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RESEARCH ARTICLE

The role of biomarkers to increase the detection of early-onset colorectal cancer

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ABSTRACT

Colorectal cancer is the third most diagnosed cancer worldwide with an estimated 1.93 million cases diagnosed in 2020. Over the past few decades there has been a dramatic rise in the incidence of early onset colorectal cancer, defined as colorectal cancer diagnosed in those aged under 50 years. The largest predictor of survival is early stage at diagnosis, therefore ways to improve prompt diagnosis of early onset colorectal cancer at an early stage is an effective way of managing the impact of this rising disease. Diagnosing colorectal cancer in younger patients has unique challenges with patients falling outside the age of most screening programs and early symptoms of colorectal cancer being common, non-specific and initially intermittent.

While colonoscopy remains the gold standard investigation, it is a limited and expensive resource, and current patterns of practice result in large numbers of patients being scoped unnecessarily. The development and use of new and novel non-invasive biomarkers may help (either alone or in combination) identify either symptomatic patients in primary care, or aid with screening asymptomatic patients to focus resources where they are needed most. This review discusses challenges around diagnosing early onset colorectal cancer, with an overview of both current and future methods that might help overcome these challenges. These include increased assessment of familial risk, and the measurement of different biomarkers including faecal haemoglobin, markers of inflammation, gut microbiota, and selected metabolites.

Keywords: early-onset colorectal cancer, diagnosis, biomarkers, screening.

Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer worldwide with an estimated 1.93 million cases diagnosed in 2020 and the second most common cause of cancer death resulting in 916,000 deaths in 2020.[1] Over the past few decades the incidence of early onset colorectal cancer (EOCRC) defined as CRC diagnosed under the age of 50 has been rising across many parts of the world, with the trend being independent to any change in overall incidence of CRC.[2] It is estimated that in 2023 13% of CRC cases diagnosed in USA will be in those younger than 50 years,[3] with some predictions estimating by 2030 10% of colon cancers and 25% of rectal cancers will be in those under 50 years.[4]

The cause for this increase is yet to be firmly established but there is an increasing awareness that most cases are sporadic and likely reflect an interaction between an individual's colonic wall (including their mucus layer), and their microbiota in combination with lifestyle and/or environmental factors.[5–7] These sporadic cases comprise at least 70% of all EOCRCs, while a smaller proportion will have an inherited predisposition.[8]

Patients diagnosed with CRC under the age of 50 tend to have a higher proportion of left sided and rectal cancers, and more commonly present with later stage 3 or 4 disease.[5] Research has shown that younger patients are more likely than older patients to experience delays to diagnosis.[9] Regardless of the cause (and until methods of prevention can be developed) timely diagnosis of EOCRC should be a major focus to reduce the impact of this rising problem, with the largest predictor of prognosis currently being stage at diagnosis.[5,10]

In this review we discuss challenges around diagnosing early onset colorectal cancer, with an overview of both current and future methods that might help overcome these challenges. These include increased assessment of familial risk, and the measurement of different biomarkers including faecal haemoglobin, markers of inflammation, gut microbiota, and selected metabolites.

Familial risk

Early diagnosis and prevention in young people at risk of developing familial EOCRC is an area where gains could be made. This is illustrated by a retrospective study of 2,473 EOCRC cases which found one in four people diagnosed with EOCRC met the criteria for early screening centred on family history-based joint guidelines put out by the American Cancer Society and US Multi-Society Task Force on colorectal cancer.[11] Of these, 98.4% would have been diagnosed earlier or prevented altogether if screening with colonoscopy had been undertaken based on these guidelines.[12] There are limitations with this approach, with research showing that family history information in patient medical records is generally inadequate to accurately assess familial risk with one study finding only 7% of patient notes recording age at diagnosis of affected first degree relatives (FDRs).[13] Improving accurate assessment of familial risk in patients and subsequent referral for screening does have the potential to help reduce the impact of rising EOCRC prevalence. In the future we may see advances in polygenic testing to a point where testing of individuals for genetic variations which confer CRC risk overtakes the reliability of family history to stratify those at risk of CRC for screening but further research here is needed.[14]

The challenge of diagnosing sporadic early-onset colorectal cancer.

