to protect the interests and welfare of the participants.

Data was imported to IBM SPSS, for Windows v.24.0 Statistical Package (IBM Corp., Armonk, NY, USA), for analysis and interpretation. Absolute numbers (N) and percentages (N%) were used to express categorical variables, while for continuous variables descriptive analysis included mean and standard deviation.

Preliminary analyses (Q-Q plots and Kolmogorov-Smirnov) were performed to check variables for normality and outliers, ensuring the adequacy of parametric tests. Based on the results, we used t-tests and ANOVA to compare mean values of GDS and MMSE scores among the different levels of the independent variables. The Pearson's coefficient was used to examine the correlation between GDS and MMSE scores. For all tests, statistical differences were determined to be significant at $p < 0.05$.

RESULTS

A total of 127 individuals participated in the study, 46% living in rural place, 49.6% were women and the mean age of all respondents was 71 years. Table 1 presents the demographic data of the studied population.

Table 1. *Demographic characteristics of the studied population.*

Characteristics	N or %
Total recorded participants	127
Male (%)/female (%)	50.4/49.6
Mean age in years ± SD	71 ± 8.1
Place of living	
Urban/ Rural	54.3/45.7
Educational level	
Elementary/Secondary (9-12 years)/ Tertiary (in %)	42.5/43.3/14.2
Financial level (per month)	
<500 E/500-1000 E/1000-2000 E/>2000	48.0/41.7/8.7/1.6
Marital status	
Married/Not married/Divorced/ Widowed (in %)	58.3/6.3/9.4/26.0
Children	
Yes/No (in %)	92.9/7.1
Supportive Environment (in %)	
Yes /No	88.61/13.4
Co-morbidity	
Yes/ No (in %)	66.4/33.6

According to GDS-15, 27.6% of the participants were screened positive for depressive symptoms. 21.3% of the elderly with moderate type and 6.3% with severe.

Depressive symptoms as estimated with GDS were more frequent (Table 2) in single/ widowed than in married individuals (p=0.000), in participants without supportive environment (p<0.001), in lower-educated (p=0.002), in the elderly with no children (p=0.022) and in older people with a history of myocardial infraction (p=0.000).

MMSE results indicate that half of older participants do not suffer from cognitive impairment and scored in the normal range. Table 3 presents the prevalence of cognitive impairment and MCI among the elderly.

Cognitive decline was strongly associated (Table 4) with advancing age (p<0.001), rural compared to urban living (p=0.007), married/ widowed status in comparison to single/divorced status (p=0.001) and a history of myocardial infraction (p=0.008). Moreover, cognitive impairment was significantly associated with depressive symptoms assessed with GDS-15 (Table 5).

No significant relationship was noticed between gender and both the presence of cognitive impairment or depressive symptoms (p>0.05).

DISCUSSION

According to our results, half of the older participants did not suffer from any type of cognitive impairment whereas 4 had moderate and 27 mild dementia (total 24.4%). Epidemiological data in Greece are sparse and show major variations of prevalence depending on geographical areas. In a study conducted in different settings in the Chrisoupolis health center (HCCh) in northern Greece, 37.6% of the men and 41.6% of the women showed various degrees of cognitive impairment [15]. A recent door-to-door study among 443 participants in a rural population in Crete showed that 9.2% of individuals suffered from dementia with or without depression [16]. Another study comprised 1792 adults 65 years of age or older, with the overall prevalence of dementia reaching 5.0% [17]. Moreover, the present study supports the findings from the literature, that risk factors for cognitive impairment include older age, and modifiable factors include low physical activity, poor social life and cardiovascular health problems [3, 17]. Although lower education is associated with a greater risk for dementia in many studies, no significant association was found in the present study. According to a systematic review in 2011, the level of education which

Table 2. *Depressive symptoms according to GDS-15 in association to various demographic and socioeconomic characteristics ([1]Student t-test, [2]Analysis of Variance).*

Demographic Characteristics (n=127)		N	Mean	SD		Sig.
	Gender					
	Male	64	6.5	3.8	-0.803[1]	0.423
	Female	63	7.0	3.9		
	Age in years					
	65 – 70	55	6.5	3.7	0.215[2]	0.930
	71 – 75	19	7.2	4.3		
	76 – 80	31	6.6	3.7		
	81 – 85	11	6.9	4.0		
	>86	11	7.5	4.4		
	Place of living					
	Urban	69	6.2	3.3	-1.593[1]	0.114
	Rural	58	7.3	4.3		
	Marital status					
	Not married	8	12.9	3.0	9.510[2]	0.000
	Married	74	6.0	3.1		
	Divorced	12	6.8	4.0		
	Widowed	33	7.0	4.2		
	Children					
	Yes	118	6.5	3.6	-2.326[1]	**0.022**
	No	9	9.6	5.4		
Depressive Symptoms (GDS-15)	***Supportive environment***					
	Yes	109	5.9	3.1	-6.675[1]	**<0.001**
	No	18	11.6	4.6		
	Educational level					
	Elementary school	54	7.4	4.3	3.971[2]	**0.002**
	High school	26	8.5	4.6		
	Lyceum	29	5.1	1.6		
	Technical Institute	3	3.7	0.6		
	University	12	4.8	0.9		
	MS/ PhD	3	6.3	3.2		
	Comorbidity					
	Hypertension					
	Yes	105	6.6	3.7	-0.777[1]	0.439
	No	22	7.3	4.6		
	Myocardial Infraction					
	Yes	17	9.8	4.4	3.744[1]	**0.000**
	No	110	6.3	3.5		
	Stroke					
	Yes	12	8.4	3.7	1.602[1]	0.112
	No	115	6.6	3.8		

Table 3. *Prevalence of cognitive impairment among the elderly.*

		N	%
Cognitive Impairmnet	***Normal***	64	50.4%
	MCI	32	25.2%
	Mild dementia	27	21.3%
	Moderate dementia	4	3.1%
	Total	127	100.0%

was associated with the risk for dementia varied by study population, 51 studies reported significant effects of lower education whereas 37 reported no significant relationship. The authors concluded that the risk for dementia was more consistent in developed compared to developing regions [18]. As a screening tool MMSE may overestimate or underestimate cognitive deficits depending on education and usual cognitive activities.

In the present study, the rates of cognitive impairment were higher in elderly rural residents. A number of epidemiologic studies have provided evidence regarding rural–urban differences in the prevalence of dementia and MCI [19,20]. Geographical differences and several sociodemographic factors may explain the higher prevalence of cognitive impairment observed in rural areas. A low education of rural residents as well as low levels of physical, intellectual and social activities that are recognized as risks factor for cognitive impairment may be associated with higher rates of dementia [19,20,21]. Moreover, the distance and limited access to health care providers and community services compared to those living in metropolitan areas, may contribute to the higher rates of dementia among older adults of remote regions [22,23]. Tountas and colleagues conducted a study in which rural patients were more likely to receive suboptimal healthcare because contacts with health care professionals were less frequent than those of urban residents [24]. Another study suggested that rural dementia patients may face barriers to effective ambulatory care and may experience unnecessary hospitalizations [25].

Almost one third of the elderly presented with mild cognitive impairment. MCI is a syndrome defined as cognitive decline greater than that expected for an individual's age and education level. Prevalence in population-based epidemiological studies among the elderly is high; it ranges from 3% to 19% [26, 27]. Tsolaki et al, in Crete estimated MCI: 15.3% and MCI with depression 8.6% among the elderly [16]. Some people with MCI seems to remain stable but more than half will convert to dementia within 2 to 5 years at an accelerated rate [28].

Based on our results, depressive symptoms and cognitive impairment appear to be associated, but the relationship between the two conditions is complex and hard to determine. It remains unclear whether a history of depression is a true risk factor for dementia or rather represents a prodromal clinical phase of cognitive decline. In a recent retrospective study, 30%-50% of dementia cases were accompanied by depression [29]. A study reported depression to be a risk factor for dementia, and found that treating depression is likely to have a great impact on reducing the prevalence of dementia [230]. Depression is a treatable mental health problem, making it a potentially modifiable factor the treatment of which can prevent or delay cognitive decline [31].

In the present study 1 out of 3 of the elderly was estimated to suffer from depressive symptoms. The prevalence of depression in people over 65-year-old shows high variability depending on study design and studied population groups. Compared to the results from a previous study that we conducted in Patras and Tripolis, Peloponnese [32] in 2015 (overall prevalence 48%) the score is lower, but in line with our findings in older ages in Athens and northern Greece, with a prevalence of depression 25% and 35%, respectively [8**,** 33].

Previous studies have identified several stressors that serve as risk factors for late-life depressive disorders, including death of a spouse or other loved one, injuries, disability and functional decline, as well as medical illness especially diseases of the cardiovascular system [6, 32, 33]. As noted previously, the loss of a loved one is one of the most significant risk factors for late-life depression and our elder windowed and not married participants were at higher risk for developing depressive symptoms. Chronic stressors, such as lower income and education level can also influence the development of depressive symptoms later in life [34], which is confirmed in the present study. In the literature female gender has been associated with increased risk of Alzheimer's disease [16, 17] and geriatric depression [32,33], but no statistical difference was observed among genders regarding dementia's forms and depressive symptoms.

This is one of the few studies on elderly residents in a rural area in Greece. It involves a real-life clinical population of patients that attend a general hospital for various health problems. While any insight into potential risk factors that might improve mental health condition of

Table 4. *Cognitive impairment in association to various demographic and socioeconomic characteristics ([1]Student t-test, [2]Analysis of Variance).*

Demographic Characteristics (n=127)		N	Mean	SD		Sig.
	Gender					
	Male	64	26.69	3.1	-.139[1]	0.434
	Female	63	26.76	3.0		
	Age in years					
	65 – 70	55	28.1	2.4	8.097[2]	<0.001
	71 – 75	19	26.9	3.0		
	76 – 80	31	25.5	2.7		
	81 – 85	11	25.5	2.5		
	>86	11	24.2	3.7		
	Place of living					
	Urban	69	27.6	2.5	3.95[1]	0.007
	Rural	58	25.6	3.2		
	Marital status					
	Not married	8	26.9	2.3	5.517[2]	0.001
	Married	74	27.4	2.8		
	Divorced	12	27.3	3.3		
	Widowed	33	25.0	2.8		
	Children					
	Yes	118	26.7	3.0	-0.170[1]	0.865
	No	9	26.9	3.4		
Cognitive Impairment (MMSE)	***Supportive environment***					
	Yes	109	26.9	2.8	1.539[1]	0.126
	No	18	25.7	4.1		
	Educational level					
	Elementary school	54	26.2	2.8	1.909[2]	0.098
	High school	26	26.3	3.4		
	Lyceum	29	27.3	3.0		
	Technical Institute	3	28.7	1.2		
	University	12	27.5	3.0		
	MS/ PhD	3	30.0	0.0		
	Comorbidity					
	Hypertension					
	Yes	105	26.9	2.8	1.729[1]	0.086
	No	22	25.7	3.6		
	Myocardial Infraction					
	Yes	17	24.9	3.1	-2.700[1]	0.008
	No	110	27.0	2.9		
	Stroke					
	Yes	12	25.4	3.2	-1.597[1]	0.113
	No	115	26.9	3.0		

older patients and reduce the number of people affected by depressive symptoms and cognitive impairment is welcome, it's important to recognize the limitations of this research. This is a cross-sectional study that cannot show the direction of cause and effect and no inference can be made. MMSE and GDS-15 are screening tests and their scores may be indicative but not evidential of the diagnoses of dementia /MCI or depression. Furthermore, this is not a clinical study and there were no laboratory results or neuroimaging data to analyze. Therefore, it was impossible to discriminate the type of dementia for each participant. Another limitation of the present study derives from the fact that, the prevalence of depressive symptoms and cognitive impairment depends on the cut-off scores which are used to distinguish between no depression/dementia, moderate/mild and severe form of the disorders respectively, and the validity of this threshold against the clinical diagnosis. Moreover, this sample compounds a small proportion of a specific region of West-Greece and may not be representative of the Greek population and cannot be generalized for the whole older population.

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Immunotherapy application in colorectal cancer. Where do we stand?

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Abstract

The landscape of contemporary cancer therapeutics has changed significantly with the advent of immunotherapy. The constantly expanding indications of immune checkpoint inhibitors have resulted in improved clinical outcomes including colorectal cancer patients. Colon cancer is listed among the most common neoplasms with a quarter of newly diagnosed patients presenting with metastatic disease while a significant proportion of localized cases will eventually develop metastatic lesions. Apart from classic cytotoxic chemotherapy, targeted therapies based on tumor molecular profiling are the mainstay in colon cancer therapeutics. Immunotherapy is incorporated in the treatment algorithms for patients with advanced colorectal cancers whose tumors are found to be microsatellite unstable or mismatch repair (MMR) deficient with significant clinical benefit. On the other hand, patients with MMR proficient/microsatellite stable tumors do not seem to respond as well to immunotherapy. Clinical trials are underway to identify potential mechanisms for improving colorectal cancer patients' outcomes, further deploy immune checkpoint inhibitors application and assess a variety of combinations of targeted therapies and immunotherapy either in the adjuvant or metastatic setting of the disease.

Key words: *Colorectal cancer; metastatic; immunotherapy; microsatellite instability; pembrolizumab; nivolumab*

INTRODUCTION

Colorectal cancer is among the most frequently diagnosed cancer types with high mortality rates [1,2]. Despite the efforts for early detection through screening programs, a quarter of all colorectal cancer patients present with metastatic disease at initial diagnosis while approximately 50% of patients will eventually develop metastases. The prognosis for advanced disease remains unfavorable despite the deployment of therapies [1]. During the last two decades, cytotoxic chemotherapy has been the backbone of treatment, while the addition of targeted therapies based on molecular profiling and identification of actionable mutations of the tumors led to increased survival rates [3]. Thus, novel therapies are under investigation to fulfill the unmet need for effective treatments for patients with advanced disease.

