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REVIEW ARTICLE

Biomarkers for Early Detection of Colitis-associated Colorectal Cancer - Current Concepts, Future Trends

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Abstract: Colitis-associated colorectal cancer (CAC) remains a critical complication of ulcerative colitis (UC) with a mortality of approximately 15%, which makes early CAC diagnosis crucial. The current standard of surveillance, with repetitive colonoscopies and histological testing of biopsied mucosa samples, is burdensome and expensive, and therefore less invasive methods and reliable biomarkers are needed. Significant progress has been made, thanks to continuous extensive research in this field, however, no clinically relevant biomarker has been established so far. This review of the current literature presents the genetic and molecular differences between CAC and sporadic colorectal cancer and covers progress made in the early detection of CAC carcinogenesis. It focuses on biomarkers under development, which can easily be tested in samples of body fluids or breath and, once made clinically available, will help to differentiate between progressors (UC patients who will develop dysplasia) from non-progressors and enable early intervention to decrease the risk of cancer development.

Keywords: Inflammatory bowel disease, ulcerative colitis, Crohn's disease, colitis-associated colorectal cancer, colorectal cancer, surveillance, biomarkers.

1. INTRODUCTION

Colorectal cancer is the fourth most commonly diagnosed cancer and the second cause of death from cancer worldwide. The 2018 incidence of colorectal cancer was 1,850,000 and mortality 880,000 [1]. The risk factors include, among others, age, male sex, smoking, family history, a diet rich in red meat and low in fiber, history of polyps, and inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) [2, 3]. Colitis-associated colorectal cancer (CAC) has a worse prognosis and higher mortality than sporadic colorectal cancer (CRC), yet in both diseases, early detection is critical [4-6].

This review work presents the current knowledge about genetic and molecular characteristics of CAC *versus* CRC and discusses easily accessible biomarkers for early diagnostics, which may help to identify the UC patients who will develop dysplasia and in which early intervention might decrease their risk of cancer development.

1.1. Inflammatory Bowel Disease as an Underlying Factor in Colorectal Cancer

Ulcerative colitis is a lifelong, resulting in disability, idiopathic inflammatory illness involving the large intestine (colon and rectum), however, it can also affect skin, eyes and joints. It is characterized by relapsing and remitting inflammation of the intestinal mucosa, starting in the rectum and extending to proximal segments of the colon [7, 8]. Crohn's disease may affect any part of the gastrointestinal tract but is most frequently located in the distal part of the small intestine and proximal part of the large intestine, which was reflected in the former name *ileitis terminalis*. UC consists of fine ulcerations in the inner mucosal lining of the large intestine, whereas in CD, the inflammation extends to the bowel wall. The key symptoms of UC are diarrhea, often with blood or pus, abdominal pain and cramping, rectal pain and bleeding, weight loss, fatigue and fever. Symptoms of CD include abdominal pain, weight loss and chronic diarrhea; systemic symptoms of malaise, anorexia, or fever are common [9]. The cause of both IBDs is unknown but studies suggest interactions between environmental factors, intestinal bacteria, immune dysregulation, and genetic predisposition [10].

Ulcerative colitis affects both sexes with no predominance and the peak incidence is between ages 30 and 40 years. The highest incidence rates are in Northern Europe, Canada and Australia (24.3, 19.2, and 17.4 per 100,000, respectively) [11-16]. The incidence in developed countries is stabilizing, however, the burden remains high as prevalence surpasses 0.3%; moreover, there is an increasing incidence in developing countries. Similar tendencies in CD epidemiology are observed [17].

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The therapy of UC focuses on obtaining and maintaining the clinical and endoscopic remission [18]. Aminosalicylates are the main choice of treatment for mild to moderate UC, topical and systemic steroids can be used to treat flares, while immunosuppressants and biological drugs are used in moderate to severe disease. 15% of patients with UC may need a colectomy [19, 20]. Treatment of CD includes immunosuppressants azathioprine and biological drugs (in particular anti-TNFα) [9].