The early diagnosis of those at risk of developing sporadic EOCRC presents a larger challenge. In simple terms this involves effective evaluation of symptomatic young patients who have no predisposing hereditary conditions or relevant family history, and this accounts for vast majority of EOCRC patients. However, symptoms and signs of CRC such as rectal bleeding, abdominal pain, altered bowel habit and anaemia are very common, often initially intermittent, and the vast majority of patients presenting with these symptoms have benign disease.

While colonoscopy remains the gold standard procedure used to diagnose CRC, the availability of endoscopy time in most health systems is limited, necessitating triaging of referrals for colonoscopy. Recent research assessing the diagnosis of over 5000 cases of EOCRC found that the presence of one or more 'red flag' symptoms (abdominal pain, rectal bleeding, diarrhoea and iron deficiency anaemia) were associated with increasing risk of CRC. Moreover, in this cohort 68.6% of patients with EOCRC had presented with one or more of these red flag symptoms between 3 months and 2 years prior to diagnosis, highlighting that early recognition may indeed aid a timelier diagnosis.[15] The non-specific, common and initial intermittent nature of these symptoms however presents a huge logistical challenge, with health systems likely unable to offer all patients who present with these red flags a colonoscopy. It has been estimated that 59,856 colonoscopies would be required to diagnose just two cases of EOCRC if every symptomatic patient was investigated.[16] There is therefore an urgent need to identify those who are at highest risk of having a cancer or significant precancerous lesion from the large numbers presenting with such symptoms. This is where the ongoing development of a wide range of biomarkers and tests may be invaluable to help prioritise those who we investigate further.

Biomarkers

Biomarkers to detect early-stage CRC fall broadly into a number of categories,[17,18] and a major consideration is the need to be able to detect early-stage neoplasia including premalignant lesions. As such, proteins and metabolites released by various cells during an active disease state may have greater utility than genetic biomarkers as a means to identify those patients presenting in primary care who should be progressed for clinical investigation. In this setting individual biomarkers need to exhibit both sensitivity and specificity to reduce under- and over-diagnosis respectively.[19] Testing for biomarkers also needs to be easily performed and relatively inexpensive. For example, while composite metabolic panels are currently being investigated as a means to triage symptomatic patients, [20] the complexity (and cost) of this approach is likely to preclude their use in routine clinical practice at least initially.

Another consideration when using biomarkers for diagnostic testing of symptomatic patients is sample choice. While a range of biological samples including blood, faeces, urine, breath and rectal colonic mucus are used, these can have limitations. Detection of a biomarker in a stool sample may be more specific and sensitive than the same biomarker measured in blood.[21] Biomarkers in stool and rectal mucosal samples however may be more indicative of distal rather than proximal disease.[22,23] Serum samples reportedly contain higher concentrations of

metabolites than urine.[24,25] This may reflect diurnal variation and/or the effect of diet on urine composition. Analysis of blood however is more complex because of the highly abundant proteins.[26] Collectively these studies highlight choice of sample may influence detection of a biomarker. Another major consideration is the threshold at which any test is reported, best illustrated by the testing of stool samples for faecal haemoglobin (f-Hb).

Faecal haemoglobin

Testing for blood in faeces has been used to assess symptomatic patients in primary care for many years, with the current faecal immunochemical tests (FIT) providing higher sensitivity than the original guaiac test for faecal occult blood (FOB). Compared to the FOB, the FIT test detects the presence of intact human haemoglobin, meaning a positive test is more specific for a colonic source. Furthermore, the FIT is not influenced by diet.[27] Using this test, f-Hb levels can also be reported quantitatively, or against a variable threshold that enables different f-Hb cut-off concentrations to be set. Positivity rate, neoplasia detection rate and sensitivity decrease as the f-Hb cut-off is increased, while positive predictive value and specificity increase.[28] This issue highlights the need for this test to be internationally standardised, particularly in symptomatic patients where the first objective is to rule out CRC.