Without a doubt, immunotherapy changed the therapeutic landscape in oncology. Immunotherapy integration in contemporary therapeutics first took place in solid tumors like melanoma and lung cancer where it managed to achieve significantly improved response rates and longer survival. It has also proved to be effective in gastrointestinal cancers, especially hepatocellular and esophageal carcinoma, renal and urothelial cancer, squamous head and neck cancers while indications continue to expand to several neoplastic diseases [4].

In 2017, immunotherapy was approved by the FDA

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for the treatment of microsatellite instability high (MSI-h) or mismatch-repair-deficient (dMMR) metastatic colorectal cancer. This population represents only a small fragment of all metastatic colorectal cancer patients [5]. On the other hand, patients with pMMR tumours do not seem to gain a similar benefit. The field of immunotherapy in colorectal cancer either as monotherapy or in combination remains challenging. This review aims to summarize the current status of immunotherapy application in CRC through the existing literature and appose future perspectives.

Rationale for immunotherapy application in colorectal cancer

Colorectal cancer not being conventionally regarded as an immune sensitive tumor, it is worth to investigate the primary implications of applying immunotherapy against it. It has been more than a decade since the significance of cytotoxic and helper immune T cells infiltrating the tumor microenvironment, has been recognised as a major prognostic factor of recurrence risk in patients with early-stage colorectal cancer [6]. Sequentially, this led to the establishment of "Immunoscore", an immunohistochemical assessment of the proportion of co-stimulatory CD3 and cytotoxic CD8 T lymphocytes present within the tumor microenvironment. Immunoscore was thereafter investigated as a prognostic marker of the recurrence probability of early-stage colorectal cancer after therapeutic surgery, as well as a probable predictive marker of adjuvant chemotherapy benefit [7], but it was not incorporated in routine clinical practice. Nonetheless, these observations set the pathway towards the deployment of the immune system against colorectal cancer [8].

In the era of gene-expression based research, molecular subtyping has been applied to colorectal cancer, identifying four subtypes: CMS1 or MSI-Immune, CMS2 or Canonical, CMS3 or Metabolic and CMS4 or Mesenchymal. CMS1, accounting for 14% of colorectal cancers, is characterized by a higher level of immune activation, probably associated with the molecular phenomenon of microsatellite instability, compared to the rest three, microsatellite stable types CMS 2, 3 and 4 [9,10]. This identification set a rational basis of employing immunotherapeutic approaches in colorectal cancer treatment, as well as for using microsatellite instability as a predictive biomarker of any probable clinical benefit.

Microsatellite instability (MSI) is a molecular characteristic implying defective DNA damage repair mechanisms, resulting in a disruption of repetitive DNA sequences, known as DNA microsatellites. The underlying mechanism is the loss or silencing of genes encoding four enzymes involved in the mismatch repair machinery, MLH1, MSH2, MSH6 and PMS2, a phenomenon described as mismatch repair deficiency (dMMR) [11,12]. MSI may be detected by PCR (Polymerase Chain Reaction) or NGS (Next Generation Sequencing) in either blood or paraffin tissue specimen, while dMMR is examined by applying immunohistochemistry on the tumor specimen, in order to check for the presence of all four mismatch repair enzymes. Although MSI/dMMR was originally identified among Lynch syndrome carriers, there has been evidence that these genetic characteristics may also arise from somatic tumor mutations and may be present in non-Lynch syndrome patients as well [13-15].

Genomic instability is thought to give rise to neoplastic neoantigens, prone to be detected and activate antigen presenting and cytotoxic immune cells, thus supporting the emerging role of pharmaceutical immune activators, in the treatment of MSI-high/dMMR CRC. Consequently, MSI/dMMR have been used as predictive biomarkers, promising to distinguish CRC patients more probable to benefit from immunotherapy agents, such as the widely employed immune checkpoint inhibitors [16,17].

MSI may as well be the result of epigenetic silencing of the involved repair genes [11,12]. Specifically, methylation of the promoter of the MLH1 gene, may result into genomic instability, due to reduced MLH1 production, without loss or mutation of the coding area, often co-existing with BRAF V600E mutation [18]. Similarly, deletion of the EPCAM (Epithelial cell adhesion molecule) protein may result to MSH2 epigenetic methylation and silencing, thus leading to genomic instability and subsequent higher tumor immunogenicity [11,12].

Tumor mutational burden (TMB), stands out as a distinctive hallmark of tumor genomic instability and the basis for increased neoantigens variability, also emerging as an alternative predictive immunotherapy biomarker, detectable with molecular sequencing. Indeed, colorectal carcinomas with high mutational load have been shown to be more responsive to immunotherapy [19-22]. Although microsatellite instability and high tumor mutational burden both account for tumors rich in neoantigens, thus easily perceived by the hosts' immune system and susceptible to immunotherapeutic agents, they should not be regarded as one and the

same. In fact, increased tumor mutational burden, may issue from genetic and molecular deficits, other than mismatch repair deficiency, such as mutations in the exonuclease domain of DNA polymerases POLE and POLD1. Such mutations, also involved in familial colorectal and endometrial cancer cases, due to their high penetrance, compromise the proofreading capacity of the mutated enzymes, leading to accumulation of DNA misallied nucleotides during the DNA duplication phase [23,24]. Evidently, POLE and POLD1 mutations may give rise to microsatellite stable but hypermutated tumors; consequently, TMB and MSI/dMMR may be regarded as distinctive hallmarks of tumor neoantigen enrichment and may be independently examined as two separate predictive biomarkers of immunotherapy susceptibility [25].

Multiple immunotherapeutic strategies have been investigated so far, including interferon administration [26], CAR T-cells engineering (Chimeric antigen/antibody receptors T-cells) [27], vaccination with antigen presenting cells exposed to tumor neoantigens [28] or with viral vectors transporting genes of immunostimulatory molecules [29] and, remarkably, immune checkpoint inhibitors (ICIs). Indeed, ICIs have been successfully incorporated in everyday practice of clinical oncology during the last decade, providing realistic therapeutic solutions against solid tumors and hematologic malignancies, insensitive to traditional chemotherapeutic approaches [30,31].

Monoclonal antibodies targeting PD-1 (Programmed Death 1) and PD-L1 (Programmed Death ligand 1), are the most widely applied, because of their effectiveness and their manageable toxicity profile. Pembrolizumab [32] and nivolumab [33], both bind and inhibit PD-1, a receptor found on cytotoxic T-lymphocytes, which, when activated, suppresses T-lymphocyte expansion and activation; thus, its inhibition by anti-PD-1 monoclonal antibodies unleashes the cytotoxic potential of T-lymphocytes, against cancer cells. Especially nivolumab, is often co-administered with an older checkpoint inhibitor, ipilimumab [34,35]; this latter, blocks another T-lymphocyte molecular brake, a surface molecule named CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4) and it has been the first immune checkpoint inhibitor ever put into clinical practice [36]. As anti-CTLA-4 monotherapy with ipilimumab had a satisfactory effectiveness level only at high doses, at the cost of severe toxicities, its administration at lower doses, combined with nivolumab, has been established as a preferable strategy [37,38]. Cemiplimab is another anti-PD-1 monoclonal antibody, which has shown to be active against squamous cell carcinomas, non-small cell lung cancer and cervical cancer [39-41].

The anti-PD-L1 antibodies, such as durvalumab, atezolizumab and avelumab were later developed, targeting the PD-1 ligand, PD-L1, a molecule often located on the surface of tumor cells. It seems that PD-L1 binds with PD-1, activating the downregulation of cytotoxic lymphocytes, impeding their antineoplastic activity [33]. Their efficacy has also been proven in several clinical trials, mostly in combination with chemotherapy [42,43], as maintenance treatments after response to first line treatment chemotherapy [44,45], as an alternative to chemotherapy when the latter is contraindicated due to patient comorbidities [46] or even as first line treatment options in non-chemosensitive neoplasms [47].

Based on the above, immunohistochemistry for PD-L1, either solely on cancer cells or in both immune and tumor cells, is employed as a predictive biomarker for ICIs against NSCLC [48], urothelial cancer [49], head and neck malignancies [50] and upper gastrointestinal tract tumors [51], despite its many controversies. Nonetheless, in colorectal cancer it is substituted by dMMR/MSI and TMB, which have been employed in the clinical trials of ICIs against colorectal malignancies, as predictive biomarkers.

Immunotherapy in the treatment of dMMR/MSI-high colorectal cancer

Given that MSI-high/dMMR has been established as an efficient predictive marker of immunotherapy benefit in colorectal cancer patients, it has served as a major patient recruitment criterion in pivotal immunotherapy trials.

The anti-PD-1 agent pembrolizumab, managed to induce objective responses in 40% of the MSI-high colorectal cancer patients, with a subsequent 20-week PFS of 78%, in a phase II single arm trial [52]. In KEYNOTE-164, monotherapy with pembrolizumab at 200mg every three weeks, induced objective responses in pretreated patients with MSI-high colorectal cancers ranging from 21 up to 46% as a second or further line of treatment; responses were durable, as the median duration of response was not reached, during a follow up lasting up to 35.6 months. Severe adverse events of grade 3 or greater, affected about 13-16% of patients [53].

In the practice-changing clinical trial KEYNOTE-177 [54], pembrolizumab (200mg every 3 weeks), managed to induce superior clinical outcome in MSI-high colo-

rectal cancer, treatment naïve patients, compared with 5-fluorouracil based chemotherapy, with or without anti-VEGF and anti-EGFR targeted agents. Pembrolizumab monotherapy reduced the probability of disease progression by 40%, prolonging median PFS from 8 to 16.5 months, and OS from 11 to 13.7 months, while it induced an overall response rate of 44% versus 33% for traditional antineoplastic treatment. 22% of patients on pembrolizumab monotherapy experienced severe adverse events, as opposed to two thirds of patients in the chemotherapy arm [54]. A later assessement of quality of life of this study population determined that patients on pembrolizumab were twice as probable to maintain their level of physical and social activities, compared to patients receiving chemotherapy [55]. Based on the above, pembrolizumab is now the recommended choice of treatment in the first line setting of metastatic patients with MSI-high colorectal cancer, being both tolerable and effective in this population.

Nivolumab has also shown significant clinical activity against MSI-high colorectal cancer. It has been examined as a second line treatment of metastatic pretreated patients, as a sole agent [56], as well as in combination with the anti-CTLA-4 agent ipilimumab [57], in the trial Checkmate 142. As monotherapy, (at 3mg/kg every 2wks), it managed to induce responses in one third of the patients, with most of them lasting beyond 3 months, with a PFS of 1 year, and a manageable toxicity profile (grade >3 AEs in up to 8% of patients) [56]. When co-administered with ipilimumab (1 mg/kg every 3weeks) for the first 4 cycles, overall response rate increased up to 52 and 57%, for patients not experiencing and experiencing immunotherapy related toxicity, respectively. Severe AEs associated with the combination were observed in 32% of patients [57]. More interestingly, in a third, more recent checkmate-142 cohort, nivolumab was administered to treatment naïve MSI-high colorectal cancer patients, achieving an ORR of 69%, with complete responses in 13% of patients, and a disease control rate of 84%. Median duration of response was not reached, while 74% were free of disease progression at 2 years of treatment [58]. Although not yet head-to-head compared to chemotherapy, the combination of ipilimumab with nivolumab is now considered as a safe and effective option for the treatment of metastatic MSI-high colorectal cancer, even in the first line setting.

As for the anti-PD-L1 agents, avelumab has been explored as a second line treatment of MSI-high/dMMR colorectal cancer, including also tumors hosting POLE mutation. It was used at 10mg/kg every 2 weeks, resulting in an ORR of 24%, with median response duration of 14 months and a median PFS of 8.1 months among MSI-high cancer patients [59]. So far, no anti-PD-L1 antibody has gained approval against MSI-high colorectal cancer.

Immunotherapy in patients with pMMR/MSS colorectal cancer.

Although patients with dMMR/MSI-H colorectal cancer, experience durable responses and prolonged survival rates, patients with pMMR disease do not seem to benefit from these therapies, either as monotherapy application or as double inhibition. Extensive research has been done so far to better comprehend the profile of pMMR colorectal cancer. The main goal is to increase tumor immunogenicity to achieve responses to immunologic therapies. Most trials investigating immunotherapy in MSS and/or mixed population mainly focus on combinations of immune checkpoint inhibitors with standard chemotherapy (5-fluorouracil, oxaliplatin, irinotecan), radiotherapy, or targeted therapies and explore potential biomarkers, other than MSI. There is evidence that chemotherapy alters the intratumoral environment through the induction of immunogenic cell death. [60]. Radiotherapy is also related to induced immunogenic cell death; it increases the number of infiltrating T cells while also having the abscopal effect [61]. Moreover, targeted therapies for metastatic CRC such as anti - EGFR and anti-VEFG antibodies (cetuximab and bevacizumab respectively), seem to enhance the immunotherapeutic effects [62].

Several international studies have been conducted to evaluate the efficacy of PD-L1 agent combined with antiangiogenesis. The researchers of the BACCI trial, a placebo-controlled randomised phase II study, assessed the efficacy of atezolizumab combined with capecitabine and bevacizumab in metastatic colorectal cancer. The study population consisted mainly of MSS metastatic colorectal cancer patients. The entire study population reached a better PFS with the addition of atezolizumab. Especially, regarding the pMMR population, the PFS benefit from atezolizumab was notable, however RR and OS remained almost the same [63].