The extent, duration and activity of IBD impact the neoplastic development [21, 22]. The estimate of CAC incidence is 0.5% per year for patients with duration of UC between 10-20 years and 1% per year thereafter [23]. Pancolitis has 5-15 times higher risk, whereas left-side disease has 3 times higher risk, both compared to the general population [24]. The CAC incidence data for CD are limited, however, the occurrence is lower than in UC [4], with an estimated cumulative risk of 2.9% at 10, 5.6% at 20 and 8.3% at 30 years, respectively [14].

The carcinogenesis in the course of IBD has distinct molecular mechanisms than those in CRC, with changes within the microenvironment resulting from chronic inflammation playing a fundamental role in the development of the former [25].

The higher incidence of CAC in males may be related to hormonal factors as estrogen receptors (ER) play a role in carcinogenesis, and ER expression tested retrospectively in UC patients with long-lasting pancolitis suggests that a decline of ER-beta expression could be used as a biomarker of UC dysplastic changes [26]. Other inflammatory changes possibly promoting carcinogenesis include higher levels of NK-1R and pEGFR in both CAC and CRC and higher levels of Cox-2 in CRC than in the normal tissue [27], or proinflammatory MUC1C-induced cytokine-mediated activation of the NF-κB signaling contributing to CAC development [28]. Another protein involved in immune regulation is a fibrinogen-like protein (Fgl2), which suppresses the CAC carcinogenesis by limiting the intestinal inflammation [29].

1.2. Genomic, Molecular and Clinical Differences between Colitis-associated and Sporadic Colorectal Cancer

1.2.1. Genomic Differences

Carcinogenesis of CAC has distinct environmental, genetic, and immunologic features in comparison with CRC [30] and the ongoing investigations keep identifying and confirming mutational differences between CAC and CRC, which may help to establish biomarkers for effective surveillance.

There is a growing body of evidence that the time and frequency of genomic alterations differ significantly between CAC and sporadic CRC [31]. For example, the mutation in the TP53 gene occurs early in CAC and may be observed already in UC patients with no dysplasia [32]. It can thus be used to detect an intermittent state between post-inflammatory regenerative changes and neoplasia.

Expression levels of p53 in dysplastic crypts may become a diagnostic biomarker in predicting the risk of evolution toward malignancy [33]. Burmer *et al.* found p53 loss of heterozygosity (LoH) in 6% of biopsy samples without dysplasia, 33% with low-grade dysplasia, 63% with high-grade dysplasia and 85% with adenocarcinoma. These changes were also present in inflamed mucosa, suggesting that chronic inflammation may be mutagenic [34, 35].

Adenomatous polyposis coli (APC) and KRAS genes were mutated at significantly lower rates in tumors from patients with colitis-associated (CAC) than in sporadic CRC tumors (for APC: 13 *vs*. 80% and for KRAS: 20 *vs*. 35% of cases, respectively), which may be of diagnostic and therapeutic importance [36, 37].

Genes mutated more frequently or uniquely in CAC tumors also include SOX9 and EP300 (encoding proteins in the WNT pathway), NRG1 (encoding an ERBB ligand), and IL16. Recurrent mutations in components of Rho and Rac GTPase network were also found, indicating a role for non-canonical WNT signaling in the development of colorectal tumors in patients with IBD [36].

A next-generation sequencing (NGS) analysis performed by Yaeger *et al.* in over 300 cancer-related genes in 47 CAC biopsy samples found 6.2 genomic alterations per tumor. It has also confirmed that changes in TP53, IDH1, and MYC were more frequent, and APC mutations less frequent than in CRC reported in The Cancer Genome Atlas database [38].

Genomic instability resulting from chromosomal instability (CIN), microsatellite instability (MSI), telomere shortening, and CpG island methylator phenotype (CIMP) accompany both CAC and CRC [39, 40], although there may be variances regarding their timing and frequency [41]. For example, the rate and timing of microsatellite instability (MSI) do not significantly differ between CAC and sporadic CRC [40, 42]. On the other hand, the telomere shortening in UC patients occurs in mucosa close to dysplastic or cancerous lesions and is more frequent in patients subsequently developing cancer [43, 44].

