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A Literature Review in Immuno-Oncology: Pathophysiological and Clinical Features of Colorectal Cancer and the Role of the Doctor-Patient Interaction

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Abstract

This review is intended to describe the features of colorectal cancer both in terms of pathophysiology and clinical features of the pathology. It also describes the anatomical and clinical features of different primary tumor locations in colorectal cancer. It is also to note how relevant it is to identify rectal cancer and colon cancer as different pathologies due to the clinical, pathophysiological and immuno-oncological features of rectal cancer compared to the ones of colon cancer while remarking the importance of medical doctors in the interaction with oncological patients. **Background**: Colorectal Cancer (CRC) is a major public health problem, representing the third most commonly diagnosed cancer in males and the second in females and it is fundamental to note and discuss doctor-patient interaction, fundamental for proper adherence and psychological status of the oncological patient, when discussing such important and impactful pathologies. **Conclusions**: This review highlights the possibility of an update in the terminology of Colorectal Cancer (CRC) into different clinically relevant pathologies within the umbrella term colorectal cancer (for instance rectal and colon cancer as different tumors). It also remarks on the importance of medical doctors in the interaction with oncological patients.

Keywords

Rectal Cancer, Colon Cancer, Colorectal Cancer, Patient-Doctor Relationship, Medical Terminology

1. Introduction

This review is intended to describe the features of colorectal cancer both in

terms of pathophysiology and clinical features of the pathology. It also describes the anatomical and clinical features of different primary tumor locations in colorectal cancer. It is also to note how relevant it is to identify rectal cancer and colon cancer as different pathologies due to the clinical, pathophysiological and immuno-oncological features of rectal cancer compared to the ones of colon cancer. It is very important for the doctor-patient interaction, fundamental for proper adherence and psychological status of the oncological patient, when discussing such important and impactful pathologies.

The etiopathological origin of colorectal cancers is the following: 70% to 80% of colorectal cancers are sporadic, 15% - 20% are familial and 5% - 10% are hereditary [1]. In the colon adenocarcinoma genesis, three main etiopathogenic pathways have been described. A mutation in the APC gene, chromosomal instability and defective Mismatch Repair (MMR) systems. Regarding hereditary CRC, the main autosomal dominant syndromes are Familial Adenomatous Polyposis (FAP) and Lynch Syndrome while autosomal recessive syndromes include MUTYH Associated Polyposis (similar phenotype to attenuated FAP, with the presence of more than 10 but lower than 100 polyps).

Focusing on sporadic colorectal cancer, one of the pathways includes the inactivation of the tumor suppressor genes APC, which together with the inactivation of p53 and activation of the oncogene Kirsten-ras (Kras) are vital determinants of tumor initiation and progression [2]. The p53 gene, localized on the short arm of chromosome 17, mutates in up to 70% of colorectal cancers [3]. The development of colorectal cancer can be attributed to at least three patterns of instability. Typically, one type will predominate in specific cancer. Chromosome Instability (CIN), which results in genetic deletions, duplications, and chromosomal rearrangements, is one of the most common patterns; it is relevant in at least 50% of colorectal cancer cases. Colorectal Cancers (CRCs) with CIN are distinguished by the presence of tumoral cells with aneuploidy. CIN results in genetic defects in genes such as APC, TP53, TGF-β, PIK3CA, EGFR, BRAF, and KRAS [4].

Another pattern of instability in the development of colorectal cancer is Microsatellite Instability (MSI), which arises in about 15% of CRCs, particularly right-sided CRCs. Microsatellites are simple repetitive DNA sequences of one to six base pairs in length that occur at various times throughout the genome. Inactivation of the DNA Mismatch Repair (MMR) system induces MSI, which results in sequences that form errors and become unusually different in length. In some instances, frameshift mutation occurs in a gene, such as a tumor suppressor gene. Lynch syndrome is the cause of 90% of MSI-mutated CRCs. An epigenetic change that requires the methylation of promoters of human genes, particularly within the CpG islands gene, is the third pattern of instability observed in colorectal cancer. These epigenetic changes can result in the silencing of certain tumor suppressor genes in CRC.

Adenocarcinoma is a type of carcinoma, a malignant neoplasm originating from

epithelial cells, specifically from glandular epithelium. This type of epithelium is present in exocrine organs (e.g. Pancreas) or in other organs containing secretive epithelial tissues (e.g. Colon). Like other types of malignant neoplasms, adenocarcinoma is a result of a complex system of mutations and changes in the tissue of origin, a process called oncogenesis.

Eight common hallmarks of cancer have emerged in the last years which explain the characteristics that cells obtain in order to transform into neoplastic malignant cells.

Infections, inflammation and genomic instability all have often a role in the process of carcinogenesis. For instance, infection by Helicobacter Pylori is considered the strongest risk factor for adenocarcinoma of the stomach.

Furthermore, the process of "Epithelial-Mesenchymal Transition" (EMT), has been shown as being an important means by which transformed epithelial cells can acquire the ability to invade and resist apoptosis (Klymkowsky and Savagner), and therefore become malignant. In the case of colorectal cancer, a particular genetic pathway of carcinogenesis has been proposed to explain the transition from healthy colonic epithelia and increasingly dysplastic adenoma to malignant cancer. This model highlights numerous relevant oncogenes and tumor suppressor genes, which cause the adenoma to undergo carcinoma transition [5]. Adenomatous Polyposis Coli (APC) and DNA Mismatch Repair (MMR) genes have been identified in this pathway [6] [7]. This carcinogenic process is further enhanced in abnormal states, in which the mucosa of the colon must also be able to repair mucosal integrity. This includes the reversible ulcer-associated cell lineage, metaplasia (irreversible constitutive change in phenotype), dysplasia (abnormal differentiated phenotype) and neoplasia (abnormal cell number and abnormal differentiated phenotype) [8].

An abnormal pattern of cell replication has been detected under several clinical conditions associated with an increased risk of colorectal malignancies. Thus, adenoma formation is induced in two main scenarios. In sporadic colonic adenomas, the initiating event appears to be a Loss of Heterozygosity (LoH) at the APC gene, followed by a second hit in the APC gene. In one model, namely the "topdown" model, mutant cells appear in the intra-cryptal zone between crypt openings. Only as the clone expands does it penetrate into the crypt. When dissecting these tumors, only the superficial cells show intense staining for β-catenin as evidence of a dysfunctional APC gene. Therefore, whereas the stem cell that is the likely oncogenic precursor must have originated in the base or depths of the crypt, the polyp originates at the top of the crypt or in the space between the crypts. There are two possibilities regarding the "top-down" model. A new source of stem cells may emerge in the intra-cryptal zone. Thus, a terminally differentiated cell may mutate into a fully competent dividing cell. On the other hand, a cell derived from a mutated stem cell may migrate to that area and, with growth potential as a "second hit" in the intra-cryptal zone, expand from this location. More likely, a stem cell with a mutational defect in growth control will proliferate in the normal course of events, and cells pushed up to the intra-cryptal area

will retain this mutation, as do all cells between the stem cell at the base of the crypt all the way up to the intra-cryptal area and beyond. Perhaps more intuitively, this can be referred to as the "bottom-up" model. Thus, only a small fraction of these lesions may evolve into malignancy, and there is evidence that a large majority (if not all) of colorectal carcinomas develop from adenomatous polyps [7]. Adenomas are well-demarcated lumps of epithelial tumor cells that can be classified into the following three major histological types: tubular, villous, and tubulo-villous adenomas. Adenomas, by definition, exhibit different grades of dysplasia and should be classified based on the portion with the most advanced grade. The word "dysplasia" is used to describe structural and cytological alterations in the epithelium that predispose an organ to cancer development. These abnormalities show varying degrees of severity, which can be graded into "mild", "moderate" and "severe" atypia [8].

