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C O N T E N T S

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Dear colleagues,

In the current issue, the editorial by Glantzounis et al. addresses the significant advances in the management of hepatocellular carcinoma, over the last 10 years. More specifically, it focusses on the progress in surgical management, the complex interventional radiology techniques, the application of minimal invasive surgery and finally liver transplantation. The original article by Karaivazoglou et al. presents the process of linguistic and cultural adaptation and psychometric validation of the Multidimensional Assessment of Interoceptive Awareness (MAIA) to a Greek-speaking setting. Another original study by Vogiatzis et al. evaluates the effects of therapy in patients with a history of syncope episodes and a positive tilt test.

Moreover, this issue includes three reviews. The first review, by Toumpanakis et al. discusses in a comprehensive way the epidemiology, prognosis, and latest advances in the field of neuroendocrine tumours (NETs), with a special focus on NETs of the digestive tract, (gastroenteropancreatic NETs, GEP-NETs). Roda et al. presents evidence on microscopic colitis, an inflammatory disease of the large intestine and describes current clinical data on disease etiopathogenesis, diagnosis and therapeutic management. Lastly, the review by Tzelepi et al. discusses the three major molecular pathways implicated in the pathogenesis of colorectal carcinoma, with an emphasis on the genes implicated, the associated cancer predisposition syndromes and the therapeutic implications of selected biomarkers.

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Effective multimodal management of hepatocellular carcinoma. An update

Georgios K. Glantzounis, Anastasia D. Karampa, George Pappas-Gogos

Liver cancer is the fifth most common cancer and the second most frequent cause of cancer-related death globally, with 854,000 new cases and 810,000 deaths per year [1]. The incidence of hepatocellular carcinoma (HCC) increases progressively with age, reaching a peak at 70 years. HCC is usually developing in chronic liver disease patients, mainly due to chronic hepatitis B and C (HBV, HCV) infection, but also to non-alcoholic fatty liver disease (NAFLD). It is estimated that 500,000-900,000 new cases of HCC in the USA may develop as a consequence of the high prevalence of NAFLD [2]. HCC has a male preponderance, with a male to female ratio estimated to be 2–2.5:1. It represents about 90% of primary liver cancers. Chronic active HBV, HCV infection, high alcohol consumption, aflatoxin exposure, NAFLD, haemochromatosis and steatohepatitis represent the main risk factors. The incidence of HCC is increasing despite effective antiviral therapy for HBV, HCV, and HBV vaccination at birth [3].

Diagnosis of HCC is based on contrast enhanced imaging methods such as multiphase computed tomography (CT) and magnetic resonance imaging (MRI). MRI has higher sensitivity compared to CT for small lesions 1-2cm [1]. HCC may also be diagnosed by ultrasound or biopsy, while PET-CT contributes slightly to the diagnosis. Without therapy, survival is ranging between 6-8 months, whereas transarterial chemoembolization (TACE) achieves 20-25 months survival [4].

HCC is characterized by phenotypic and molecular heterogeneity. Biomarkers represent a non-invasive way to detect HCC at early stages and have the potential to estimate disease prognosis and recurrence. The specificity of αFP for HCC is close to 100% but the sensitivity falls below 45% [5]. For this reason, it is imperative to find other more sensitive biomarkers for the diagnosis and identification of recurrence. More specifically, autophagy's molecules, such as beclin-1, LC3-II and p62 seem to play a significant role in HCC [6]. Basal autophagy acts as a tumor suppressor by maintaining genomic stability in normal cells. However, once carcinogenesis is established, unbalanced autophagy will promote tumor growth. According to multicenter studies increased autophagy has been detected in advanced HCC and is closely related to low survival. Moreover, autophagy contributes to the chemoresistance of HCC cells [6]. Another serological and histochemical marker that is specific for HCC is glypican GPC3. Recent studies report higher levels of GPC3 expression in poorly differentiated HCC [7]. Other biomarkers involved in the development and progression of HCC is β-catenin, cell free DNA (cfDNA) and circular RNAs (such as cSMARCA5 and circZKSCAN1). The latter have been used in clinical trials as biomarkers for diagnosis, early recurrence detection and treatment of HCC [8].

Advances in the surgical management of hepatocellular carcinoma

Surgery (liver transplantation, resection and ablation) can offer potential cure and long-term survival. Liver transplantation is the ideal treatment for liver cirrhosis and HCC, but has several limitations, as it is mainly applied in patients which fulfil the Milano criteria (single

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Key words: *Hepatocellular carcinoma; surgical management; intermediate and advanced stage; minimal invasive surgery; living related liver transplantation; radiological simultaneous portohepatic vein embolization (RASPE)*

tumor <5 cm, 3 tumors <3cm each, absence of vascular infiltration) [9].

Liver resection is the treatment of choice for large HCCs with preserved liver function. Among more comprehensive staging systems, six have been thoroughly tested, three European (the French classification, the Cancer of the Liver Italian Program [CLIP] classification and the Barcelona- Clínic Liver Cancer [BCLC]) and three Asian (the Chinese University Prognostic Index [CUPI] score, the Hong-Kong Liver Cancer [HKLC] staging system and the Japan Integrated Staging [JIS]. The Barcelona Clinic Liver Cancer (BCLC) is the most commonly accepted system for prognosis and study comparisons. It is an evolving system that links tumor stage with treatment. It entails prognostic variables related to tumor status, liver function and health performance status along with treatment-dependent variables obtained from randomized trials. It is an evolving system that correlates tumor stage with treatment strategy in a dynamic manner, enabling the incorporation of novel advances in the understanding of the prognosis and management of HCC [1]. Nevertheless, BCLC proposes only conservative treatments for the intermediate and advanced stage, excluding these patients from liver transplantation and resection [1]. The guidelines of TACE, as the only management option for the intermediate stage, according to BCLC algorithm, has been heavily criticized by the international hepatobiliary surgical community. An observational multicenter study showed that 36% of patients who underwent liver resection for HCC were classified as intermediate stage and a 5years overall survival of 57% was achieved [4].

A recent systematic review has shown that liver resection may broaden its indications as it can be applied in intermediate and advanced stages of the disease (multinodular HCCs, HCCs with limited macrovascular invasion) with satisfactory long-term survival [10]. Recently the Pan-Asian adapted ESMO clinical practice guidelines have included liver resection as a reliable option for multinodular HCC and for advanced stage HCC with intrahepatic macrovascular invasion without extra-hepatic metastases [11]. Patients with HCC and pre-existing liver disease often present the problem of small future liver remnant (FLR). The gold standard for patients with HCC and inadequate FLR is portal vein embolization (PVE) [12]. Recently a new technique named radiological simultaneous portohepatic vein embolization (RASPE) has been developed which aims to rapidly increase the FLR in order to perform major hepatectomy. During RASPE the right hepatic vein (HV) and the right portal vein are embolized simultaneously [13]. Recent studies showed that RASPE is safe and induces faster and greater FLR, with better functional capacity, in comparison to PVE [13]. The increase in regeneration rate versus PVE could be due to the following reasons: embolization of the hepatic vein could reduce portal inflow and minimize porto-portal collaterals. Furthermore, RASPE can increase liver injury by reducing the flow in the hepatic artery through the hepatic arterial buffer response.

RASPE has the potential to overcome the disadvantages of PVE and ALPPS, since it increases FLR rapidly, is safe and has low post-operative mortality. However, until nowadays most studies are conducted on patients with metastatic liver lesions without pre-existent liver disease [14]. Therefore, new trials should be carried out with HCC, as the regeneration process differs significantly.

Minimally invasive liver resection (MILR) gains more and more ground on a global scale. MILR includes laparoscopic liver resection (LLR) and robotic liver resection (RLR). For patients with resectable HCC, LLR has many advantages over the open approach. The main advantages are lower incidence of ascites and postoperative liver failure, as the abdominal trauma is smaller and the surgical stress significantly less [15]. According to a recent systematic review LLR for HCC is feasible and offers improved short-term outcomes in respect to complication rate, blood loss, and duration of hospital stay, as well as comparable long-term outcomes to those of the open approach [16]. Several studies have shown the feasibility of LLR for HCC in cirrhotic patients and reported reduced complications rates and shortened hospital stay [17]. As regards to the size and location of the mass, they do not represent contraindications for LLR in specialized centers [17]. However, LLR remains a technically demanding procedure that requires advanced laparoscopic technology and an experienced surgical team.

Moreover, the introduction of robotic surgery might bridge the gap of conventional laparoscopy. The most significant clinical benefit of the robotic system over conventional laparoscopy is presumably the performance of minor resections in difficult located liver lesions. Also, the endo-wristed instruments make the robotic system appropriate for parenchymal-sparing resection, and parenchymal preservation [18]. It seems that robotic liver resection maintains the benefits of minimally invasive surgery, but its superiority over laparoscopy has not been proved yet [18]. On the other hand, robotic surgery has much higher cost in comparison with LLR.

Liver transplantation (LT) is the ideal therapy since it may cure both cirrhosis and HCC. Until nowadays, LT has been offered to patients with HCC within the Milan criteria and preserved liver function. However, there is a lack of potential donors for deceased donor liver transplantation (DDLT). Latest data indicate that many patients with HCC have low probability of receiving DDLT before tumor progression [19]. Therefore, Living Donor Liver Transplantation (LDLT) is emerging as an additional therapeutic option, since it has remarkable advantages: (1) The transplantation can be performed on an elective basis before serious decompensation of the recipient or tumor growth, (2) waiting time can be short minimizing the risk of dropout, (3) grafts are in excellent condition and (4) LDLT provides immunological benefits. Due to the technical complexity of the LD allograft, LD recipients have higher complication rates, including bleeding, hepatic artery thrombosis, biliary complications and late biliary strictures. Another complication often associated with LDLT is small for size syndrome (including coagulopathy, cholestasis, encephalopathy and ascites), which can increase mortality. Studies from Asian centers demonstrate that with the incorporation of biological markers in the selection criteria, in order to eliminate biologically aggressive HCCs, LDLT may contribute to better survival rates for HCC patients [20].

In conclusion, significant advances have taken place in the surgical management of hepatocellular carcinoma, over the last 10 years, such as liver resections in the advanced stages of the disease, complex interventional radiology techniques for the management of the small liver remnant, broad application of minimal invasive surgery and living related liver transplantation. All these, along with the conservative management (TACE, targeted therapies, immunotherapy) can offer long term survival with good quality of life and can transform an aggressive disease to chronic disease. It should be emphasized that the management of HCC should be done in specialized hepatobiliary centers with harmonic collaboration of different specialities, the cases should be discussed in the multidisciplinary tumor boards and an individualized approach should be followed.

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Linguistic and cultural adaptation and psychometric validation of the Multidimensional Assessment of Interoceptive Awareness in Greek individuals

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Abstract

Background: Interoception refers to the processing of stimuli from within the body and has been linked to several mental and physical health conditions. The Multidimensional Assessment of Interoceptive Awareness (MAIA) is a 32-item self-report instrument, used to assess several dimensions of bodily awareness. The current study's aim was to present the process of linguistic and cultural adaptation and psychometric validation of the MAIA in a Greek-speaking setting.

Methods: The forward-backward translation methodology was employed including cognitive debriefing interviews with 6 Greek-speaking adults to assess content validity. The final form of the translation was subsequently administered to a larger group of participants to determine the translated questionnaire's factorial structure and its internal consistency.

Results: Following the translated version's pilot testing, the revised version was administered to 107 Greek-speaking adults, 54.2% males with a mean age of 39.4 (12.3) years old. Exploratory factor analysis (EFA) revealed the existence of 8 factors similar to the original version, accounting for 70.6% of the total variance. 31 items presented with satisfying factor loadings (0.396-0.987) to the same factors as the original version, while only 1 item had a lower loading of 0.255 to its theoretical subscale. All MAIA subscales exhibited satisfactory or high internal consistency (Cronbach's alpha ranging between 0.64 and 0.88). In addition, most MAIA subscales exhibited moderately high subscale-subscale correlations.

Conclusion: The Greek version of the MAIA exhibited satisfying content validity, a factorial structure similar to the original version and high reliability and may be useful in assessing interoceptive sensibility in Greek-speaking individuals.

Key words: *Interoception; MAIA; linguistic adaptation; psychometric validation*

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INTRODUCTION

Interoception is defined as the sensing of internal bodily states and is considered crucial for physiological homeostasis [1]. It reflects the brain's capacity to focus inwards, on stimuli derived mainly from the gastrointestinal, respiratory and cardiovascular system and is clearly differentiated from the other senses (vision, audition, taste, smell, touch and proprioception) which process external information or use bodily stimuli to describe the body's relation to the external environment [2]. Research has linked impairments in interoceptive processing with mental and physical health disturbances including sickness behavior, fatigue, obesity, diabetes, depression, autism spectrum, anxiety and eating disorders and for this reason there is an emerging interest in studying interoception in several chronic disease populations [1,3].

Interoception constitutes a multidimensional concept encompassing neurophysiological mechanisms, emotional and behavioral correlates, and metacognitive processing. Interoceptive ability consists of interoceptive accuracy which refers to the objective perception of interoceptive sensations, interoceptive sensibility which includes the self-report (subjective) sensitivity to interoceptive sensations and interoceptive awareness which refers to the correspondence between a person's interoceptive sensibility and his/her interoceptive accuracy [4-6]. The Multidimensional Assessment of Interoceptive Awareness (MAIA) is a 32-item questionnaire which measures interoceptive sensibility and has been widely used in interoception research in various linguistic and cultural settings exhibiting satisfying psychometric properties [7]. Its multidimensional nature has broadened interoceptive assessment and has contributed to the discrimination between maladaptive and adaptive aspects of body awareness [8]. However, the use of any self-reported questionnaire requires its translation and adaptation to the socio-cultural characteristics of the research population [9]. This process is fundamental to ensure the instrument's suitability to be used in a cultural setting different from the setting of its original development and validation, in order to exhibit its optimal psychometric properties. In this context, the present study's aim is to perform the linguistic and cultural adaptation and psychometric validation of MAIA in a Greek-speaking setting. We strongly believe that our findings will reveal valuable information regarding Greek people's interoceptive abilities and will provide researchers with a promising and highly reliable psychometric instrument that might further advance research on interoception and its correlates.

PARTICIPANTS AND METHODS

The current study was conducted at the Division of Gastroenterology of the Internal Medicine Department of the University Hospital of Patras with the collaboration of the Department of Psychiatry and the Department of Public Health. Study participants were treated in accordance with the Declaration of Helsinki and the study protocol and all relevant procedures were approved by the University Hospital's Ethical Committee. All participants provided written consent prior to study entry, after being thoroughly informed about the study's aim and methods.

Adult individuals recruited from outpatients, hospital personnel and a community convenience sample were invited to participate. Individuals with major psychopathology, severe cognitive or neurological deficits, malignancies, or severe chronic diseases, as well as individuals who were not fluent in the Greek language were excluded. All collected data were sealed in envelopes and the names of participants were assigned to numbers, insuring blinding of participants and personnel. Data procession was performed by an independent researcher, who had exclusive access to the specially designed storage space of envelopes.

Interoception questionnaire

The original MAIA is a 32-item self-reported questionnaire used to assess multiple dimensions of interoceptive sensibility. It consists of 8 sub-scales, namely the (1) Noticing subscale which reflects awareness of uncomfortable, comfortable, and neutral body sensations, the (2) Not-Distracting subscale which reflects the tendency not to ignore or distract oneself from sensations of pain or discomfort, the (3) Not-Worrying subscale which reflects the tendency not to worry or experience emotional distress with sensations of pain or discomfort, the (4) Attention Regulation subscale which represents the ability to sustain and control attention to body sensations, the (5) Emotional Awareness subscale which refers to awareness of the connection between body sensations and emotional states, the (6) Self-Regulation subscale which reflects the ability to regulate distress by attention to body sensations), the (7) Body-Listening subscale which refers to the active listening to the body for insight, and the (8) Trusting subscale which refers to the experience of one's body as safe and trustworthy. MAIA items are rated on a 6-point Likert Scale (0-5) and higher scores are suggestive of greater interoceptive sensibility [7].

Translation process and linguistic validation

We followed a forward-backward translation process**.** Two independent translators, who were native-speaking

Greek language, translated separately the original MAIA subscales from English into Greek (forward translation). A prior detailed analysis of the original questionnaires was conducted by translators to clarify their context and they were informed about the questionnaires' targets and their use in research. A third independent translator and Greek native-speaking reconciled the two forward translations to one single target language translation, creating a revised version of the questionnaire. A native-speaking English language translator, who speaks Greek language fluently and had never read the original version of the questionnaire translated independently and separately the reconciled version of the questionnaire from Greek into English (backward translation). A review of the back-translated and reconciled version of the questionnaire was performed by an evaluation committee comprised of three native Greek-speaking experts in mixed fields [a healthcare professional (psychiatrist), specialized in the context of the questionnaires, a scientist specializing in the methodology of questionnaire's validation and an independent translator of the questionnaire scientist, specialized in biomedical sciences] who examined independently all the translation process steps, selecting the most appropriate terms or suggesting new terms of translation for each questionnaire item.