Epigenetic changes: DNA methylation, histone modification, chromatin remodeling, and small non-coding microRNAs also contribute to the development of colon cancer [45]. Changes in DNA methylation have been identified in CAC more frequently in comparison to CRC, and several genes have been identified as potential biomarkers for CAC based on DNA methylation in non-tumor adjacent tissue. These are: EYA4, ESR, RUNX3, MINT1, MYOD and p16 exon 1 (all more highly) and COX2 (less highly) methylated [46, 47]. Other studies showed that the hypermethylation of APC, CDH13, MGMT, MLH1 and RUNX3 in the colonic mucosa with no neoplastic changes may be used as predictive biomarkers and help in early detection of CAC carcinogenesis by selecting patients for closer surveillance. The chronic inflammation tissue can be transformed into a dysplastic also as a result of epigenetic aberrations, which silence the tumor suppressor genes. These aberrations may result from excessive methylation of VIM, TFPI2 and ITGA4 promoters, however, the well-established VIM proved neither specific nor sensitive enough to distinguish between the non-neoplastic and neoplastic tissue. Both methylation markers ITGA4 and TFPI2 might be better biomarkers to assess the risk of CAC development instead [48]. Finally, the phosphorylated sphingosine kinase 1

(pSphK1) expression is upregulated in CAC more than in CRC patients [49].

Dysregulation of microRNAs (miRNAs), regulating gene expression, has been confirmed in both UC and colorectal cancer and has the potential of biomarkers for carcinogenesis as non-invasive tests are available [50]. This group of biomarkers can be well-suited for diagnostic and prognostic purposes, with relevant characteristics such as small size, stability of their biological samples (including in body fluids), small total number, compared to the hundreds of mRNAs they regulate and should be further investigated as candidate biomarkers to monitor the CAC risk [45].

Eleven miRNAs were expressed differently inactive UC in comparison to normal colonic mucosal tissue, which was confirmed by further studies - with miR-21 and miR-135b as examples of common key oncogenes in sporadic and IBD-related carcinogenesis [51]. Feng explored the predictive potential of miR-449a for CAC patients and shown its decreasing expression during carcinogenesis [52]. A study by Lewis *et al.* showed that the expression of miR-200 family, especially miR-200b-3p, is increased in UC dysplasia lesions and may be used as an early biomarker of CAC and possibly therapeutic target as well [53]. Another microRNA, miR-19a, has been found to promote inflammation and CAC development by stimulating nuclear factor NF-κB signaling and production of tumor necrosis factor-α (TNF-α) [54].

Of note, the methylation levels of specific miRNAs (MIR1, MIR9, MIR124, and MIR137) in rectal mucosa were significantly higher in UC patients with dysplasia or CAC than in patients without neoplasia in the study by Toiyama *et al.* and it was associated with patient age as well. The level of methylation of MIR137 was an independent risk factor for CAC [55].

1.2.2. Other Molecular and Clinical Differences

The colitis-associated carcinogenesis is initiated and maintained through inflammatory insult: the repetitive inflammation [56, 57], and increasing duration of the disease cumulatively increase the risk of CAC, on the other hand, the anti-inflammatory treatment decreases the risk [58-61]. The high levels of cytokines accompanying a longstanding inflammation, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interferon-gamma (IFN-γ), chemokines (chemoattractant cytokines) and metabolites of arachidonic acid lead to activation of nuclear transcription factor kappaB (NK-κB), an important mediator of cancer development [39, 62]. For example, IL-6 activates the JAK/STAT signaling pathway [63] inhibiting apoptosis and promoting proliferation – the latter mostly through hyperphosphorylation of the transcription factor STAT3 [64, 65]. Higher expression of both IL-6 and STAT3 has been detected in epithelium of biopsy samples with neoplastic lesions taken from patients with active UC compared with biopsy samples from non-active UC or control patients [66] and the activation of IL-6/STAT3 pathway is linked to development of ileitis and cancer [67]. On the other hand, the activity of the enzyme cyclooxygenase-2, COX2, involved in cell proliferation, apoptosis and antiproliferation is increased by pro-inflammatory cytokines, IL-1 and TNF-α. Noteworthy, the levels of COX2 mRNA are increased not only in the inflamed mucosa but also in dysplastic lesions from CAC patients [68]. Garrity-Park *et al.* found significantly higher expression of TNF-α, IL-1β and methylation levels of RUNX3, MINT1, and COX2 in the non-dysplastic, nonadjacent colonic samples from UC patients with neoplasmic changes than in UC controls, esp. the RUNX3 methylation is significantly higher in progressors *vs*. non-progressors patients with low-grade dysplasia [69].