1.1. Anatomy of the Colon and Rectum: Embryological Origins

Another important topic to discuss is the embryological origins of parts of the lower gastrointestinal tract, emphasizing the difference between colonic and rectal tissues. The Gastrointestinal (GI) tract's development begins at the start of the third week of embryonic development (in the gastrulation phase, when the three germ layers begin to form, namely the ectoderm, mesoderm, and endoderm, which form the embryonic disc). This embryonic disc has a cranial end (which will form the mouth) and a caudal end (which, with the cloacal membrane, will form the anus). In the fourth week, the primordial GI tract begins its formation from the endoderm, which creates a gut tube suspended by a mesentery (double peritoneum layer). The gut tube is then divided into a foregut, midgut, and hindgut. The foregut gives rise to the esophagus, stomach, liver, gallbladder, bile ducts, pancreas, and proximal duodenum. The midgut develops into the distal duodenum, jejunum, ileum, cecum, appendix, ascending colon, and proximal two-thirds of the transverse colon. The hindgut becomes the distal one-third of the transverse colon, descending colon, sigmoid colon, and upper anal canal (Reference: Christine Waasdorp Hurtado).

The hindgut forms the distal one-third of the transverse colon, the descending colon, the sigmoid colon, and the rectum (as well as the superior portion of the anal canal). The hindgut's cloaca also forms part of the urogenital tract (the anal canal is formed by both the ectoderm and endoderm; the pectinate line marks the junction). There are genes associated with the development of the gastrointestinal tract, including the sonic hedgehog (in the endoderm, for mucosa, submucosa, and muscularis propria development). Additionally, the Homeobox (HOX) genes and NODAL gene are important for GI tract development (potential role in sphincter formation and GI musculature, as well as cranio-caudal formation pf the GI tract, thanks to genes expressed in the mesoderm).

Anatomically, the rectum has further peculiar features. The layers of the rectum include the mucosa (which itself includes the simple columnar epithelial cells, lamina propria, and muscularis mucosa), submucosa, muscularis propria,

and serosa. The rectum begins at the vertebral S3 level, at the end of the large intestine. The rectum is pivotal in fecal continence and the storage of feces. Directly above the pelvic diaphragm, or the levator ani, and the anococcygeal ligament is a dilation of the terminal rectum known as the ampulla. This is where feces accumulate and remain until defecation. The ampulla of the rectum is highly distensible and capable of regulating the movement of fecal material into the anal canal for expulsion. The shape of the rectum, in conjunction with the flexures created along the way, also aids in the mechanism of fecal continence (Nakashima & Zulfiqar, s.d.) [9].

1.1.1. Anatomy of the Colon

The colon is part of the distal GI tract, beginning at the caecum and ending at the anal canal. Anatomically, the colon (averaging 150 cm in length) can be divided into four parts: ascending, transverse, descending, and sigmoid (Gray's Anatomy).

The colon begins as the ascending colon, being retroperitoneal and ascending superiorly from the cecum. At the level of the right lobe of the liver, it turns 90 degrees to move horizontally. This turn is known as the right colic flexure (or hepatic flexure), and it marks the beginning of the transverse colon. The transverse colon extends from the right colic flexure to the spleen, here turning another 90 degrees to point inferiorly (the left colic flexure or splenic flexure).

At this point, the colon is attached to the diaphragm by the phrenicocolic ligament. The transverse colon is the least fixed part of the colon (it can dip into the pelvis in tall, thin individuals). The transverse colon is intraperitoneal, as it is enclosed by the transverse mesocolon. After the left colic flexure, the colon moves inferiorly towards the pelvis and is called the descending colon. It is retroperitoneal in most individuals but is located anterior to the left kidney.

When the colon begins to turn medially, it becomes the sigmoid colon. The 40-cm long sigmoid colon is located in the left lower quadrant of the abdomen, extending from the left iliac fossa to the level of the S3 vertebra. This gives the sigmoid colon its characteristic "S" shape. The sigmoid colon is attached to the posterior pelvic wall by the mesentery, namely, the sigmoid mesocolon. The long length of the mesentery permits this part of the colon to be particularly mobile (Sobotta Anatomy).

The neurovascular supply to the colon is closely linked to its embryological origin, namely the ascending colon and proximal two-thirds of the transverse colon, derived from the midgut. The distal one-third of the transverse colon, descending colon, and sigmoid colon is derived from the hindgut. Typically, midgut-derived structures are supplied by the superior mesenteric artery, and hindgut-derived structures are supplied by the inferior mesenteric artery. The ascending colon receives arterial supply from two branches of the superior mesenteric artery, specifically the ileocolic and right colic arteries. The ileocolic artery gives rise to the colic, anterior cecal, and posterior cecal branches, all of which supply the ascending colon. The transverse colon is derived from both the

midgut and hindgut; it is therefore supplied by branches of the superior mesenteric artery and inferior mesenteric artery: the right colic artery (from the superior mesenteric artery), the middle colic artery (from the superior mesenteric artery), and the left colic artery (from the inferior mesenteric artery). The descending colon is supplied by a single branch of the inferior mesenteric artery, namely the left colic artery. The sigmoid colon receives arterial supply from the sigmoid arteries (branches of the inferior mesenteric artery). The marginal artery (of Drummond) is a clinically important vessel that provides collateral supply to the colon, thereby maintaining arterial supply in the case of the occlusion or stenosis of one of the major vessels. As the terminal vessels of the superior mesenteric and inferior mesenteric artery approach the colon, they split into many branches, which anastomose with each other. These anastomoses form a continuous arterial channel, namely the marginal artery, which extends the length of the colon. Long, straight arterial branches (called vasa recta) arise from the marginal artery to supply the colon. Similar to the arterial supply, the innervation to the colon is dependent upon its embryological origin; midgut-derived structures (ascending colon and proximal two-thirds of the transverse colon) receive their sympathetic, parasympathetic, and sensory supply via nerves from the superior mesenteric plexus, while hindgut-derived structures (distal one-third of the transverse colon, descending colon, and sigmoid colon) receive their sympathetic, parasympathetic, and sensory supply via nerves from the inferior mesenteric plexus, parasympathetic innervation via the pelvic splanchnic nerves, and orthosympathetic innervation via the lumbar splanchnic nerves.

The venous drainage of the colon is similar to the arterial supply, with the ascending colon being drained by the ileocolic and right colic veins, emptying into the superior mesenteric vein. The transverse colon is drained by the middle colic vein, which empties into the superior mesenteric vein. The descending colon is drained by the left colic vein, which drains into the inferior mesenteric vein. Lastly, the sigmoid colon is drained by the sigmoid veins into the inferior mesenteric vein. The superior mesenteric and inferior mesenteric veins then empty into the hepatic portal vein. This allows toxins absorbed from the colon to be processed by the liver for detoxification. The lymphatic drainage of the ascending and transverse colon goes into the superior mesenteric nodes. The descending and sigmoid colon drain into the inferior mesenteric nodes. Attached to the surface of the large intestine are omental appendices, which are small pouches of peritoneum, filled with fat. Running longitudinally along the surface of the large bowel are three strips of muscle, known as the teniae coli. These include the mesocolic, free, and omental coli. The teniae coli contract to shorten the wall of the bowel, producing sacculations known as haustra. Furthermore, one difference between the large and small intestine is that the large intestine has a substantially wider diameter than the small intestine. These features cease at the rectosigmoid junction, where the smooth muscle of the teniae coli broadens to form a complete layer within the rectum. Most of the lymph from the superior

mesenteric and inferior mesenteric nodes passes into the intestinal lymph trunks, and then to the cisterna chyli, where it ultimately empties into the thoracic duct (Anatomy References: Gray's Anatomy and https://teachmeanatomy.info/abdomen/gi-tract/rectum/, s.d.).