Cognitive debriefing

A pilot-testing of the first consensus of the questionnaire was performed, including cognitive debriefing interviews with 6 native Greek-speaking adults, who were given the questionnaire and were asked about the understanding of questions, whether or not there were any clarity issues, inappropriate context, irrelative terms or unpleasant feelings caused by the questions. Participants were also asked to suggest alternative wording regarding some translated terms. The evaluation committee analyzed all participants' comments and suggestions to ensure the comprehension of terms by the target language population and revised the translated version leading to the final version.

Statistical analysis

Content validity was assessed based on the participants' answers to the cognitive debriefing interviews regarding the content of each question. A descriptive statistical analysis was performed to describe the sample's background characteristics. In order to detect the underlying factor structure of the Greek MAIA we conducted an exploratory factor analysis (EFA) of the 32 items. Initially, we computed the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and the Bartlett's test of sphericity to assess whether our data were factorable. Estimation of the factors was performed by factoring the Pearson correlation matrix by the maximum likelihood (ML) method. The number of extracted factors was based upon eigenvalues >1.0. Internal consistency was estimated with the calculation of Cronbach's alpha for each MAIA subscale. Scales means and range of item-total correlations were also elicited and Pearson correlations were conducted to determine subscale-subscale associations. Statistical analyses were performed with the SPSS version 20.0 software for Windows.

RESULTS

During the MAIA pilot-testing, 6 adults (3 females) were initially administered the questionnaires and were interviewed regarding their content and the administration process. Based on the analysis of the cognitive debriefing interviews, all 6 participants reported that the questionnaire was administered in a visually satisfying form, item scoring was easy, and they found no question disturbing or embarrassing. However, 5 individuals reported that items 12, 13 and 16 were hard to comprehend and asked for clarifications. Based on these comments, the evaluation committee properly rephrased items 12, 13 and 16 and suggested that the MAIA should be administered in the presence of a mental health professional familiar with its content and scope in order to provide appropriate clarification if needed.

Subsequently, we proceeded with the next part of the study which included 107 adults, 58 (54.2%) males with a mean age of 39.4 (SD: 12.3 years). Participants' demographic characteristics are depicted in Table 1.

Exploratory factor analysis

The Bartlett's test of sphericity was significant, χ^2 (496) = 2041.89, $p < 0.001$, and the Kaiser–Meyer–Olkin (KMO) sampling adequacy was 0.78, which together indicate that the MAIA items had sufficient common variance for factor analysis. Exploratory Factor Analysis revealed the existence of 8 factors with an eigenvalue exceeding 1.0, and the extracted factors explained 70.6% of the total variance. Supplementary Table 1 includes the factor loadings of all 32 items, with items 10 ("I can notice an unpleasant body sensation without worrying about it") and 16 ("I can maintain awareness of my whole body even when a part of me is in pain or

Table 1. *Socio-demographic characteristics of the study population.*

Total participants	107
Gender, male, N (%)	58 (54.2)
Age, mean (SD)	39.4 (12.3)
Education, mean (SD)	14.3 (2.6)
Family status, N (%)	
Single	40 (37.4)
Married without children	6 (5.6)
Married with children	50 (46.7)
Divorced, widow/-er	7 (6.5)
Occupation status, N (%)	
Unemployed	8 (7.5)
Private sector	24 (22.4)
Public sector	29 (27.1)
Free lancer	10 (9.3)
Student	10 (9.3)
Retired	7 (6.5)

discomfort") presenting with the lowest loadings (0.255 and 0.396, respectively), while all the remaining items presented with significantly higher loadings ranging between 0.444 and 0.987.

Internal consistency

The Noticing, Attention Regulation, Emotional Awareness, Self-regulation and Body Listening subscales exhibited high internal consistency with Cronbach's alpha ranging between 0.80 and 0.88. The Not distracting, Not worrying and Trusting subscales exhibited satisfying internal consistency (Cronbach's alpha 0.66, 0.64 and 0.65, respectively). Table 2 includes Cronbach alphas, mean scale scores and ranges of item-total correlations for each Greek translated MAIA subscale.

Correlations among the 8 subscales ranged between 0.002 and 0.688 indicating independence (Table 3). The strongest correlations were observed between Body Listening and Self-Regulation (0.688) and Emotional Awareness (0.654) and between Self-Regulation and Emotional Awareness (0.610). In contrast, the weakest correlations were observed between Not Distracting and Not Worrying (-0.031), Attention Regulation (-0.012), Emotional Awareness (-0.015) and Body Listening (-0.002) and between Not Worrying and Attention Regulation (-0.024), Self-Regulation (0.005) and Body Listening (-0.034).

DISCUSSION

The present study's findings confirm that the Greek version of the MAIA is a well-accepted and valid instrument and can be reliably used to assess interoceptive sensibility in a Greek-speaking setting. All subscales exhibited high (5 scales) or satisfying (3 scales) internal consistency and for 7 of them, namely the Noticing, Not Distracting, Not Worrying, Attention Regulation, Emotional Awareness, Self-regulation and Body Listening, the Cronbach's alpha indices were equal or even higher to those of the original version.

Exploratory factor analysis confirmed the factorial structure of 8 subscales which in general loaded the same items as the original version. For items 4, 16, 17, 18, 19, and 24 factor analysis revealed slightly higher loadings to a different factor compared to the original version; however, this difference was rather small and factor loadings to their theoretical subscales were quite

Table 2. *Scale means, range of item-total correlations and Cronbach's alphas for the MAIA subscales.*

	# of items	Items numbers	Scale means (SD)	Range of item-total correlations	Alpha original MAIA	Alpha Greek validation
Noticing	4	1-4	3.11 (1.28)	0.45-0.73	0.69	0.80
Not-distracting	3	5-7	1.77 (1.09)	0.36-0.56	0.66	0.66
Not-worrying	3	8-10	2.50 (1.14)	0.20-0.65	0.67	0.64
Attention regulation	7	11-17	2.92 (1.08)	0.48-0.75	0.87	0.85
Emotional awareness	5	18-22	3.28 (1.37)	0.59-0.80	0.82	0.88
Self-regulation	4	23-26	2.52 (1.32)	0.51-0.79	0.83	0.84
Body listening	3	27-29	2.34 (1.29)	0.65-0.69	0.82	0.82
Trusting	3	30-32	3.79 (0.97)	0.44-0.53	0.79	0.65

MAIA: Multidimensional Assessment of Interoceptive Awareness

Supplementary Table 1. *Exploratory factor analysis (EFA) loadings of the Greek MAIA (all 32 1items).*

	Factor							
	1	2	3	4	5	6	7	8
Noticing								
Item1	,220	-,103	**,723**	,230	,347	,415	,309	-,128
Item2	,231	-,208	**,986**	,232	,132	,306	,138	,022
Item3	,371	-,047	**,623**	,340	,260	,252	,349	,248
Item4	,505	-,362	**,466**	,438	,191	,480	,142	,038
Not distracting								
Item5	,105	,161	-,233	-,042	-,189	-,094	**-,481**	,250
Item6	,110	,100	-,216	,051	-,035	,029	**-,519**	,105
Item7	-,029	,018	-,125	-,069	,031	,046	**-,552**	-,124
Not worrying								
Item8	-,137	**,750**	-,167	-,196	-,076	-,110	,004	,289
Item9	-,178	**,987**	-,180	-,112	,041	-,188	-,018	,145
Item10	,087	**,255**	,048	,056	,189	,033	,516	,071
Attention regulation								
Item11	,276	-,126	,114	**,637**	,402	,361	,157	,071
Item12	,268	-,051	,228	**,629**	,246	,312	,217	,001
Item13	,394	-,081	,164	**,730**	,082	,383	-,034	,179
Item14	,399	-,194	,284	**,920**	,076	,243	-,141	,233
Item15	,406	-,143	,280	**,752**	,187	,361	,084	,365
Item16	,363	,073	,204	**,396**	,219	,139	,318	,533
Item17	,451	,103	,205	**,496**	,326	,423	,254	,555
Emotional awareness								
Item18	**,452**	-,223	,412	,488	,322	,634	,165	,171
Item19	**,509**	-,384	,404	,361	,123	,706	-,003	-,138
Item20	**,733**	-,306	,341	,488	,177	,663	,002	,104
Item21	**,985**	-,181	,329	,416	,305	,432	,016	,190
Item22	**,801**	-,219	,295	,492	,298	,651	-,009	,127
Self regulation								
Item23	,301	,089	,148	,336	**,444**	,367	,325	,353
Item24	,483	-,011	,145	,410	**,605**	,654	,238	,335
Item25	,451	-,090	,352	,391	**,822**	,496	-,019	,211
Item26	,424	-,077	,357	,403	**,866**	,590	,086	,223
Body listening								
Item27	,378	-,115	,334	,378	,397	**,767**	,012	,094
Item28	,383	,090	,213	,318	,533	**,652**	,109	,330
Item29	,417	-,049	,348	,468	,295	**,677**	-,140	,319
Trusting								
Item30	-,016	,213	-,011	-,007	,298	,028	,120	**,533**
Item31	,308	,270	-,057	,169	,180	,173	-,038	**,545**
Item32	,209	,175	,021	,225	,034	,166	-,101	**,631**

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Table 3. *Pearson product-moment correlations among the eight MAIA subscales.*

	1	2	3	4	5	6	7	8
Noticing								
Not-distracting	-0.268**							
Not-worrying	-0.196*	-0.031						
Attention Regulation	0.423**	-0.012	-0.024					
Emotional Awareness	0.578**	-0.015	-0.282**	0.566**				
Self-regulation	0.452**	-0.065	0.005	0.555**	0.610**			
Body Listening	0.467**	-0.002	-0.034	0.517**	0.654**	0.688**		
Trusting	0.055	0.088	0.290**	0.294**	0.096	0.339**	0.275**	

MAIA: Multidimensional Assessment of Interoceptive Awareness
* p<0.05, ** p<0.001 (bilateral)

satisfactory (equal or exceeding 0.40) and for this reason, these items were grouped to the initial subscale in accordance with the original version. Item 10 loaded significantly higher to the Not Distracting compared to its original Not Worrying subscale (0.516 vs 0.255), which suggests that item 10 might be more suitable to be added to the Not Distracting subscale. However, removing this item from the Not Worrying subscale would leave only 2 items in that scale, an alteration that would significantly weaken the subscale's internal consistency. It should be noted that, the vast majority of the translated items presented high factor loadings ranging between 0.444 and 0.987, suggesting that the MAIA translation provides a valid and reliable instrument to assess the 8 dimensions of interoceptive sensibility described by Mehling et al (2012) in the original validation study [7].

Subscale-subscale correlations analysis revealed moderately high correlations among the MAIA subscales, except for the Not Distracting subscale which negatively correlated only with the Noticing subscale and the Not Worrying subscale which correlated only with the Emotional Awareness and the Trusting subscale. These moderately high correlations confirm the anticipated associations among the subscales as subdimensions of the same construct (interoceptive sensibility) and the fact that these correlation coefficients did not exceed 0.80 confirm the validity of each subscale as a measure of a distinct aspect of interoception. Our findings corroborate earlier studies [10,11] which have shown that the Not Distracting and the Not Worrying subscales are not significantly associated with the remaining MAIA subscales.

In conclusion, the Greek version of the MAIA was well accepted by Greek-speaking adults and exhibited satisfying psychometric properties, providing a reliable and useful instrument in the field of interoception research. Clinicians and researchers are encouraged to use this linguistically and culturally adapted version in a variety of clinical settings to improve its qualities and expand its usefulness.

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Effect of counseling on Neurocardiogenic Syncope Treatment

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Abstract

Background: Neurocardiogenic syncope is a common cause of syncope and is found in 50% of patients hospitalized because of syncope. Neurocardiogenic syncope is not a life-threatening situation; however, it may lead to injuries and an impaired quality of life. Initial treatment of neurocardiogenic syncope consists of adequate fluid and salt intake, regular exercise and implementation of special exercises. The aim of the study is the evaluation of the effects of therapy in patients with a history of syncope episodes.

Methods: Sixty-eight patients (33 men and 35 women, mean age 45.8±15.6 years) with a history of syncope episodes and positive tilt test, with a diagnosis of neurocardiogenic syncope, entered the study. All participants followed a non-pharmacological therapeutic intervention which included counseling to change lifestyle along with daily special exercises. The effectiveness of this non-pharmacological treatment in the reduction of syncope episodes and improvement of quality of life, using a general questionnaire (EQ-5D) was evaluated.

Results: Our sample had fewer syncope episodes on average, at 3, 6 and 12 months of non-pharmacological treatment compared to the last year before treatment (0.3±0.48 versus 3.9±0.9 / p=0.003). Quality of life was improved over time with greater improvement in patients who had fewer recurrences.

Conclusion: In patients with a history of neurocardiogenic syncope, non-pharmacological therapy has the benefit of reducing new episodes and ameliorating quality of life.

Key words: *Neurocardiogenic syncope; non-pharmacological treatment; quality of life*

INTRODUCTION

Syncope is a sudden and transient loss of consciousness and postural tonus followed by a quick and spontaneous recovery. It is caused by an acute decrease in systemic arterial blood pressure and cerebral blood flow [1]. Reflex syncope is caused by systemic arterial hypotension resulting from reflex vasodilation, bradycardia, or both [2]. Neurocardiogenic syncope, mediated by emotional or orthostatic stress, is the most common cause of reflex syncope [2-3]. Neurocardiogenic syncope (NS), also known as vasovagal or neurally-mediated syncope, is a common cause of syncope and is found in 50% of patients hospitalized because of syncope. Neurocardiogenic syncope is not life-threatening and its only consequences are injuries and reduced quality of life. Recently, it was found that quality of life (QOL) in patients who suffered from NS was poor compared with healthy people [4]. It is typically triggered by environmental, physical or mental stress, with an estimated lifetime prevalence of 35% [5]. It is diagnosed by obtaining a detailed history and performing a head-up tilt test, with or without drug provocation. Widely accepted measures, not confirmed to be effective, include explanations of the

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underlying mechanism, patient education, reassurance emphasizing the generally benign nature of the disorder, recognition of premonitory manifestations and avoidance of triggers. Several studies have been performed about its management. Initial treatment of neurocardiogenic syncope consists of adequate fluid and salt intake, regular exercise and implementation of special exercises (physical counterpressure maneuvers i.e. muscle tensing) which are recommended as the first line of treatment for neurocardiogenic syncope in current syncope management guidelines [6-8].

The aim of the present study is the evaluation of the effects of treatment in patients with a history of syncope episodes and positive tilt test.

METHODS

Sixty-eight patients (33 men and 35 women, mean age 45.8 ± 15.6 years), with a history of recurrent syncope episodes (>2 episodes and positive head-up tilt-table test - HUT-test) were studied prospectively, in whom the diagnosis was neurocardiogenic syncope (based on the definition of the syncope management guidelines of the European Society of Cardiology). Patients were referred to the Syncope Unit in the Department of Cardiology. Patients with orthostatic hypotension, suspected or confirmed heart disease with a high likelihood of cardiac syncope, steal syndrome, episodes of loss of consciousness due to other reasons than neurocardiogenic syncope, were excluded. In the same manner, patients receiving medications that could interfere with treatment response or patients with orthopedic and functional limitations that could prevent them from performing exercises, were excluded.

Diagnosis was based on the head-up tilt (HUT) test. Tilt test has become a widely accepted method in the clinical evaluation of patients with syncope. Its duration is 30 to 45 min at 60 to 80 degrees and is widely accepted in laboratories for evaluating adult patients. Diagnostic specificity is 80 to 100%, however sensitivity, in contrast, does not exceed 40 to 70%. Isoproterenol or nitrates are the most common agents applied for pharmacologic provocation [9].

After diagnosis, patients followed a program of non-pharmacological therapy, with counseling to change lifestyle alongside daily special exercises [10]. Initially, all patients were informed about the benign nature of their condition and the potential risks, such as accidents, downfalls, etc. and were advised with general guidelines on the way they could avoid everything it could trigger syncope (eg emotional stress). They were asked to avoid certain conditions, such as alcohol, caffeine or nicotine consumption and to drink at least 2.0 L of water per day [11].