The tumor necrosis factor ligand-related 1A (TLlA) is not only linked to the development of IBD but can also promote the carcinogenesis of CAC by activating Wnt/β-catenin pathway and inducing tumor cell proliferation [70]. High expression of CD44v9 antigen on the proliferative tumor stem cells stimulates carcinogenesis, however, anti-inflammatory treatment of UC with sulfasalazine may reduce the risk of CAC resulting from CD44v9 expression [71].

Expression of activating transcription factor 6 (ATF6) showed diagnostic accuracy as a biomarker distinguishing between low-grade dysplasia and inflammatory epithelium undergoing regeneration in patients with UC, having significantly higher levels in dysplasia and CAC than colitis mucosa without neoplastic changes [72]. C-Jun N-terminal kinases (JNK) activities impact carcinogenesis and increased levels of JNK-regulated proteins, p21WAF1 and γ-H2AX, induce cell cycle arrest, whereas their down-regulation increases proliferation and DNA damage leading to carcinogenesis. Restoring the p-JNK2 expression levels can be used to early prevent the CAC development [73].

NOD-like receptor protein regulating immunity, NLRX1, suppresses carcinogenesis of both CAC and CRC by limiting cellular proliferation, NF-κB, MAPK, STAT3 activation, and IL-6 levels which all are known factors promoting oncogenesis through various regulatory pathways. NLRX1 expression can be utilized as a biomarker of colon cancer, and IL-6 inhibitors could be used for the treatment of either CAC or CRC with low NLRX1 expression [74].

Caspases, the proteolytic enzymes, especially caspase-1, are involved in both IBD and CAC and a correlation has been found between the increased expression levels of stromal caspases-4 and -5 and the inflammation or dysplasia in UC patients, while the epithelial expression is limited to the neoplastic tissues only [75].

The signaling path of vitamin D is believed to be important in the CAC carcinogenesis and its potential influence on the microbiome is being investigated. The protective effect of vitamin D supplementation on CAC development still needs to be better understood, though [27, 76].

The intestinal microbiota is increasingly considered as playing an important role in inflammation of the colon mucosa, and disturbances in gut microbiota composition (dysbiosis) are observed in IBD, which may ultimately lead to carcinogenesis [39]. Rodents treated with feces obtained from CAC mice had higher CAC prevalence than rodents colonized with healthy mouse feces [77]. The composition of the intestinal microbiota is strongly influenced not only by dietary habits but by antibiotics as well, and there is an increased risk of CRC in elderly patients associated with antibiotics treatment early in life, suggesting a long-term impact of dysbiotic microbiota. Dysbiosis is a significant misbal-