1.1.2. Anatomy of the Rectum

Regarded as the most distal part of the large intestine, the rectum is in direct continuation with the sigmoid colon, terminating in the anal canal. It begins at the level of the S3 (as a continuation of the sigmoid colon). It is macroscopically different from the colon, lacking the *Taenia coli*, haustra, and omental appendices. The rectum serves primarily as a reservoir for feces and tumors in the rectum that are less than 16 cm from the anal verge and located at least partially within the supply of the superior rectal artery [10]. The rectum has two main flexures: the sacral flexure, which is an anteroposterior curve with concavity anteriorly (following the curve of the sacrum and coccyx), and the anorectal flexure, an anteroposterior curve with convexity anteriorly. This flexure is formed by the tone of the puborectalis muscle and contributes significantly to fecal continence. The final segment of the rectum, the ampulla, relaxes to accumulate and temporarily store feces until defecation occurs. It is continuous with the anal canal, which passes through the pelvic floor to end as the anus. In the superior third of the rectum, the anterior surface and lateral sides are covered by peritoneum. The middle third only has an anterior peritoneal covering, and the lower third has no peritoneum associated with it. In males, the reflection of the peritoneum from the rectum to the posterior bladder wall forms the rectovesical pouch. In females, the peritoneum reflects to the posterior vagina and cervix, forming the rectouterine pouch (pouch of Douglas). The rectum is located within the pelvic cavity and is the most posterior structure of the pelvic viscera. The rectum receives arterial supply through three main arteries, namely the superior rectal artery (which is the terminal continuation of the inferior mesenteric artery), the middle rectal artery (a branch of the internal iliac artery), and the inferior rectal artery (a branch of the internal pudendal artery).

Venous drainage involves the superior, middle, and inferior rectal veins. The superior rectal vein empties into the portal venous system, while the middle and inferior rectal veins empty into the systemic venous system. Anastomoses between the portal and systemic veins are located in the wall of the anal canal, making this a site of portocaval anastomosis. Importantly, the rectum is also closely anatomically associated with the rectal venous plexus; however, this structure is more functionally related to the anal canal. The rectum receives sensory and autonomic innervation. The orthosympathetic nervous supply to the rectum is from the lumbar splanchnic nerves and the superior and inferior hypogastric plexuses. Parasympathetic supply is from S2-4 via the pelvic splanchnic nerves and inferior hypogastric plexuses. Visceral afferent (sensory) fibers follow the parasympathetic supply. The lymphatic drainage of the rectum occurs through the pararectal lymph nodes, which drain into the inferior mesenteric

nodes. Additionally, the lymph from the lower aspect of the rectum drains directly into the internal iliac lymph nodes (Anatomy References: Gray's Anatomy and https://teachmeanatomy.info/abdomen/gi-tract/rectum/, s.d.).

1.1.3. Microanatomy of the Large Intestine

The mucosal layer of the large intestine consists of columnar epithelium, which forms the innermost layer up to the lower part of the anal canal (stratified squamous epithelium). In the mucosa, unlike in the small intestine, there are no villi or microvilli, nor are there circular folds. Instead, there are intestinal crypts containing goblet cells, which are the most relevant originators of mucous in the large intestine.

Therefore, a transection of the colonic wall consists first of a mucosa (which is smooth, as there are no villi or circular folds) with its columnar epithelium. In the mucosa, there are intestinal glands, called crypts of Lieberkühn, which extend throughout the complete thickness of the mucosal layer. The epithelium has an oval basal nucleus and an apical brush border, being the microscopic representation of microvilli (*Proteinatlas.org*). As stated in the previous section, numerous goblet cells are the main mucinous secretory components of these glands. Beneath the epithelial layer is the lamina propria, with connective tissue and the presence of cells belonging to the immune system. The mucosa ends at the lamina muscularis mucosae, where it marks the submucosal layer, which is composed primarily of loose connective tissue, containing vessels and nerves along with occasional lymphoid follicles. The submucosal layer contains blood vessels, lymphatics, and terminal nerve fibers. It is an important layer with regards to the genesis of cancer because once a tumor has invaded into this region of the bowel wall, it can enter the blood supply and lymphatic system, thereby spreading widely throughout the body [11].

The muscularis layer (muscularis externa) consists of an inner circular smooth muscle layer and an outer one, which is not continuous as in the rest of the GI tract. The muscular layer is divided into three thickened bands called *Taenia coli*.

Through the histological and microscopic structural assessment of the colon, four layers have been discovered: the mucosa, the submucosa, the muscularis externa, or propria (containing circular and smooth muscle layers), and the serosa [11]. The earliest genetic changes mostly occur in the mucosa cells due to the continuous cell division of the normal cells to replenish those that are shed from the bowel wall into the lumen. The rectal mucosa is similar to that of the colon except for the presence of more numerous goblet cells.

Thus, it must be remembered that during the metastatic formation of CRC, several steps occur. In the primary tumor site, the transformed tumor cells begin to grow and secrete angiogenic factors, promoting angiogenesis, which, along with their capacity to invade the vessels through the activation of proteases, allow the cancer cells to enter the blood stream (Rei Mizuno *et al.*, 2019 Feb) [12].

Notably, the presence of plexuses provides innervation to the gut. The myenteric plexus (or Auerbach's plexus) provides motor innervation to both layers of

the muscular layer of the gut, having both parasympathetic and sympathetic input, whereas the submucosal plexus has only parasympathetic fibers and provides secretory and motor innervations to the mucosa nearest the lumen of the gut.

1.2. Colorectal Cancer

Colorectal Cancer (CRC) is located in the colon or rectum, and it is the third deadliest solid tumor in the world. Most of these cancers arise from pre-existing adenomas (in the form of polyps), which develop in the lining of the large intestine, or the rectal area. Ninety-six percent of all CRC are comprised of adenocarcinomas often involving APC genetic mutation [7]. Ten percent of these adenocarcinomas are mucinous, while 1% are signet ring (both types are more aggressive than classical adenocarcinomas). Other types of CRCs include Gastrointestinal Stromal Tumors (GISTs), colorectal lymphomas (a type of non-Hodgkin's lymphoma), melanomas, leiomyosarcomas, and carcinoid tumors (neuroendocrine tumors).

The location of colon cancer ranges from the caecum to the sigmoid (approximately 15 cm above the anal verge), while rectal cancer ranges from the recto-sigmoid to the anus [13].

CRC is also the third most common solid tumor in both men and women [14] (Colorectal Cancer: Statistics, 2019). CRC accounts for 13% of new cancer diagnoses in Europe. Approximately 1.8 million new cases per year and 900,000 deaths per year (Giovannucci, 2019 August 27) [15] occur due to CRC. The incidence of CRC around the world, particularly in the developed world, has been continually rising in recent years (Araghi M1, 2019 Jun 15) [16]. Among men, it is the third most frequently diagnosed form of cancer after prostate and lung cancer (663,000 cases, 10.0%), and the second most common cancer type after breast cancer in women (570,000 cases, 9.4%) [17]. The estimated number of deaths from colorectal cancer in 2008 was 608,000, which represented 8% of all cancer deaths. This therefore renders CRC the fourth most common cause of cancer deaths.

Approximately half of patients diagnosed with colorectal cancer die from the disease within five years. In the Western world, the lifetime risk of developing colorectal cancer is 4% to 5% [18]. Nevertheless, the incidence of colorectal cancer is increasing in developing.

Fifty-four percent of Colorectal Cancers (CRCs) originate from the sigmoidal and rectal areas (with the sigmoid colon being the location of 18% of CRC, the sigmoid-rectal junction 7%, and the rectum 29%) (Toms, 2004) [19].

1.3. Clinical Presentation of Patients Affected by CRC

Knowledge regarding the clinical presentation of patients affected by CRC is vital for the correct diagnosis of CRC and the implementation of the necessary diagnostic clinical examination. Patients with early-stage cancer are typically diagnosed via screening, as these patients are mostly asymptomatic. Common symp-

toms associated with colorectal cancer include abdominal pain, rectal bleeding, altered bowel habits, and involuntary weight loss [20].