Another study aiming to evaluate the use of bevacizumab and atezolizumab in this setting based on biomarkers was the MODUL trial, a randomised phase III international umbrella trial. Patients with wild-type BRAF colon cancers underwent therapy with FOLFOX and bevacizumab followed by maintenance therapy of

fluorouracil and bevacizumab either with or without atezolizumab as first-line treatment for mCRC. This study was a negative trial as PFS and OS were similar in both study arms [64].

The IMBlaze 370 trial assessed the efficacy of atezolizumab in addition to the MEK inhibitor cobimetinib. The study included 363 patients previously treated for metastatic colorectal cancer, stratified into three arms. The first one received the combination atezolizumab - cobimetinb, the second arm received atezolizumab alone and the third was on regorafenib. Most of the patients harbored microsatellite stable tumors. The median overall survival was 8.9 months for the atezolizumab - cobimetinib arm, similar to regorafenib which was 8,5 months. Atezolizumab monotherapy failed to improve mOS. In general, none of the three arms achieved significant differences in terms of OS, PFS, OR [65]. Moreover, in a small study combining the anti-PD-1 agent SHR-1210 with apatinib, in MSS mCRC patients, no benefit was achieved, either in OS or PFS [66].

Anti-PD-1 agent nivolumab has been tested in different combinations, in a series of clinical trials. As mentioned above, in the CheckMate 142 phase II study, the nivolumab plus ipilimumab combination achieved objective response rates as high as 69%, among MSI high mCRC patients [56-58]. As for MSS/pMMR mCRC, Li J. et al, in a retrospective review of 23 pretreated patient cases of MSS/pMMR mCRC, noticed that the combination of variable anti-PD1 monoclonic antibodies, with the VEGFR inhibitor regorafenib, induced a disease control rate of 78.3%, although without any benefit in terms of overall response rate and a modest median PFS of 3 months [67]. Furthermore, the Japanese REGONIVO phase Ib study showed that the combination of nivolumab and regorafenib had emboldened results in response rate [68]. On the contrary, Fakih M et al recently reported the results of a single-arm phase II study, where the same combination, resulted in worse outcomes in the North American population [69].

Moreover, a significant number of clinical trials is evaluating the synergistic effects of immunotherapy and anti-EGFR antibodies combination in MSS CRC patients. The CAVE colon phase II trial analyzed the effectiveness of avelumab combined with cetuximab as a rechallenge in pretreated, RAS wild type, pMMR metastatic colorectal cancer patients. The trial met the primary endpoint reaching a median OS of 11,6 months, suggesting that the combination represents an active, well-tolerated therapeutic option [70]. Similarly, in the AVETUX trial, mFOLFOX6 combined with cetuximab and avelumab was tested in patients with RAS/BRAF wild type, mCRC. From a total study population of 43, 40 patients harbored pMMR tumors. The results of this single-arm phase II study, indicate a high response rate in MSS patients [71].

Preclinical data indicating a potential synergistic effect between immunotherapy and radiotherapy application led to the investigation of the combination in small scale clinical trials. Published results suggest a manageable toxicity profile and noticeable responses in patients with advanced pretreated pMMR metastatic disease and guarantee the further exploration of this strategy [72].

Future perspectives

Currently, numerous clinical trials are exploring the possibilities of immunotherapy in the treatment of metastatic colorectal cancer [73]. Tolerability and efficacy of administrating immune check point along with targeted treatments, is still under investigation, in phase I/II trials, such as NCT03657641, combining pembrolizumab with regorafenib, in colorectal cancer patients beyond the 2nd line of treatment. The MAP kinases pathway inhibitors encorafenib (BRAF inhibitor) and binimetinib (MEK inhibitor), have already proven their value, showing clinical benefit in patients carrying the BRAF V600E mutation, resulting in the recent approval of combination of the anti-EGFR monoclonal antibody cetuximab and encorafenib for the treatment of BRAF mutated colorectal cancer [74]. At present, co-administration of encorafenib and binimetinib along with nivolumab (NCT04044430) is under examination, in MSS stable, BRAF V600E mutation carriers.

Chemotherapy in combination with immunotherapy is also an intriguing option; temozolomide is a well-established alkyliotic agent, applied against glioblastoma multiform. It seems that resistance against temozolomide is mediated by a DNA repair enzyme, known as O6-methylguanine DNA methyltransferase (MGMT), whose methylation and epigenetic silencing confer susceptibility to temozolomide [75]. Building on that, temozolomide is now tested in combination with nivolumab and ipilimumab, against MSS stable but MGMT methylated metastatic colorectal cancer (NCT03832621). Similarly, the combination of avelumab with irinotecan and cetuximab, (NCT03608046) as well as of pembrolizumab with oxaliplatin, capecitabine and bevacizumab are also under investigation

(NCT04262687), against treatment refractory, pMMR stable colorectal cancer.

Immune checkpoint inhibitors may also be combined with novel agents; ALX148 is a new immune checkpoint inhibitor, binding on CD47, a molecule found on cancer cells, serving to the suppression of immunostimulatory potential of myeloid cells [76]. Phase II trial NCT05167409 is using triple blockade with AXL148 (anti-CD47), pembrolizumab (anti-PD-1) and cetuximab (anti-EGFR), against chemotherapy refractory, MSS stable colorectal cancer. More recently, the comparative study NCT04854434, has started recruiting colorectal cancer patients carrying RAS mutations, aiming to assess the potential benefits of combined treatment with selinexor and pembrolizumab. Selinexor is a novel oral agent, inhibiting exportin 1, an intracellular protein involved in the transport of oncogenic mediators from the nucleus to the cytosol, promoting oncogenesis, already used against hematologic malignancies [77].

Immunotherapy has not yet been incorporated in the adjuvant/neoadjuvant treatment setting of colorectal cancer. Nonetheless, a recent, small exploratory phase I trial [78], showed that the administration of ipilimumab plus nivolumab, was associated with pathologic response rates of 100% and 27% among MSI-high and MSS stable patients, respectively. Moreover, pembrolizumab, together with vactosertib, an inhibitor of the TGF-beta oncogenic pathway [79], is now examined in patients having undergone hepatic metastasectomy (NCT03844750), in addition to classic perioperative chemotherapy.

CONCLUSION

Colorectal cancer still imposes major therapeutic challenges on patients and physicians, regardless of the broad variety of antineoplastic treatment choices nowadays available. Up to date, microsatellite instability has served as the cornerstone of applying immunotherapy against colorectal cancer, without providing realistic solutions for pMMR, non-hypermutated colorectal cancer types. Ongoing research aims to overcome this barrier, as well as to provide clinicians with proficient, evidence-based treatment algorithms, so that immunotherapeutic, targeted, and cytotoxic agents may be administered, sequentially or contemporarily, in a way that maximizes clinical benefit.

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PET/CT in the management of cancer patients

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Abstract

PET/CT is a new generation, hybrid, whole-body imaging modality, which combines the functional imaging of cellular metabolism with Positron Emission Tomography (PET) plus the detailed depiction of human anatomy with Computed Tomography (CT). Nowadays, it is an established modality with approved clinical indications in oncology, while it has been incorporated in evidence-based diagnostic algorithms and guidelines. By reviewing recent literature, the current paper illustrates, concisely, the main strengths, limitations, and whole spectrum of clinical applications of PET/CT in the management of oncological patients.

Key words: *Positron emission tomography (PET); PET/CT; hybrid imaging; cancer; oncology*

INTRODUCTION

PET/CT is a state-of-the art, hybrid, whole-body imaging modality, which combines two methods in a single session: the functional tracing of cellular metabolism with Positron Emission Tomography (PET) plus the detailed, high-resolution depiction of human anatomy with Computed Tomography (CT). Since its initial experimental introduction in the 90's, PET/CT has considerably evolved and been implemented into routine oncological practice. Nowadays, it is an established modality with miscellaneous clinical indications, while it has been incorporated into various evidence-based algorithms in Oncology [1]. PET/CT is no longer regarded as a "luxury" but as a mainstay of routine clinical practice affecting treatment decisions in oncological patients.

More than 90-95% of PET/CT studies are performed with the use of the radioactive tracer ^{18}Fluoro-Deoxy-Glucose (FDG). FDG is a non-specific tracer, yet it has proven extremely efficient in clinical practice. It is a glucose analogue labelled with radioactive ^{18}F, which emits positrons. FDG is injected intravenously, rapidly distributes throughout the body and shows avid uptake by cancerous cells (Figure 1). Malignant cells have increased metabolic needs, which are fulfilled by anaerobic glycolysis; hence, these cells show avid uptake of glucose and glucose analogues like FDG. Within the cancer cell, FDG is not metabolized, but it continuously accumulates, being trapped into the cytoplasm.

FDG-PET/CT imaging is performed in two stages. First, CT is performed for attenuation correction of the PET images and anatomical localization. Then, PET is conducted detecting the photons emitted from FDG. Both PET and CT images are finally combined together into fusion images [2,3]. Fusion provides us with valuable information regarding the size, morphology, location, and extent of malignant lesions plus their metabolic activity. Fused PET/CT exhibits inherent advantages in oncological imaging:

- It shows high sensitivity for the detection of malignant lesions even in regions with normal anatomy;

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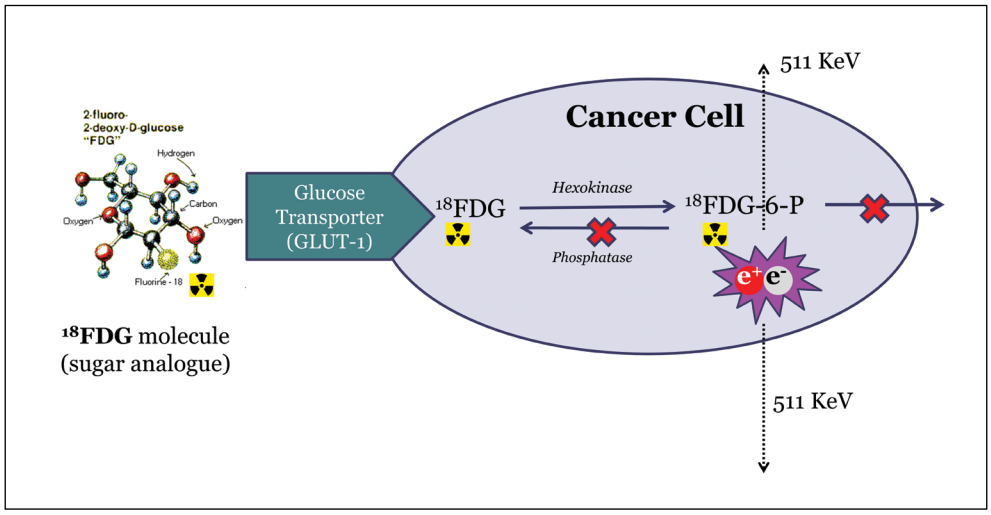

Figure 1. FDG uptake by the malignant cell. ^{18}Fluoro-Deoxy-Glucose (FDG) is avidly taken up by the cancer cell. This transport is facilitated by the Glucose Transporter enzymes (GLUT). Inside the cell, FDG is phosphorylated via the hexokinase, but, unlike normal glucose, it does not enter the Krebs cycle and does not undergo further metabolism. Thus, FDG is continuously trapped inside the cytoplasm, and positrons are emitted by the radioactive ^{18}F. The positrons annihilate with human body electrons and two gamma-ray photons with energies of 511 KeV are emitted in the opposite directions. The photons penetrate the human body and are detected by PET crystals of the scanner.

hence, PET/CT implementation may result in early cancer detection.

- It detects neoplastic lesions in regions, not easily evaluated by conventional anatomical imaging methods (CT/MRI) due to distorted anatomy after previous surgery, radiotherapy or other treatments.
- Post treatment, it may accurately distinguish between metabolically active, viable neoplastic tissue and non-viable, necrotic or fibrotic residual tissue.

PET/CT achieves high diagnostic accuracy, which translates into significant changes of the therapeutic strategy in almost 30% of oncological patients. The major clinical indications of PET/CT are summarized into: a) initial staging of neoplastic disease, b) evaluation of treatment response and c) cancer restaging plus timely and accurate detection of disease recurrence [1] (Table 1).

The main limitations of FDG-PET are the false positive findings in cases of active inflammation. FDG is a non-specific tracer and may be taken up by macrophages and lymphocytes, which accumulate in inflammatory regions. As a result, various inflammatory entities

Table 1. *Main Clinical Applications of PET/CT.*

Initial Diagnosis	evaluation of solitary pulmonary nodule, investigation for cancer of unknown primary especially in head-neck cancers with cervical nodal metastases
Initial Staging	Lymphomas, Non-Small Cell Lung Cancer, Head-Neck Cancer, Advanced-Stage Melanoma, Oesophageal Cancer, locally advanced Cervical or Breast Cancer, Bone Sarcomas
Treatment Response Evaluation	Lymphomas, Lung Cancer, Head-Neck Cancer, GISTs, Oesophageal Cancer, Breast Cancer, Melanoma, Seminoma
Restaging – Evaluation for Recurrence & Disease Follow-up	Lung & Head-Neck Cancer, Melanoma, Gastrointestinal & Gynecological Cancers
Neoplastic disease detection in case of increased tumor marker levels	ovarian, colorectal, breast & thyroid (increased thyroglobulin levels with negative iodine whole body scan) neoplasms
Guidance of Biopsy	e.g., in suspicious nodal or distal metastases in lung cancer
Radiotherapy Treatment Planning	in cases of lung cancer to differentiate between tumor and adjacent atelectasis

may act as sources of false positive findings such as sarcoid, tuberculosis and other infections, abscesses, abdomino-pelvic inflammations, and post-traumatic/ post-surgical changes. There are also benign tumors and other entities showing false-positive, increased FDG uptake including Warthin tumors, benign bone tumors, inflammatory pseudo-tumor, thyroid adenomas, and uterine fibroids.