ance in the composition of microbiota; the recent research suggests overgrowth of some bacterial strains: *Fusobacterium nucleatum* (Fn), *Escherichia coli* (*E. coli*), *Bacteroides fragilis* (Bf) or *Porphyromonas* in CRC. Much less is known about the impact of the decreased levels of protective bacterial species, for example fiber-fermenting *Clostridia*, on intestinal tumorigenesis. Noteworthy is the pro-inflammatory role of Fn in IBD and the abundance of Fn in periodontal disease, which is linked to an increased CRC risk, there is also a pre-clinical and clinical evidence of Fn relevance in CRC. The role *E. coli* plays in tumorigenesis is unclear; however, some strains may promote inflammation and produce toxins with oncogenic potential, such as colibactin. Bf species and Bf-derived toxin are also considered to cause the inflammation-derived tumorigenesis. Metagenomics studies supported the observation that certain gut microbiome composition is associated with CRC and overrepresentations of *Bacteroides* spp., *E.coli*, Fn, *Parvimonas*, *Bilophila wadsworthia*, *Solobacterium moreii*, *Roseburia intestinalis*, *Clostridium hathewayi*, *Peptostreptococcus*, *Gemella* and *Alistipes* have been found, suggesting the potential to test certain bacterial strains for early CRC diagnostic in the future. Details regarding differences in microbiota composition and possible impact on inflammation as well as on carcinogenesis are out of the scope of this review, however, have been thoroughly investigated recently [78-81].

1.2.3. Surveillance Diagnostic

1.2.3.1. Current Practices

Regular endoscopic examinations with considerable biopsy sampling [82, 83] are the standard recommendation and cancer prevention management is driven by histological results, allowing to reduce the risk of colorectal cancer and lower mortality rates [84]. It is important to differentiate UC patients, who will develop dysplasia (progressors) from patients who will not (non-progressors) [85].

Dysplastic changes identified in IBD patients unmistakably indicate a high risk of malignancy in IBD patients [86-88], however, the outcome of the assessment depends strongly on the quality of endoscopy, sampling procedures, and experience of the pathologist [89, 90]. Dysplasia, *i.e*. intraepithelial neoplasia [45], develops as raised (polypoid), adenoid-like mucosal lesion, easier to be detected, or as flat patchy dysplasia-associated lesions or masses (DALM), which may be overlooked even when high-definition endoscopy is used [32, 45, 88, 91]. The low-grade (LG) dysplasia has a much lower association with synchronous cancer (3% of cases) than the high-grade (HG) dysplasia (29%) [92]. The histological diagnosis, strongly influenced by inter-observer variability, has a critical impact on the treatment of dysplasia patients after proctocolectomy, so reliable biomarkers are needed to establish the early neoplastic transformation and assess the high risk of cancer [93]. Additional challenges, related to the complexity of CAC genetic background, the impact of gut microbiota on CAC and difficulties in drawing conclusions from biopsy samples obtained during endoscopy and transformed into formalin-fixed paraffin embedded (FFPE) specimens, make it difficult to come to practical conclusions [45].

1.2.3.2. Next Generation Diagnostics

Material from biopsies obtained during colonoscopy (or colectomy), with immunohistochemical staining of FFPE tissue provides information about the etiology of the disease and what biomarkers could be looked for. The next step is the isolation of the genetic material (DNA, RNA, miRNA) and proteins for proteomic analysis using next-generation diagnostics to identify alterations suggesting UC dysplastic or cancerous lesions, which are found in non-neoplastic regions. The ideal location of such lesions would be a rectum as rectal biopsies require less-invasive procedure (proctoscopy) and do not require specialist equipment or clinic.

1.2.4. Biomarkers Under Development

1.2.4.1. Colonic Samples

Genetic material from colonic epithelium is a promising source of biomarkers but CAC has different loss of gene pathways than CRC. The research has thus focused on genome-wide, not locus-specific, instability. A bacterial artificial chromosome (BAC) array detects pan-colonic chromosomal instability early in UC [43].

Calcium-sensing receptor (CaSR), with anti-inflammatory and anti-carcinogenic roles, may be a biomarker for colonic cancer progression. Activation of CaSR reduces the risk for both UC and CAC, which has also been confirmed by supplementation of calcium, which reduces the risk of colorectal cancer. Decrease of CaSR expression occurs in carcinogenesis and cancer development. Targeting CaSR should limit the inflammation of colonic mucosa and prevent carcinogenesis, especially favorably in colitis-associated colorectal cancer [94].