Cancers of the proximal part of the colon (right-sided CRC) rarely cause gross rectal bleeding because the blood tends to mix with the stool and degrade during colonic transit. This occult blood loss means such patients often present with iron deficiency anemia [21] of a microcytic type. In contrast, distal rectal tumors and left-sided CRCs may present with fresh rectal bleeding, pelvic pain, or tenesmus [22]. Tumors of the transverse colon and on the left side of the colon, generally invade the colonic wall in a ring-shaped pattern, mainly producing obstructive symptoms (cramping pain after meals, changes in stool form, occasional sudden ileus paralyticus symptomatology development, and even toxic megacolon with bowel perforation). Symptoms of tumors confined to the recto sigmoid portion typically include tenesmus and hematochezia. In rare cases, patients without recent symptoms present with an emergency involving intestinal obstruction, fistulation, or perforation. Up to 20% to 25% of colon cancer cases present as emergencies, while only a small number of rectal cancer cases present as emergencies. In the case of rectal cancer, a palpable mass may be detected through a digital rectal examination in over 50% of cases.

1.4. Current Diagnostic Approaches to CRC

The current and most common diagnostic procedure for the diagnosis of colorectal cancer is endoscopy or colonoscopy. This type of examination allows for a histological analysis to be performed on the target tissue for the diagnosis of the CRC histological type. However, the diagnostic workup for colorectal cancer depends on the mode of presentation. If a patient present with an emergency with symptoms and clinical signs of peritonitis, such as Blumberg sign positivity, a diagnosis of colorectal cancer may only be made incidentally during operative intervention. However, in the elective setting, CRCs can be diagnosed either through direct endoscopic visualization or through a radiological investigation (Barium enema, Computerized Tomography (CT), or CT colonography). In most cases, histological confirmation is obtained through an endoscopic biopsy. A histological diagnosis should be made and the disease fully staged before treatment is initiated.

The importance of the endoscopic appearance of the lesion during the diagnostic endoscopic procedure can provide an initial, approximate assessment of the depth of infiltration (into the mucosa or beyond) and can guide the subsequent management. The Paris classification was developed to allow the morphological classification of superficial lesions.

The Paris classification of early and superficial tumors in the GI tract was developed by an international consortium in a two-stage process. Superficial, or early, neoplasia in the entire GI tract are primarily assessed based on their endoscopic appearance and are defined as Type 0. Type I indicates polypoid forms, Type II indicates flat or superficial forms, and Type III indicates excavated forms (thus, Type II and Type III are non-polypoid lesions) (Endoscopic Classification

Review Group, Endoscopy 2005).

A further point must be addressed with regards to rectal uTNM staging: Intrarectal ultrasound examination can improve preoperatory preparations for surgery involving rectal cancer patients by allowing an understanding of the infiltrative depths of the tumor.

Finally, the endoscopic procedure can also be curative when, for instance, polyps containing non-metastatic, low-stage, and low-grade malignancy are removed.

Histologically, further staging can also be implemented in the target samples, such as Kikuchi staging (which include SM, or submucosal, staging).

In addition to the diagnostic procedure, which is based on the microscopical examination of a tumor specimen, immunohistochemistry can be used to determine the colorectal origin of a metastasis or to visualize the spread of tumor cells in surrounding tissues. Antibodies that exhibit high sensitivity and specificity for tumors of colorectal origin include Cytokeratin 20, CDX-2, SATB2, and Cadherin-17. Chromogranin-A antibodies can be used to distinguish endocrine tumors in the bowel from common adenocarcinomas (*Reference*: *Proteinatlas.org*).

1.5. Risk Factors and Preventative Measures for CRC Development

Known risk factors that contribute to the increased incidence of CRC include obesity, abdominal fat, sedentary life style, processed foods, red meat, age > 50 years, and the presence of Inflammatory Bowel Disease (IBD), such as Crohn's disease (Prashanth Rawla, 2019) [23].

Colorectal cancer occurs in two major forms: sporadic and inherited. Approximately 75% of new cases of CRC are sporadic (occurring in people with no family history of the disease). In these patients, the oncogenetic changes occur de novo, caused by etiologic factors such as age, chronic inflammation, diet, and environmental contributors. The risk of developing CRC increases with age, although it affects people of all ages. In terms of the age of presentation, in recent years, there has been an increase in cases of CRC in younger individuals. The age-related exponential increase in CRC incidence can be explained by the accumulating genetic events in aging tissues. After the age of 40, the probability of being diagnosed with CRC increases gradually, rising sharply after age 50. Over 90% of colorectal cancer cases are diagnosed in people aged 50 or older. The incidence rate is over 50 times higher in individuals aged 60 to 79 years compared to those younger than 40 years old.

In the United States, colorectal cancer is now one of the top 10 most commonly diagnosed cancers among men and women between the ages of 20 and 49 years. In recent years, diet has been considered an important risk factor for the development of CRC. In fact, low BMI (Body Mass Index) and physical activity have been proven to diminish the incidence of colon cancer [24]. An association

has been established between low fruit and vegetable intake and an increased risk of colorectal cancer. It has been hypothesized that the fiber, antioxidant vitamin, folic acid, micronutrient, or phytochemical (flavone) content in vegetables and fruits may exert a protective effect. Differences in dietary fiber intake have been proposed as a reason for geographical differences in CRC incidence rate, since the increased intake of dietary fiber may increase fecal bulk and reduce colonic transit time [25]. Furthermore, some observational studies have uncovered an inverse relationship between dietary fiber consumption and the risk of colorectal adenomas or CRC [26]. Other studies have also suggested that a diet high in red meat, animal fat, or cholesterol may be associated with CRC development, especially in the left colon [27]. One potential etiological factor is that the typical Western diet favors the development of bacterial flora, which is capable of degrading bile salts to potentially carcinogenic N-nitroso compounds. For red meat, the mechanism has been attributed to the presence of heme iron in red meat [28]. In addition, some meat cooked at high temperatures results in the production of heterocyclic amines and aromatic hydrocarbons, which are believed to have carcinogenic properties [28]. The role of exercise in reducing the risk of colorectal cancer is now well established as well [29], although differences in its impact on the development of CRC in the colon and the rectum have been found. In 2009, a meta-analysis concluded that regular exercise reduced the risk of colon cancer by almost 25% in both men and women. Various mechanisms have been proposed to explain the positive effect of exercise in reducing CRC. These include lowered levels of prostaglandins, diminished gut transit time, and improved immune function [30].

Connected to a high-fat and low-fiber diet and a lack of physical exercise is the issue of obesity, which has also been proven to be a risk factor for the development of CRC. A study by Moghaddam *et al.* [31] estimated that individuals with a BMI of over 30 kg/m^2 had a 20% greater risk of developing CRC compared to normal weight controls. Hypertension, elevated blood glucose, hyperinsulinemia, and Type 2 diabetes are metabolic abnormalities associated with insulin resistance syndrome that are also independently associated with colorectal cancer [32]. The findings of a recent meta-analysis [33] suggested that Type 2 diabetes increases the risk of colorectal cancer by 30%. The underlying mechanisms linking insulin resistance syndrome with colorectal cancer are not fully understood, but the available evidence suggests that hyperinsulinemia is the most likely cause.

Long-term cigarette smoking has also been demonstrated to be associated with colorectal cancer. Tobacco smoke is known to contain carcinogens such as heterocyclic amine, nitrosamines, and polycyclic hydrocarbons, which have been proven to spur cancer growth in the colon and rectum and increase the risk of being diagnosed with CRC [34]. Cigarette smoking is important to both the formation and growth rate of adenomatous polyps, which are precursor lesions for CRC [35]. Studies have also demonstrated that cigarette smokers have an

earlier average age of onset of CRC. The EPIC trial investigated the impact of alcohol consumption on a cohort of almost half a million subjects over a six-year period and concluded that both lifetime and baseline alcohol intake increased the risk of colon and rectal cancer [36]. Furthermore, a pooled analysis of 14 separate studies suggested that a high alcohol intake (defined as more than 100g per week) was associated with a 19% increase in the risk of colon cancer in both men and women [36]. A direct effect of acetaldehyde, a compound known to alter DNA, has been implicated in the mechanism by which alcohol influences tumor development [37]. A further study reported that the risk of colorectal cancer was significantly lower in postmenopausal women who were on Hormone Replacement Therapy (HRT) compared to those who had never received such treatment [38]. The reason for this is that estrogens reduce the production of bile acids, which have been implicated in initiating and promoting malignant change in colonic epithelium. Estrogens also decrease serum levels of Insulin-like Growth Factor 1 (IGF1), an important mitogen required for cellular proliferation and subsequent transformation into cancerous cells [39]. There is convincing evidence that patients taking Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) reduce their risk of developing colorectal cancer. In 2003, a randomized controlled trial of over 1000 patients concluded that daily aspirin reduced the risk of colorectal adenoma formation in patients with a history of polyps. These findings are supported by epidemiological data which suggests that NSAIDs not only reduce the incidence of adenomas but can also reduce the risk of progression to adenocarcinoma [40].