Moreover, they were instructed to a daily training program consisting of isometric contraction maneuvers (Figure 1, Figure 2) and standing in a special position for 10 minutes (Figure 3) [10,12]. The whole training program lasted for about 20 minutes daily. The combined effect of information, lifestyle changes and the training program was estimated.

Follow-up

Patients were asked to note the date and the symptoms of probable recurrences. At 1, 3, 6, and 12 months after inclusion, patients informed the medical personnel about syncopal recurrence, the frequency of physical exercises and also their effectiveness. Patients were contacted by telephone or were seen at the outpatient department of the Syncope Unit.

Quality of Life (QoL)

At the same time, QoL was estimated using a general questionnaire (EQ-5D), before intervention initiation and at 1 month, 3 months, 6 and 12 months of follow-up.

The questionnaire consists of two parts, the descrip-

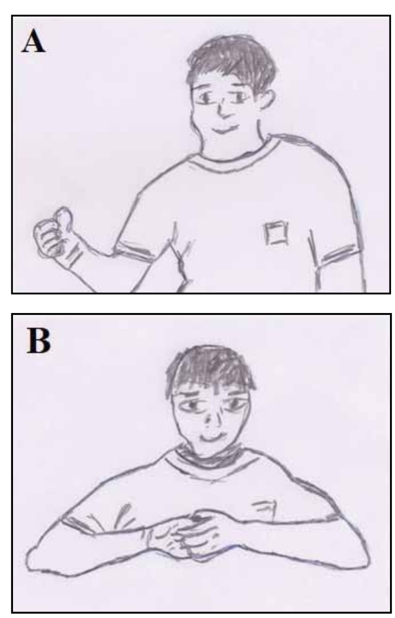

Figure 1. Isometric Exercises of the arms. A: Grip trial, B: Tension of the arms.

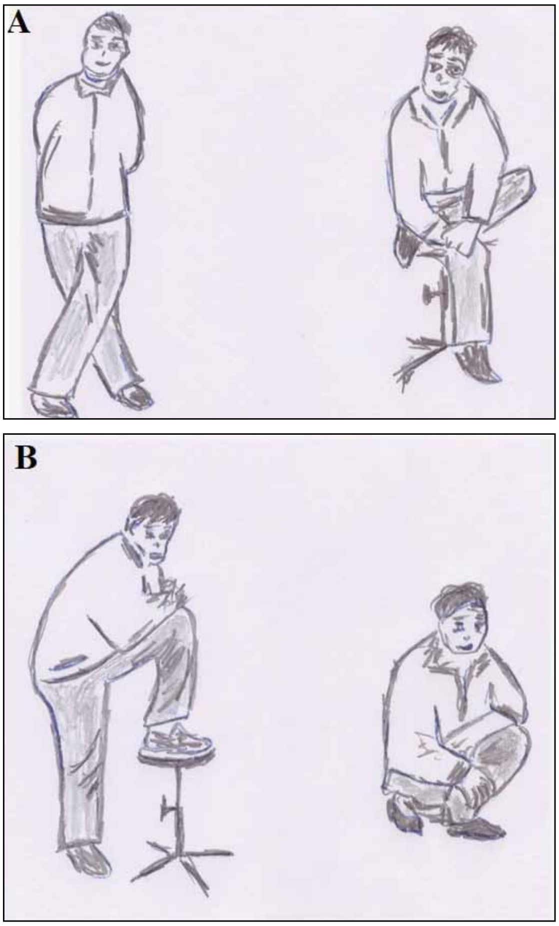

Figure 2. (A, B): Isometric Exercises of the legs.

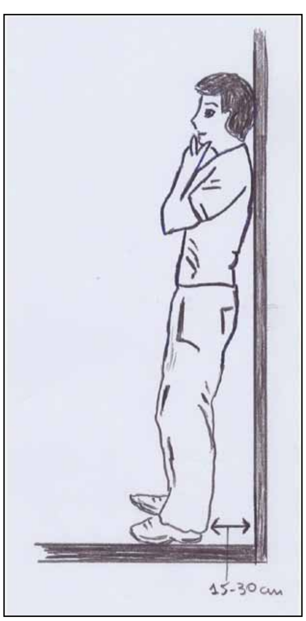

Figure 3. Standing trial. The back of the patient lays on the wall and the legs lie 15-30 cm from the wall.

tive system which includes 5 dimensions (mobility, self-care, usual activities, pain - discomfort, anxiety - depression each of which is rated in a 3-point scale) and the EQ visual analogue scale (EQ-VAS) to estimate the patients' current health status from zero to one hundred. EQ-5D has been extensively used in a large number of clinical studies of cardiovascular disease patients and is one of the most reliable instruments for measuring quality of life, both in the general population and in particular subgroups suffering from specific diseases [14]. The structure and the features of the EQ-5D in recording quality of life have been extensively described [13].

Each patient completed the questionnaire at his/hers first visit, before application of the program and then at one, three, six and twelve months, during the follow-up period. Patients completed the questionnaires on their own, in a separate room, or with the assistance of laboratory staff on the days they had to come to their appointment during the follow-up period. The values calculated using the EQ-5D range between -0.594, which indicates serious problems in mobility, self-care, usual activities, pain - discomfort, stress - depression, and 1 which indicates the absence of any problems. On the contrary, death which in our case was not observed has a value of 0 [15].

In case of lost values or questionnaires, the last observation was used. About 12% of the questionnaires had missed responses, but due to their small number it was not considered to affect the overall evaluation model.

Moreover, patients during their visits were completed a set of questions referring to:

1) Their general health status
2) Frequency and impact of symptoms due to recurrences of arrhythmia (0 = never, 1 = rarely, 2 = sometimes, 3 = often, 4 = always).
3) Severity of symptoms due to recurrences of arrhythmia (1 = mild, 2 = moderate, 3 = severe).
4) Number of visits to Health Units and Health Services consumption (Hospitals, Health Centers, outpatients services).

This information was used as a specific tool to measure quality of life, in accordance with a recent study evaluating the impact of atrial fibrillation recurrences on quality of life [16].

The study's protocol was approved by the Ethical Committee of the Hospital of Veroia (13/2010) and all patients provided written informed consent.

Statistical Analysis

Analysis was performed using the statistical package SPSS 19.00 (SPSS Inc., Chicago, Ill, USA). Initially, an estimation of the normality of the distribution of quantitative variables using the Kolmogorov-Smirnoff test (population >50 individuals) was performed. For comparison of continuous variables, the t-test and the non-parametric Mann-Whitney test were used, while the x^2 test and the Fischer test were used to assess differences in the distribution of categorical variables. The visualization of the time of syncope recurrences was achieved with the Kaplan-Meier curve. The variables that were significantly associated with syncope recurrences were introduced in a multivariable regression model (Cox regression model) to calculate the relative risk and 95% confidence interval (95% CI). The probability $p < 0.05$ (2- way) was considered statistically significant.

RESULTS

The sample's baseline characteristics are shown in Table 1. The mean age of the population was 46 years and 48.5% were males. The mean follow-up time was 12 months.

Patients had fewer syncope episodes on average, at 1, 3, 6 and 12 months of non-pharmacological treatment compared to the last year before treatment (0.3±0.48 versus 3.9±0.9 / p=0.003). In the same way, trauma and fractures due to syncopal episodes were decreased significantly. Thirty patients (44.12%) had experienced a syncopal episode during the follow-up period. The mean time until a new syncopal episode after the counseling period was 46.5 days. Using Cox regression analysis, it was found that the number of syncopal episodes before the counseling period were independent factors for syncopal recurrence (>3 episodes per year, HR=1.34 – CI=1.15-2.56, p=0.03).

Forty-two patients (80.77%) reported that they followed the instructions and all of them found them beneficial.

Quality of life was improved during the follow-up period compared with the baseline (Figure 4), with a greater improvement in patients who had fewer recurrences. These patients reported an improvement in severity and frequency of symptoms in questionnaires (Figure 5).

DISCUSSION

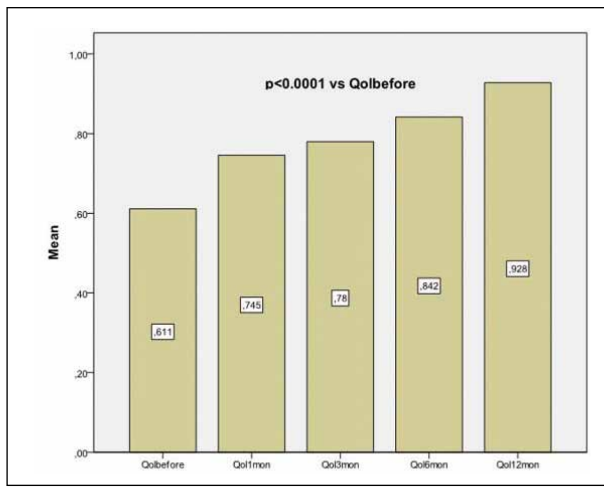

Figure 4. Quality of life index before intervention and during the follow-up period as it was estimated with EQ-5D questionnaire.

Table 1. *The basic characteristics and parameters of the patients.*

	Before intervention	After counseling	p
Age (years)	45.8±15.6		
Onset age (years)	19.64±5.62		
Gender (Man) n (%)	33 (48.53)		
The elapsed time of symptoms	18.18±10.31		
Syncopal episodes in life time (n)	8.54±4.58		
Syncopal episodes last year (n)	3.9±1.17	0.3±0.7	**<0.001**
Trauma n (%)	27 (51.92)	11 (21.15)	**0.01**
Fractures n (%)	12 (44.44)	2 (18.18)	
Time until a new syncopal episode (Days)		46.5±32.8	
Patients followed the instructions		42 (80.77)	

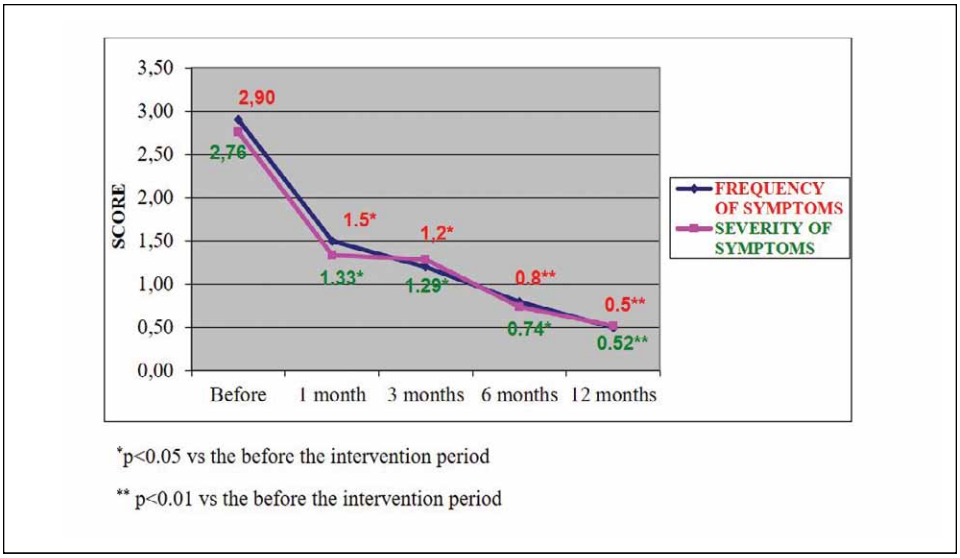

Figure 5. Alterations of frequency and severity of symptoms during the follow-up period according to the questionnaires.

Neurocardiogenic syncope (NCS) is a temporal functional abnormality of the Autonomic Nervous System (ANS) which affects mainly younger persons, especially women, presenting without signs of structural heart disease or other neurological abnormality. Patients complaining about frequent episodes, present with limitations of daily activities and especially their jobs, significantly affecting their quality of life [17].

Various medications have been proposed as a definite therapy including b-blockers, fludrocortisone, serotonin reuptake inhibitors. The results are poor and disappointing on many occasions [18-20].

In addition, a variety of non-pharmacological measures have been proposed, as patient education and lifestyle modifications, including the avoidance of triggering factors such as hot environment, humid atmosphere and prolonged standing, an increase of water consumption during the day, normal food intake and the use of exercises that prevent syncopal episodes. An informative and instructive discussion with the patient about the benign nature and prognosis represents the first step in the management of neurocardiogenic syncope [21,22]

A multicenter, randomized clinical trial, showed that vasovagal syncopal patients who received conventional therapy plus training in exercise protocols had a lower syncope burden than the control group [23]. This means that the exercise protocol is a safe, effective, and low-cost intervention, which should be used as first-line treatment for patients with neurocardiogenic syncope.

The efficacy of non-pharmacological measures in managing NCS was obvious in the current study. The frequency and severity of symptoms were decreased and as a result, patients' quality of life was improved.

In various studies [24,25] the favorable effect of programs encompassing physical counterpressure maneuvers was clear and marked in comparison with patients that received only lifestyle advice or pharmaceutical therapy. Studies [4,26] have shown that the results of these therapies are disappointing.

After a positive tilt test, the patients were given instructions to perform lifestyle modifications and an exercise program. A session protocol [maximum duration of 30min- hour] includes a presentation of the program's purpose and session structure, an analysis of simple physiology and vasovagal reflexes and a demonstration and explanation of the maneuvers. In various studies, physical exercise and all these measures have shown a decreased or elimination of syncopal recurrences [24,25]. These exercises and protocols besides their favorable effect on the impact of the autonomic nervous system on the cardiovascular system in patients suffering from NCS, may also expand the circulatory blood volume and increase the muscle tone in the lower limbs. As a result, the systolic and diastolic volumes are increased and the excitation of ventricular C fibers, responsible for triggering NCS is obvious [27]. In our study general instructions for the avoidance of triggering factors, an increase of water, other liquids and salt intake and the performance of physical counterpressure maneuvers were advised. It is concluded that these measures could be used as first-line treatment for patients with neurocardiogenic syncope. In the above study 40 patients (58.82%) did not present any symptoms during the 12-month follow-up period in contrast to other studies where patients relapsed during the follow-up period. This could be explained by the fact that the patients included were severely affected, with several syncopal episodes before their participation to the study. It is known that the burden of syncopal episodes is a predictor of relapses [26].

On the other hand, it is widely known that physical and psychosomatic functions are altered in patients with NCS. Sheldon et al. [28] reported a proportional decrease of syncopal episodes due to better management, better knowledge regarding the pathophysiology of syncope, greater reassurance and advice around the management of episodes and vigorous application of the exercises.

As a result, patients were assured that symptoms are decreased or disappeared and this leads to quality-of-life improvement as it is recorded in self-report questionnaires, at least six months after the beginning of the program. In previous studies, [29,30] Qol was assessed only after treatment initiation in groups of patients with different health issues and different diagnoses and treatment. In our study, Qol was assessed in different time intervals during the follow-up period.

General Qol, as it was estimated with the generic questionnaire EQ-5D, was significantly improved during the follow-up period. Moreover, the severity and frequency of symptoms were improved gradually. All Qol parameters are expected to improve if patients are reassured that they will have no syncopal episode or if they can manage them successfully.

Limitations of the study. In the study, none of the patients received any pharmacological treatment. Syncopal episodes before and after the application of the program were compared and this requires a certain caution from the patients in recording the episodes. Many of them, suffering from NCS, come to the hospital after symptom deterioration. As a result, any improvement in symptomatology would resemble a success. The collected data were subjective, as the self-reported measures of QoL, which could be subject to bias and misinterpretation.

Conclusion: In patients with a history of neurocardiogenic syncope, non-pharmacological therapy has the benefit of reducing new episodes and enhancing quality of life. This kind of therapy should be recommended to all patients with NCS, however, some of them need additional therapy.

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Gastroenteropancreatic neuroendocrine tumours (GEP-NETs). A comprehensive review

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Abstract

Neuroendocrine tumours (NETs) are a heterogeneous group of epithelial tumours arising from the diffuse endocrine system throughout the body. NETs are considered to be rare tumours, however their incidence is increasing, while their pathophysiology is yet poorly understood. Moreover, given their heterogeneity, they remain a challenging disease to diagnose and treat. In this review we aim to delineate in a comprehensive way the epidemiology, prognosis, as well as the latest advances in diagnosis and management in the field of NETs, with focus on NETs of the digestive tract, (gastroenteropancreatic NETs, GEP-NETs).