There are a growing number of studies that report using FIT in symptomatic patients as a means to rule out advanced colorectal neoplasia.[18,29–36] Based on these findings, the National Institute for Health and Care Excellence (NICE) diagnostic guidelines now recommend a threshold of 10 µg Hb/g faeces to guide referral for colorectal cancer in primary care.[37] When the threshold is reduced further to the lowest level of detection (2 µg/g), a negative FIT is shown to effectively rule out colorectal cancer in 99.5% of symptomatic patients under 50 years of age.[40] The absence of anaemia and a palpable abdominal or colorectal mass in these patients further supports this.[22,36,38–40]

Early use of the FIT test was restricted to patients without rectal bleeding, however there is now good evidence that shows its ongoing efficacy in this group. A study of 3143 patients referred to the NHS with rectal bleeding found 56% of patients were FIT negative. Moreover, the sensitivity of the test was preserved. The authors hypothesised that undetectable FIT in patients with rectal bleeding can be explained by sporadic bleeding in both significant and non-significant bowel disease.[41] Given that outlet rectal bleeding is generally rare in patients with proximal cancers, in FIT negative patients who have rectal bleeding without concurrent anaemia or abdominal mass (both of which are suggestive of proximal CRC) flexible sigmoidoscopy may be a reasonable means to further exclude most cases of CRC. This approach is cheaper, and easier than complete colonoscopy.[39]

Another issue is the growing awareness that the diagnostic accuracy of the FIT means a positive test (even at a threshold of 150 µg/g) does not necessarily distinguish patients with CRC (early or late onset) from other serious bowel diseases.[32] This is illustrated by the finding that the number needed to scope (NNS) to detect one CRC in symptomatic young patients (< 50) when the f-Hb threshold is set at 150 µg/g is 8.8 colonoscopies,[40] whereas the NNS in symptomatic patients of

all ages at the same FIT threshold is reportedly 2.8-3.3.[42] This may reflect a higher incidence of inflammatory bowel disease in the younger patient, given that detection of f-Hb is suggested to also indicate systemic inflammation associated with longer-term conditions. [43] As such, this has the potential to underlie the observed lack of specificity of the FIT when used as a diagnostic test to identify symptomatic patients with early-stage CRC.[44] Collectively, these studies highlight that while valuable as an adjunct to clinical history, FIT is not a diagnostic test in itself to identify all patients with early stage disease,[36] and still has significant limitations when used to triage the large numbers of patients presenting with symptoms suggestive of CRC.

Biomarkers of inflammation

While long considered a potential biomarker of colorectal polyps and cancer,[45] measurement of faecal calprotectin (FC) actually appears to have limited diagnostic accuracy for identifying patients with CRC, irrespective of stage.[45–48] This is reinforced by studies that have compared the sensitivity and specificity of FC to quantitative FIT in this setting.[22,30,31] Measurement of chitinase-3-like protein 1 (CHI3L1), a glycoprotein released by macrophages, neutrophils and tumour cells, can likewise predict colon cancer in patients without co-morbidity[49] and analysis of faecal levels identifies CHI3L1 as a good discriminatory marker of CRC.[21] Levels in symptomatic primary care patients however are not significantly different (p=0.193) from those detected in the healthy controls. Faecal levels of CHI3L1 also have limited ability to discriminate between patients who do or don't have evidence of lesions (AUC=0.52, p=0.74), and do not reliably identify those symptomatic primary care patients who subsequently present with early-stage disease (polyps and adenomas) or CRC. Moreover, the discriminatory power of FIT was not increased by incorporating the CHI3L1 results in this setting,[21] possibly reflecting the observation CHI3L1 is also considered a biomarker of IBD.[50]

The negative predictive value of FC is reported as between 97.2-98.7 for CRC, and 93.2-97.2 for high risk adenomas.[30,48,51,52] Given that NICE guidelines accept a 3% risk in missing CRC in setting symptom criteria for referral,[53] levels of these biomarkers below an established threshold may help rule out younger patients who more commonly present with non-specific lower GI symptoms,[54] which is not dissimilar to the growing awareness that a negative FIT may likewise rule out colorectal cancer in 99.5% of symptomatic patients under 50 years of age.[40]

