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Assessing the value of matrix metalloproteinase 9 (MMP9) in improving the appropriateness of referrals for colorectal cancer

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Background: A blood test may be an effective means of improving the appropriateness of referrals for symptomatic patients referred to specialist colorectal clinics. We evaluated the accuracy of a serum matrix metalloproteinase (MMP9) test in indicating colorectal cancer or its precursor conditions in a symptomatic population.

Methods: Patients aged over 18, referred urgently or routinely to secondary care following primary care presentation with colorectal symptoms completed a questionnaire and provided a blood sample for serum MMP9 estimation. Univariate analysis and logistic regression modelling investigated the association between presenting symptoms, MMP9 measurements and the diagnostic outcome of patient investigations, in order to derive the combination of factors which best predicted a high risk of malignancy.

Results: Data were analysed for 1002 patients. Forty-seven cases of neoplasia were identified. Age, male gender, absence of anal pain, diabetes, blood in stools, urgent referral, previous bowel polyps and previous bowel cancer were significantly associated with neoplasia. Matrix metalloproteinase 9 measurements were not found to be associated with significant colorectal pathology.

Conclusion: This study, despite robust sampling protocols, showed no clear association between MMP9 and colorectal neoplasia. Matrix metalloproteinase 9 therefore appears to have little value as a tool to aid referral decisions in the symptomatic population.

Colorectal cancer (CRC) is the third most common cancer in the United Kingdom, accounting for around 13% of new cancer cases each year (Cancer Research UK, 2010a). The annual total cost of diagnosing and treating CRC in England is estimated to be ~£1.1 billion, with the cost of diagnosis forming the largest component of this expenditure (University of York Health Economics Consortium and University of Sheffield, 2007). In 2009, there were over 40 000 new cases of CRC diagnosed in the United Kingdom, and over 16 000 deaths (Cancer Research UK, 2010b). Survival is closely associated with stage at diagnosis; the current 5-year relative survival rate for patients diagnosed with Dukes stage A is over 90%, compared with <7% for those diagnosed with late-stage CRC (National Cancer Intelligence Unit, 2009). A national programme of biennial CRC screening to detect CRC in

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asymptomatic individuals aged between 60 and 69 (extended in 2012 to include those aged up to 75) is now in operation (Atkin, 2006; Department of Health, 2007).

Despite the introduction of screening, the majority of CRCs continue to be diagnosed clinically because most cancers occur outside of the screening age range (Cancer Research UK, 2010a); around half of those invited to participate in screening currently decline (Logan *et al*, 2012) and screening cannot detect all cancers. Thus, many CRC patients are diagnosed following symptomatic presentation in primary care. Guidelines have been introduced to facilitate appropriate referral between primary and secondary care for patients with suspected cancer, with referral type (urgent or routine) determined by the symptoms reported and the clinical suspicion of malignancy (National Institute for Health and Clinical Excellence, 2005). All suspected cancer patients should be seen within 2 weeks of referral ('urgent referrals'); however, the application of guidelines may be problematic. The non-specific nature of many symptoms associated with CRC (Hamilton, 2009), and their variable interpretation by general practitioners mean that many patients referred as urgent do not meet referral guideline criteria; the majority of patients with bowel symptoms who meet criteria for urgent referral will not be found to have CRC, and some patients who are subsequently found to have cancer will have been referred routinely rather than urgently (Flashman *et al*, 2004).

There is some evidence that matrix metalloproteinases (MMPs), enzymes associated with tissue remodelling in both normal and pathological processes, could have potential application as tumour biomarkers (Collins *et al*, 2001). Overexpression of MMPs, and in particular MMP9, has been found in patients with colorectal adenomas (Parsons *et al*, 1998) and CRCs (Garbett *et al*, 1999; Heslin *et al*, 2001). Serum MMP9 measurements have been shown to be significantly higher in CRC patients compared with disease-free controls, and higher in late-stage colorectal neoplasia compared with early stage disease (Baker and Leaper, 2003; Dragutinovic *et al*, 2011). If serum MMP9 concentration could be used to identify patients with significant colorectal pathology and malignancy, this would allow diagnostic investigations to be focused on the relevant patient population, and would reduce pressure on NHS resources within secondary care. It could also reduce the morbidity and mortality associated with subjecting a normal population to invasive tests such as colonoscopy and sigmoidoscopy.

We undertook a pilot study of 300 urgent referrals to a specialist colorectal clinic that showed that a predictive model incorporating serum MMP9 levels indicated colorectal neoplasia in 77.3% of cases (sensitivity 77.9%, specificity 77.1%) (Hurst *et al*, 2007). These results suggest that MMP9 may be an effective screening test, but the study was undertaken in a selected population with an unusually high prevalence of neoplasia and as such had limited generalisability. As the performance of a diagnostic test can vary according to a condition's severity and clinical presentation (Goehring *et al*, 2004), research into the association between MMP9 and colorectal neoplasia should be undertaken in a setting that provides a more representative spectrum of disease. This paper reports the findings of a large study that aimed to investigate whether a blood test that measured serum MMP9 levels could improve the appropriateness of referrals for symptomatic patients referred to a specialist colorectal clinic via either the urgent or routine referral pathways, and to determine the combination of factors (e.g., MMP9 and symptoms) that best predict whether a patient has CRC or its precursor conditions.