Key words: *Neuroendocrine tumours; somatostatin analogues; octreotide; lanreotide; malignant carcinoid syndrome*

INTRODUCTION

The discovery of neuroendocrine tumours (NETs) dates back to 1870, when the German physiologist Rudolf P.H. Heidenhain identified a group of cells that were different from the enteric, chief, and parietal cells of the gastrointestinal (GI) tract. A few years later, in 1907, the German pathologist S. Oberndofer was the first to use the term "carcinoid" - from the German word for "cancer-like"- to describe NETs of the gastrointestinal tract. In 1995, a revised classification of NETs was published suggesting to avoid the use of the term "carcinoid tumours", as it fails to encapsulate their malignant potential and promotes the misconception that all NETs lead to carcinoid syndrome. Instead, the use of the term "neuroendocrine tumours" for all NETs was established. Throughout this period, physicians have continuously studied neuroendocrine cells in an effort to pinpoint their intricacies, analyse their clinical presentation, and manage their symptoms [1,2].

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NETs are a group of heterogeneous epithelial tumours originating from secretory cells of the neuroendocrine system. They are indolent neoplasms that secrete a range of peptide hormones and monoamines [3]. The "neuro" component refers to their dense core granules (DCGs), which are organelles commonly found in serotonergic neurons that store biogenic amines. The "endocrine" component refers to their ability to synthesize and secrete these monoamines [4]. The neuroendocrine system includes the parathyroid, pituitary, and adrenal glands, as well as thyroid and pancreatic endocrine islet cells. The neuroendocrine cells within these glandular organs, together with scattered cells found in the gastrointestinal, respiratory, or genitourinary tracts, constitute the diffuse neuroendocrine system [4].

The most common primary tumour sites for NETs are the lungs, GI tract and pancreas. However, given the extensive distribution of NE cells, NETs can also be found in a plethora of other organs, such as the prostate, breast, skin, thymus, and genitourinary system [5,6].

This review will primarily focus on gastroenteropancreatic NETs (GEP-NETs), which consist of tumours of the GI tract and pancreatic tumours (pNETs). Although

GI tumours arise from enterochromaffin cells of the gut, pNETs originate from the islet of Langerhans or precursors of the ductal/acinar system [5]. By classifying NETs by their embryonic origin, a distinction can be made between foregut (gastric and duodenal), midgut (jejunal, ileal, and caecal), and hindgut (distal colonic and rectal) tumours [5].

EPIDEMIOLOGY

According to the Surveillance, Epidemiology, and End Results (SEER) program in the United States, the age-adjusted incidence rate of NETs has increased 6.4-fold from 1973 (1.09 per 100000) to 2012 (6.98 per 100000) in all stages, grades, and sites of the disease [7]. The incidence has been increasing in a 3-10% rate depending on the tumour subtype [8]. This increase could be attributed to the advancements in disease detection via imaging and recognition of neuroendocrine histology [9].

GEP-NETs are the second most prevalent gastrointestinal tract cancer [10]. The most common primary site of GEP-NETs is the small intestine (30.8%), with the rectum (26.3%), colon (17.6%), pancreas (12.1%), stomach (8.9%), and appendix (5.7%) following. Small intestinal NETs are more common amongst Caucasians, whereas NETs originating from the rectum are more prevalent in African American, Asian, and Native American populations [7]. Although NETs of the stomach, appendix, and cecum develop more frequently in females, jejunal, ileal, duodenal and rectal NETs are more common amongst the male population [7]. As far as disease progression is concerned, 53% of patients have localized disease, 20% present with locoregional disease, and 27% present with distant metastases at the point of diagnosis [11]. In patients with a family history of NETs in a first-degree relative, the risk of developing NETs increases by 3.6-fold [12].

Although the vast majority of GEP-NETs are sporadic, approximately 10% of pNETs can be found within the context of genetic syndromes such as Multiple-Endocrine-Neoplasia type 1 (MEN-1), von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1), and tuberous sclerosis complex (TSC). Of those, MEN-1 is the most common one, consisting of hyperparathyroidism (associated with parathyroid adenomas in over 90% of cases), pNETs (in up to 70% of cases) and pituitary adenomas (in 30-40% of cases). Other manifestations of MEN-1, include bronchial NETs and type-II gastric NETs [13].

HISTOPATHOLOGIC FEATURES

The histopathologic morphology and immunohistochemical profile form the basis for the diagnosis of NETs [15]. Depending on their morphology and degree of proliferation, NETs can be categorized into poorly differentiated neuroendocrine carcinomas (NEC) and well differentiated neuroendocrine tumours. Well differentiated NETs are composed of bland, benign looking, monomorphic neoplastic cells. The intermixing of coarse chromatin with finely granulated chromatin gives these cells their characteristic "salt and pepper" appearance. Despite their predictable cytologic and nuclear features, well differentiated NETs demonstrate wide-ranging morphological growth patterns. These include trabecular, glandular, solid or gyriform growth patterns, with tumour cells sometimes arranged in pseudorosettes.

Poorly differentiated neuroendocrine carcinomas (NEC) are particularly aggressive in nature and lack resemblance in morphology, clinical presentation, and genetic makeup to well differentiated NETs. Depending on their nuclear size, they are divided into large and small cell neuroendocrine tumours.

Small cell carcinomas morphologically resemble their well-differentiated NETs counterparts, as they both exhibit the characteristic salt and pepper chromatin. Their cell nuclei are near each other because of their thin cytoplasm, thus giving them the distinctive appearance of nuclear impressions ("nuclear moulding"). Small cell carcinomas commonly demonstrate confluent growth patterns, with cells arranged in solid sheets in a streaming pattern. Single cell necrosis as well as large necrotic areas are frequently identified.

Large cell NETs demonstrate a clumpy chromatin appearance with prominent nucleoli. They consist of highly pleomorphic and hyperchromatic tumour cells with a markedly pronounced cytoplasm. They are characterized by a solid growth pattern, with extensive necrotic areas centrally and palisading peripherally.

The immunohistochemical markers of NETs include synaptophysin, chromogranin A (CgA), cluster of differentiation 56 (CD56), neuron-specific enolase (NSE) and Ki-67. Poorly differentiated tumours are often positive for synaptophysin and NSE expression, whereas well differentiated tumours usually demonstrate high levels of synaptophysin and CgA expression. Thyroid transcription factor 1 (TTF1), caudal type homeobox 2 (CDX2) and insulin gene enhancer protein 1 (ISL1) can be immunohistochemically labelled to track the primary site of metastatic tumours, as these proteins are typically found in the lung, small intestine, and pancreas respectively. Ki-67 is a proliferation marker used for grading GEP-NETs as well as for predicting the course of the disease [14].

CLASSIFICATION AND STAGING (TABLE 1)

There are several ways of approaching the classification of GEP-NETs. One classification, based on embryonic derivation, distinguishes between foregut (gastroduodenal), midgut (jejunal, ileal, and cecal), and hindgut (distal colic and rectal) tumours. GEP-NETs can be subclassified into two groups: carcinoid tumours of the luminal GI tract and pancreatic NETs. In addition, the grade and degree of differentiation of GEP-NETs are of paramount importance in determining the clinical behaviour of the disease. Grade refers to how rapidly the neoplastic cells divide, proliferate and grow. It is measured by the Ki-67 index or the mitotic rate. Each tumour receives a numerical grade, with grade 1 (G1) tumours having a Ki-67 index from 0% to 2% or a mitotic rate from 0 to 1 per 10 high power fields (HPF), G2 tumours having a Ki-67 index from 3% to 20% and mitotic rate from 2 to 20 per 10 HPF, and G3 tumours having a Ki-67 index over 20% and mitotic count higher than 20 per 10 HPF [15]. Importantly, it is advised that tumour grades should be measured at the areas of the histopathology specimen with the highest levels of mitotic activity, as GEP-NETs are considered to have a high degree of intratumor heterogeneity when it comes to morphology and proliferative rate. Specifically, it is recommended that 40-50 high-power fields should be used for the mitotic count, and at least 2000 cells should be counted in the areas of highest labelling for an accurate measurement of the Ki67 index [16]. Importantly, according to the WHO newest classification (2017), there is a distinction between well-differentiated (G1, G2, or G3) NETs and poorly-differentiated NECs (found primarily in the pancreas), which are considered high-grade by definition [17].

The prognostic significance of the current grading systems has been demonstrated in a study by Karakas et al. [18]. In particular, the 5-year survival rates for G1 and G2 NETs were 97% and 82% respectively; however, the prognostic significance of G3 tumours could not be evaluated due to the low number of patients presenting with G3 tumours. Larger, long-term studies with well-balanced patient populations should be performed to effectively evaluate the prognostic significance of the 2017 WHO classification system.

The TNM staging system for NETs was first introduced by Rindi et al in 2006 and was later adopted by the European Neuroendocrine Tumour Society and the American Joint Committee on Cancer in 2010 [19]. The latest version (2017) features separate staging systems for well-differentiated NETs of the appendix, stomach, colorectal, duodenal, jejunal, and ileal primary sites. A new TNM staging system is also used for pNETs, which is separate from the one used for exocrine pancreatic cancers [20].

Importantly, case reports have also demonstrated that NETs can rarely co-exist with pancreatic and colorectal adenocarcinomas, giving rise to unique therapeutic challenges [21–23]. Accordingly, it is recommended that patients with NET diagnosis should undergo meticulous screening to prevent late-stage diagnosis of synchronous tumours [24].

CLINICAL PRESENTATION AND SYMPTOMS (TABLE 2)

GEP-NETs

GEP-NETs clinical presentation depends greatly on the hormonal status of the tumour. Non-functioning GEP-NETs are usually incidentally discovered during surgery, as they are commonly asymptomatic [25]. Nonspecific symptoms such as abdominal discomfort

Table 1. *Classification of GEP-NENs*

Grade	Differentiation	Ki-67 %	Mitotic rate/10 HPF
G1 NET	Well	<3	<2
G2 NET	Well/moderate	3-20	2-20
G3 NET	Moderate	>20	>20
G3 NEC	Poor	>20	>20

Abbreviations: GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; HPF, high power field.

Table 2: *Secretory syndromes in patients with hormonally active GEP-NENs*

Tumor type	Symptoms/Findings	Substances responsible
Midgut NETs with liver metastases	Carcinoid syndrome (diarrhea, flushing, wheezing, carcinoid heart disease)	Serotonin, bradykinin, histamine, prostaglandins
Insulinoma	Hypoglycemia	Insulin
Gastrinoma	Multiple gastric ulcers, abdominal pain, diarrhea	Gastrin
VIPoma	Watery diarrhea, hypokalemia, achlorhydria (WHDA syndrome)	VIP
Glucagonoma	Diabetes, diarrhea, stomatitis, weight loss, necrolytic migratory erythema	Glucagon

Abbreviations: GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; NET, neuroendocrine tumor; VIP, vasoactive intestinal peptide.

may also be present, but do not contribute to earlier diagnosis due to their vague nature. As a result, diagnosis may delay up to a decade, and often symptoms are attributed to irritable bowel syndrome or other benign gastroenteric disorders. On the other hand, functioning tumours may present with a variety of symptoms, based on the anatomic location of the tumour, as well as the type of produced hormones [26,27]. For example, small bowel/midgut carcinoids metastatic to the liver are responsible for the carcinoid syndrome, characterized by flushing, diarrhoea, wheezing and carcinoid heart disease, via releasing serotonin and other vasoactive substances in the circulation [5]. Similarly, functioning pNETs (VIPoma, glucagonoma, gastrinoma etc.) are responsible for the development of various clinical syndromes which will be described in detail below.

1. Appendiceal NETs

Appendiceal NETs are usually benign and not associated with any hormonal-related symptoms. They are commonly diagnosed incidentally after examining the specimens of appendicectomies. Women have higher prevalence of appendiceal NETs, possibly due to the fact that pre-menopausal females undergo diagnostic laparoscopies more frequently in order to differentiate between gynaecologic and other reasons of lower abdominal pain [28].

2. Gastric NETs

Gastric NETs are rare and can be subdivided into three categories. Type I gastric NETs have an association with chronic atrophic gastritis and pernicious anaemia. Due to the loss of the gastric glands and long-term achlorhydria, antral G cells are forced to secrete excessive serum gastrin, thus causing hyperplasia of the gastric neuroendocrine cells [Entero-Chromaffin-Like (ECL) cells] and development of multifocal, polypoid NETs [31]. Type II gastric NETs are associated with Zollinger-Ellison and MEN-1 syndrome. They are commonly small in size, multifocal, and relatively unaggressive. Patients usually suffer from symptoms of Zollinger-Ellison syndrome, such as diarrhoea, heartburn, and peptic ulcers. Type III gastric NETs are large, sporadic, solitary tumours that are not associated with gastrin excess. They are more invasive than their Type I and II counterparts and can occasionally present with an "atypical carcinoid syndrome" mainly due to histamine production. This can be clinically distinguished from the typical (serotonin-associated) carcinoid syndrome by the patchy red, serpiginous, highly pruritic flush patients usually present with [29,30].

3. Small bowel NETs

The majority of small bowel NETs are located in the distal ileum [32], with around 25% of patients presenting with multifocal tumours clustered close to each other at the time of diagnosis. Despite the fact that the malignant potential of GEP-NETs is associated with tumour size, even small bowel NETs less than 1cm in size have the ability to metastasize [33]. Common sites of metastases include the liver, mesentery, and peritoneum. Mesenteric desmoplasia and intestinal ischemia can occur when the tumour metastasizes to the lymph nodes at the root of the mesentery [34]. Accordingly, patients may present with colicky or intermittent abdominal pain and intestinal obstruction [34]. Small bowel NETs originating from the duodenum are rarely syndromic. Up to 30-40% of advanced small bowel NETs produce and secrete serotonin and other vasoactive substances, causing "carcinoid syndrome". Carcinoid syndrome occurs due to the hypersecretion of vasoactive amines and peptides, such as serotonin. Serotonin is synthesized from dietary tryptophan in specialized neuroendocrine cells called enterochromaffin (Kultchisky) cells [35]. The classical "carcinoid syndrome" symptoms include diarrhoea (73%), flushing (65%), and bronchospasm (8%) [5]. Other symptoms include hypotension (as part of "carcinoid crisis") and valvular heart disease ("carcinoid heart disease"). Excess serotonin is primarily responsible for the development of diarrhoea, whereas flushing can be mainly attributed to substance P, kallikrein, and a range of other prostaglandins and tachykinins [36,37]. Flushing may be triggered by alcohol consumption, stress, spices, and tyramine-containing foods. It usually manifests in the face, neck, and thorax. High levels of serum serotonin can also lead to "carcinoid heart disease". In this case, serotonin receptors in the subendocardial cells of heart valves are activated [38], leading to fibrosis of the tricuspid and pulmonary valves and consequently to tricuspid regurgitation and pulmonary stenosis [39] (Figure 1). The left side of the heart is usually unaffected as serotonin is metabolised while passing through the lungs [40]. Similarly, since serotonin is secreted from small bowel NETs, it is drained in the portal circulation and metabolized by monoamine oxidases in the liver before entering the systemic circulation [41]. Thus, carcinoid syndrome only occurs in patients with liver or other distal metastases. Carcinoid syndrome patients also commonly present with hypoproteinaemia, as tryptophan is the precursor for serotonin synthesis [42]. Pellagra-like symptoms like diarrhoea, dermatitis, and dementia can also manifest, as niacin production is reliant on tryptophan [43]. Rarely, carcinoid syndrome can be associated with pancreatic (<1% of pNETs), bronchial, and ovarian NETs [44].

Figure 1. Tricuspid valve, almost replaced by fibrotic plaque ("carcinoid heart disease").

4. Colorectal NETs

Colorectal NETs are rare but have poorer prognosis than adenocarcinomas due to their aggressive clinical course [45]. They can manifest with rectal bleeding, pain, and change in bowel habit. Most colorectal NETs are small, located in the submucosa, and are incidentally discovered during lower endoscopy [46]. In particular, small (<1cm), nonaggressive rectal NETs have low metastatic potential and are often endoscopically or transanally excised. On the other hand, large (>2cm), high-grade rectal NETs present with stage IV disease in more than half of the patients. The tendency of intermediate-size tumours to metastasize depends on the depth of tumour invasion of the muscularis propria [47]. Tumours originating distal to the cecum are more malignant in nature than rectal NETs, as they are commonly poorly differentiated [48].