Gut microbiota

The gut microbiota is increasingly recognised to have a role in influencing the biology of CRC, an association that has been demonstrated using a number of different approaches. The simplest is screening faecal samples for molecular evidence of known bacterial virulence factors considered to have a role in initiating CRC. For example, enterotoxigenic *Bacteroides fragilis* (ETBF) express a toxin[55] that is associated with promotion of carcinogenesis in mice[56] and humans.[57] However, taking such a targeted approach is not without limitations. The molecular tools to detect the ETBF toxin in patient-derived stool samples potentially lack the specificity and sensitivity needed of a reliable biomarker in a diagnostic setting.[58,59] Bacterial species other than ETBF are also likely potential drivers of CRC possibly through similar pathways.[60]

A broader approach is recognising that environmental changes in early stage disease allows some bacterial species to out-compete others.[60] While this suggests that it may be possible to predict colon tumorigenesis on the basis of a CRC-associated molecular microbial signature, evidence that environmental metabolites drive CRC-associated dysbiosis[61] suggests that dysbiosis may be a consequence rather than a cause of CRC. This would explain shifts in the relative abundance of different members of the gut microbiota seen through progression to adenoma,[62,63] carcinoma[64,65] and CRC.[66] As such the development of premalignant and malignant lesions may create a distinctive microbiotic pattern that may help with the diagnosis.

Metabolites

CRC is increasingly considered a metabolic rather than a genetic diseased,[67] evidenced by a 2009 study that showed metabolic profiling of biopsied CRC tumours and matched normal tissue could discriminate normal from malignant samples, as well as colon from rectal cancers.[68] Since then, identifying metabolic biomarkers that can identify patients with suspected CRC has become an increasingly active area of investigation. Broadly, this approach uses different analytical platforms to search for metabolic signatures that reflect bacterial dysbiosis and/or altered metabolic pathways that occur in CRC. Metabolic biomarkers are usually measured in in the liquid phase of blood, urine and/or faecal samples. There is however a subset of metabolites able to move from the liquid phase into the gas phase that are detectable as volatile organic compounds, best illustrated by the sensitivity of canine scent detection in detecting CRC-related VOCs in patient breath and stool samples.[69] CRC-associated VOCs have now been detected in the headspace of exhaled breath,[70–72] urine,[31,73,74] blood,[75] and faecal samples.[76–79]

Presently the metabolomic (metabolic and VOC) profiles generated by various investigations differ. This may reflect the method of detection and/or the analytic platforms used to identify metabolic biomarkers for CRC[80] or it may also reflect population-based diversity including interindividual differences in diet and/or gut microbiota. Other variables to consider are the impact that factors such as colonic transit time,[81] smoking,[82] age and gender[83] has on an individual's metabolic profile. Collectively these variables may underlie the heterogeneous results across studies to date.[80] Additionally, a comprehensive systematic review and meta-analysis of the VOC signature of CRC raises questions regarding the sensitivity of this approach.[84] Despite this, studies showing that metabolites in serum[85,86] are able to discriminate between patients with adenoma and disease-free controls suggesting this approach could be considered for use in primary care. Likewise, evidence that VOC profiles can also detect advanced adenomas[87] as well as improving CRC detection in FIT-negative patients[31] warrants further investigation.

Among the range of metabolites identified across the different platforms and samples types, two stand out as having potential in the context of identifying young people at increased risk of developing EOCRC. These are D-glucose and N1,N12-diacetylspermine, identified as upregulated in a systematic review and meta-analysis of urinary metabolites in patients with CRC and advanced adenomas

versus healthy controls.[84] Glucose is linked to consumption of a Western style diet that is shown to significantly increase the risk of young onset advanced adenomas, particularly in the colon and rectum[88] while increased levels of N1,N12-diacetylspermine may indicate increased polyamine synthesis by gut bacteria[89] and/or consumption of a polyamine-rich diet.[90]