Another important risk factor for the onset of CRC is the presence of genetic syndromes which cause non-sporadic CRC, such as familial adenomatous polyposis, an autosomal dominant syndrome wherein the APC tumor suppressor genes (genes which prevent the uncontrolled growth of cells and which, in contrast to protooncogene, can occur in germ lines) are mutated and which involves the formation of polyps in the colon. This requires careful monitoring, including an endoscopy procedure every one to three years and, in some cases, prophylactic colectomy if more than 100 polyps are present. Another genetic syndrome which influences the development of CRC is Lynch syndrome, also called Hereditary Non-Polyposis Colorectal Cancer (HNPCC), a further autosomal dominant syndrome. The primary feature of HNPCC is defective DNA mismatch repair, which leads to microsatellite instability (changes in the length of dinucleotide repeats in MSI-high tumors). Abnormal mismatch repair proteins at immunohistochemistry are stained in MSI-high tumors. In HNPCC prophylactic, hysterosalphingectomy can be performed in women (with a risk of endometrial and ovarian cancer), and anti-PD1 Ab can be used as immunotherapy in treating CRC caused by HNPCC.

The most common of these syndromes is hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome), which is present in about 3% of people with colorectal cancer with a lifetime CRC risk to be 50% to 80% [41]. Other syn-

dromes that are strongly associated with colorectal cancer include Gardner syndrome (an autosomal dominant form of polyposis characterized by the presence of multiple polyps in the colon, along with tumors outside the colon) and Familial Adenomatous Polyposis (FAP), this last one with a 95% risk of developing CRC by the age of 50 [41].

Prevention is crucial to the decreasing mortality of CRC in the developed world. The hallmark of prevention in Italy is colonoscopy procedures, starting when the patient is 50 years old, and then every 10 years if no other risk factors are present. A fecal occult blood test is also performed annually in many countries to search for occult lesions in the lower gastrointestinal tract, since these may indicate the presence of a colonic neoplasm.

1.6. Current Treatments for CRC (Surgery and Adjuvant Therapies)

Various treatments have been implemented against CRC, primarily including surgical resection, chemotherapy (adjuvant and neoadjuvant), and radiotherapy.

Surgery was one of the first methods of treatment against solid tumors, already implemented by the first surgeons in the 1700s, such as by the Scottish surgeon and anatomist Alexander Monro. The only curative strategy in the treatment of colorectal cancer is the complete surgical resection of the tumor. Despite the figures indicating that approximately 70% to 80% of patients are eligible for curative surgical resection at the time of diagnosis [42], five-year overall survival is only 50% to 60% [43]. Seventy-five percent of patients who undergo curative resection will experience local recurrence or distant metastases. In 85% of patients, relapse is diagnosed within the first 2.5 years after surgery [44]. When a diagnosis of metastatic disease is made in patients with advanced colorectal cancer, the median survival rate is only six months. During this period, many patients suffer from severe physical and psychological tumor-associated symptoms that drastically diminish their quality of life. Quality of life among these oncological patients is measured using various criteria, such as the ECOG Performance Status. Therefore, post-operative systematic treatment of colorectal cancer seeks to prevent local recurrence or metastatic disease after complete surgical resection, or adjuvant therapy. Additionally, systematic treatment seeks to prolong survival, control symptoms, and improve quality of life. TNM staging such as >T3, positive nodal involvement, or M1 influence the choice of surgical resection, or the enactment of protocols such as the use of neo-adjuvant therapy [45].

Neoadjuvant treatment with radiation (with or without chemotherapy), followed by surgery, is current practice for managing most mid-low rectal cancers that are staged preoperatively as at least T3 and/or at least N1 (*i.e.* Stage II or Stage III), in individuals well enough to tolerate it [46].

Regarding surgery, there are three main types of surgical approaches to patients affected by CRC. Open surgery, laparoscopic surgery and the new robotic surgery. Laparoscopic approach is increasingly used in CRC. It has been sug-

gested to carry short-term benefits in safety, recovery, and preservation on immune function for patients with CRC. Patients who undergo laparoscopic procedure have a decreased incidence of adhesional obstructions and of incisional hernia compared to the patients who undergo the more invasive open surgery [47]. Robotic surgery has also been shown to be feasible and safe in the treatment of CRC [48] even though a longer operative time has been recorded for this approach [49].

Regarding adjuvant therapy, Oxaliplatin-based chemotherapy (e.g. FOLFOX regimen, which includes 5-Fluorouracil, Oxaliplatin and Folinic Acid) is now considered the standard adjuvant therapy for Stage III and high-risk Stage II colon cancer [50]. It must also be noted that the main agents for chemotherapy are therefore: 5-fluorouracil (mainly a Thymidylate Synthase (TS) inhibitor), folinic acid (aiding the activation of partial normal DNA replication processes), and oxaliplatin (cytotoxic effects). Furthermore, the introduction of monoclonal antibodies that target Epidermal Growth Factor Receptor (EGFR), e.g. cetuximab, or that target Vascular Endothelial Growth Factor (VEGF), e.g. bevacizumab, has expanded the agents available for use in the adjuvant setting. Anti-VEGF agents have the potential to suppress angiogenesis to prevent tumor growth and metastasis, while there is an improved delivery of anticancer therapy to the tumor, which would enhance tumor cell killing. Ultimately, anti-VEGF therapy may also disrupt the existing tumor blood supply, further enhancing tumor cell killing [50].

This blockade, through the use of the EGFR mAb, has exhibited clinical efficacy in patients without downstream activating KRAS mutations. The KRAS mutation status is analyzed before beginning an anti-EGFR treatment (*Proteinatlas.org*).

New therapies in the field of targeted antibody therapy and immunotherapy have also been used to combat CRC. Other than the EGFR targeted therapy mentioned before, immunotherapy has been proven to be clinically effective for certain CRC types. Two antibodies, specifically Programmed-cell Death 1 (PD1) blocking antibodies, namely pembrolizumab and nivolumab, have proven effective in treating patients with metastatic CRC of the microsatellite instability-high type (mismatch repair deficient), and both antibodies have been granted FDA approval.

Adjuvant chemotherapy aims to destroy micrometastases following surgery, and neoadjuvant chemotherapy is aimed at reducing the tumor mass to allow surgery for either the primary tumor or distant metastases (typically to the liver or lungs).

In patients with easy resectable primary and colorectal liver metastasis, perioperative FOLFOX before and after liver resection is the recommended treatment strategy, and in selected cases, the primary may be resected concurrently. Recommendations are to administer chemotherapy for a total of at least 6 months [51].

Despite major advances in diagnosis and surgery and despite global and national prevention programs, about 50% of colorectal carcinomas are diagnosed in advanced stages. Advanced disease is largely refractory to conventional therapy, and five-year survival remains poor. Patients with advanced disease suffer symptoms which are implications for diagnostic and therapeutic procedures and can heavily disturb the process of chemo immunotherapy and radiotherapy. Patients with suspected colon cancer and laboratory abnormalities should have routine blood tests, including a hemogram with platelet count determination, serum electrolytes and glucose determination, evaluation of routine serum biochemical parameters of liver function test (transaminase, gamma-gt, ALP), and a routine coagulation profile. About half of patients with colon cancer are anemic [52]. Anemia, however, is highly common, so that only a small minority of patients with anemia have colon cancer. Iron deficiency anemia of undetermined etiology, however, warrants evaluation for colon cancer, particularly among the elderly. Levels of the serum lactate dehydrogenase may also increase with colon cancer.