5. Pancreatic NETs (pNETs)

The vast majority (90%) of pNETs are hormonally non-functioning. Hormonally silent neoplasms appear to have worse prognosis than hormonally active tumours, possibly because they are diagnosed late in the disease progression [49]. Insulinomas are the most common type of hormonally functioning pNETs. They are commonly small (<2 cm), solitary, hypervascular tumours, with low malignant potential. They usually manifest with low blood glucose levels, symptomatic hypoglycaemia, reversal of symptoms after administrating glucose (Whipple triad) [50], and hypokalaemia due to excessive insulin secretion [51]. Gastrinomas commonly present in the pancreas and duodenum and are responsible for the development of Zollinger-Ellison syndrome. Their clinical features include peptic ulceration, heartburn, and diarrhoea. High-dose proton pump inhibitors can help in alleviating these symptoms [52]. VIPomas are another subtype of pNETS. Given that vasoactive intestinal polypeptide (VIP) inhibits electrolyte and water absorption and stimulates intestinal secretion, VIPomas usually present with profuse, watery diarrhoea and electrolyte disturbances, such as hypokalaemia [53]. Glucoganomas, on the other hand, are extremely rare and manifest with hyperglycaemia, weight loss, deep vein thrombosis, dermatitis (necrolytic migratory erythema), and depression [54]. Somatostatinomas are characterized by excessive secretion of somatostatin. Patients usually present with steatorrhea, hyperglycaemia, cholelithiasis, diabetes, and reduced gastric acid levels. ACTH, PTHrP, growth hormone-releasing hormone, serotonin, and cholecystokinin, may infrequently be secreted by pNETs, leading to the development of the corresponding syndromes [55].

DIAGNOSIS

GEP-NET diagnosis requires a high index of suspicion and is based on their clinical presentation, histopathologic morphology, immunohistochemical profile, and imaging modalities. Traditionally, carcinoid syndrome diagnosis is largely dependent on detection of elevated urinary 5-hydroxyindoleacetic acid (5-HIAA) over 24 hours [56]. Recently, however, it has been found that serum and plasma 5-HIAA can be used as an alternative for the diagnosis and monitoring of carcinoid syndrome [57]. Hormone levels should be measured in patients presenting with symptoms of hormonally functioning pNETs. Hormone concentration can then be monitored and used as a marker of disease progression or treatment response [58]. On the other hand, Chromogranin A (CgA) is the diagnostic biomarker of choice for non-functioning NETs [59]. CgA has a high sensitivity (53%-91%) but low specificity (<50%) [59]. Endoscopic imaging also plays an important in the diagnosis of NETs. In particular, endoscopic ultrasonography is the most sensitive test for the diagnosis of pNETs (sensitivity 82%-93%), especially for detecting tumours smaller than 2 cm and for the localization of insulinoma [60,61]. Colo-rectal NETs are usually identified at colonoscopy. Importantly, the entire colon needs to be examined to detect any potential synchronous tumours [62].

Cross sectional imaging is of paramount importance for the identification of tumour location and the assessment of the extent of invasion of GEP-NETs. In particular, computed tomography (CT) and magnetic resonance imaging (MRI) of the abdomen and pelvis are employed for the detection of pNETs and midgut carcinoids respectively. A triple-phase helical CT is recommended for the diagnosis of liver metastasis [63]; however, a RCT by Baudin et al showed that MRI may be superior to CT [64]. Functional imaging modalities also play a key role in the diagnosis of GEP-NETs. Specifically, somatostatin receptor scintigraphy (Octreoscan) is commonly employed, during which a $^{[111]}$indium-labeled somatostatin analog like octreotide is used to detect tumours expressing somatostatin receptors. Lately, gallium-68 (^{68}Ga)-DOTATATE PET/CT has become the preferred imaging modality, due to increased patient satisfaction, high sensitivity (97%), specificity (95.1%) and accuracy (96.6%), as well as decreased radiation exposure (Figure 2) [65]. Gallium-68, is a positron emitter that can be linked to somatostatin analogues and can be localized with positron emission tomography (PET) imaging [66]. (^{68}Ga)-DOTATATE PET/CT contributes to staging the disease, identifying potential lymph node or bone tumour invasion, and detecting previously unknown primary tumours in complex cases [67]. A ^{18}F-fluorodeoxyglucose PET/CT scan is usually employed for imaging of the Grade 3 NETs and poorly differentiated NECs [68]. Combination of (^{68}Ga)-DOTATATE PET/CT and ^{18}F-fluorodeoxyglucose PET/CT at follow-up of GEP-NETs patients is needed, when tumour heterogeneity or co-existence of GEP-NETs with adenocarcinomas is suspected [69].

GEP-NETs: APPROACHES IN MANAGEMENT

The European Society of Medical Oncology [70] and European Neuroendocrine Tumour Society [65–68] have developed an evidence-based approach on the manage-

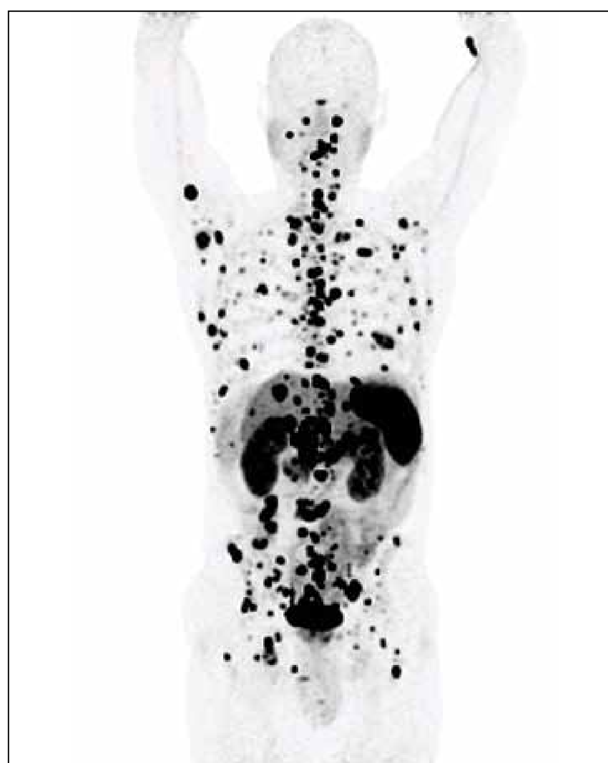

Figure 2. Gallium-68 (68Ga)-DOTATATE PET of a patient with metastatic small bowel NET with multiple hepatic mesenteric and skeletal metastases.

ment of GEP-NETs, including gastric, SI, pancreatic and colorectal NETs which is delineated below. In summary, the main goals of management of GEP-NETs include: a) control of hormonal-related symptoms (in functioning tumours), b) consideration of surgery in localized and sometime in metastatic disease (if technically feasible and clinically appropriate) and c) control of tumour growth with systemic treatments and prolong patients' survival in cases with advanced disease. The selection of treatment is generally affected by the extent of disease (locoregional vs. locally advanced/metastatic) with the latter being the most common presentation, whilst tumour histology and status (stable/progressing), as well as patient's performance status and comorbidities need to be taken into account. Patients should be referred to a Specialized NET Unit. Management decisions should be individualized and made in Multi-Disciplinary-Team meetings, aiming not only to control the disease, but also to improve and maintain patients' quality of life.

1. Management of localized/locoregional disease

Surgery is the treatment of choice for local or locoregional disease in NET G1 and G2. For small bowel NETs (SB-NETs), radical resection in combination with mesenteric lymph node resection is recommended. Surgery may also be considered in cases of locally advanced SB-NETs, for palliative purposes and avoidance of complications like acute/subacute/chronic small bowel obstruction and intestinal ischemia in the presence of a large mesenteric mass [70,71]. For pNETs, surgery is also recommended in locoregional disease, especially in tumours >2cm (standard pancreatectomy, pancreaticoduodenectomy or distal pancreatectomy). Regional lymph node resection should also be considered given high risk for nodal metastases. For non-functioning pNETs <2cm, a conservative watch-and-wait approach may be considered, with yearly imaging [70,71].

In terms of localized gastric NETs, a surveillance approach is recommended for type I, with potential endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) to be considered in tumours ≥10mm. For type II, an individualized treatment approach is recommended, given the possibility of concomitant tumours as part of MEN-1 (e.g. pan-NETs). Local or limited excision can be recommended, however a referral to a NET center of excellence should be strongly considered for further management. For type III, a partial or total gastrectomy with lymph node resection is recommended, given that they are considered to be more invasive than their I and II counterparts [72].

Treatment of colorectal NETs depends on site and size. For colonic lesions of any size, a localized colectomy with lymph node resection should be considered. For rectal NETs, lesions <1 cm can be resected endoscopically, carrying a low risk of developing metastatic disease in the future. Lesions >2cm have a higher metastatic potential and radical surgery (anterior excision) is often recommended. For borderline lesions 1-2cm with unclear metastatic potential, approach is individualized based on certain tumour characteristics like histologic features (e.g. high mitotic index) and disease extent, which can be assessed with a combination of CT/MRI/PET or endoscopic ultrasound (EUS) techniques [73,74].

2. Management of locally advanced/metastatic disease

Management of locally advanced/metastatic disease revolves around the role of systemic treatments with antiproliferative effect and/or symptomatic control of carcinoid syndrome, resection of primary site and/or metastatic deposits, and utilization of locoregional treatments, mainly with palliative intent. Below a summary of ESMO Consensus Guidelines for management of locally advanced/metastatic disease is presented [71].

A. SYSTEMIC THERAPY

Systemic therapy has a dual role in GEP-NETs; it is used not only to inhibit tumour growth (antiproliferative) but also to control symptoms related to hormonal production (antisecretory), and especially carcinoid syndrome (CS) [71].

Antiproliferative treatments

In terms antiproliferative treatment options, SSAs can be considered as first-line especially in slowly-growing G1 and G2 GEP-NETs with Ki-67 up to 10% and demonstrated somatostatin receptor (SSTR) positivity on functional imaging modalities (octreoscan, Ga-Dotatate PET scan). Octreotide and lanreotide are the most commonly utilized SSAs and are mainly used in long-acting formulations, requiring intramuscular administration in 4-week intervals. It should be noted that in patients with stable advanced SB-NETs, low disease burden and very low Ki-67 (<2%) an active surveillance strategy can be considered. Other lines of antiproliferative treatment include IFN-a, which could be considered in patients with midgut NETs, where SSAs have failed or functional imaging shows SSTR-negative tumours. Everolimus, a selective mTOR inhibitor, is another antiproliferative agent approved by FDA for use on G1 and G2 advanced well-differentiated GEP-NETs and bronchial NETs, progressing on prior treatment lines, based on the results of the RADIANT-1 trial [75]. In addition, sunitinib, a multi-targeted tyrosine kinase inhibitor (TKI), is another option for patients with pNETs progressing on prior lines of treatment, based on the results of SUN 1111. This was a phase 3 randomized control trial comparing sunitinib to placebo in patients with advanced well-differentiated pNETs, with disease progression <12 months before baseline, demonstrating a statistically significant superior median progression-free survival for sunitinib (11.4 months compared to 5.5 months with placebo) [76]. It should be noted that both sunitinib and everolimus are not valid treatment options in G3 tumours.

An important breakthrough regarding the management of advanced GEP-NETs has been the development of peptide-receptor radionuclide therapy (PRRT). PRRT is a targeted form of systemic radiotherapy, utilizing the attachment of a radioactive agent such as Yttrium- 90 or Lutetium-177 to a somatostatin analogue, which then binds to somatostatin receptors of GEP-NETs and directs the radionuclides inside the tumour cells [77]. Based on the results of NETTER-1 trial, a phase 3 randomized controlled trial accruing patients with advanced, progressive, somatostatin-receptor-positive G1 and G2 midgut NETs, 177Lu-DOTATATE plus octreotide LAR demonstrated superior PFS and response rate compared to octreotide LAR alone [78]. 77Lu-DOTATATE is FDA-approved as a second-line therapy for patients with midgut G1 and G2 NETs and disease progression on SSAs. It may also be used in pan-NETs, after failure of approved therapies, as well as in carefully selected patients with NET G3 [71]. Guidelines recommend that PRRT should ideally be used in conjunction with SSAs in patients with functioning NETs and CS to prevent CS flares, which are expected to occur in the setting of PRRT [71].

Systemic cytotoxic chemotherapy is generally advised in NETs G3 of any site. Cisplatin or carboplatin plus etoposide is considered first line in NEC G3. Data on second-line regimens are conflicting and several combinations have been used, mainly extrapolated from the treatment of GI adenocarcinomas [5-FU/leucovorin/irinotecan (FOLFIRI), 5-FU/leucovorin/oxaliplatin (FOLFOX), capecitabine plus temozolomide]. In metastatic disease from G1/G2 pan-NETs regimens like streptozotocin with 5FU can also be considered [71].

Antisecretory treatments

SSAs are the mainstay of antisecretory agents for CS control in GEP-NETs as well as other hormonal syndromes, such as those associated with functional pNETs (VIPomas, glucagonomas). For patients with CS refractory to standard doses of SSAs, next line options include SSA treatment escalation with increased doses or increased frequency of administration, peptide-receptor radionuclide therapy (PRRT) and telotristat ethyl [71]. Telotristat ethyl, a novel inhibitor of tryptophan hydroxylase, which is implicated in the production of serotonin, has been developed, for patients with GEP-NETS and carcinoid syndrome [79]. An international, multicenter, randomized, double-blind, placebo-controlled phase III trial (TELESTAR) reported a reduction of approximately 40% of bowel movements per day using telotristat ethyl doses of 750–1500 mg in those patients [79]. Other antisecretory treatments also specifically target functional pNETs. In the case of insulinoma, which is characterized by excessive production of insulin, diazoxide, a benzothiadiazide derivative that inhibits insulin secretion via ATP-dependent potassium channels in pancreatic β-cells, can be utilized [79]. Similarly, proton pump inhibitors (PPIs) are frequently used to suppress gastric acid hypersecretion in the case of gastrinomas [79].

B. THE ROLE OF SURGERY

Surgery for primary NET site can be considered in the palliative setting for Stage IV NETs, especially in the case of SB-NETs, to prevent or treat complications related to small bowel obstruction and intestinal ischemia. A similar approach can be followed in advanced functional pNETs with uncontrolled hormonal symptoms. On the other hand, surgical removal of metastases has a limited role, and is primarily indicated in the case of liver metastases, in patients with exclusive/predominant liver metastatic disease. Other options for treatment of liver metastases includes liver transplantation, while locoregional liver treatments (e.g. selective internal radiation therapy – SIRT) can be considered a more conservative approach in patients with otherwise rejectable liver deposits, while locoregional liver treatments (as below) can be considered a more conservative approach in patients with otherwise rejectable liver deposits.[71].

C. LOCOREGIONAL TREATMENTS

Locoregional treatments are mainly targeted against liver metastatic deposits, aiming to control liver tumour burden and sometimes also improve symptoms of carcinoid syndrome. They are divided into two main categories; ablative and transarterial.

Ablative approach

Ablative treatments include radiofrequency, microwave, cryoablation and alcoholization. Of those, radiofrequency ablation (RFA) and microwave ablation are the most commonly utilized in the management of liver metastases. During RFA, liver tumours are ablated with the heat generated from medium-frequency alternating current between 350-500 kHz. Microwave ablation utilizes microwaves, a non-ionizing form of radiation used to generate heat, in order to ablate liver metastatic tissue, and it is an appropriate alternative to RFA [80].

Transarterial approach

Transarterial treatments are directed against highly vascular liver metastases from GEP-NETs, which are mainly perfused by the hepatic artery. The aim is to induce ischemia and necrosis of metastatic liver lesions by occluding their arterial supply. These interventions are performed by accessing the arterial liver circulation via the femoral artery, followed by transarterial embolization (TAE) of the hepatic artery with gelatin beads, often combined with intraarterial administration of cytotoxic chemotherapeutic agents (transarterial chemoembolization, TACE), or drug-eluding beads. (TACE-DEB). Another interesting transarterial approach is the so-called transarterial radioembolization (TARE) with yttrium-90 (Y-90) microspheres which are injected through the hepatic artery to the pre-capillary level of the liver metastases, attaching to the microcirculation and releasing radiation [80].

CONCLUSION

In this review, we delineate in a comprehensive way the latest data on epidemiology, histopathologic features, clinical presentation, diagnosis and management of patients with GEP-NETs. We demonstrated that GEP-NETs are a rather heterogeneous group of tumours with differences as well as many similarities in terms of incidence, diagnostic and therapeutic approach. Patients' management is based on a multi-disciplinary approach and needs to be individualized. Further research is still needed in the field of NETs to further elucidate the pathogenesis of these malignancies as well as define new diagnostic methods and novel treatments in the field.

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REVIEW

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Microscopic colitis: Overview

Giula Roda

Abstract

Microscopic colitis (MC) is an inflammatory disease of the large intestine that causes persistent watery diarrhea especially in older patients. Microscopic colitis encompasses 2 different subtypes: lymphocytic colitis and collagenous colitis. MC is characterized by a nearly normal-appearing colonic mucosa. Diagnosis is based on histology. Risk factors for MC include increasing age, female sex, presence of other autoimmune diseases and possibly use of certain drugs, including proton pump inhibitors, nonsteroidal anti-inflammatory drugs, selective serotonin reuptake inhibitors, and statins. In the last decade, emerging evidence regarding disease pathogenesis has provided advances in the management strategies for this disease. This is a comprehensive review on disease etiopathogenesis, diagnosis and therapeutic management.