N1,N12-diacetylspermine is an acetylated form of spermine, a polyamine formed by the intracellular decarboxylation of amino acids. Polyamines are associated with a wide range of intracellular physiologic functions but excess levels can derange cellular metabolism,[91] resulting in dysregulation of polyamine metabolism reflected by increased production of-reactive oxygen species (ROS) that is in turn linked with carcinogenesis.[92] The production of N1,N12-diacetylspermine is driven by the enzyme spermine -N1-acetyltransferase (SSAT) and is associated with increased oxidative damage.[91] Intracellular levels of spermine oxidase (SMO) are also increased during cellular stress.[93] Intriguingly SMO expression, which is associated with measurable oxidative stress and DNA damage, is shown to be increased in colonic epithelial cells following exposure to the *B. fragilis* toxin[94] and may be a mechanism linking long term carriage of enterotoxigenic strains of *B. fragilis* with increased risk of colon carcinogenesis.[57] N1,N12- diacetylspermine measured in urine can discriminate between benign and malignant colon cancer whereas only 2 of 15 adenoma cases were positive for this biomarker.[95] This may reflect the sensitivity of the ELISA used in this study and/or the failure of the authors to define the adenomas as high- or low-risk.[44]

D-glucose was the second urinary metabolite found to be significantly different between CRC patients or patients with advanced adenoma and healthy controls. [84] Despite evidence that the post-prandial glucose response is highly variable (likely in part, reflecting an individual's unique gut microbiota),[96] unrelated studies report higher fasting blood glucose levels and glycosylated haemoglobin (HbA1c) levels are associated with higher risk of colon cancer in men [97] and colorectal adenoma risk in the non-diabetic 40–50-year-olds[98] respectively. The idea that chronic dysglycaemia may increase risk of colon carcinogenesis in young patients is further strengthened by epidemiological evidence of an association between long-term consumption of sugar-sweetened beverages (SSBs) and increased incidence of proximal CRC in women, [99] including those under 50 years of age. [100] Increased risk might be explained by sugar intake exceeding the digestive capacity of the small intestine, leading to rapid sugar fermentation in the proximal colon[101,102] at the expense of butyrate[103] and mucus production [104] that respectively help maintain normal colonocyte and intestinal barrier function. As neoplasia develops, colonocytes rely on glucose processing via glycolysis to support more rapid growth.[105] The dimeric M2 form of the pyruvate enzyme (M2-PK) plays an integral role in this increased metabolic activity but the failure to identify precancerous bowel lesions or CRC in a subset of symptomatic patients[44] suggests glucose levels may have greater utility than M2-PK levels as a biomarker in young patients.

Implications for screening

Lastly, it is worth making mention of the potential benefit of widespread population-

based screening, particularly as the above-mentioned methods are refined they may be able to be applied to asymptomatic 'average' risk individuals. Traditionally screening in most countries around the world does not include patients under the age of 50.[106] This however is changing, with major American organisations now recommending cancer screening begins at the age of 45 years, something which is now becoming widespread in certain countries including USA.[107] These programmes involve screening with FIT testing and follow up colonoscopy for positive tests and have shown to improve outcomes and be cost effective.[108] While this approach will help detection of a large proportion of EOCRC diagnosed between 45-49 years it will still miss all cases in those aged under 45 years. As risk of CRC in people younger than 50 years is still most strongly associated with increasing age, screening with FIT remains effective down the age of 45 but evidence of its efficacy at younger ages declines, due to reducing incidence necessitating larger and larger numbers of screening tests to be done per cancer found.[107] As technology around diagnosis with biomarkers are improved, we will be able to improve the accuracy of our screening methods and therefore reduce the numbers of those requiring investigation to only those at highest risk of having underlying malignancy. There is already some promise with research showing that combining the measurement of faecal haemoglobin with levels of certain faecal protein biomarkers (including calprotectin and serpinF2) improves overall sensitivity, with further research underway to establish its efficacy in screening.[109] It is foreseeable that as these technologies evolve screening will thus become effective from younger ages, allowing early diagnosis before symptoms have arisen, improving outcomes in these patients.

Conclusion

The prompt diagnosis of colorectal cancer in young patients presents health systems with a unique challenge. The symptoms of colorectal cancer are varied, non-specific, often initially intermittent and extremely common, requiring further triaging of the large numbers presenting to primary care to allow health systems to focus limited resources such as colonoscopy on patients more likely to have underlying malignancy. Biomarkers are likely to play an increasing role in this process improving workup of symptomatic patients and may evolve to a stage where they can be used effectively in population screening of asymptomatic individuals.

Conflict of Interest Statement:

The authors have no conflict of interest to declare.

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