MATERIALS AND METHODS

Patient recruitment and data collection. The design of this study is reported in full elsewhere (Ryan *et al*, 2006). In brief, eligible patients were those aged 18 or over who were a new urgent or routine referral to the colorectal clinic at University Hospitals Birmingham NHS Foundation Trust within the study period. Eligible patients were identified via a search of Trust information systems, and those who met the inclusion criteria were sent an information leaflet and covering letter describing the research and a symptom questionnaire $\sim$2 weeks before their scheduled clinic appointment. The questionnaire collected patient sociodemographic information; asked patients about bowel symptoms (derived from symptoms cited in the National Institute for Health and Clinical Excellence guidelines for referral of suspected CRC; NICE, 2005) and their duration; any injuries or other chronic illnesses experienced in the 3 months before questionnaire completion (as these can increase the levels of MMP9 in the blood), and collected data on personal or family history of CRC.

Invited patients who wished to take part in the study were asked to bring their completed questionnaire to their clinic appointment, and on arrival, those who indicated their desire to participate in the research were asked to see a research nurse prior to their appointment with the consultant. The research nurse answered any questions about the study and took consent, excluding any patients who were deemed unable to provide informed consent. Once a patient had consented to participation, the research nurse checked the questionnaire, providing non-directive assistance for completion of any unanswered questions and obtained a blood sample for MMP9 estimation. The results of each patient's clinic examination and any subsequent investigations were collected from hospital records using a standard proforma at least 2 months after the clinic appointment in an attempt to ensure that all relevant investigations had been completed and diagnoses documented. Patients without a definitive diagnosis within this 2-month period were followed up by study staff for as long as possible; all patients were tracked via the NHS Central Register (until April 2012) to maximise the ascertainment of malignancy in participating patients.

Storage and handling of blood samples. Blood samples were stored and handled in accordance with the study protocol. One 5-ml blood sample was collected from each participating patient for MMP9 determination. Each sample was stored in a red-topped Vacuette tube (Greiner Bio-One Ltd., Gloucester, UK) with no additive, labelled, put into a plastic bag and kept on ice in an insulated polystyrene container until the end of the colorectal clinic. The container was then transported by hand to the laboratory at the Institute for Cancer Studies at the University of Birmingham and handed over to named personnel. Once received at the laboratory, each sample was immediately centrifuged and the serum fraction separated and stored at $-80\,^{\circ}C$. Circulating MMP9 levels were determined on duplicate aliquots of each sample by ELISA assay kit (R&D Systems, Abingdon, UK). No other haematology data were collected. The technician undertaking the ELISA assay was blinded to the symptoms and diagnosis of the patient to minimise bias, and all samples were analysed in the same laboratory to ensure that measurement and reporting were standardised.

Justification of sample size. The sample size calculation was based on the confidence interval and precision with which the sensitivity of MMP9 could be estimated. A sensitivity of 99% was obtained in study pilot work, and to estimate this sensitivity to within 2.5% precision (95% confidence), blood samples from 60 people with colorectal neoplasia were required. Conservatively assuming a 6% prevalence of colorectal neoplasia (Thiis-Evensen *et al*, 1999; Lieberman *et al*, 2000), a sample of 1000 patients would allow a specificity of 63% to be estimated, with 4.0% precision (95% confidence). Of these 1000 patients, we would expect 940 not to have colorectal neoplasia.

Data analysis. Based on their 'final outcome' diagnosis, patients were classified into two pre-specified groups: a high-risk 'neoplasia' group consisting of patients diagnosed with adenocarcinoma of the colon or rectum, unspecified cancer of the colon/rectum, or high-risk polyps, and a low-risk 'non-neoplasia' group that consisted of patients diagnosed with low-risk polyps, cancer at sites other than colon/rectum, benign inflammatory disease of the colon/rectum, other benign disease of the colon/rectum, other benign conditions, or no abnormality.

All continuous variables (MMP9 measurements, age and body mass index (BMI) – derived from patient-reported height and weight) are summarised by medians and interquartile ranges (IQR) and univariate comparisons of these variables across the neoplasia/non-neoplasia groups used the non-parametric Mann–Whitney *U*-test, as normality testing indicated that the data for these continuous variables were not normally distributed ($P \leqslant 0.01$). Univariate odds ratios (OR) were calculated for each categorical sociodemographic, symptom-related and clinical variable to test the association between variable subgroups (e.g., presence or absence of a specific symptom) and colorectal neoplasia. In addition to analyses undertaken using MMP9 measurements as a continuous variable, an extra variable was created which categorised MMP9 by quintile.