Key words: *Microscopic colitis; diarrhea; collagenous colitis; lymphocytic colitis*

INTRODUCTION

Microscopic colitis (MC) is characterized by the presence of non-bloody diarrhea with normal colonoscopy and microscopic evidence of mucosal inflammatory changes of the colonic tissue [1]. Microscopic colitis encompasses two different disorders: lymphocytic (LC) and collagenous colitis (CC) [1]. Microscopic colitis may occur in patients of any age but typically emerges in late middle age and the elderly and is more prevalent in women. The gender difference is more significant for collagenous colitis. Non-bloody diarrhea is the predominant symptom, although abdominal discomfort and weight loss may also occur. Both diseases run a benign course and there is no risk of malignancy. Sometimes patients present a relapsing course with a need for immunosuppressive therapy and rarely for surgery [1, 2].

Because both lymphocytic and collagenous colitis, manifest with histologic evidence of chronic mucosal inflammation, in the absence of endoscopic or radiologic abnormalities of the colon, diagnosis is only possible through histological analysis. Patients with lymphocytic colitis have an increased number of intraepithelial lymphocytes in the colonic epithelial layer and increased number of sub-epithelial chronic inflammatory cells compared with healthy individuals. Patients with collagenous colitis have a thickened subepithelial collagen layer that can vary between 7 to 100 μm. Similar changes in inflammatory cell populations such as increased number of intraepithelial lymphocytes also occurs in collagenous colitis [1,2].

Microscopic colitis' etiology and pathogenesis remain unknown. No genetic factors have been identified, although some familial cases have been described [3,4]. Several hypotheses have been advanced, including autoimmune dysfunction or an abnormal immune or inflammatory response to an unknown luminal antigen or luminal factor. This later hypothesis is supported by the regression of inflammation following diversion of the fecal stream and recurrence of inflammation following restoration of intestinal continuity in some patients [5]. However, the identity of the inciting antigenic factors is uncertain. A variety of luminal factors have been implicated in the pathogenesis of MC, including drugs, bile salts, bacterial products, and toxins. NSAIDs, aspirin, proton pump inhibitors, ticlopidine, SSRI, acarbose and statins, are some of the drugs that have been more frequently associated with the disease.

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Autoimmunity is another condition that has been proposed to have a role in the pathogenesis of microscopic colitis. In epidemiological studies a strong association with other autoimmune diseases has been reported in patients with microscopic colitis, including celiac disease, thyroiditis and rheumatoid arthritis. Budesonide, the only drug that has been tested in multiple randomized controlled trials (RCT), is highly effective and achieves clinical remission in approximately 80% of patients. However, symptoms' relapse occurs in 60% to 80% of patients after treatment withdrawal [6].

EPIDEMIOLOGY

Previously considered to be a rare diagnosis, microscopic colitis accounts nowadays for 4 to 13% of patients investigated for chronic diarrhea [7, 8]. The incidence of microscopic colitis seems to be increasing. In the United States, the overall prevalence of microscopic colitis is around 103 per 100,000 persons. The reason might be a more accurate diagnosis with biopsies, or possibly increased incidence of immune-mediated disorders. MC is nearly as common as classic Inflammatory Bowel Diseases (IBD) [7,8].

A strong female and elderly predominant have been identified. However, 25% of MC patients are younger than 45 [1].

CLINICAL PRESENTATION

The key clinical feature is chronic non-bloody diarrhea, which is typically watery, leading to urgency in 70% of patients and, ultimately, fecal incontinence in 40% of patients [9, 10]. The two forms of MC have similar symptoms. Relapses occur in 60%–80% of the cases after discontinuation of budesonide treatment, indicating that the course of the disease if often chronic. Abdominal discomfort or cramps may occur in up to 50%. Of note, a differential diagnosis between MC and irritable bowel syndrome may be challenging in these patients. Moreover, weight loss is observed in 50% of patients with active disease [9,10].

ETHIOPATHOGENESIS:

Auto-immunity in microscopic colitis

There is some evidence in the literature pointing to a possible role of autoimmunity in microscopic colitis. In some case series, CC is more frequent in women, as other autoimmune diseases. An overrepresentation of autoimmune diseases is found in microscopic colitis [11]. Epidemiological studies have shown an association with autoimmune diseases that reaches 30-40%. The most common diseases which have been associated with microscopic colitis are celiac disease and several forms of arthritis. Sjögren's syndrome, scleroderma, Raynaud's disease, recurrent iritis, giant cell arteritis, systemic lupus erythematosus, diabetes mellitus, sarcoidosis, psoriasis, myasthenia gravis, Crohn's disease, ulcerative colitis have also been reported to be associated with MC. In patients with collagenous colitis there have been reports of a significant increase in mean serum concentration of immunoglobulin M and a non-significant trend toward increased concentrations of antinuclear antibodies and perinuclear antineutrophil cytoplasmatic antibody in collagenous colitis. TNFα gene polymorphisms were found to be more frequent in patients with MC than in controls; these polymorphisms have been associated with susceptibility to several autoimmune diseases, such as juvenile idiopathic arthritis, systemic lupus, dermatitis herpetiform and celiac disease. Some studies have revealed an association with the HLA genes. Three HLA alleles [HLA-B*08:01, HLA-DRB1*03:01, and HLA-DQB1*02:01], related to the ancestral haplotype 8.1, were significantly associated with increased CC risk [12]. These HLA alleles were not associated with LC. Moreover, lymphocyte infiltration at the site of inflammation can be found and the majority of patients respond to steroid therapy.

Role of bacteria

There is some evidence supporting a role for bacteria or for bacterial dysregulation (dysbiosis) in the pathogenesis of microscopic colitis, although no specific causative agent has been identified. The strongest argument for a luminal agent, which could be a bacterial agent or a bacterial toxin, comes from the fact that the diversion of fecal stream in patients with medically refractory diarrhea results in the resolution of histological inflammation, that recurs upon transit reconstruction [5, 13].

Recent evidence has also suggested the contribution of an infective agent as risk factor for microscopic colitis. Indeed, gastrointestinal infection has been associated with collagenous colitis [14]. In a small case series, patients with collagenous colitis presented Yersinia antibodies more commonly than healthy controls, leading the authors to speculate that in some cases, Yersinia might have been the triggering factor in the development of collagenous colitis [15].

Genetics

Familial occurrence of MC has been reported, but the exact role of genetic factors remains to be defined. Allelic variation of the matrix metalloproteinase-9 gene does appear to be associated with CC [16]. Three HLA alleles (HLA-B*08:01, HLA-DRB1*03:01, and HLA-DQB1*02:01), related to the ancestral haplotype 8.1, were significantly associated with increased CC risk but not LC [12].

Risk factors

Smoking is a risk factor for MC both for men and women and smokers develop the disease earlier than nonsmokers (by a median of 14 years) [17-19]. Drugs such as acarbose, aspirin, cyclo3 fort, lansoprazole, nonsteroidal anti-inflammatory drugs, ranitidine, sertraline, and ticlopidine have been suggested to act as an environmental risk factor in causing or triggering MC [20]. Of note, nonsteroidal anti-inflammatory drugs and proton pump inhibitors were identified as the 2 drugs with the highest likelihood to cause MC [21]. Of note, given the increased incidence of MC in postmenopausal women, sex hormones disturbances have been suggested as risk factors in the development of inflammatory bowel disease and other immune-mediated diseases as well as in MC [22].

THERAPEUTIC INTERVENTIONS

Currently the primary goal of therapeutic interventions in MC is to achieve clinical remission, whereas the role of histological remission is still unknown [23]. Budesonide is the only drug with strong evidence of response rates up to 80%. Moreover, improvement of quality of life under budesonide treatment has been shown by a small number of studies [24, 25]. No evidence-based alternatives to budesonide have been proposed. There are no RCTs for antidiarrheals drugs. Budesonide has been shown to be superior to prednisolone [26]. Indeed, patients treated with budesonide were less likely to experience a recurrence compared to those under prednisolone [26]. Of note, RCTs have shown that MC patients achieve clinical remission within 4 weeks on induction therapy with 9mg budesonide or maintain clinical remission on 6mg or less of budesonide. 10% to 20% of these patients are non-responders and may be candidates for immunosuppressive therapy [24]. No sufficient data are available for bismuth subsalicylate and data have shown that mesalazine should not be used as induction therapy [25]. Although evidence is limited, biologics should be considered when symptoms worsen, and patients are non-responders to budesonide. Moreover, data are limited on long term use of biologics in MC.

An algorithm for the treatment of MC has been proposed by the European Microscopic Colitis Group. Antidiarrheals and/or cholestyramine may be use if there are mild symptoms. In active disease short-term budesonide (6–8 weeks) should be initiated and readministered in case of relapse. In more severe cases, biologics should be considered and as maintenance treatment, immunomodulators such as AZA or mercaptopurine. In patients refractory to medical therapy, surgical treatment is a therapeutic option.

CONCLUSION

Microscopic colitis is a chronic disease for which several data on genetics, autoimmunity and microbiome influences have been generated in the last decade. Overlaps with inflammatory bowel disease have offered new insights into the etiopathogenesis of MC as well as into treatment options. Emerging studies suggest a role for biologicals and immunosuppressive therapies for the management of budesonide-refractory or budesonide-dependent disease. MC can have a substantial negative effect on patient quality of life and therefore well-designed clinical trials are mandatory to assess novel therapeutic interventions.

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Molecular pathways in colorectal cancer

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Abstract

Colorectal cancer is a common cause of cancer-related deaths. Significant advances have been made in recent years regarding the understanding of its pathogenesis. Colorectal carcinomas develop through the serial accumulation of genetic and epigenetic events along two pathways: the chromosomal instability pathway, where adenomas are the precursor lesions and *APC* and *KRAS* mutations represent early events, and the CpG island methylator pathway, where serrated lesions are the precursor lesions, *BRAF* or *KRAS* mutations represent early events and epigenetic MLH1 silencing is a frequent occurrence activating the microsatellite instability pathway (MSI). Carcinomas in patients with familial adenomatous polyposis develop along the chromosomal instability pathway, whereas in Lynch syndrome mutations in mismatch repair genes (that is *MLH1, MSH2, MSH6, PMS2*) result in microsatellite instability. These developments have important therapeutic implications and testing for the presence of *KRAS/BRAF* mutations and MSI is recommended in patients with colorectal carcinomas to guide therapeutic decisions in the era of precision medicine.

Key words: *Colon cancer; microsatellite instability; KRAS; BRAF; CIMP*

INTRODUCTION

Colorectal cancer (CRC) is the second (women) and third (men) most common malignant neoplasm [1], with its incidence increasing in the last years, especially in low and middle income countries [2], probably due to lifestyle changes. It also represents the third (women) and fourth (men) most common cause of cancer-related death in humans [1]. Increased cancer screening and development of newer therapeutic modalities has resulted in a decrease in mortality rates (in patients older than 50 years) [3]. There has been an increasing understanding of colorectal carcinogenesis pathways in the last two decades; cancer predisposition inherited syndromes, notably familial adenomatous polyposis and Lynch syndrome, that consist 5% of all CRC cases, have played a major role in this progress [4].

Colorectal cancer develops through a stepwise accumulation of genetic and epigenetic abnormalities than enable cells to bypass proliferation control, evade apoptosis, avoid immune destruction, promote angiogenesis, and survive and proliferate at metastatic sites [5]. Along this serial accumulation of genetic and epigenetic events, recognizable lesions are formed (i.e. adenomas, serrated lesions) that progress to more advanced lesions through additional mutations or epigenetic events, a process that requires 10 to 15 years [6]. The molecular pathways that have been linked to the pathogenesis of colorectal carcinoma are the chromosomal instability (CIN) pathway, the microsatellite instability (MSI) pathway, and the CpG island methylator phenotype (CIMP) pathway [7]. A fourth pathway, the polymerase proofreading aberrations pathway is responsible for a minority (<3%) of CRC tumors [3]. In this review we summarize the three major pathways of colorectal carcinoma pathogenesis, with an emphasis on the genes implicated, the associated cancer predisposition syndromes and the therapeutic implications of selected biomarkers. Preneoplastic lesions are also mentioned in brief.

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Chromosomal instability (CIN

The CIN pathway consists of gains and losses of whole chromosomes or fractions of them and it usually occurs as a result of mutations in proto-oncogenes or tumour suppressor genes [8,9]. Tumours developed through this pathway are characterized by aneuploidy (abnormal number of chromosomes in a cell, that is 46+/-n) and loss of heterozygosity (LOH, the somatic loss of wild-type alleles, concerning the entire gene and the surrounding chromosomal region). Genes frequently altered in tumours developing through CIN can be divided in three categories, that represent different stages of cell cycle progression: chromosomal segregation, telomere stability and DNA damage response [9–11].

Chromosome segregation results in separation of the duplicated chromosomes into the daughter cells during mitosis by the mitotic spindle [11]. This procedure is briefly paused by the spindle checkpoint, taking place in prometaphase, until all chromosomes have established bipolar connections (*bioriented chromosomes*) to the mitotic spindle. On instances where specific pairs of sister chromatids are not properly aligned on the metaphase plate, usually due to kinetochores not being properly attached to microtubules or not under enough tension by spindle-pulling forces [12,13] a signal is generated so that anaphase is delayed [14]. This signal is received by a series of spindle-checkpoint proteins, including *MAD1, MAD2, BUB3, BUB1, BUBR1*, and *CENP-E* (centrosome protein E). The checkpoint inhibits the *APC/C* (anaphase-promoting complex/cyclosome) and its coactivator Cdc20, (APC/C(Cdc20)). When all chromosomes are properly attached and aligned, the *APC/C* complex initiates ubiquitin-dependent degradation of *securin* and activation of *separase*, which in turn dissolves the cohesion between sister chromatids, by cleaving a multiprotein complex termed *cohesin*. Cohesin is responsible for the cohesion between the sister chromatids and plays an important role in chromosome segregation in dividing cells [15].

Mutations in genes that participate in this process lead to errors in segregation and, occasionally, carcinogenesis. More specifically, mutations in kinetochore proteins *hRod/KNT, hZw10* and *hZwilch/FLJ10036*, which contribute to the spindle checkpoint, have been identified in colorectal cancer cases [16]. Aurora kinases, which consist of *AURKA* (Aurora A), *AURKB* (Aurora B) and *AURKC* (Aurora C), are serine/threonine kinases that also participate in regulating the process of chromosomal segregation, each one having their own role and localization [17]. *AURKA* is associated with centrosome maturation and bipolar spindle formation. When overexpressed, it leads to amplified centrosomes and defective spindle formation. Subsequently, mitosis is inhibited and the incomplete cytokinesis results in multinucleation [9,18,19]. *AURKA* has been associated with some instances of CIN in colorectal tumours [20]. *AURKB* is a passenger protein and participates in cytokinesis, chromatid segregation and the modification of histones. Its overexpression relates to advanced stages of colorectal cancer [21].

Telomere dysfunction has also been recognized as a causative factor for CIN. Chromosome ends are protected by telomeres, which are short DNA sequences with their associated proteins. Their main function is to protect the chromosome ends from double-strand breaks, occurring during segregation, and to prevent them from fusing [9,22]. However, after each replication round, a part of telomeric DNA is lost, which leads to telomere shortening (*end-replication problem*). This occurs as a result of the inability of DNA polymerase to fully form the 3′ end of linear chromosomes. When telomeres reach a critically short length, the cell enters senescence, while the ones that fail to complete this path undergo massive cell death, after entering a crisis-state. Activation of telomerase, the enzyme that is responsible for elongating telomeres, or the ALT (Alternative Lengthening of Telomeres) mechanism, occurs in cells that manage to survive the aforementioned crisis [9].

DNA-damage response (DDR) mechanisms have evolved in order to detect structural DNA alterations, occurring mainly because of environmental agents, reactive oxygen species and spontaneous hydrolysis of nucleotide residues [23,24]. These protein complexes play an important role in identifying an error in a DNA sequence, so that the cell cycle is paused, allowing the damage to be repaired, or, in cases where the damage is beyond repair, halting cell's growth or initiating its apoptosis.

As expected, cells with abnormal DDR are more sensitive to DNA damaging sources, and exhibit genomic instability. Specifically, some of the genes whose protein products participate in these signalling and repair processes have been reported to relate to certain syndromes that predispose humans to cancers. Some examples are *ATM* (ataxia telangiectasia mutated) and *ATR* (ataxia telangiectasia and Rad3-related) protein kinases, involved in Louis-Bar syndrome and Seckel syndrome respectively, *BRCA1, BRCA2* proteins, whose mutations lead to hereditary ovarian and breast cancer and *WRN* (Werner syndrome protein) linked to Werner syndrome. Last but not least, mutations in *TP53* protein have been causatively related to Li-Fraumeni syndrome, which has been linked to CIN in colorectal cancer [9,25].