On the flip side of PET-positive inflammation, there are neoplasms with slow metabolic rate, low glucose metabolism, thus low FDG uptake: differentiated thyroid cancer and prostate cancer with some exceptions, indolent lymphomas, low-grade sarcomas and neuro-endocrine tumors (NETs) and some mucinous carcinomas. These may result in false negative findings.

Reporting of PET/CT studies is mainly based on visual image interpretation. The nuclear medicine physician evaluates the intensity of FDG uptake by suspicious target lesions and compares this uptake with adjacent background FDG activity in order to, finally, decide whether the lesion is abnormal/malignant. To improve this subjective, reporter-dependent approach, quantification of FDG uptake is applied by means of a numeric ratio, Standardized Uptake Value, SUV=Activity in a specified region of interest/ Total injected Activity normalized by body weight. Higher SUV values correspond to higher probabilities of malignancy; however, they cannot, alone, substitute for visual, qualitative interpretation. SUV values show considerable overlap between malignant and benign-inflammatory lesions with no absolute cut-off thresholds being specific for malignancy. Moreover, SUV calculations show significant variability dependent on multiple factors including body habitus, blood glucose levels, uptake time, type of PET/CT scanner and software, image reconstruction algorithms, timing of treatment and size of the lesion.

Clinical Applications

PET/CT is the imaging method of choice in the initial staging of the majority of lymphomas, with the most frequent types of Hodgkin's, Diffuse Large B cell and follicular lymphomas showing increased FDG uptake [4]. PET/CT brings increased sensitivity in the detection of lymphomatous nodal disease even in small/normal-sized nodes. It is characterized by higher sensitivity than CT in the detection of extra-nodal disease, specifically in the spleen and bone marrow. PET/CT findings lead to upstaging in up to 25% of Hodgkin lymphomas, and this translates into intensified therapy. It has excellent Negative Predictive Value (NPV>95%) in the detection of bone marrow involvement in Hodgkin's lymphoma [5], essentially replacing bone marrow biopsy [6,7]: a negative PET rules out bone marrow disease in Hodgkin's patients. PET/CT is superior to other imaging methods in the initial staging of aggressive non-Hodgkin lymphomas detecting occult disease in sites not previously suspected [8].

Apart from staging, PET/CT is applied in the early therapeutic evaluation of Hodgkin's lymphoma in the form of interim PET/CT performed after 2-3 initial cycles of chemotherapy [9]. Patients with negative interim PET and no hypermetabolic lesions identified may continue with the same effective treatment or switch to less aggressive, less toxic protocols. On the contrary, patients who do not show PET response may be subjected to more aggressive treatment in order to eradicate hypermetabolic active disease. Randomized controlled trials have proven that interim PET/CT shows high NPV for final treatment response and for increased progression free survival in Hodgkin's lymphoma [10-12]. Recently, the accuracy in reporting and interpretating interim and post-treatment PET/CT studies has increased by applying specific objective criteria: Deauville 5-scale criteria [13]. High Deauville uptake score of 4-5, in case FDG uptake in lesions exceeds liver activity, corresponds to active neoplastic disease. FDG uptake in lesions, which is equal or lower than in the mediastinal blood-pool, is interpreted as negative for disease: Deauville score of 1-2.

PET/CT is the imaging method of choice for final post-treatment assessment of lymphomas showing excellent NPV and superior diagnostic accuracy compared with CT [14]. After treatment, a significant proportion of patients show residual anatomic lesions on CT; yet, in only a small minority of cases, these lesions correspond to residual disease. PET/CT has high diagnostic accuracy in the evaluation of residual tissue and may distinguish between PET-negative fibrotic/necrotic tissue and PET-positive, active residual disease (Figure 2). The modality also has high NPV in the evaluation of megatherapy before stem cell transplantation. In this setting, a favorable PET response is associated with better progression free survival and overall survival [15].

PET/CT has valid clinical roles and several applications in the evaluation and management of lung cancer. In the assessment of solid pulmonary nodules of sufficient size (≥8-10 mm), it shows high sensitivity (~90%) in detecting lung cancer. The specificity is somewhat lower (~80-85%) due to inflammatory, false positive nodules

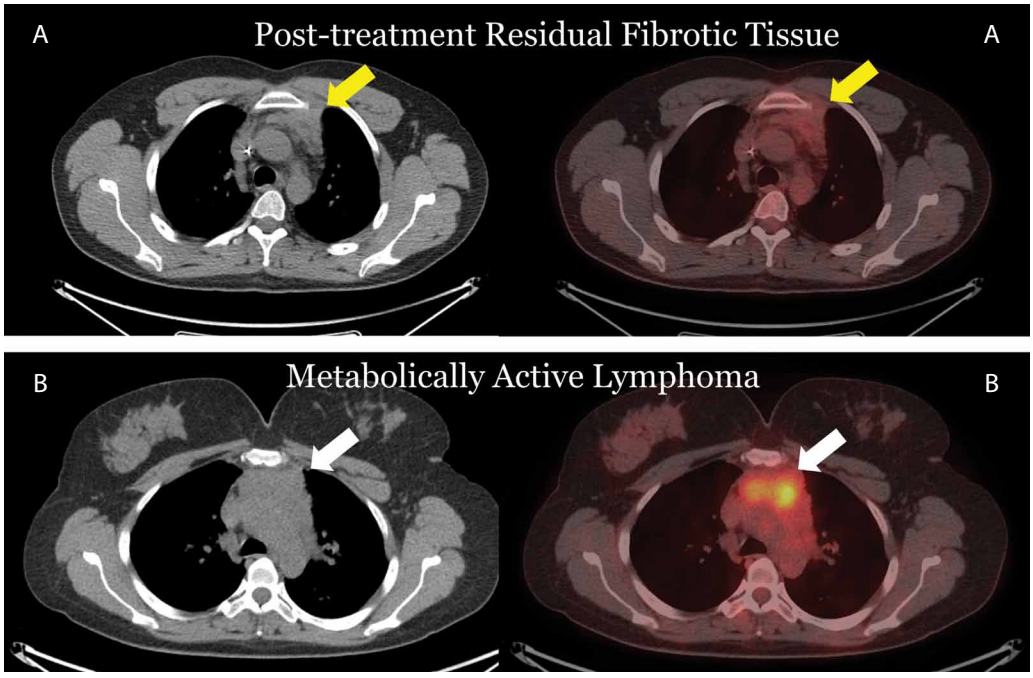

Figure 2. Hodgkin's Lymphomas post treatment. A. Treatment response with residual anterior mediastinal mass not showing increased FDG uptake (yellow arrow). B. Case of residual active disease with lobulated mediastinal soft-tissue mass showing increased metabolic activity (white arrow).

in cases of sarcoid, tuberculosis or other granulomatous disease and infections [16,17]. Pulmonary carcinoids and adenocarcinomas in situ may be false negative on PET, especially when the latter appear as pure ground-glass or semi-solid nodules [18].

PET/CT brings improvements in the accurate staging of lung cancer and optimizes therapeutic decisions [19]. It shows superior diagnostic accuracy compared with CT in the evaluation of mediastinal nodes with sensitivity >80% and specificity >90%. PET helps in overcoming the limitations of CT, the latter relying on size (short axis >1 cm) and morphological criteria: small and normal-sized nodes may harbor neoplastic disease, while slightly enlarged nodes may be reactive, not associated with malignancy. PET can, however, miss occult mediastinal disease, especially in large central tumors [20,21]. Therefore, it cannot replace minimally invasive methods of nodal sampling in all patients, but rather guide sampling to specific suspicious nodal stations.

PET/CT is the modality of choice for the detection of distal metastases with sensitivity and specificity ≥ 90%. Almost 20-35% of newly diagnosed lung cancers are already metastatic, with 40% of these patients having skeletal metastases (Figure 3). PET can detect hypermetabolic bone lesions in early stage before inducing anatomical skeletal changes such as bone destruction-lysis or sclerosis. The modality may distinguish between adrenal metastases and benign adenomas with high accuracy. It detects metastases in unsuspected regions such as soft-tissue deposits and hypermetabolic subcutaneous nodules. PET/CT implementation leads to changes in clinical staging in a significant proportion of patients (25-60%), thus resulting in corresponding treatment modifications: avoidance of futile thoracotomies, accurate definition of treatment volumes in radiotherapy planning, change of therapeutic plan from a curative to a palliate approach etc.

In the evaluation of treatment response, PET/CT is increasingly being applied as it shows inherent advantages: it may distinguish between viable tumor and post-treatment changes, it evaluates the whole tumor burden and may reveal metabolic treatment response irrespective of anatomical changes which may underestimate treatment effect. In restaging, PET/CT is applied as a problem-solving tool to detect residual or recurrent disease, especially when there are equivocal or difficult to interpret findings by other imaging methods.

PET/CT is not typically indicated for the initial diagnosis and detection of primary head-neck squamous cell carcinomas with the exception of unknown primary

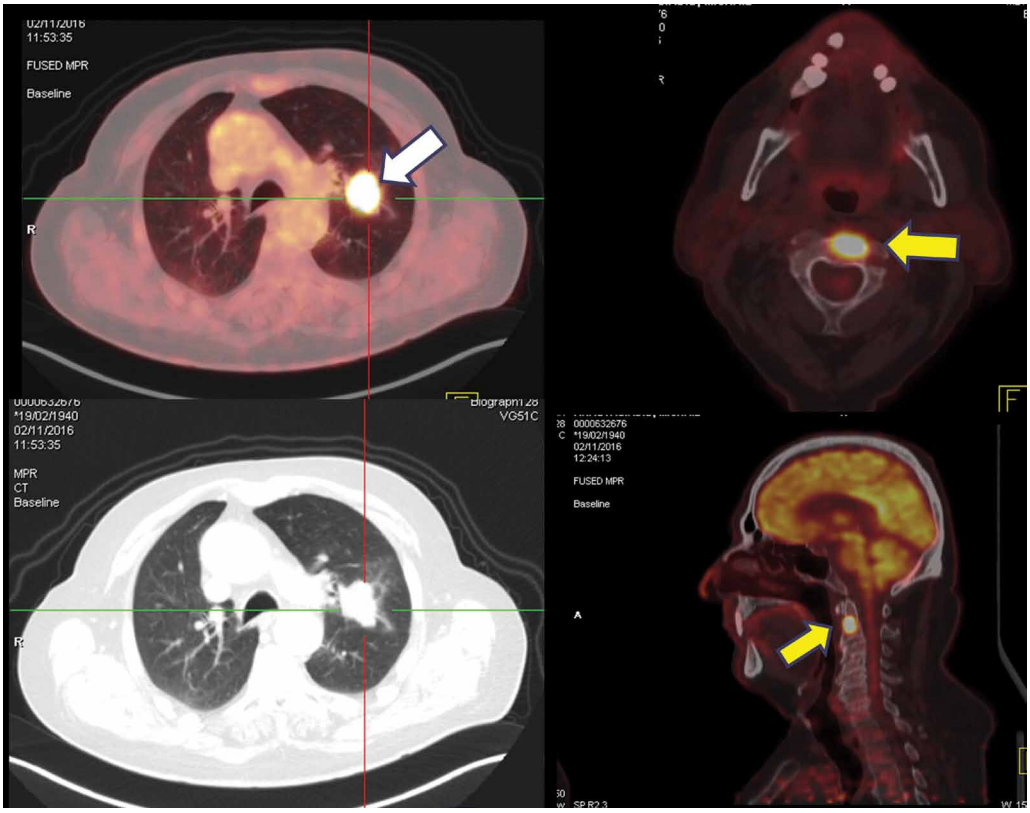

Figure 3. Metastatic lung cancer. Hypermetabolic left lung cancer mass (white arrow) with a solitary FDG-avid metastatic bone lesion in C2 vertebra (yellow arrow).

cancer presenting with cervical nodal metastases [22-24]. The diagnostic detection yield, in this setting, is around 25-55%. Most frequently, the primary tumor resides in the palatine or lingual tonsils or in the tongue base. The detection of the primary tumor has critical prognostic and therapeutic implications, since it can guide surgical planning, mode of neck dissection or definition of radiotherapy volumes [25]. PET/CT has superior diagnostic accuracy compared with other modalities for the evaluation of disease-involved nodes in head-neck cancer [22,26] (Figure 4). It is the modality of choice for the detection of distal metastatic disease and other synchronous tumors [24,27]. It is indicated by oncological guidelines for the accurate evaluation of post-treatment neck, after radical chemo-radiation therapy [22]. It is highly accurate in the assessment of post-treatment neck, which is hindered by extensive post-surgical changes including flap reconstructions and neck dissections, distortion of normal anatomy, oedema, asymmetries, and obliteration of fat planes. PET/CT has high NPV post radical chemo-radiation therapy sparing these patients from unnecessary morbid nodal dissections [28,29]. In the differentiated thyroid cancer, PET/CT has only one major clinical application: the evaluation of patients with increased Thyroglobulin levels and negative whole-body iodine scans indicating disease de-differentiation and more aggressive clinical behavior [30].