1.7. CRC Primary Location Sites

In recent years, a new focus on CRC site localization has emerged. Various studies have revealed not only anatomical, but also genetic, immunological, therapy-response, and prognostic differences between colon cancer (in the right-sided colon, which is the ascending colon, as opposed to the left colon, which is the descending colon) and rectal cancers.

The five-year survival rate for localized stage colon cancer is 90%, while the five-year survival rate for rectal cancer is 69% (Colorectal Cancer: Statistics, 2019) [14]. Furthermore, many differences have been found between right-sided, left-sided, and rectal CRC. For example, evidence has shown that in left-sided CRC and Rectal Cancers (RCs), the mechanism of invasive cancers is skewed toward a progression of preexisting mucosal lesions, while in right-sided CRC, a de-novo pathway has also been implicated in the development of invasive cancers (Konishi, 1999 Dec 1) [8].

Other differences include the presence of Microsatellite Instability (MSI) in, for instance, Lynch syndrome (hereditary non-polypoid CC) mutations higher on right-sided CRC (Burcin Baran, 2018 Aug) [53]. Conversely, polypoid lesions from APC mutations in, for instance, familial adenomatous polyposis is more common in distal and rectal CRC.

The colon is approximately 1.5 meters in length from the ileocecal valve to the anus [54]. The difference between the right-sided and left-sided colon is based on embryological origin: The right-sided colon includes the cecum, appendix, ascending colon, hepatic flexure, and the proximal two-thirds of the transverse colon, and all of these structures embryologically originate from the midgut. While the left-sided colon includes the distal one-third of the transverse colon, the splenic flexure, the sigmoid colon, the descending colon, and the rectum all

originate from the hindgut. These embryological differences also mean that CRC of different sites have different histologies: sessile serrated or mucinous adenocarcinoma for right-sided CRC, and tubular, villous, and typical adenocarcinomas for right-sided CRCs. Additionally, increased immune infiltration, regarded as higher immunogenicity with higher T-cell infiltration, is observed in right-sided CRCs (high MSI in right-sided) (Burcin Baran, 2018 Aug) [53]. While on left-sided CRCs cancer is more typical, Chromosomal Instability (CIN) of carcinogenesis is present.

Other differences include response to therapy, better response to immunotherapy in right-sided CRC, and age and sex differences (females and older age in the case of right-sided CRC) and metastases site (peritoneal for cases of right-sided CRC, while liver and lung for left-sided CRC) (Burcin Baran, 2018 Aug) [53].

Furthermore, in the left-sided colon, scientific studies have uncovered rectal differences compared to the rest of the left-sided colon and the right-sided colon area.

Topographically, the rectum is a large bowel up to the edge of 16 cm from the Anocutaneous line (AC line), where it is partly intraperitoneal and partly extraperitoneal, in the small pelvis (variations based on sex are also present). The lower part of the rectum includes a particular mesorectal structure, fascia, and both nerval and vascular anatomy, including an internal iliac artery supply of the lower two-thirds of the rectum, as opposed to the inferior mesenteric artery supply to part of the left-sided colon, including the sigmoid colon and upper third of the rectum (Stephan Paschke, 2018 Sep) [24]. Other differences to consider in relation to CRC location relate to the microbiome and how it differs in the different parts of the colonic lumen (Nakamura, 2010) [25].

Aspirin, low BMI, and minimal physical activity have been shown to diminish the incidence of colon cancers but not rectal cancers (Stephan Paschke *et al.*, 2018) [24].

1.8. Tumor Immunological Microenvironment

Since Virchow's times, in 1863, inflammation has been associated with tumor genesis and progression (Balkwill, 2001 Feb 17) [55]. Since then, chronic inflammation has become a key hallmark of cancer.

Since the 1800s, scientists and clinicians have attempted to exploit the human immune system in the fight against cancer. William Coley first found the relationship between infection and cancer regression, which led him to experiment in treating cancer-affected patients with deliberate bacterial infection of *Streptococcus Pyogenes* and *Serratia Marcescens*.

Cancer arises from uncontrolled cells' growth and the immunoevasion of these cells. The relationship between cancer cells and the immune system is described in the dynamic process known as immunoediting [56].

The immune system can distinguish between normal, healthy, and stressed

cells by recognizing a variety of "danger" signals including Danger-Associated Molecular Patterns (DAMPs), self-induced antigens (being a marker of abnormal cells, be it infected or tumoral), tumor-specific antigens (like abnormal products of p53 mutation, present only on tumoral cells), tumor-associated antigens (which can be present also on non-tumoral cells) and onco-fetal antigens (proteins which are physiologically present only during fetal development such as Carcioembryonic Antigen, CEA). Cells may be unhealthy due to infection or because of cellular damage caused by non-infectious agents such as sunburn or cancer. Infectious microbes such as viruses and bacteria release another set of signals recognized by the immune system, called pathogen-associated molecular patterns (PAMPs) (*Niaid.nih.org*).

Immunoediting consists of three phases. Once cells are transformed into cancer cells through the process of carcinogenesis, the immune system can extrinsically suppress the growth of these, functionally preventing the further advancement or formation of cancer.

In the first phase, elimination, previously known as cancer immunosurveillance, innate and adaptive immune cells and molecules recognize transformed cells and destroy them, resulting in a return to normal physiological tissue. If this phase fails, surviving tumor cells may enter the equilibrium phase, wherein cells and molecules of adaptive immunity prevent tumor outgrowth. These variants may eventually acquire further mutations that result in the evasion of tumor cell recognition, killing, or control by immune cells; they may then progress to clinically detectable malignancies in the escape phase.

The actions of the immune system within the tumor microenvironment are controlled and determined by tumoral cells' interactions with immune cells. In tumor formation and progression, immune cells and inflammatory cytokines fail to enact their anti-tumoral capabilities and may even further reinforce tumoral growth and invasion, for instance by the remodeling of the Extracellular Matrix from neutrophil's elastase [57] or by the stimulation of angiogenesis on the part of tumor associated macrophages [58]. In addition to tumor cells, the tumor microenvironment contains cells of the immune system, the tumor vasculature, and lymphatics, as well as fibroblasts. The cells within the tumor microenvironment can be identified by cell-type-specific markers, which are often cell surface molecules [59]. The tumor microenvironment contains several immune cell phenotypes, including T-cells (such as regulatory-T cells, CD 8+), B-lymphocytes, NK cells, tumor associated macrophages and tumor associated neutrophils. Regulatory T cells and Myeloid derived suppressor cells (a cell line derived from a pathological status on the myeloid-line) when combined with a constant activation of NFkB, they have been proposed to provide a mechanism for tumor evasion from the immune system (Whiteside, 2008).

The evolution, structure, and activities of the cells in the tumoral microenvironment involve many parallel processes in relation to those involved in wound healing and chronic inflammation, including fibroblast recruitment and activa-

tion, Extracellular Matrix (ECM) component deposition, infiltration of immune cells, neovascularization, and cellular lineage plasticity [60].

The different immune cell populations and their roles in the tumor microenvironment have been closely investigated by research teams worldwide. Regarding the adaptive immunity cell family, their role has been shown to be of different prognostic relevance depending on the cancer type and cell phenotype. Different T cell populations within the tumor microenvironment infiltrate the tumor areas, at the invasive tumor margin and in draining lymphoid organs. Among these, cytotoxic CD8+ memory T cells (CD8+), which are normally antigen "experienced" and capable of killing tumor cells, are strongly associated with a positive prognosis [61]. Instead, IL-10-producing B cells, known as regulatory B cells (Bregs) [62], have been demonstrated to increase tumor burden and inhibit tumor-specific immune responses in inflammation-induced skin cancer [59]. Conversely, B cell infiltration within the tumoral microenvironment is associated with good prognosis in breast and ovarian cancers [63].