Aberrant DNA methylations, especially differentially methylated regions (DMRs), found by Toiyama *et al.* in the rectal mucosa of UC patients, may be used as screening biomarkers for CAC and prospective studies need to validate this approach [95]. Beggs *et al.* identified an abnormal methylation of TUBB6 during epigenome analysis and validated it as a potential biomarker of UC-associated dysplasia [96].

Blood vessel epicardial substance (BVES), underexpressed in epithelial malignancy, has been shown to promote inflammatory carcinogenesis and decreased levels of BVES mRNA *via* promoter hypermethylation can be used as a biomarker of CAC, especially, that it was also present in distant non-malignant-appearing mucosa [97].

1.2.4.2. Stool

Biomarkers present in stool are easy to apply, non-invasive and inexpensive methods for CAC surveillance and more research is still needed to identify an optimal biomarker. The frequent changes in DNA methylation occur early during carcinogenesis and are good candidates for surveillance as detectable both in the blood and in the stool. Significant differences in methylation of *EYA4*, *BMP3* and *NDRG4* between CAC neoplasia and IBD controls and more *SLIT2* gene methylation in IBD patients with high-risk for neoplasia than in low-risk patients were found in stool samples [98].

RNA are less studied than DNA as potential biomarkers, however, miRNAs are involved in carcinogenesis, angiogenesis and metastasis, and are also more stable than mRNAs in the stool. Unique patterns of miRNA expression in stool samples have been identified by Link *et al.* [99]. The overexpression of various mi-RNA (miR-17–92 and miR-135) has been found in CRC patients in comparison to healthy individuals [100]. miR-21 and miR-92a had significantly higher levels [101] and significant changes in miRNA expression (increased miR-21, miR-106a, miR-96, miR-203, miR-20a, miR-326 and miR-92 and decreased of miR-320, miR-126, miR-484–5p, miR-143, miR-145, miR-16 and miR-125) were detected in the stool samples of CRC patients compared with UC patients [102].

Proteins in stool are another biomarker approach and calprotectin and lactoferrin are being used for monitoring of IBD patients. Colon-cancer screening with fecal calprotectin and M2 pyruvate kinase (M2-PK) has not yet provided consistent results, although a pooled sensitivity of 79%, specificity of 80% and accuracy of 85% has been shown in a meta-analysis [103]. A well-known stool biomarker is haptoglobin, an acute-phase response protein, with 92% sensitivity and 98% specificity, with an increased sensitivity to 100% when combined with occult blood testing, for CRC detection [104]. Methylation of DNA markers (bone morphogenic protein 3, methylated N-Myc downstream-regulated gene 4, and mutant KRAS) can also be detected in stool samples and their levels were significantly higher in IBD patients with small adenomas and serrated lesions than in IBD controls [105].

Gut microbiome is getting more attention recently and alterations in its composition before any macroscopic neoplastic changes occur are believed to play a critical role in carcinogenesis [106, 107]. The composition of microbiota in stool samples from the gut shows disappearance of the dominant band in the *Bifidobacterium* group in stool samples of all CRC patients, but were present in colitis and internal hemorrhoid samples [108]. Significant increase of *Fusobacterium nucleatum* (*Fn*) in CRC, [109] increase of *Fusobacterium* and *Porphyromonas*, as well as decrease of protective bacteria such as *Bacteroides*, *Lachnospiraceae and Clostridiales* in CRC patients compared to healthy individuals has also been found [110].

1.2.4.3. Urine

The diagnostic relevance of urinary biomarkers for early CRC detection has not yet been shown; in the Polish study Rozalski *et al.* found oxidatively modified DNA bases/nucleosides in urine samples of CRC patients, however, its diagnostic performance was insufficient [111]. Potential biomarkers assessing the risk of CAC development could be the increased levels of d-2-hydroxyglutarate (D2HG) in urine and decreased levels of d-2-hydroxyglutarate dehydrogenase (D2HGDH) in colonic tissues of IBD patients [112]. Periodic measurement of prostaglandin E-major urinary metabolite (PGE-MUM; 7-hydroxy-5,11-diketotetranor-prosta-1,16-dioic acid), correlated with mucosal inflammation, may improve control of the chronic disease and decrease the risk of subsequent CAC development [113].