The Wnt/β-catenin pathway plays an important role in the pathogenesis of colorectal cancer, primarily affected by mutations of the *APC* (Adenomatous Polyposis Coli) gene (5q21). The protein encoded by this gene participates in many cell functions, but especially noteworthy is its participation in the Wnt signalling pathway, as it is crucial for tumorigenesis. The purpose of the Wnt pathway is to control the translocation of β-catenin to the cell nucleus. After its migration, β-catenin, interacts with the *TCF/LEF* (T-cell factor/lymphoid enhancer factor) family of transcription factors and, thus, transcriptionally activates various genes, which are involved in cancer growth. When Wnt ligands are absent, a complex consisting of *APC, Axin, CK1* (casein kinase) and *GSK-3β* (glycogen synthase kinase) mediates β-catenin degradation. More specifically, the amino terminal area of β-catenin is phosphorylated by the two kinases of the complex, which allows β-Trcp, a protein with ubiquitin ligase activity, to recognize it. Subsequently, *β-catenin* is ubiquitinated and subjected to degradation by proteasomes [26].

When a Wnt ligand is present, it binds to *FZD* (Frizzled) and its co-receptors *LRP5* or *LRP6* (low-density lipoprotein). Subsequently, a complex called 'signalosome' is formed, which interacts with the *Dvl* protein (Dishevelled) and causes *LRP6* phosphorylation and *Axin complex* activation. This mechanism inhibits *β-catenin* phosphorylation. β-catenin is, then, translocated to the nucleus where it participates in transcriptional gene activation [26]. When *APC* is mutated, the β-catenin degradation complex is not formed and, the pathway is constantly activated, even in the absence of a signal, leading to uncontrolled cell proliferation [26–28].

It has been reported that ***APC* mutations** are an early event in colon carcinogenesis. *APC* appears somatically mutated in 5% of dysplastic aberrant crypt foci, 30%–70% of sporadic adenomas and 70% of sporadic tumors [9,29–31]. Another, albeit not as common, mechanism responsible for *APC* gene inactivation is the hypermethylation of the gene's promoter (seen in 18% of primary colorectal carcinomas and adenomas) [32]. In addition, germline mutations of *APC* have been directly linked to FAP (Familial Adenomatous Polyposis), an inherited colon cancer predisposition syndrome (see below) [33,34].

Apart from *APC*, gain-of-function mutations have been reported in the gene encoding β-catenin (*CTNNB1*) in 50% of colorectal tumours without *APC* mutations [32,35]. It has been shown that *CTNNB1* mutations are more frequent in small adenomas (12.5%) than they are in large ones (2.4%) and invasive cancers (1.4%), [36], a finding which suggests that they are not as prone to induce malignancy as APC mutations. Finally, *AXIN* and *AXIN2/conductin* gene mutations have been found in CRC, albeit not in those developing through the CIN pathway, but rather those with an MSI high phenotype (see below) [37,38].

In the adenoma to carcinoma sequence, in the CIN pathway of CRC carcinogenesis, a relatively early event following *APC* mutations and WNT pathway activation, with significant clinical implications, is *KRAS* mutation [9]. Proteins that belong to the **RAS** family (three isoforms; *KRAS, NRAS,* and *HRAS* with >80% homology) possess the ability to bind guanosine triphosphate (GTP) and guanosine diphosphate (GDP), cycling between their active and inactive alternative states, respectively [39]. Their role is to mediate a number of signalling pathways, so that extracellular signals are transduced to the cell nucleus, and enhance gene transcription, leading to initiation/regulation of cellular procedures, like cell proliferation and growth, differentiation and migration [40]

RAS, in its activated form, is involved in many signal transduction pathways, including the **RAS/RAF/MAPK pathway** (also known as the mitogen-activated protein kinase (MAPK) cascade) and the PI3K/AKT pathway [40]. These pathways participate in regulating cell cycle, migration and apoptosis, tissue healing and angiogenesis, important hallmarks of carcinogenesis [5].

Following ligand (i.e. epidermal growth factor-EGF) binding to its receptor (i.e. epidermal growth factor receptor-EGFR), the receptor is dimerized, auto-phosphorylated and activated, thereby activating adaptor proteins which enable RAS to exchange its GDP with GTP. GTP-bound RAS interacts with RAF and activates a phosphorylation cascade. RAF as a family consists of three serine/threonine kinases, A-RAF, B-RAF, and C-RAF (first recognized as retroviral oncogenes in the avian retrovirus Mill Hill 2 (MH2), and the murine sarcoma virus (MSV) 3611 isolate) [41] which are activated by RAS-GTP and in turn, phosphorylate and activate MEK1 and MEK2. The latter are MAPKs (Mitogen-Activated Protein Kinases), four different kinds of which have been recognized: ERK, c-Jun N-terminal kinase (JNK), ERK5 and p38 MAPK (p38) [42]. MEK1 and MEK2, which are characterized by substrate specificity, catalyse the phosphorylation of ERK1 and ERK2, which proceed to phosphorylate multiple substrates (because as opposed to their activators, they have a wider specificity), leading to the regulation of several transcription factors and, as a result the expression of multiple genes [9,43,44].

KRAS is mutated in 30-50% of colorectal cancers [9,45] and is generally considered to be one of the oncogenes that are most frequently mutated in human cancers (also frequently mutated in carcinomas of the lung, pancreas, breast, and oesophagus) [46–49]. In colon cancer, KRAS mutations are a relatively common event as they have been identified in 60-95% of aberrant crypt foci (the earliest morphologic manifestation of adenomas) [9,50,51]. *KRAS* mutations are not limited to carcinomas developing through the chromosomal instability pathway, but are an early event in serrated carcinogenesis too (through a traditional serrated adenoma precancerous lesion) (see below).

KRAS mutations usually occur in exon 2, followed by mutations in exons 3 and 4. Most of its mutations consist of amino acid substitutions (caused by single nucleotide point mutations) in codons 12 and 13 of exon 2, amounting for 88% of recurrent mutations in all types of cancers. Mutations may also appear in codons 59 and 61 of exon 3, and in codons 117 and 146 of exon 4, albeit less frequently. On the other hand, *NRAS* and *HRAS* mutations are much less frequent than KRAS mutations and are usually located in codons 61 and 59 (exon 3), followed by codons 12 and 13 of exon 2 and 117 and 146 of exon 146 [43,52]. These alterations have the same effect, diminishing the molecule's endogenous GTPase activity and inducing a constant GTP-bound state, leading to continuous activation of the cascade and finally promoting cell survival, proliferation and migration and inducing carcinogenesis.

A therapeutic choice for metastatic colorectal carcinoma is EGFR inhibition. In contrast to lung cancer, EGFR mutations are not common in colorectal cancer and their presence does not predict therapeutic response. In contrast, the presence of *KRAS/NRAS* mutations has a negative predictive role as they predict lack of response to EGFR targeting therapy [53], since activation of the pathway is due to a genetic event downstream of the receptor. Initially, exon 2 *KRAS* mutations were identified as predictive [52,54,55], but further research [56,57] has shown that all the mutations mentioned above are associated with lack of response to anti-EGFR targeting therapy (monoclonal antibodies *cetuximab* and *panitumumab,* targeting the extracellular domain of the receptor). Thus, current guidelines from the American Society of Clinical Oncologists, the College of American Pathologists, and the Association for Molecular Pathology recommend that extended ras analysis, including *KRAS* [exons 2 (Codons 12,13), 3 (codons 59, 61) and 4 (codons 117, 146)] and *NRAS* [exons 2 (Codons 12,13), 3 (codons 59, 61) and 4 (codons 117, 146)], should be performed to all patients with metastatic colorectal carcinoma considered for anti-EGFR therapy [58,59]. Only patients with wild-type KRAS are candidates for this type of therapies [52,60] as those have a higher probability of responding to the treatment. This way patients not likely to respond are excluded and saved unnecessary toxicity, and cost. An estimate of 7500$ per patient is saved when these predictive markers are used in therapy selection [61,62].

TP53 is another gene frequently mutated in tumours associated with CIN, albeit this happens relatively late in the pathway. This gene encodes a nuclear transcription factor which acts as a tumour suppressor, inducing cell cycle arrest when the DNA appears damaged. That way, DNA can be repaired or, in case of irreversible damage, the cell is led to apoptosis. Loss of function mutations in *TP53* have been reported in more than 50% of cancers, so *TP53* dysfunction is generally considered a hallmark in human tumours [63]. Regarding colorectal cancer, loss of function in TP53 has been increasingly found with progression of the lesion as it is seen in 4%–26% of adenomas, 50% of early carcinomas developing in adenomas, and in 50%–75% of late carcinomas [9,64], making TP53 mutations a defining event in the adenoma to carcinoma progression. Most of its mutations are missense: transitions of GC to AT principally occurring in five hotspot codons (175, 245, 248, 273, and 282) [65].

Other abnormalities frequently encountered in CRC associated with CIN are COX-2 overexpression leading to overexpression of its product, PGE2, which regulates proliferation, tumorigenesis and angiogenesis [9,66], and loss of 18q (where SMAD2 and SMAD4, mediators of the TGFb pathway are located) [67,68]. Mutations in PIK3CA leading to its activation, occur late in the adenoma to carcinoma progression in a small proportion of cancers [69] and even though there are some reports for a positive predictive function of their presence in regards to aspirin effect in reducing CRC recurrence [70,71], data are conflicting [72] and current guidelines do not include PIK3CA mutational analysis as necessary for CRC patients [58].

Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome, caused by germline *APC* (5q21) mutations (frequency 1 in 6,850 to 29,000 people) [73]. Patients with FAP develop hundreds to thousands of adenomas, morphologically indistinguishable from sporadic adenomas. One or more adenomas will eventually progress to carcinoma and the lifetime probability of developing colorectal cancer in FAP individuals is 100%, unless a colectomy is performed (usually at an age between 15 and 25 years old) [74]. This syndrome presents with various degrees of penetrance and, thus, different phenotypes, which is not surprising considering the fact that causative mutations can occur at different loci in the gene and that environmental factors may alter the disease phenotype [74,75].

The hallmark feature of this disease is the development of adenomatous polyps along the GI tract beginning in early adolescence, with a rapid increase in number and size with age, and progression to colorectal cancer by the fourth decade [33]. The adenomas that develop in FAP are histologically similar to sporadic adenomas (Figure 1) [76]. Almost 75% of patients suffering from FAP have already developed colorectal carcinoma by the age of 30 [73].

Lesions can develop not only in the colon and rectum, but also throughout the GI tract. In the stomach mostly fundic gland polyps are seen, usually benign and morphologically similar to their non-syndromic counterparts, but, unlike sporadic polyps, exhibiting dysplasia in 25% of the cases [77]. In the small intestine adenomas are seen in 30-70% of patients with FAP, most commonly in the periampullary region of the duodenum [74,78]. Adenomas vary in size, from 1mm to > 1cm, and their number ranges from 100 to more than 5.000. Attenuated FAP is characterized by <100 polyps, increased risk of CRC development (albeit a little less than classic FAP and at an older age) and mutations involving the 5′ or 3′ part of the gene [75]

Patients with FAP occasionally present extracolonic manifestations [74,75] in the bones (osteomas in 65-80% of patients), teeth (dental abnormalities found in 30-75%

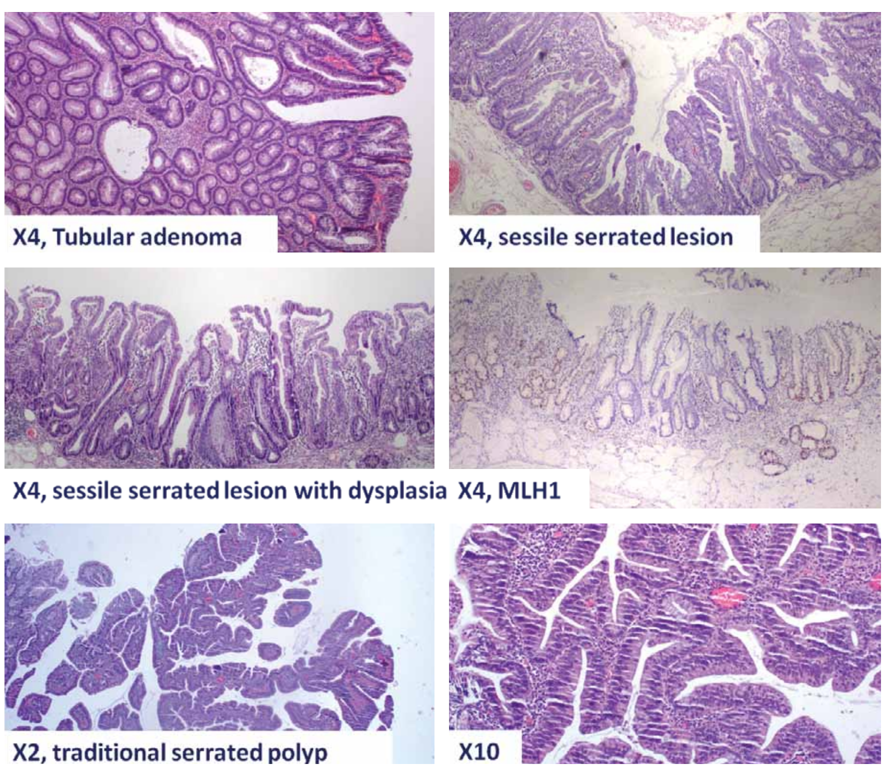

Figure 1. Preneoplastic lesions of the colon. Tubular adenomas develop through the CIN pathway. Sessile serrated lesion is characterized by crypt serration that extends to their base. MLH1 promoter hypermethylation and protein expression loss is characterized by dysplasia morphologically. Traditional serrated polyp with the characteristic villous architecture and ectopic crypt formation. This lesion harboured a *KRAS* exon 2 mutation (G12X).

of patients: impacted or unerupted teeth, tooth ankylosis, congenitally missing teeth, supernumerary teeth, compound odontomas), retina (Congenital Hypertrophy of Retinal Pigment Epithelium, CHRPE, the most common and earliest extraintestinal manifestation of FAP), thyroid (cribriform-morular variant of papillary thyroid carcinoma), liver (hepatoblastoma), central nervous system (brain tumours in general, especially patients with *APC* mutations between codons 697 and 1224) [79].

After FAP has been confirmed (clinical criteria or APC mutation confirmed by genetic testing) [33], treatment can be surgical (colectomy with or without proctectomy) or non-surgical (NSAIDS, COX-2 inhibitors). However, delaying the former with the use of medication that reduces the number of adenomas has limited results [75]. Annual screening is recommended even after the patient has undergone surgery [73,74].

MSI (Microsatellite instability)

Microsatellites represent repetitive DNA sequences (1 to 8 nucleotide units long), found throughout the human genome [80,81]. Alterations in their length are caused by DNA polymerase slippage, resulting in insertion or deletion of base pairs, thus, altering the number of repeats [81–83]. Responsible for recognition and correction of this type of errors (insertion/deletion mispairs) is the DNA mismatch repair (MMR) system, which also repairs base mismatches [84].

The MMR system comprises of several proteins: hMutSα (formed by heterodimerization of MSH2 with MSH6) and hMutSβ (MSH2-MSH3 heterodimer) recognize mismatches and single base insertions/deletions loops or 2-8 bases insertions/deletions loops, respectively. Then, they recruit hMutLα (MLH1-PMS2 heterodimer), hMutLß (MLH1-PMS1 heterodimer) or hMutLγ (MLH1-MLH3 heterodimer), along with replication factors and other proteins [83–85]. EXO1, single-strand DNA-binding protein RPA, proliferating cellular nuclear antigen (PCNA), DNA polymerase d (pol d), and DNA ligase I, are also involved in this process and mediate the excision of the most recently synthesized helix (the one containing the error), its re-synthesis (with the correct sequence) and ligation of the new helix with the rest of the DNA [84].

Loss of action of components of the DNA MMR system (known as MMR deficiency), is associated with a propensity for multiple point mutations across the genome (hypermutability), as well as insertions and deletions in microsatellite sequences, the latter accounting for the name of this state as microsatellite instability (MSI or MSI-high) [86,87]. Almost 15% of all colorectal cancers have been reported to display MMR deficiency and MSI [80,86]. Defects in this system occur as a result of germline mutations in MMR genes (in Lynch syndrome), or epigenetic inactivation by promoter hypermethylation (in sporadic MSI high tumours).