Regarding the innate immune system cells' role in the tumoral microenvironment, they have been shown to be of immense importance to the internal milieu of the system. For instance, Tumor-Associated Macrophages (TAMs) are abundant in most human cancers, and their activities are typically pro-tumorigenic [64] [65], which is also explained by the fact that most of these TAMs are generally of the IL-10 high phenotype (immunosuppressive). Tumor Associated macrophages (TAMs) are a common component of tumor related inflammation. Cells of the monocyte-macrophage lineage exhibit considerable plasticity and diversity. TAM populations in murine tumors can be quite diverse and hypoxia may be one driver of this. Subsets have been identified between mouse and human monocytes. It remains to be determined whether diversity of TAM reflects their origin from different monocyte precursors or microanatomical differences (e.g. oxygen tension in different part of the cancer tissue) [55]. Thus, inflammation and immunity have become an important focus in cancer research. TAMs also have particular polarization phenotypes, including the M2 alternative phenotype, which has been shown to be anti-inflammatory and pro-tumoral (driven by IL-4 or IL-13) [58] and are considered the main population of macrophages in TAMs, as these are driven by tumor-derived and T-lymphocytes derived cytokine, shifting the macrophages polarization to M2. These, M2 TAMs are a fundamental role in inhibiting the adaptive immune response and promote tumor growth and progression [58].

The role of Tumor-Associated Neutrophils (TANs) in primary tumor growth and metastasis is controversial. There is evidence that neutrophils promote primary tumor growth in mouse cancer models and have pro-tumorigenic effects by enhancing angiogenesis [66]. Furthermore, CD11b+ bone-marrow-derived cells, a heterogeneous myeloid cell population which includes neutrophils, have been associated with the priming of the premetastatic lung and the enhanced seeding of circulating tumor cells. By contrast, in a different study, an antitumor function of these cells has been observed following immunological [10] or cyto-

kine activation [52], leading to activation and invasion of neutrophils in the tumor, together with an increase in surface contact zones between neutrophils and cancer cells [10]. Under these conditions, neutrophils can actively eliminate disseminated tumor cells [67], as they do in situations of TGF-β blockade in the tumor microenvironment [68].

Other non-malignant cells that can be found in the tumoral microenvironment are fibroblasts. They have been discovered to be capable of secreting growth factors, such as the EGF family members Hepatocyte Growth Factor (HGF), Fibroblast Growth Factor (FGF), and Insulin-like Growth Factor 1 (IGF1), which are mitogenic for malignant cells [69].

The extracellular matrix within the tumor microenvironment also plays an important role in tumor progression and evasion, as it contains key growth factors, such as angiogenic factors and chemokines, that interact with cell surface receptors and give each tissue its tensile and compressive strength and elasticity [17] by means of myofibroblasts depositing large quantities of ECM proteins, secreting GFs and exerting strong contraction forces on the ECM [17].

In CRC, a great focus has been placed on immunity and its effect on therapy response and prognostic relevance. A great example of this is the Immunoscore, wherein sections of CRC from patients are taken and are stained through a process of Immunohistochemistry (IHC). Staining of CD3+, and CD8+ T lymphocytes, both intratumorally and in the invasive margins of the sections is performed [70]. This type of score has been proven to be a reliable index of reoccurrence in CRC patients, and thus something worth considering as an addition to TNM staging (Pagès *et al.*, May 26, 2018) [70]. A high immunoscore has been shown to correlate with prolonged survival, and differences in immunoscore have been revealed between CRC sites; specifically, it is lower in right-sided CRC (Guo, 2019) [71].

Therefore, macrophages, along with neutrophils, have been shown to be an important part of the tumor microenvironment, and different procedural interventions, such as VEGF modulation, can alter this environment through these cells by for instance hindering the recruitment and development of M2 TAMs, which have poor antigen-presenting capacity and a decreased cytotoxic capacity due to the weak NO production [72]. TAMs can also hinder T-cell activation and proliferation by releasing IL-10, TGF-β, and prostaglandins. Therefore, the innate immune system has also become an important field of interest in cancer research. For instance, a further example of this is that in recent studies, one of the parameters used as a secondary biomarker for therapy success in cancer treatment is the neutrophils-to-lymphocytes ratio (Halama, 2019) [73], illustrating clinics' implementation of immune-oncological discoveries in the field of the role of immunity in tumoral microenvironments.

1.9. Neutrophils and the Tumor Microenvironment

Neutrophils are granulocytes, comprising 40% to 60% of the total white blood cells in humans (aafp.org). Their role in the innate immune system has been re-

garded as mainly present in the resistance against extracellular pathogens, such as fungal and bacterial infections [74]. However, various studies have uncovered new aspects of the function of neutrophils. For instance, they have been shown to be polarized into two different phenotypes according to environmental signals. In fact, TGF-β plays a significant role in the determination of neutrophils' phenotype, by shifting the balance from an antitumor (N1) toward a more permissive (N2) phenotype [75]. They are classically characterized based on their ability to induce phagocytosis, release lytic enzymes, and produce Reactive Oxygen Species (ROSs). In the context of the tumor microenvironment, recent studies have demonstrated the prominent role of neutrophils in infiltrating tumor tissues to promote their growth, invasion, angiogenesis, and metastasis in various types of cancers, although they were initially considered to have a defensive function against tumor cells [76].

Recent studies have suggested that Tumor-Associated Neutrophils (TANs) also exhibit considerable plasticity and are capable of polarization into either an anti-tumorigenic N1 phenotype or a pro-tumorigenic N2 phenotype [77]. Neutrophils are known to secrete several inflammatory, immunoregulatory, and angiogenic factors, including neutrophil elastase, Matrix Metalloproteinases (MMPs), and Vascular Endothelial Growth Factor (VEGF), which can exhibit paracrine effects on the tumor microenvironment. N1 neutrophils exhibit increased cytotoxicity and reduced immunosuppressive ability through the production of Tumor Necrosis Factor (TNF)-α, Intercellular Adhesion Molecule (ICAM)-1, ROS, and Fas, and by diminishing arginase expression [78]. In contrast, "N2" neutrophils support tumor expansion by producing MMP-9, VEGF, and numerous chemokines including CCL2, CCL5, and CXCL4. These N2 phenotypes express high levels of arginase with respect to the N1. The phenotype of TANs depends on the signals encountered in the tumor microenvironment (by molecules such as Interferon-β for N1 phenotype and TGF-β for N2 phenotype) (Rei Mizuno *et al.*, 2019 Feb) [12].

2. Differences between Rectal and Colon Cancer

In the last years, a new focus on CRC site localization has started. Different studies revealed not only anatomical, but also genetical, immunological, therapy-response and prognostic differences between colon cancer (in the right-sided colon, being the ascending colon vs left colon, being the descending colon) and rectal cancers. 5-year survival rate for localized stage Colon Cancer is 90%, while the 5-year survival rate for rectal cancer is 69% [14]. Furthermore, many differences were found between Right-sided, Left-sided and Rectal CRC. For example, there were evidences which were found showing that in left-sided CRC and Rectal Cancers (RCs), the mechanism of invasive cancers was skewed towards a progression of preexisting mucosal lesions, while in right-sided CRC a de-novo pathway was also implicated in the invasive cancers' generations [8]. The colon is approximately 1.5 meters in length, from the ileocecal valve to the anus. The

difference between right-sided and left-sided colon is based on embryological origin: the right-sided colon includes the cecum, appendix, ascending colon, hepatic flexure, proximal two thirds of transverse colon, and all of these structures embryologically originate from the midgut. While the left-sided colon includes the distal one third of the transverse colon, the splenic flexure, the sigmoid colon, the descending colon and rectum, which all originate from the hindgut. These embryological differences also mean that CRC of different sites have different histologies. Sessile serrated or mucinous adenocarcinoma for the right-sided CRC while tubular, villous and typical adenocarcinomas for the right-sided CRCs. Also, increase immune infiltrate, seen as higher immunogenicity with higher T-cell infiltrate, is seen in right sided CRCs (high MSI in right-sided) [53]. While on left-sided cancer a more typical Chromosomal Instability (CIN) path of cancerogenesis is present.

Other differences include response to therapy, better response to immune-therapy in right-sided CRC, age and sex differences, females and older age in case of right sided CRC, and metastases site, peritoneal for the case of right sided CRC, while liver and lung for left-sided CRC [53]. Furthermore, in the left-sided colon, scientific studies have shown rectal differences compared to the rest of the left-sided and colon and the right-sided colon area.