1.2.4.4. Exhaled Breath Samples

Volatile organic compounds (VOCs) of metabolome present in exhaled breath to screen colorectal cancer (CRC) - cyclohexane, methylcyclohexane, 1,3-dimethyl-benzene and decanal can be used as the signature for CRC screening [114]. Prior studies have shown promising results using exhaled propanal for detecting CRC (96% sensitivity/76% specificity) [115]. The Colorectal BReath Analysis (COBRA), NCT03699163, examined the discriminatory breath compounds and analysis of this preliminary dataset, presented by Woodfield at ESMO 2018, is expected to reveal compounds of interest for colorectal cancer diagnosis once the study has been completed [116].

1.2.4.5. Blood

Blood is easily available and inexpensive source of many biomarkers. The techniques used for the assessment of protein levels are enzyme linked immunosorbent assays (ELISA), modified ELISAs, targeted proteomics, and nanotechnology. Levels of serum p53 in UC patients were higher than in normal controls, but only 8/13 of CAC patients had elevated p53 [117]. As described earlier, the increase of p53 levels occurs early in CAC carcinogenesis, however, has limited sensitivity as biomarker for high-risk UC patients.

Testing of microRNAs (miRNAs) in blood samples from colonoscopied UC patients revealed 28 miRNAs with abnormal expression, including significant upregulation of miR-375 in CAC *versus* active UC cohort. Also Patel *et al.* found higher levels of miR-375 in blood samples in patients with colitis-associated cancer cohort compared with active ulcerative colitis [118].

Another miRNA of interest, miR-26b, showed increased expression in both tissues and serum of UC and CAC patients and adding the Ki-67 expression levels to the panel allowed to predict the CAC. As miR26b expression is decreased in CRC this biomarker could be used to distinguish between colitis-associated and sporadic colorectal cancer [119]. Assessment of circulating miRNA levels in plasma or serum of IBD patients could emerge as a convenient tool for early diagnosis with predictive and prognostic features.

The increased levels of inflammatory biomarkers in serum, such as C-reactive protein as well as erythrocyte sedimentation rate, are linked to a higher risk of CAC [120], so is the high C-reactive protein (CRP)-albumin score. Koutroubakis *et al.* identified significantly higher levels of long-term serum inflammatory biomarkers, including CRP, erythrocyte sedimentation rate, hemoglobin, platelets, and albumin in UC patients with dysplasia when compared with UC patients without dysplasia [121].

AUTHOR'S INSIGHT ON THE TOPIC AND CONCLUSION

Despite progress in recent years, the early diagnosis of CAC remains an unmet medical need. Effective surveillance, in addition to cumbersome and expensive biopsies during colonoscopies, should include increasing usage of biomarker tests from low burden sources, such as blood, stool, urine or breath. The ultimate objective should be the possibility to differentiate UC patients, who will develop dysplasia (pro-

gressors) from patients who will not (non-progressors). There are multiple examples of effective predictive biomarkers of CAC carcinogenesis (expression of p53, various miRNAs, stromal level of some caspases or DNA methylation levels and many others), however none of the tests has been approved for the routine practice in early CAC detection so far so further research is needed.

LIST OF ABBREVIATIONS

CAC	=	Colitis-associated colorectal cancer
CD	=	Crohn's disease
CRC	=	Colorectal cancer (sporadic)
IBD	=	Inflammatory bowel disease
NGS	=	Next generation sequencing
UC	=	Ulcerative colitis
VOCs	=	Volatile organic compounds

AUTHORS' CONTRIBUTIONS

All authors provided the overall concept and framework of the manuscript; TM and JF researched and identified appropriate articles, and wrote the manuscript; all authors revised the manuscript and approved its final version.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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