PET/CT is not routinely indicated in early stage I-II melanomas offering no significant diagnostic or prognostic information [31]. The method has unacceptably low sensitivity (30-50%) in the determination of regional lymph node status, since it cannot detect metastatic burden in small nodes <5-10 mm; hence, it cannot substitute for sentinel node biopsy, which remains the clinical standard of care in the evaluation of locoregional nodal status [32]. The diagnostic benefit of PET/CT implementation increases as the clinical stage increases. In melanomas with high Breslow thickness (>4,0 mm), the modality can detect disease-involved regional lymph nodes and additional distal metastases in almost 30% of patients affecting subsequent treatment strategy. PET/CT is indicated by clinical guidelines in stage III-IV melanomas and has been included in the correspond-

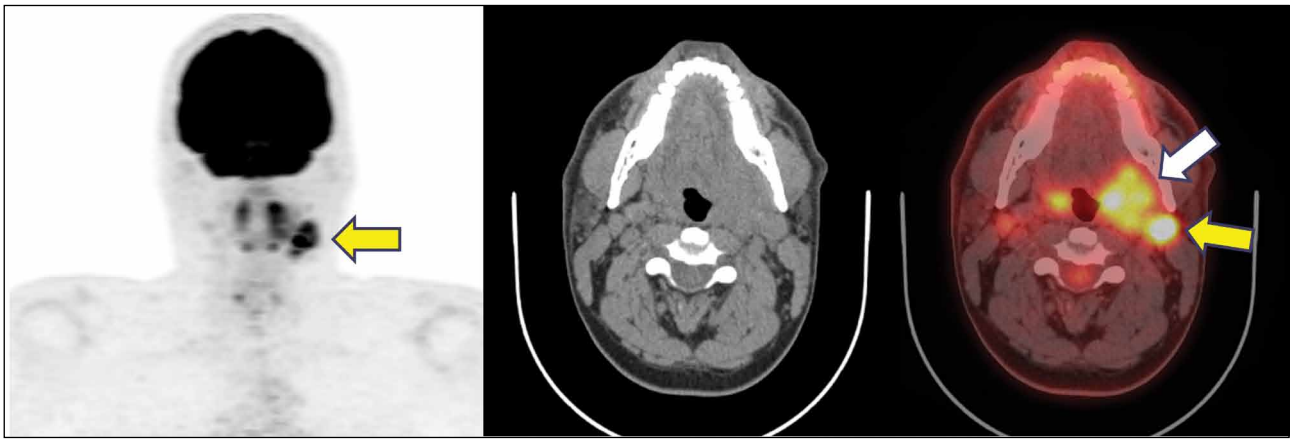

Figure 4. Oropharyngeal cancer. Case of oropharyngeal cancer with hypermetabolic enlargement of the left palatine tonsil (white arrow) obliterating the adjacent parapharyngeal space. There are FDG-avid, disease-involved ipsilateral jugular nodes (yellow arrow). Oropharyngeal cancer is the most common head-neck cancer in the western world, often arising in the tongue base or in the palatine tonsils. It, often, shows a predictable pattern of nodal spread from superior to inferior, first involving the upper jugular chain and then the middle and lower nodes.

ing algorithms. It also appears as a promising tool in the evaluation of patients treated with targeted therapy and immunotherapy as it may verify metabolic treatment response irrespective of anatomical changes [33,34]. The assessment of immunotherapy response is challenging, since, unlike conventional chemotherapy, the neoplastic lesions may, initially, enlarge before shrinking or the final response may occur despite the presence of new lesions; thus, new appropriate criteria for response evaluation are needed. PET/CT is also the modality of choice for detecting distal metastases in the staging and restaging of melanoma patients [31].

PET/CT is not a proper modality for the initial diagnosis and T-staging of oesophageal cancer because it misses small superficial tumors and cannot, accurately, determine the exact depth of tumor penetration through the eosophageal wall. However, PET/CT may depict advanced T-stage tumors (T3-T4) with the CT component of the study showing stranding of the adjacent peri-oesophageal fat, obliteration of fat planes and displacement or indentation of the mediastinum or other structures [35]. The modality has low sensitivity in the detection of regional nodes, which may be obscured by FDG activity in the adjacent, hypermetabolic primary tumor, yet it shows potential to detect distal nodes in the mediastinum, abdomen or supra-clavicular regions [35-37] (Figure 5). Guidelines appreciate the strengths of PET/CT to detect distant metastases with high specificity and metastatic lesions not identifiable by other methods. By doing this, it affects treatment decisions and selects patients suitable for radical treatment. Clinical guidelines suggest PET/CT for the evaluation of treatment response after pre-operative or radical chemoradiation. In gastric cancer, the primary tumor evaluation is hampered by low FDG uptake in certain histologic types such as mucinous, signet-ring and diffuse cancers and by incidental normal or inflammatory FDG uptake in the gastric wall. PET nodal staging shares the same properties as in oesophageal cancer. The method is not accurate in the evaluation of peri-gastric nodes, yet it may detect distal nodes outside of the typical lymphadenectomy bed, thus altering treatment plan [35]. It is also highly accurate in the detection of distant metastases in the liver or peritoneum. Gastric cancer often gives metastases in the peritoneum appearing as hypermetabolic soft-tissue nodules, peritoneal plaques or diffuse infiltrating stranding in the omentum, mesentery or other peritoneal spaces.

PET/CT it is not appropriate for the initial evaluation of primary pancreatic adenocarcinoma, because it cannot define by itself any encasement or infiltration of major vessels namely the superior mesenteric artery or the celiac axis. Of note that primary pancreatic adenocarcinomas show variable, sometimes low or moderate FDG uptake due to adjacent abundant fibrotic stroma, while inflammation-pancreatitis can be a source of false-positive findings. The role of PET/CT is reserved as a complementary tool in the assessment of probable metastatic disease and, in the restaging setting, to differentiate between metabolically active disease

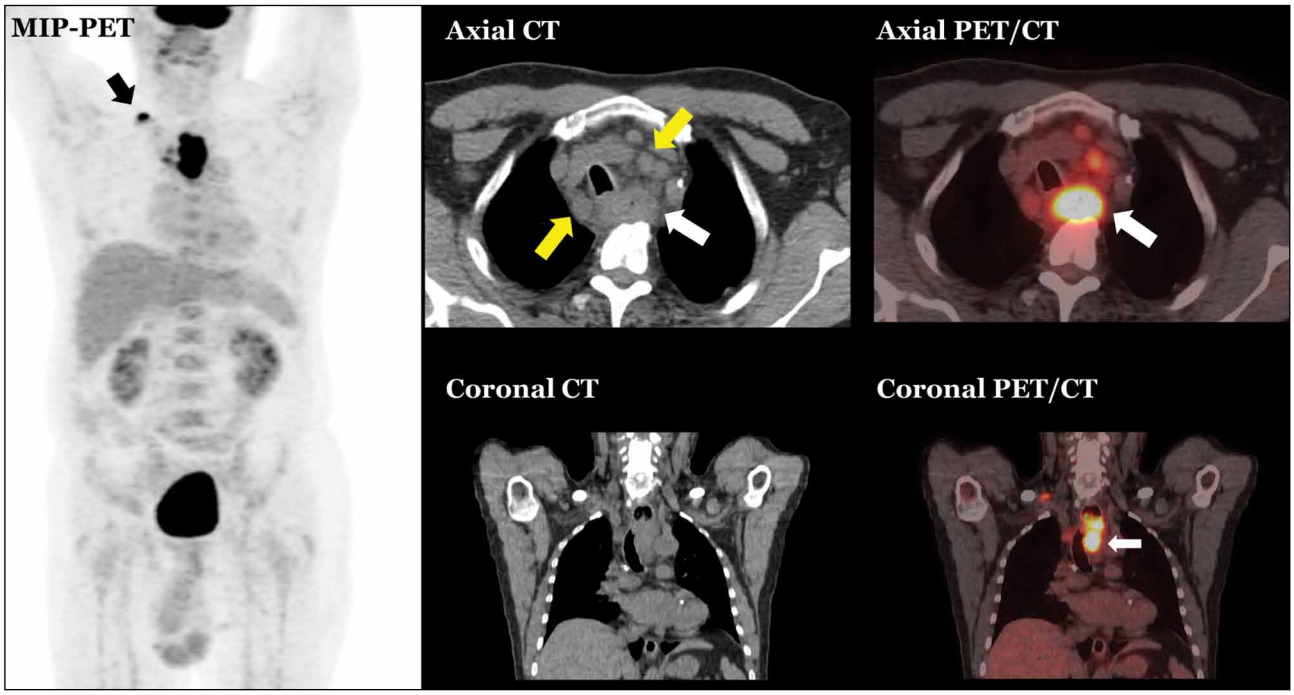

Figure 5. Advanced T-stage oesophageal cancer with nodal metastases. Primary tumor (white arrow) presenting as abnormal concentric wall thickening with adjacent peri-oesophageal fat stranding and displacement of the trachea to the right. There are FDG-avid nodes in the mediastinum (yellow arrow) and in the right supraclavicular fossa (black arrow).

and post-treatment necrotic or fibrotic charges [38,39].

PET/CT is not suitable for the initial diagnosis and staging of hepatocellular cancer, because this malignancy shows heterogenous metabolic behavior with low FDG uptake in some cases due to low glucose transporter expression or due to increased phosphatase activity which metabolizes FDG.

Regarding colorectal cancer, PET/CT has limitations in T- and N-staging and strengths in the detection of distant metastases [35,40]. Primary tumor may appear as hypermetabolic abnormal mural thickening with concomitant luminal narrowing or as a polypoid intraluminal lesion. The modality may be useful in certain clinical scenarios such as: i) in selected candidate patients before the radical treatment of hepatic metastases. PET/CT may detect extrahepatic lesions altering treatment plan ii) in patients with equivocal CT/MRI findings which affect treatment decisions iii) in selected patients with high tumor marker levels in whom previous imaging is negative iv) in selected rectal cancer patients with high probability of distant metastatic disease v) in post-treatment rectal cancer, to evaluate residual pre-sacral tissue and differentiate between fibrosis or recurrent disease. In Gastrointestinal Stromal Tumors (GISTS), the method is useful in the evaluation of response to imatinib therapy not always fulfilling the typical criteria of tumor size reduction [41,42].

In gynecological cancers, PET/CT has certain, discrete roles. It is suitable for nodal and distal metastatic evaluation in cervical cancer and may modify treatment plan [43]. Thus, it is suggested by clinical guidelines in advanced-stage FIGO II-IV disease both for initial staging and for restaging/follow-up. In ovarian cancer, PET/CT has complementary role in staging of abdominopelvic nodes and distant metastases [44,45]. Of note that, ovarian cancer often metastasizes in the peritoneum in the form of peritoneal nodules, plaques and thickening or "haziness" of peritoneal fat. In endometrial cancer, PET/CT has high specificity in the detection of nodal disease and high diagnostic accuracy in detecting distant metastases; hence, it is applied and offers diagnostic benefit in high-risk patients [46].

PET/CT has no role in the initial diagnosis of breast cancer with very low sensitivity (≤ 50%) in primary breast cancer detection, low spatial resolution for the detection of small sub-centimeter lesions and considerable number of false positives in cases of inflammation, abscesses, fat necroses and fibroadenomas [47]. Despite all previous limitations, focal incidental PET-positive breast findings need further evaluation with mammography, because

they bear a considerable probability of malignancy, around 30-40% based on retrospective studies. FDG uptake in the primary tumor depends on histopathological characteristics: lobular carcinomas show lower uptake than infiltrative ductal ones, while high-grade, highly proliferative and triple-negative tumors show intense uptake [48]. PET/CT has unacceptable, low sensitivity in the evaluation of axillary nodes (55-60%) and inability to detect micrometastatic burden, hence it cannot substitute for sentinel lymph node biopsy procedure, which is the standard of care in the evaluation of the axilla. The main strengths of PET/CT are: i) the potential to detect and depict hypermetabolic nodes beyond axillary levels I-II (typical nodal clearance is performed in these levels) and ii) the high sensitivity in distal metastatic evaluation even in sites not suspected by previous imaging. Thus, PET/CT is suggested in breast cancer stages ≥IIB-III and in triple-negative tumors [48-50]. It is not routinely suggested in early stage I-IIA tumors as it is unlikely to induce significant clinical impact. PET may detect hypermetabolic bone lesions with no corresponding anatomic abnormalities on the CT component of the study. Of note that breast skeletal metastases may, occasionally, appear sclerotic not showing increased metabolic activity.

In seminomas, PET/CT is suggested, by clinical guidelines, to evaluate any post-treatment residual tissue and differentiate between residual active neoplastic disease and necrotic scar tissue with high NPV>90% [51]. PET has very few clinical applications in renal-urinary cancers hampered by high normal FDG excretion into the urinary tract. It shows, however, high diagnostic accuracy in the detection of distal metastatic lesions from invasive bladder tumors [52]. In sarcomas, FDG activity in primary tumors depends on tumor grading [53]: osteosarcomas, Ewing sarcoma and high-grade chondrosarcoma show avid uptake, while high-grade liposarcomas show more intense uptake than myxoid and well-differentiated subtypes. Clinical guidelines include PET/CT in the staging of bone sarcomas and suggest this modality as complementary method in staging of soft-tissue sarcomas [54,55]. In research setting, PET/CT is applied to evaluate neoadjuvant chemotherapy and distinguish between responders and non-responders. The modality is also applied in sarcoma restaging to detect recurrent disease in areas with distorted anatomy by previous surgical treatment.

FDG dominates the clinical applications of PET in oncology, yet it is not the sole efficient radioactive tracer available. Other PET tracers following different molecular pathways rather than cellular metabolism or having high affinity for specific cellular receptors have been developed. These tracers have fulfilled unmet clinical needs and, nowadays, they have found their place into diagnostic and therapeutic algorithms.

Neuroendocrine tumors (NETs) over-express Somatostatin Receptors (SSTRs) on their cellular membranes. These receptors are effectively targeted with positron-emitting peptides (DOTA-octreotides e.g., ^{68}Ga-DOTATATE), which show high selective affinity for SSTRs. DOTA-peptides are indicated in the staging and restaging of NETs, showing high diagnostic accuracy in mapping the whole disease burden, thus affecting therapeutic decisions [56]. DOTA-peptides have another inherent advantage in the evaluation of NETs: diagnostic verification of avid SSTR expression with PET provides the basis for further NET targeted treatment by use of the corresponding therapeutic DOTA-peptides (e.g., ^{177}Lu-DOTATATE) emitting lethal beta radiation. The concept of applying the same molecular structure (octreotide) both for diagnosis and therapy is known as theranostics, a field where Nuclear Medicine meets Precision Medicine. The theranostic treatment of NETs by means of DOTA-peptides has been approved in clinical practice as it shows survival benefit verified by NETTER-1 randomized controlled trial [57].