Topographically, the rectum is large bowel up to the edge of 16 cm from the Anocutaneous line (AC line), where it is partly intraperitoneal and partly extraperitoneal, in the small pelvis (variations for the sexes also present). The lower part of the rectum includes particular mesorectal structure, fascia and both nerval and vascular anatomy, including internal iliac artery supply of the lower two thirds of the rectum vs the inferior mesenteric artery supply to part of the left-sided colon, including sigmoid colon and upper third of rectum [24]. Other differences to take in consideration when talking about CRC location are regarding the microbiome and how it differs in the different parts of the colonic lumen.

An important note to discuss is also the embryological origin emphasizing the difference between colonic-rectal tissues. The Gastrointestinal (GI) tract development begins at the start of the third week of embryonic development (in the gastrulation phase, where the three germ layers begin to form, ectoderm, mesoderm and endoderm, which together form the embryonic disc). This embryonic disc has a cranial end (which will form the mouth) and a caudal end (which, with the cloacal membrane, will form the anus). In the fourth week, the primordial GI tract starts its formation from the endoderm, which creates a gut tube suspended by a mesentery (double peritoneum layer). The gut tube is divided then in foregut, midgut and hindgut. Foregut gives rise to the esophagus, stomach, liver, gallbladder, bile ducts, pancreas and proximal duodenum. The midgut develops into the distal duodenum, jejunum, ileum, cecum, appendix, ascending colon, and proximal 2/3 of transverse colon. The hindgut becomes the distal 1/3 of the transverse colon, descending colon, sigmoid colon and the upper anal canal [79] [80].

The hindgut forms the distal one-third of the transverse colon, the descending colon, the sigmoid colon, and the rectum (also the superior portion of the anal canal). The hindgut's cloaca, forms also part of the urogenital tract (anal canal is form both ectoderm and endoderm, the pectinate line marks the junction). There are genes associated with the development of the gastrointestinal tract, including the sonic hedgehog (in the endoderm, for mucosa, submucosa, and muscolaris propria development). Also, the Homeobox (HOX) genes and NODAL gene are important for GI tract development (possible role in sphincter formation and GI musculature, and cranio-caudal formation, genes expressed in mesoderm).

Anatomically, the rectum has further peculiar features. The layers of the rectum include the mucosa (which itself includes the simple columnar epithelial cells, lamina propria, and muscularis mucosa), submucosa, muscularis propria, and serosa.

Various treatments have been implemented against CRC, primarily including surgical resection, chemotherapy (adjuvant and neoadjuvant), and radiotherapy.

It must also be noted that the main agents for chemotherapy are therefore: 5-fluorouracil (mainly a Thymidylate Synthase (TS) inhibitor), folinic acid (aiding the activation of partial normal DNA replication processes), and oxaliplatin (cytotoxic effects).

3. Tumor Microenvironment in Colorectal Cancer

Since Virchow's time, in 1863, inflammation has been associated with tumor genesis and progression [55]. Since then, chronic inflammation has become a key hallmark of cancer. The immunological tumor microenvironment is controlled and determined by tumoral cells interactions with immune cell. Immune cells and inflammatory cytokines fail to enact their antitumoral capabilities and may even promote tumoral growth and invasion. T regulatory cells and Myeloid Suppressor cells enrich the milieu, and together with a constant activation of NFkB, might provide a mechanism for tumor evasion from the immune system.

A great focus has also been put on immunity and its effect on therapy response and prognostic relevance in CRC. A great example of this is the immunoscore, where sections of CRC from patients are taken and through a process of Immunohistochemistry (IHC), CD3+ and CD8+ T lymphocytes, both intratumorally and in the invasive margins of the sections, are stained. This type of score has been proven to be a reliable index of reoccurrence in CRC patients, and thus something worth considering as an addition to the TNM staging [70]. High immunoscore has been shown to correlate with prolonged survival and also differences in immunoscore have been revealed between CRC sites namely, lower in right-sided CRC [71].

Thus, inflammation and immunity have become an important focus in cancer research, not only adaptive immunity, but also innate immunity. An example can be the Tumor-Associated Macrophages (TAMs) and their polarizations, including the M2 alternative phenotype which has been shown to be anti-inflammatory

and pro-tumoral driven by IL-4 or IL-13 [81].

Innate immunity has also shown great importance in CRC, as in other solid tumors. For example, neutrophil granulocytes, together with macrophages, have been shown to be an important part of the tumor microenvironment and that different interventions, such as VEGF, can modulate this environment through these cells. In fact, one of the parameters used as a secondary biomarker for therapy success is the neutrophils-to-lymphocytes ratio [73].

4. Doctor-Patient Interaction and Intercultural Sensitivity

In an ever-changing world, where the exponential evolution of technology led to the globalization of virtually everything, including workforce, it is certain that any kind of work environment, in this case, the medical one, will have people with different cultural backgrounds and different ethnicities. It is important to focus on the medical environment as a lack of understanding or of acceptance of other people's cultures and miscommunication could lead to a loss of lives. As an example, to show how important is the doctor-patient relationship (not just from a clinical standpoint, but also from a more general understanding of the patient's situation), the author is going to talk about the important topic of intercultural sensitivity.

As shown in the Milton Bennett's DMIS (Developmental Model of Intercultural Sensitivity) model (Bennet, 1986, 1993) [4] [5], in order to have an intercultural sensitivity one must first pass from an ethnocentric perspective to an ethno-relative perspective. Especially for health practitioners, it is important to learn to listen to a patient coming from a different cultural background and therefore understand and try to negotiate a correct treatment which allows the clinician to perform his/her job as a medical professional and on the other hand, makes the patient feel more comfortable and that his or her cultural background is respected. Otherwise, what could happen is that the patient, or his or her family, feeling a lack of respect towards their cultural background, will negate any kind of treatment or suggestions. Therefore, the job of the physician is not only to make the correct therapies, but also to have a sense of empathy toward his or her patients. To reach this stage, health practitioners could follow the LEARN rule, which is: listen, explain, acknowledge, recommend and finally negotiate with the patient to create the correct environment for a good therapeutic intervention. This model is not intended to replace the traditional skeleton of the medical interview but it is intended for it to be an addition to it. This is a very important point as the physician interacts with patients who have emotions and feelings (including a lot of fear and uncertainty especially in the field of oncology), who come from different cultural backgrounds and therefore it is the physician's job to understand the true cause of the disease and try to find if the patient is comfortable in listening and talking to the doctor, especially during history taking. A patient's background is very important, especially if their cultures or religions are different than the doctors' and it is important to understand the pa-

tients in order to avoid them not trusting the clinicians because of a lack of knowledge of their culture.

5. Final Considerations

This review sheds new light on the state of the research and the need for new classification criteria, dividing the too-broad terminology of CRC (Colorectal Cancer) into colon cancer and rectal cancer as two different entities with different clinical and etiopathological courses. As researchers and clinicians, we should push towards a clinical shift of considering rectal and colon cancer as different pathologies and abandoning the term Colorectal Cancer (CRC) often used as an umbrella term to band together very different pathologies in terms of the current knowledge and of the unknown one. Much is still to be validated and researched and there is a growing need for new discoveries and sharing of research data and results in order to push forward the current knowledge in this field, leading to discussions, validations, and sharing of open-access science which can hopefully in the future save lives and aid in the prevention and treatment of these diseases. This is fundamental and as Plos.org puts it, to share raw scientific data is "to enhance their understanding of published research, for purposes of verification, replication and reanalysis, and to inform future investigations. Proactively sharing data ensures that your work remains reproducible over the long term. Sharing data demonstrates rigor and signals to the community that the work has integrity. Making data public opens opportunities to get academic credit for collecting and curating data during the research process. Access to data accelerates progress. According to the 2019 State of Open Data report, more than 70% of researchers use open datasets to inform their future research". I, as a scientist and intellectual, completely agree with this and as a scientist, it is my strong belief to support this.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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