Screening patients for MSI in colorectal carcinomas and determining MMR functionality is important, as it can help identify individuals with Lynch syndrome. This has profound consequences not only for the patients themselves (increased colon surveillance for the development of subsequent tumours, screening for the development of tumours in other organs frequently affected in this syndrome), but also for their family members that may also be affected and need to be enrolled in screening programs [82,88]. In addition, MSI high carcinomas are associated with better prognosis [89] and according to the 2021 NCCN guidelines adjuvant treatment is not needed for patients with MSI high stage II tumours [90]. However, if adjuvant therapy is needed, MSI-H tumours do not respond well to therapy based on traditional cytotoxic agents (such as 5-FU, oxaliplatin, irinotecan) and different regimens should be used. Lastly, in the metastatic stage, MSI can predict response to immune checkpoint inhibitors [80,81] such as anti-PD-1 antibodies (nivolumab, pembrolizumab) and the anti-CTLA-4 antibody (ipilimumab). Consequently, pembrolizumab has been FDA approved for use as first line treatment in patients with dMMR/MSI-H CRC [91,92]. Nivolumab (alone or in combination with ipilimumab) [93] has been FDA approved for use in patients with dMMR/MSI-H CRCs that have progressed following treatment.

Tumours that are MSI-H have been linked to certain characteristics, such as location in the proximal part of the colon, mucinous (i.e mucins pools with neoplastic cells floating within them) or medullary (solid nested or trabecular syncytial growth with tumor infiltrating lymphocytes) histology, poor differentiation, lower rates of *KRAS* and *TP53* mutations, and increased immune cell infiltrates (sometimes with a Crohn-like reaction) [94,95]. However, the predictive value of the histologic characteristics in regards to MSI status is rather low and, thus, histology is no longer used to guide decisions regarding MSI testing [96]. All newly diagnosed colorectal cancers, regardless of family history, should be subjected to MMR or MSI testing, according to the College of American Pathologists, the American Society for Clinical Pathology, the Association of Molecular Pathology, and the American Society of Clinical Oncology [58] and the National Comprehensive Cancer Network (NCCN) [90].

MSI and MMR status can be detected by various methods: MSI-PCR, immunohistochemistry and next-generation sequencing (NGS). Regarding MSI-PCR, the National Cancer Institute (NCI) has recommended five microsatellite sequences as markers, known as the Bethesda panel: *BAT25, BAT26, D2S123, D5S346* and *D17S250* [97], albeit additional microsatellite markers are now commercially available. In principle, the length of each marker is compared between tumour tissue and normal tissue. Tumours can therefore be classified in three categories, based on MSI status: MSI-high (**MSI-H**), indicating a difference in the length of two or more of the five markers in tumour DNA, MSI-low (**MSI-L**), when only one marker exhibits a difference in its length, and microsatellite stable (**MSS**), when all markers have the same length in tumour and healthy tissue [81,98,99].

Another method for determining MSI status is detecting the absence of the expression of one or more of the four MMR proteins (MLH1, MSH2, MSH6 and PMS2) with immunohistochemistry (IHC), a state known as defective MMR (dMMR). Because these proteins function as heterodimers, PMS2 and MSH6 are usually unstable without MLH1 and MSH2 expression (their dimer partners), respectively. Thus, when MLH1 expression is lost, PMS2 (its dimer partner) is also lost (same with MSH2 and MSH6) (Figure 2). In contrast, MLH1 and MSH2 are stable even when PMS2 and MSH6 are absent. Therefore, loss of PMS2 and MSH6 is not accompanied by loss of their partners MLH1 and MSH2, respectively [99,100].

Comparison between the two methods (MSI-PCR and immunohistochemistry) has shown a high level of concordance [99,101]. IHC advantages are that it is fast, low-cost and readily available in most laboratories, it has low requirements in terms of tissue quantity and is the preferred method in cases with low tumour content (i.e. intense inflammation) [102]. In addition, it can specifically indicate which MMR gene is mutated. However, in up to 10% of the cases, mutations in the MMR genes although affecting their function (thus, the cells are dMMR), they do not affect the protein's expression (thus, immunohistochemistry is falsely positive) [103]. In addition, technical issues and previous therapy may affect the IHC results [102]. Nonetheless, both methods are crucial as they complement each other in regards to recognizing defective MMR [104]. Newer techniques, such as next generation sequencing are also effective in determining MSI status, with comparable results to PCR and immunohistochemistry [105] and the advantages of simultaneous analysis of multiple genetic aberrations [106,107], and, in some instances, not requiring paired normal tissue [108]. NGS challenges include high cost, increased technical demands, difficulties in data interpretation and poor diagnostic yield in samples with poor DNA quality, but technology is continually improving [109,110] and, in the future, it may lead to its more widespread use.

Lynch syndrome

Lynch syndrome, formerly known as hereditary nonpolyposis colorectal cancer (*HNPCC*, a term not currently preferred), is inherited by an autosomal dominant pattern and is characterized by a high risk of developing various types of tumours, especially, colorectal and endometrial carcinomas [111,112]. Other types of tumours that have a high probability of developing in individuals with Lynch syndrome are carcinomas of the breast, ovary, stomach, pancreas, small bowel, liver, bile duct, kidney, prostate, and urinary tract. In addition, brain tumours, namely medulloblastomas, and certain types of skin cancers develop in variants of the disease (Turcot and Muir-Torre syndrome respectively [88], the

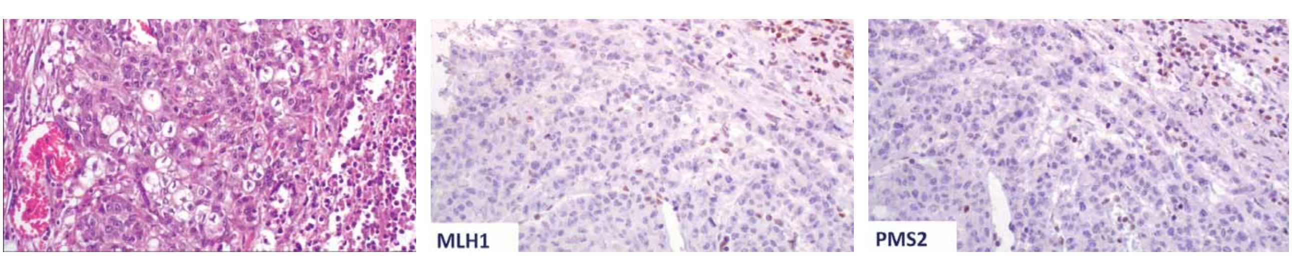

Figure 2. This tumour from a 75-year-old female patient was located in the cecum, and displayed poor differentiation and advanced T stage (T4b) histologically. Loss of both MLH1 and PMS2 was seen immunohistochemically. A *BRAF* V600E mutation was detected. This is a prototype case of sporadic MSI-H CRC (original magnification X20).

former also termed constitutional mismatch repair deficiency syndrome and is usually seen with homozygous mutations of one of the MMR genes [113].

Inherited alterations in MSH2 (40%) and MLH1 (30%) are responsible for the largest proportion of Lynch syndrome cases, followed by PMS2, and MSH6. Another genetic event recognized as causative of Lynch syndrome is the deletion of the EPCAM gene, resulting in MSH2 methylation and, thus, loss of its expression [114]. There are some differences in the phenotype of the disease depending on the affected gene, in regards of the risk and type of cancer that is developed [76]. The risk of cancer development is higher in MSH2 and MLH1 mutations, followed by MSH6 mutations and the least when PMS2 is affected. In addition, the risk of extracolonic manifestations is null with certain EPCAM deletions [76].

CIMP (CPG ISLAND METHYLATOR PHENOTYPE)

CpG islands are regions where CpG dinucleotide (a cytosine nucleotide followed by a guanine nucleotide) clustering is observed and are commonly found in gene promoters. DNA methyltransferases mediate the transfer of a methyl group to the C-5 position of the cytosine ring of DNA, resulting in CpG methylation which negatively regulates gene expression [115]. The opposite process, DNA demethylation, results in increased gene transcription. DNA methylation is an epigenetic mechanism of gene expression regulation. Epigenetic refers to the change in gene expression without any alteration in DNA sequence [116]. Tumours developing through this pathway are believed to harbour a methylator phenotype, meaning that there is a progressive increase in the methylation of CpG islands, leading to tumour suppressor gene silencing and, thus, tumorigenesis. An overlap with the MSI pathway is observed, as one of the genes frequently (although not always) undergoing hypermethylation in CIMP high carcinomas is MLH1 [117–119]. In fact, in sporadic colorectal cancer, the MSI phenotype, arises as a result of epigenetic silencing of the MLH1 gene by aberrant methylation of CpG islands in its promoter region. Reportedly, epigenetic silencing of MLH1 has been documented in more than 95% of MSI-H (MSI high) sporadic carcinomas [81,82,84,111,120].

Approximately 20–30% of all CRCs exhibit the CIMP phenotype and they are classified in three categories based on their hymermethylation level: low (CIMP-L), high (CIMP-H) or negative (CIMP-0) [121]. Two panels of genes are now widely used to investigate the CIMP status of tumours (p16, hMLH1, MINT1, MINT2 and MINT31, described by Toyota and CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1 described by Weisenberg) [121,122]. CIMP-H is characterized by activation of the WNT/β-catenin pathway, probably induced by non-APC mutations [121], frequent *BRAF* mutations and MLH1 methylation, with *TP53* mutations rarely encountered. On the other hand, CIMP-0 exhibits a high *TP53* mutation rate, while CIMP-L is usually associated with *KRAS* mutations.

Serrated polyps, characterized by a saw-toothed appearance under the microscope (epithelial infolding) are the precancerous lesions in tumours developing through this pathway, hence, also known as the serrated pathway. They are further classified in three categories based on their morphology: *hyperplastic polyps, sessile serrated lesions and traditional serrated adenomas (TSAs) or polyps*. Most of colorectal cancers with serrated lesions as precursors have been reported to harbour BRAF mutations (with KRAS being less frequently mutated) and have been connected with sporadic MSI and CpG island methylator phenotype (CIMP) [117,121].

Based on their morphology, polypoid lesions have also been associated with specific mutations and CIMP category. Hyperplastic polyps (further classified into microvesicular HP, goblet cell HP and mucin poor HP) usually harbour a V600E mutation in BRAF and belong to the CIMP-H category. BRAF is often mutated in sessile serrated lesions which have been characterized as MSS and CIMP-H with an unmethylated MLH1. The development of dysplasia coincides with the appearance of MSI-H phenotype, MLH1 methylation and higher risk of progression (as mentioned above). As for traditional serrated adenomas or polyps, they often present with KRAS or BRAF mutations, and can either be CIMP-L or CIMP-H, and are MSS [121].

More specifically, the pathway usually starts with *BRAF* mutations (V600E point mutation) [123], which lead to continuous signalling in the RAS/RAF/MAPK pathway (described in previous paragraphs). BRAF belongs to the RAF (rapidly accelerated fibrosarcoma) family of kinases, originally identified through the cloning of a viral mouse gene that induced transformation of NIH3T3 cells. BRAF is a non-receptor serine-threonine kinase that is located downstream of KRAS. Once activated, (from KRAS) it participates in phosphorylation cascades of the MAPK pathway and, thus, transcriptional activation of genes involved in cell growth, proliferation, survival and migration. When mutated, it is locked in its active form and mediates continuous activation of the pathway [124]. However, after the first wave of proliferation, that results in the development of hyperplastic polyps, one of the earliest manifestations of this pathway, the cell reaches a senescence state, and may remain there for a very long period; in fact, it may never progress. Cell cycle regulators, such as *p53* and *p16INK4a*, play an important role in this oncogene-induced senescence [125], as they halt further proliferation. In some lesions however, silencing of these molecules or their regulators, for example *IGFBP7,* may ensue, resulting in cell's escape from senescence and in following bursts of proliferation [117]. A sessile serrated lesion is the morphologic analogous of these molecular events. Again, the lesion may remain stable for a long period, until MLH1 is hypermethylated and dysplasia is encountered morphologically (sessile serrated lesion with dysplasia) (Figure 1). From this stage, evolution of the lesion to invasive cancer (through additional epigenetic events) is usually quicker than in the previous stages and BRAF mutated, CIMP-H, MSI-H (sporadic MSI-H) carcinomas develop (Figure 2 and 3) [121]. In some cases, the pathway may progress though epigenetic silencing of alternative genes (not *MLH1*), and then, BRAF mutated, CIMP-H, MSS carcinomas develop [121,126,127].

BRAF mutations are seen in 5-15% of the patients with CRC, with V600E being the most common and characterized by the substitution of valine by gutamic acid at the 600 position (located at the activation site of the molecule). *BRAF* mutations are more common in female patients, in tumours located in the right side of the colon and in MSI-H carcinomas [128]. Testing for *BRAF* mutations is necessary for all MSI-H carcinomas (their absence indicates that the tumour has developed in the setting of Lynch syndrome and should prompt germline genetic testing) [33]. *BRAF* mutations are associated with worse prognosis (in MSS tumours) and low response to EGFR targeting therapy [128,129]. Based on the results of the BEACON trial [130], double inhibition of BRAF (encorafenib) and EGFR (cetuximab/panitumumab) is a therapeutic option for *BRAF* V600E mutant CRC after prior treatment [90].

In some instances, where the initiating event may be a *KRAS* (instead of *BRAF*) mutation, precancerous lesions are characterized as traditional serrated adenomas (Figure 1), methylguanine methyltransferase (MGMT) gene is methylated [131] and carcinomas that develop have a *KRAS* mutated, CIMP-L, MSS/MSI-L molecular phenotype [121,127].

CRCs have also been classified into five molecular subtypes [132,133], according to their MSI-CIMP status and the mutational profiles of KRAS and BRAF: (1) type 1: MSI+, CIMP+, *BRAF*- mutated, *KRAS*- wildtype; (2) type 2: MSI–, CIMP+, *BRAF*- mutated, *KRAS*- wildtype; (3) type 3: MSI–, CIMP–, *BRAF*- wildtype, *KRAS*- mutated; (4) type 4: MSI–, CIMP–, *BRAF*- wildtype, *KRAS*- wildtype; and (5) type 5: MSI+, CIMP–, *BRAF*- wildtype, *KRAS*- wildtype. These distinct categories also exhibit a different prognosis, with type 1 exhibiting the best, type 2 the worst and 5-4-3, with this order, having an intermediate prognosis [126]

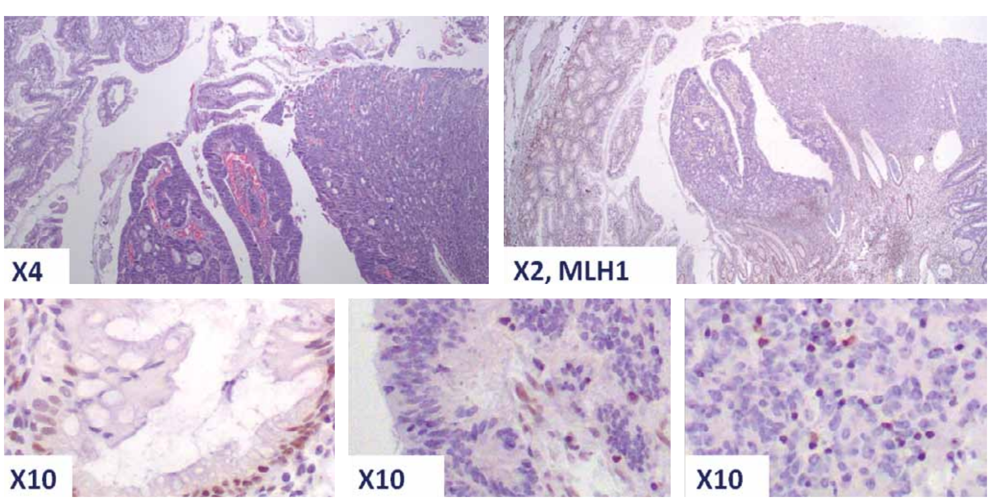

Figure 3. The serrated pathway of carcinogenesis. Poorly differentiated carcinoma developing in a sessile serrated lesion with dysplasia. Note the loss of MLH1 in the dysplastic and neoplastic epithelium while residual sessile serrated lesion retains its expression.

CONCLUSION

This review aims to describe the molecular pathways that have been implicated in the pathogenesis of colorectal carcinoma. As this type of cancer still comprises a major health risk nowadays, it is important to understand and analyse the underlying mechanisms, not only because they offer insights regarding the pathogenesis of the disease, but also because some of them have predictive and prognostic implications and form the basis for personalized therapy in CRC patients.

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