Nowadays, various effective PET tracers, namely Prostate-Specific Membrane Antigen (PSMA)-ligands, are applied in the imaging of prostate cancer. PSMA is a specific trans-membranic glycoprotein with 100-1000fold over-expression in prostate cancer compared with normal prostatic tissue [58]. PSMA expression is particularly high in high-grade, metastatic and castration-resistant tumors. Specific PET tracers (i.e., ^{68}Ga-PSMA-11, ^{18}F-PSMA-1007) have been developed exhibiting high selective affinity for PSMA. PSMA-PET gives images of exceptional quality showing avid tracer uptake in cancerous lesions with high target to background ratios. It is the Nuclear Medicine method of choice for imaging of prostate cancer. PSMA-PET shows high sensitivity in the detection of small lesions such as sub-centimeter nodes and early bone lesions with no concomitant sclerosis. It is clinically useful in staging of high-risk patients showing higher diagnostic accuracy compared with conventional CT and bone scan imaging [59,60]. PSMA-PET is indicated in cases of biochemical recurrence with high detection rates even in low trigger PSA values <1 ng/ml [61]. In these patients, it may differentiate between loco-regional recurrence, which can be treated with radiotherapy, and metastatic disease, which is going to be subjected to

systemic treatment. To evaluate local recurrence, MRI is still the modality of choice, while distal disease is more effectively assessed with PSMA-PET. In PSMA imaging, the theragnostic concept applies: metastatic lesions exhibiting avid tracer uptake may be, selectively, targeted with therapeutic ^{177}Lu-PSMA. The latter emits beta radiation which destroys cancer cells and has already shown favorable results in clinical trials [62,63].

To sum up, PET/CT has evolved to be an established method in everyday oncological practice. It has been incorporated into clinical algorithms and guidelines altering therapeutic decisions in oncological patients. Besides the approved clinical indications, the quest for technological improvements, new applications and novel tracers continues, and the future of PET molecular imaging appears promising.

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BRAF positive colorectal cancer

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Abstract

Colorectal cancer constitutes a clinical entity affecting many people with a risk increasing through somebody's lifetime. Early diagnosis is still the most significant factor for a successful outcome thus the role of screening colonoscopy in asymptomatic individuals remains paramount. As clinical experience and research on this disease becomes broader and deeper, we are becoming increasingly aware of the distinct biological phenomena that take place and the various patients' subgroups. This expanding knowledge sheds light on the diversity of the clinical scenarios and outcomes we observe in real practice. One of the molecular and pathophysiological events that takes place is the dysregulation of the EGFR/MAPK pathway, which involves molecules such as the RAS and the BRAF proteins. The significance of these molecules and their accountable genes' mutations is now ever more studied and understood. Gene expression analysis has classified CRC according to the various molecular alterations and their clinical associations in four distinct groups (consensus molecular subgroups, CMS1-4). *BRAF* mutations, especially the dominant *V600E* mutation, has been correlated to a more aggressive phenotype and poor outcome (CMS1). Fortunately, great steps in the management of this unique patients' group have been achieved and novel successful approaches have been found while research is ongoing.

Key words: *Colorectal cancer; molecular alterations; BRAF mutation*

INTRODUCTION

Colorectal cancer (CRC) is the third commonest cancer in males and females respectively, following breast, lung and prostate, and the second commonest cause of death among all cancer patients worldwide [1]. Our understanding regarding colorectal cancer's etiology has been evolving over the last 20 years leading to changes and advances in its treatment. We have reached now the era of individualized and tailored management, where apart from the classic and paramount clinical judgement various specific genomic alterations help to select the appropriate strategy for the different patient sub-populations and each individual patient accordingly.

The main biological pathway of CRC carcinogenesis is through the signaling cascade RAS/RAF/MEK/extracellular signal-regulated kinase (ERK), also known as the mitogen-activated protein kinase (MAPK) pathway that starts from the transmembranic epidermal growth factor receptor (EGFR) pathway (Figure 1). In normal cells, this pathway drives cell proliferation and differentiation and additionally their migration, survival and angiogenesis. This cascade is composed of the RAS small proteins [guanidine triphosphatase (GTPase)], which activate the RAF family proteins (mainly BRAF) and subsequently lead to the phosphorylation and activation of MEK1/2 proteins and ERK. Dysregulation of this pathway often leads to uncontrolled proliferation and tumorigenesis [2,3].

The percentage of RAS mutations' detection in all colorectal cancers varies from 9%–30% whereas BRAF mutations are found in 7% of all cancers (including early-stage disease) but in 8%-12% of metastatic CRC, with BRAF V600E accounting for >90% of mutations in BRAF-mutated cancers [4,5]. The mutated gene mimics regulatory phosphorylation with a 10-fold increase in BRAF activity compared with the wild-type. In contrast to the dominant activating BRAF V600E mutation, there

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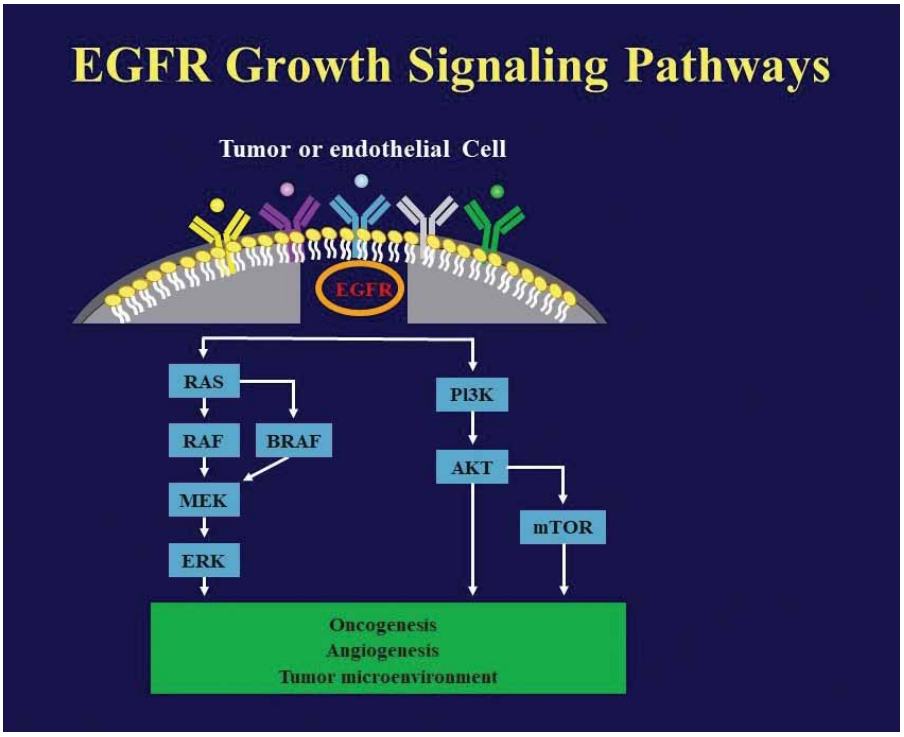

Figure 1. The molecular pathways of EGFR.

are other less common ones such as BRAF D594G or G596N, which are kinase-impairing mutations. Patients with $^{non\text{-}V600E}$BRAF-mutant metastatic colorectal cancer are younger, with fewer high-grade and right sided tumors. They also show a significant longer median overall survival and a better prognosis [6,7]. In this review though, we will focus on the commonest V600E mutation and its role in CRC.

Genetic abnormalities and molecular classification in CRC

Most colon cancer cases, namely about 80% of cases, are sporadic. The remaining 20% are familial or related to specific genetic syndromes such as familiar adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal carcinoma (HNPCC) which account for about 1% and 5% of all CRC cases respectively. FAP is associated with mutations of the *adenomatous polyposis coli* (*APC*) gene whereas HNPCC with germline mutations of mismatch repair (MMR) genes (mainly *hMSH2*, *hMSH6, hMLH1*) [8].

The majority (85%) of genetic changes occurring in CRC are due to chromosomal instability (CIN). CIN has long been reported as a key genetic abnormality having a dominant effect in colorectal carcinogenesis following the traditional adenoma-carcinoma model [9]. The alternative serrate adenoma to adenocarcinoma pathway (around 10% of cases) is characterized by microsatellite instability (MSI).

Serrated tumours are not chromosomal instable but often exhibit extensive DNA methylation of CpG islands. This methylation may occur in the *MLH1* promoter (a gene of the mismatch repair system) leading to the 'sporadic' microsatellite instable (MSI) phenotype.

Mutations in the *BRAF* gene in CRC pathogenesis develop within the serrated pathway. Tumours with *BRAF* V600E mutations are often associated with a high mutational burden, microsatellite instability (MSI), and a CpG island methylator phenotype (CIMP), with high levels of epigenetic modulation of gene expression through DNA methylation. Quite recently, using cutting edge technology (Next generation sequencing, NGS) four distinct subgroups [known as the consensus of molecular subtypes (CMS)] were identified in CRC based on intrinsic gene expression profile patterns (Table 1). The majority of *BRAF*-mutant CRCs are CMS subtype 1 (MSI high, immune) and are associated with deficient DNA repair, hypermethylation, and a high mutational burden [9,10].

Additionally, two new subtypes of *BRAF*-mutant CRC

Table 1. *Consensus Molecular Subtype Classification (CMS).*

Subtype	Biological findings	Clinical findings	Prognosis	Incidence
CMS 1 Immune	MSI high BRAF mutated Hypermutations (high TMB) Immune activation	Right sided Females Older age	Intermediate to poor survival	~ 14%
CMS 2 Canonical	CIN high ↑ EGFR activation MSS TP53 mutation ↑↑ WNT/MYC activation	Left sided	Good	~ 37%
CMS 3 Metabolic	CIN low KRAS mutation PI3K mutations Metabolic dysregulation		Intermediate	~ 13%
CMS 4 Mesenchymal	CIN high Notch3/VEGFR2 overexpression ↑ TGF-beta activation Stroma infiltration Angiogenesis	Younger age	Poor survival and worse relapse free survival	23%
Mixed	Intratumoral heterogeneity	Transition phenotype		13%

classification, BM1 and BM2, have also been proposed based on differential gene expression with distinct molecular patterns [10]. BM1 is characterized by *KRAS/AKT* pathway activation, mTOR (mammalian target of rapamycin) deregulation, and epithelial–mesenchymal transition-related (EMT) processes with *KRAS* signaling and immune response, whereas BM2 is characterized by deregulation of the cell cycle and cycle checkpoint-related processes [10]. The presence of two subgroups of *BRAF*-mutant CRC may help explain the differences in response to treatment among various patients and their diverse outcome. BM1 has a worse prognosis and a different approach in treatment is recommended compared to BM2. For instance, targeting the EGFR downstream cascade may provide greater benefit to BM1 compared to checkpoint-CDK inhibition that may offer more benefit to BM2 [10].

Finally, overlapping between MSI and *BRAF* mutation often occurs in this population. In the era of immunotherapy in cancer, anti-PD1 drugs have been approved in MSI tumours including mCRC. However, the role of these antibodies in MSI *BRAF*-mt mCRC is still to be determined. Therefore, the best sequence (targeted therapy or checkpoint inhibitors) is still to be determined in the future.

Clinical implications of BRAF mutations

Clinically, *BRAF*-mt CRC has been associated with a more advanced age of diagnosis and female sex, proximal (right) colon tumors, poorer differentiation, mucinous histology, MSI high and larger primary tumors. The pattern of metastatic spread seems also to differ compared to *BRAF* wildtype (*BRAF*-WT) tumours with more peritoneal metastases seen in BRAFmt and fewer liver-only and lung metastases [11,12].

BRAF mutation confers worse prognosis in the metastatic setting. In a pooled analysis of some of the largest phase III studies in metastatic CRC (the FOCUS, COIN and CAIRO I and II trials) worse OS for *BRAF*-mt CRC as compared to the *BRAF* wild type (WT) counterparts has been reported [13].

Survival in *BRAF*-mt populations after lung or liver metastasectomy has been also studied and results confirm worse prognosis and shorter OS after surgery compared with *KRAS*-mt or -BRAF WT tumors [14,15].

The poor prognosis of *BRAF V600E*-mutant mCRC has

been attributed to various biological phenomena such as aberrant programmed cell death or the suppressed expression of CDX2 (caudal type homeobox 2) [16]. CDX2 is a tumor suppressor and transcription factor involved in the regulation of intestinal epithelial cell differentiation, cell adhesion, and polarity and the loss of CDX2 has been associated with metastasis and poor prognosis in CRC [17]. Given their overall favourable prognosis in earlier stages, MSI-H tumors may attenuate the adverse prognostic impact of *BRAF* mutations [18]. *BRAF*-mutated MSI-H tumors have a less aggressive clinical phenotype and improved OS compared to *BRAF*-mutant MSS tumors [13].

Although chemotherapy has significantly improved overall survival (OS) in CRC, response and treatment benefit appear lower for *BRAF*-mt tumors both at earlier and advanced disease stages.

Whether BRAF can serve as a predictive biomarker of response to chemotherapy this has been long debated with early evidence from retrospective and phase II data suggesting patients with *BRAF*-mt CRC do better with intensive regimens such as FOLFOXIRI-bevacizumab though no confirmation from the phase III TRIBE 3 study was found for this particular population. It has been postulated that the small numbers of BRAFmt patients in these studies don't help us draw safe conclusions [19,20].

On the other hand, we know that treatment with monoclonal antibodies targeting the EGF receptor (cetuximab and panitumumab) in the *RAS*-WT patients' population is not that effective in the presence of *BRAF* mutations according to meta-analyses of many clinical trials [21,22].

Treatment of BRAF mutant CRC

Since no data have shown any role of targeting the BRAF protein during the early stage of the disease we will focus here on the metastatic setting where intensive research has taken place over the last decade.

Based on the positive experience and the successful results with BRAF inhibitors in BRAF V600E positive melanoma many clinical studies tested their usefulness in metastatic CRC. Disappointingly enough there was no similar to melanoma benefit from monotherapy with the BRAF inhibitors vemurafenib, dabrafenib or encorafenib in pretreated CRC patients probably due to the presence of resistance mechanisms or the complexity of involved pathways rather than a targetable point mutation. The main studies in BRAF mutant CRC patients and the efficacy of the tested agents or regimens are summarized in Table 2. Since monotherapies did not produce positive results, combinational strategies were planned and indeed a better outcome was reported when BRAF inhibitors were combined with EGFR inhibitors (cetuximab – panitumumab), MEK inhibitors (trametinib, binimetinib) or a PI3K inhibitor (alpelisib) in double or triple regimens.

The first meaningful clinical results were derived from the phase II SWOG 1406 study where vemurafenib with cetuximab plus irinotecan showed that triple therapy was associated with an objective response rate of 16% and a PFS of 4.4 months [23]. The addition of an MEK inhibitor to BRAF inhibition has also been found to increase inhibition of the MAPK pathway and produce potentially greater antitumor activity in preclinical and initial clinical studies [24].

Triplet combinations have been evaluated in an attempt to improve outcomes for patients with *BRAF*-mutant mCRC. The combinations of dabrafenib plus panitumumab, dabrafenib and trametinib plus panitumumab, and trametinib plus panitumumab showed a better response rate (ORR) for the triplet therapy, but at the cost of more adverse events mainly grade 3/4 diarrhea compared to the doublet treatment [25]. Lately, combination of encorafenib and cetuximab versus encorafenib, cetuximab, and the PI3K inhibitor alpelisib were evaluated in a phase Ib dose-escalation study in 28 patients with refractory *BRAF*-mutated CRC. The authors reported an 18% ORR and a disease control rate of 93% for the triplet regimen of encorafenib, cetuximab, and alpelisib [26]. These results were reproduced in a subsequent phase II study in 52 patients treated with these regimens and additionally the PFS was numerically higher for the triplet compared to the doublet regimen (5.4 months versus 4.2 months) [27]. As expected, the frequency of adverse events with the triplet was higher, mostly anaemia, hyperglycemia, and increased serum lipase levels [27].

Finally, the most significant results available today came from the phase III BEACON 3-arm trial that was published in 2019, in patients with *BRAF* V600E-mutated mCRC who had had disease progression after one or two previous treatment regimens (28). A total of 665 patients were randomized 1 : 1 : 1 to receive encorafenib, cetuximab, and binimetinib (a MEK inhibitor) (arm A) versus encorafenib and cetuximab (arm B) versus irinotecan or FOLFIRI plus cetuximab (arm C) [28]. Almost all patients had previously received oxaliplatin and half of patients had previously received irinotecan before enrolment

Table 2. *Main studies of BRAF inhibitors in metastatic CRC.*

Regimen (Author, reference)	RR, %	mPFS, mo
Single/Doublet BRAF/MEK		
Vemurafenib (Kopetz S. JCO 2015)	5	2.1
Dabrafenib (Falchook GS. Lancet 2012)	11	NR
Encorafenib (Gomez-Roca C. ESMO 2014)	6	4
Dabrafenib + Trametinib (Concoran R. JCO 2015)	12	3.5
Doublet with EGFR		
Vemurafenib + Panitumumab (Yaeger R. Clin Ca R 2015)	13	3.2
Vemurafenib + Cetuximab (Tabernero J. ASCO 2014)	20	3.2
Encorafenib + Cetuximab (van Geel R. Canc Disc 2017)	19	3.7
Dabrafenib + Panitumumab (Atreya CE, ASCO 2015)	10	3.4
Triplet with EGFR		
Vemurafenib + Cetuximab + Irinotecan (Hong D. Cancer Discov 2017)	35	7.7
Dabrafenib +Trametinib + Panitumumab (Atreya CE, ASCO 2015)	26	4.1
Encorafenib + Cetuximab + Alpelisib (van Geel R. Cancer Discov 2017)	18	4.2
Encorafenib + Cetuximab +/- Binimetinib vs Cetuximab + Irinotecan or FOLFIRI (Kopetz S. NEJM 2019)	26.8 / 19.5 vs 1.9	4.5 / 4.3 vs 1.5 **[mOS: 9.3/9.3 vs 5.9]**
Encorafenib + Cetuximab + Binimetinib (1st line) (Van Cutsem E. ESMO 2021)	47.5	5.8

into this study. This trial is the largest ever conducted in this population and the first phase III trial to show both a survival and response advantage in pre-treated *BRAF*-mutated CRC patients. The primary endpoints for the BEACON CRC study were overall survival (OS) and blinded central review confirmed objective response (ORR) for the triplet combination (arm A) compared with the control arm C. A key secondary endpoint was OS for the encorafenib plus cetuximab (doublet) regimen versus control. Other secondary endpoints included progression free survival (PFS), duration of response, and safety [28].

The mature results of the BEACON CRC study showed that the encorafenib plus cetuximab regimen significantly improved OS compared to the control group, with a median OS of 9.3 months (95% CI 8.0-11.3 months) compared with 5.9 months (95% CI 5.1-7.1 months) for the control regimens (HR 0.61; 95% CI 0.48-0.77) [29]. Efficacy was similar when binimetinib was added to the encorafenib plus cetuximab regimen (9.3 months OS) and both regimens (arms A & B) had significantly improved efficacy and quality of life (QoL) assessments relative to the control in patients with *BRAF* V600E-mutated mCRC whose disease had progressed after one or two prior regimens [29]. On the Patient Global Impression of Change scale, more than 20% of the patients in arm B and arm A said they were "very much improved," compared with 10% of those on the control arm C [30]. In the updated analysis, confirmed ORR results by blinded independent review based on all randomized patients were 26.8% (95% CI 21.1% to 33.1%) for triplet, 19.5% (95% CI 14.5% to 25.4%) for doublet, and 1.8% (95% CI 0.5% to 4.6%) for control. For median PFS, the updated results were 4.5 months (95% CI 4.2-5.4 months) in arm A, 4.3 months (95% CI 4.1-5.4 months) in arm B, and 1.5 months (95% CI 1.5-1.9 months) in arm C, respectively,

with HRs of 0.42 (95% CI 0.33-0.53) and 0.44 (95% CI 0.35-0.55) for arms A and B, respectively, compared with the control arm C. These data are comparable to earlier results from studies of irinotecan and cetuximab with or without vemurafenib. The safety and tolerability profiles of both investigational combinations were consistent with the known profiles of the involved agents with more grade 3 or higher adverse events being seen in arm A (58%) than arm B (50%) but almost similar to standard arm C (61%). Binimetinib as part of the triple combination does actually add some additional toxicity associated with MEK inhibition. Overall, side effects such as anaemia, dermatitis acneiform, diarrhoea, nausea, and vomiting were reported at a higher incidence of more than 10.0% difference in arm A than in arm B, whereas headache and melanocytic nevus were reported at a higher incidence in the doublet arm than in the triplet arm [29].

The results of the BEACON CRC study set the basis of a new standard of care in this pre-treated patient population as it is the first trial that provided a meaningful survival benefit and an improvement over the till now standard of care. Based on the more tolerable toxicity profile the American and European Authorities approved the doublet regimen for the treatment of *BRAF* V600E-mutated mCRC after prior therapy.

A single-arm phase II first-line study (ANCHOR CRC) [encorAfenib, biNimetinib and Cetuximab in subjects witH previOusly untreated BRAF-mutant ColoRectal Cancer] was recently completed and evaluated the triplet regimen in this setting [31]. The findings after 92 patients from ANCHOR CRC were assessed were very positive, and the investigator-assessed confirmed ORR was 47.5% (95% CI 37.3 - 58.2), the disease control rate (DCR) reached 88% while the median PFS was 5.8 months (95% CI 4.6-6.4 months) and the reported OS 17.2 months (95% CI 14.1-NE). Grade 3 or higher adverse events were reported by almost 70% of patients, in particular anaemia (10.5%), diarrhoea (9.5%), nausea (8.4%) bowel obstruction (6.3%) and renal injury (5.3%) and have been consistent with those observed in prior studies [31].

These encouraging results emphasize the need for further exploration and confirmation thus the phase III study BREAKWATER is now in progress and will test the efficacy of encorafenib plus cetuximab with or without chemotherapy as a first line treatment of BRAF V600E mutant untreated CRC patients [32].

As far as the $^{\text{non-V600E}}$ BRAF mutant patients are concerned, better outcome and potential response to antiEGFR therapy has been suggested in preclinical, early phase studies and case reports [33-35].

CONCLUSIONS

The role and significance of BRAF mutations in colorectal cancer is now well accepted. The treatment of *BRAF*-mutated CRC has evolved rapidly over the last several years. Combination strategies involving MAPK pathway blockade have shown promising results for the treatment of patients with *BRAF* V600E-mutated mCRC. The BEACON CRC study represented the largest phase III study in this population to date and has given strong clinical evidence to support BRAF and EGFR inhibition with the combination of encorafenib plus cetuximab. Based on these results we have a new standard of care in 2nd or 3rd line treatment, in *BRAF* V600E-mutated patients.

The ANCHOR phase II study suggested similar activity of the doublet (encorafenib/cetuximab) in the first line setting. So, it will be much anticipated to see the outcomes of the phase III BREAKWATER first line study [BRAF V600E-mutant colorectal cancer study evaluating encorafenib taken with cetuximab plus or minus chemotherapy (NCT04607421)] and if positive the BEACON doublet regimen may even deserve an evaluation in the adjuvant setting. In future, other potential targets might be explored, taking advantage of other unique molecular characteristics of *BRAF*-mutated mCRC tumors as defined by the gene expression profiling. Given the enrichment of *BRAF* V600E mutations within CMS subtype 1 CRCs, there is a significant interest in combining anti-programmed cell death protein 1 (PD-1) treatments with BRAF/EGFR-targeting therapies (e.g. NCT0404430). Additional investigations incorporate various combinations of BRAF, MEK, ERK, CRAF, SHP2, and PD-1 inhibitors (e.g. NCT04294160). Future research should focus on developing treatments that overcome mechanisms of resistance. An enhanced understanding of the role of the *BRAF* V600E mutation in the pathogenesis of mCRC will eventually expand recent treatment advances and further improve outcomes for patients. When possible, the non-V600E BRAF mutations should be sought and when clinically appropriate, patients may be given the opportunity of anti-EGFR treatment. In any case this sub-population requires separate clinical studies.

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CASE REPORT ACHAIKI IATRIKI | 2022; 41(3):154–158

Post-COVID Multisystem Inflammatory Syndrome in an adolescent: A case report

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Abstract

Multisystem Inflammatory Syndrome in Children (MIS-C) is an emerging clinical entity which was first described in the pediatric population in April 2020. MIS-C syndrome can occur in children and teens under 21 years of age and is characterized by hyperinflammatory illness and severe extrapulmonary multiorgan dysfunction, particularly cardiovascular, occurring within 2 to 6 weeks of antecedent coronavirus disease 2019 (COVID-19) or exposure to a person with diagnosed COVID-19 in the past month. We report a case of an 18-years-old Asian male, vaccinated for SARS-CoV-2 and with a history of COVID-19 44 days ago, that was admitted to the emergency department with persistent fever, pharyngeal pain, nausea and vomiting. Clinical examination revealed skin rash all over the body, bilateral conjunctival injection, pharyngeal erythema, lip redness and swelling. Laboratory tests and imaging revealed myocarditis, elevated inflammatory markers, liver and kidney dysfunction, bilateral ground-glass opacities at lung bases, ascites and lymphadenopathy. Thorough investigation ruled out infectious causes and a diagnosis of MIS-C was made, as all six criteria were fulfilled. Intravenous immunoglobulin and methylprednisolone were administered along with aspirin. After 5 days of treatment the patient showed prompt clinical and laboratory improvement and was discharged. MIS-C is rarely seen in vaccinated children and adolescents after SARS-CoV-2 infection. It is still unknown whether the type of COVID-19 vaccine or the Delta variant of SARS-CoV-2 may play a role in the development of MIS-C. Physicians and not only pediatricians should be aware of this rare clinical entity, as it has also been described in adults (MIS-A).

Key words: *Multisystem inflammatory syndrome in children (MIS-C); Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2); Coronavirus disease (COVID-19)*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) led to millions of new cases every day and up to 5 million deaths worldwide. A rare but serious complication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents was first described in April 2020, known as Multisystem Inflammatory Syndrome in Children (MIS-C) [1]. MIS-C is an emerging clinical entity that can occur in children and teens under 21 years of age and is characterized by hyperinflammatory illness and severe extrapulmonary multiorgan dysfunction, particularly cardiovascular, occurring within 2 to 6 weeks after SARS-CoV-2 infection [2]. MIS-C is mainly reported in unvaccinated children and adolescents. However, a recent study found a small number of vaccinated individuals aged 12-20 years diagnosed with MIS-C, most of them with laboratory evidence of past or recent SARS-CoV-2 infection [3]. We

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report a case of MIS-C in an 18-years-old Asian male, who was diagnosed with mild COVID-19 in August 2021 and had received one dose of a COVID-19 vaccine (Ad26. COV2. S; Janssen) in June 2021.

CASE PRESENTATION

An 18-years-old Asian male was admitted to the emergency department with persistent fever (>38°C for the last 72 hours), pharyngeal pain, nausea and vomiting. Clinical examination revealed a skin rash all over the body, bilateral conjunctival injection, pharyngeal erythema, lip redness and swelling (Figure 1, Figure 2). The patient had been in his usual state of health until 3 days before admission, when fever developed, with a temperature of up to 39.6 °C, associated with the skin rash. He started azithromycin after consultation with his primary care physician. The patient had a history of SARS-CoV-2 infection first diagnosed on 23rd August 2021 (laboratory confirmed with antigen test) and received one dose of a COVID-19 vaccine (Ad.26. COV2.S; Janssen) on 30th June 2021. Furthermore, he had a medical history of asthma. He was a non-smoker and did not use alcohol. Medications included salbutamol and budesonide/formoterol for inhalation.

On examination, body temperature was 37.8°C, blood pressure was 99/38 mm Hg, heart rate was 98 beats per minute, respiratory rate was 35 breaths per minute, and oxygen saturation was 94% while the patient was breathing ambient air. The patient had moderate shortness of breath and diffuse coarse crackles at lung bases. Laboratory test results showed elevated inflammatory markers [White Blood Cells (WBC) 11.000 K/μL, Erythrocyte Sedimentation Rate (ESR) 85 mm/hr, C-Reactive Protein (CRP) 307 mg/dl, ferritin 853 ng/ml and procalcitonin (PCT) 1,24 ng/ml], elevated troponin (7648,6 pg/ml), B-type natriuretic peptide (BNP) 621 pg/ml, d-dimers (3869 ng/mL) and fibrinogen (571mg/dL), as well as renal and liver dysfunction. Other laboratory test results on admission are shown in Table 1.

A chest radiograph obtained in the emergency department showed bilateral multifocal patchy opacities. Antigen test for SARS-CoV-2 infection was positive. Subsequently, two nasopharyngeal swab samples for SARS-CoV-2 with a 12hour difference revealed a negative Real-Time Reverse-Transcriptase Chain Reaction (RT-PCR) result. Two liters of intravenous lactated Ringer's solution were administered, along with ceftriaxone, doxycycline, proton pump inhibitor and bemiparin 3500IU. The patient was admitted to the hospital and was examined by a cardiologist who suggested the conduction of an echocardiogram, which revealed typical findings of myocarditis with a depressed left ventricular ejection fraction (LVEF) around 50%. Bisoprolol was administered. Blood cultures were obtained, followed by a detailed laboratory investigation for infectious diseases (Hepatitis A virus, B virus, C virus, Human immunodeficiency virus, Varicella zoster virus, Cytomegalovirus, Epstein-

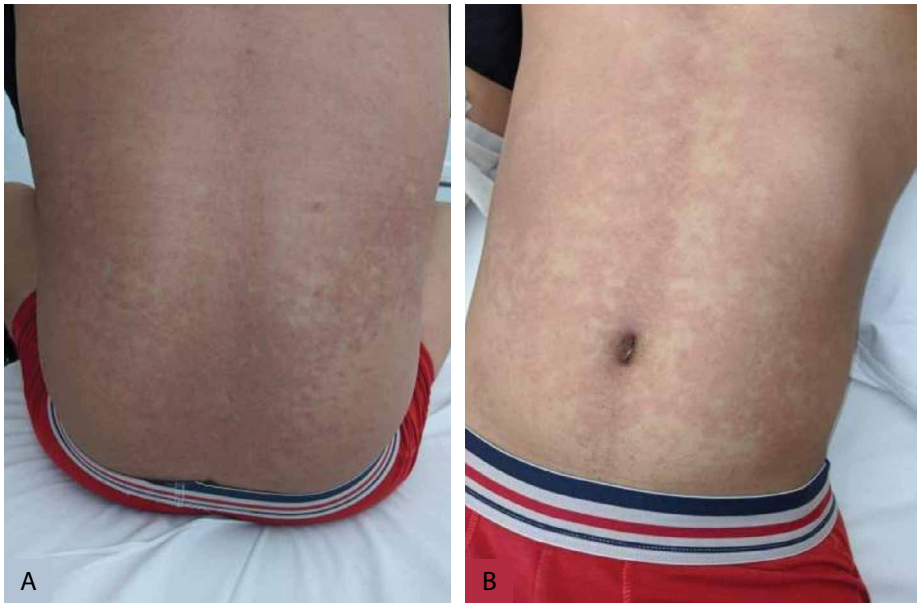

Figure 1. A, B: Skin rash all over the body associated with itch.

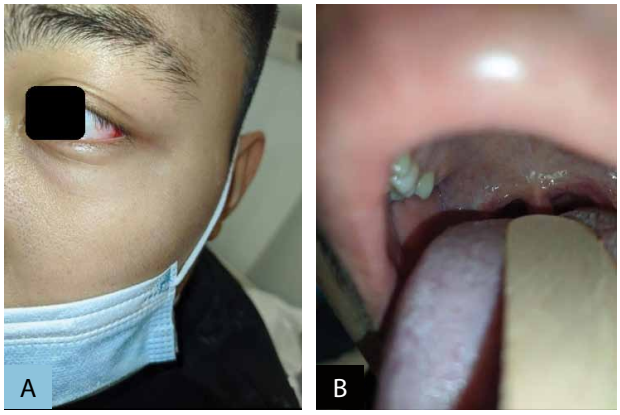

Figure 2. A: Conjunctival injection. B: Pharyngeal erythema, lip redness and swelling.

Barr virus, Herpex Simplex virus, Coxsackie virus, Parvo B19 virus, *Toxoplasma gondi, Mycoplasma, Legionella, B hemolytic Streptococcus*), which was negative.

On hospital day 3, the fever persisted. Neck stiffness and pain along with altered mental status were noted at clinical examination. Subsequently, a lumbar puncture was conducted with no evidence of infectious diseases at cerebrospinal fluid (CSF) analysis. A Computed Tomography (CT) scan of the abdomen, chest and brain was performed after oral and intravenous contrast administration. Findings were suggestive of bilateral groud-glass opacities at lung bases, pleural and pericardial effusion, mediastinal and hilar lymphadenopathy, ascites, mesenteric and retroperitoneal lymphadenopathy. The patient was examined by an infectious diseases specialist who suggested the diagnosis of MIS-C, as all six criteria were fulfilled and alternative plausible diagnoses were ruled out. Intravenous immunoglobulin, methylprednisolone and oral aspirin were administered and ceftriaxone and doxycycline were stopped.

On hospital day 7, the patient showed prompt clinical and laboratory improvement (Table 1). Echocardiogram was repeated with no findings of myocarditis and an improved estimated LVEF around 55-60%. After 8 days of hospitalization the patient was discharged. Methylprednisolone was continued for 15 days along with aspirin, until the conduction of a follow-up echocardiogram to rule out coronary artery aneurysms. At 30 days follow-up he had no suggestive findings of coronary artery aneurysms and aspirin administration was stopped. His laboratory test results were normal.

Table 1. *Laboratory test results at admission and at discharge.*

Laboratory tests	At Admission	At Discharge	Reference Range
White Blood Cells	18.000	15.200	3.800 - 10.500 10^3 K/μL
Neutrofils	92	68,2	45 - 75 %
Lymphocytes	4,9	21,4	20 - 51 %
Hemoglobin	13,8	15,4	14 -18 g/dL
Hematocrit	41,3	48,5	40 - 52 %
Platelets	124	540	150 - 450 10^3/μL
Aspartate aminotransferase	69	132	10 - 37 U/L
Alanine aminotransferase	72	279	10 - 45 U/L
C-Reactive Protein	303,7	18	mg/L
Urea	31	37	10 - 43 mg/dL
Creatinine	1,31	0,89	0,84 – 1,25 mg/dL
Gamma-glutamyl transferase	115	237	< 55 U/L
Creatine phosphokinase	1908	74	< 170 U/L
B-type natriuretic peptide	621		< 100 pg/ml
Procalcitonin	1,24		< 0,5 ng/ml
Troponin	7648,6	189,2	< 19,8 pg/ml
D-dimers	3869	2810	< 500 ng/mL
Fibrinogen	571	293,8	180 – 350 mg/dl

DISCUSSION

MIS-C is a rare complication of SARS-CoV-2 infection among persons younger than 21 years (occurs in less than 1% of children with COVID-19 infection) [3,7]. Herein we report a case of an adolescent with MIS-C who was admitted to the hospital for the management of an acute inflammatory syndrome. The diagnostic work-up and the management of the patient is presented in detail. Study cohorts revealed a mortality rate around 2% of MIS-C patients [4,6]. A recent epidemiological study found an overall incidence of MIS-C 5.1 persons per 1,000.000 person-months. However, the overall incidence of MIS-C was higher in SARS-CoV-2 infected patients (316 persons per 1,000.000 SARS-CoV-2 infections) [7]. Among those who did have prior health conditions across studies, the most common comorbidities were being overweight (10–39%) and having a prior history of asthma (5–18%), as in our case [8]. In most study cohorts the median age of patients with MIS-C syndrome was 9 years old with a male predominance (60%) [4,5]. Subsequently, internal medicine doctors in contrast with pediatricians are not familiar with the diagnosis and the management of this rare clinical entity which tends to increase as the COVID-19 pandemic continues. We feel that our case adds to the field as it outlines the possibility of MIS-C occurrence in young adults.

According to the US Centers for Disease Control and Prevention (CDC) Health Advisory, the case definition for MIS-C syndrome is an individual aged under 21 years of age presenting with fever, laboratory evidence of inflammation, clinically severe illness requiring hospitalization, multisystem (involving at least two systems) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological), with no alternative plausible diagnoses and laboratory confirmed current or recent SARS-CoV-2 infection (positive RT-PCR, serology or antigen test) or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms[1]. Our case fulfilled all these six criteria for MIS-C. Evaluation for current or previous infection with SARS-CoV-2 is necessary in order to distinguish MIS-C syndrome from biphasic COVID-19.

Except from CDC classification there are also the World Health Organization (WHO) and the Royal College of Pediatrics and Child Health (PCRCH) diagnostic criteria. According to the WHO criteria, a child up to 19 years old with persistent fever for at least 3 days, elevated inflammation markers, evidence of COVID-19, no other obvious microbial cause of inflammation and at least two of the following clinical manifestations (rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs, hypotension or shock, features of myocardial dysfunction such as pericarditis, valvulitis, or coronary abnormalities, evidence of coagulopathy and acute gastrointestinal symptoms) is diagnosed with MIS-C syndrome [13]. According to the RCPCH criteria, MIS-C syndrome is diagnosed in a child presenting with persistent fever, evidence of both inflammation and single or multi-organ dysfunction with the additional occurrence of several other features [14]. Any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes and infections associated with myocarditis must be excluded and SARS-CoV-2 PCR testing may be positive or negative.

The patient met both WHO and PCRCH diagnostic criteria. We mainly relied on the CDC criteria as more clinical and laboratory parameters are included and consequently the diagnostic accuracy is considered higher.

MIS-C has a wide spectrum of clinical signs and symptoms, though it most commonly presents with fever (97-100%). Other commonly seen signs and symptoms include abdominal pain (69%), vomiting (67%), diarrhea (54%), skin rash (55%), conjunctival injection (55%), shortness of breath (28%), mucocutaneous lesions (23%), neck pain (22%), altered mental status (11%), and, in severe cases, hypotension (52%) and cardiovascular involvement (80%). Among laboratory markers of inflammation, CRP and ferritin were frequently elevated (99% and 87%, respectively) [5,8]. MIS-C may be associated with acute kidney injury in one-in-five cases and is characterized by a self-limiting time course, as noted in our patient [11]. An epidemiological study revealed that nearly all MIS-C patients who had serologic testing for SARS-CoV-2 antibodies performed tested positive (98%), almost half had SARS-CoV-2 RT-PCR positive (53%) and 67% had a positive viral antigen test [5]. Our patient had an antigen test for SARS-CoV-2 positive, but two RT-PCR negative results.

Imaging studies found that MIS-C associated with COVID-19 is characterized by cardiovascular abnormalities, although solid visceral organ, gallbladder, and bowel abnormalities as well as ascites are also seen, reflecting a multisystemic inflammatory process, as in our case [10]. While there are currently no standard clinical practice guidelines regarding treatment for MIS-C, current management and treatment plans have generally yielded favorable outcomes. Similar to standard Kawasaki Disease (KD) treatment, intravenous immunoglobulin (IVIG) therapy was the most commonly reported treatment provided to patients

(55–100%), followed by corticosteroids (10–96%), aspirin and anticoagulation therapy, as were administered in our patient. In severe cases mechanical ventilation has also been reported [5,12].

Current studies support the hypothesis that SARS-CoV-2 may act as a trigger or immunomodulatory factor in MIS-C pathogenesis, however the exact mechanism is still unknown. Eliminating the transmission of SARS-CoV-2 not only serves to prevent COVID-19 but also presents an effective strategy for MIS-C prevention [9]. A recent study found that MIS-C without evidence of SARS-CoV-2 infection is rare after COVID-19 vaccination (reporting rate lower than 1 per million vaccinated individuals aged 12–20 years) [3]. Our patient was vaccinated and after mild SARS-CoV-2 infection he was diagnosed with MIS-C. It is still unknown whether the type of COVID-19 vaccine or the Delta variant of SARS-CoV-2 may play a role in the development of MIS-C.

CONCLUSION

In conclusion, MIS-C is a rare but serious condition which is associated with previous SARS-Cov-2 infection in most children and adolescents and rarely seen in vaccinated individuals, as in our case. Physicians and not only pediatricians should be aware of this rare clinical entity, as it has also been described in adults (MIS-A). The transmission of SARS-CoV-2, the emergence of potentially more severe and highly transmissible variants, such as the Delta variant, and the number of unvaccinated individuals is likely to have contributed to the increased incidence of MIS-C following increased SARS-CoV-2 transmission all over the world.

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