Finally, binary logistic regression modelling was used to derive the combination of factors that best predicted a high risk of malignancy (e.g., invasive disease or high-risk polyps). All variables for which data had been collected were considered in the regression analyses. The model was derived using a backward elimination stepwise method whereby at each model iteration, variables with a statistical significance of >0.2 were removed, and the model re-run containing the remaining variables. Two regression models were generated: one in which MMP9 measurements were 'forced' to remain in the model, and one in which MMP9 measurements were removed from the model once the statistical significance of the MMP9 variable met the >0.2 criteria for variable removal. All statistical analyses were undertaken using Stata (version 11, StataCorp LP, College Station, TX, USA).

RESULTS

Response rates. Searches of appointment lists identified 2204 patients scheduled to attend the colorectal clinic who met the study inclusion criteria; all of whom were sent a study information leaflet and questionnaire prior to their appointment date. The flow of participants through the study is outlined in Figure 1. Of the 2204 patients invited to participate in the study, 185 (8.4%) cancelled or did not attend their clinic appointment. Of the remaining 2019 eligible patients, 987 (44.8%) either did not wish to see a research nurse about the study, or were unable to do so because their clinic appointment took place before a consent interview could be undertaken. Eight patients (0.4%) were excluded from participation at the consent interview stage, leaving 1024 patients who gave consent to participate in the study. Of these, 20 patients did not give a blood sample, and a further two patients gave a blood sample which was unsuitable for analysis. The final number of study participants was 1002 (45.5%).

A comparison of proportions test was undertaken to compare the characteristics (gender, age and referral type) of those consenting to participate in the study with those who did not participate. There was no significant difference between participants and non-participants on the basis of patient gender ($\chi^2 = 0.98$; df = 1; $P = 0.322$). Patients in both the youngest and oldest age groups (18–39 years and 70+ years) participated in lower than expected numbers than those aged between 40 and 69 ($\chi^2 = 28.25$; df = 3; $P \leq 0.0001$), and patients who had been referred as urgent were significantly more likely to participate in the study than those who had been referred routinely ($\chi^2 = 5.89$; df = 1; $P = 0.013$).

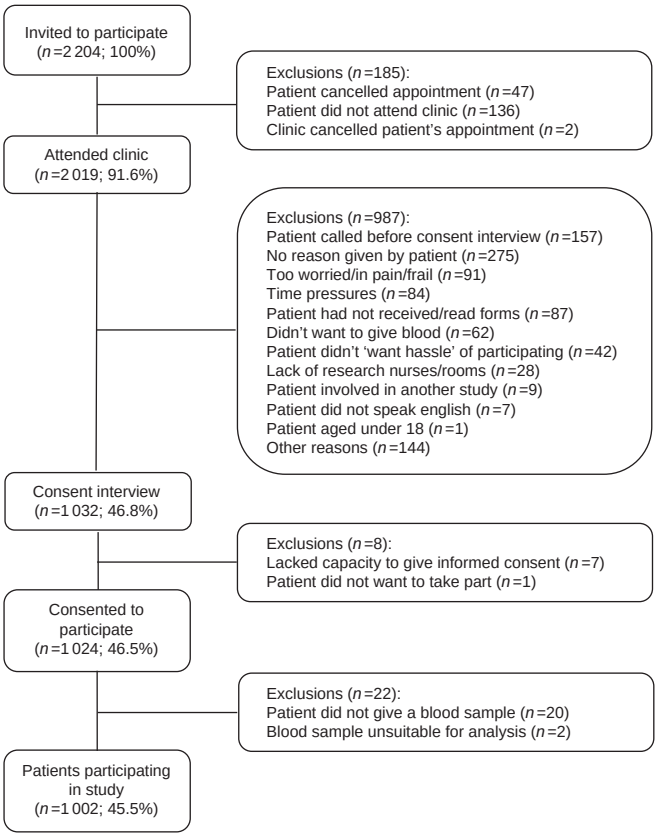

Figure 1. Patient recruitment flowchart.

Sample demographic and clinical characteristics. The median age of participants was 57 years (IQR: 43–68). In all, 48.7% of patients were males ($n = 488$), and the majority of participants were in the 'white' ethnic group ($n = 891$; 88.9%). Around a quarter of participants had been referred urgently ($n = 249$; 24.9%), (Table 1).

The most commonly reported colorectal symptoms were anal pain (54.5%; median duration of symptoms: 14+ weeks; IQR: 8–14+ weeks), tiredness (50.9%; median duration: 14+ weeks; IQR: 8–14+ weeks), change in bowel habit to looser stools (50.4%; median duration: 12 weeks; IQR: 6–14+ weeks), rectal bleeding (48.3%; median duration: 12 weeks; IQR: 3–14+ weeks), and the need to open bowels more frequently (44.9%; median duration: 12 weeks; IQR: 6–14+ weeks). Symptoms reported by the fewest patients were weight loss ($n = 142$; 14.2%) and the need to open bowels less frequently ($n = 231$; 23.1%), (Table 2).

A number of patients reported co-morbid conditions within the 3 months prior to their participation in the study. In all, 32.0% ($n = 321$) had arthritis and 29.2% (293) had high blood pressure. In all, 92 patients (9.2%) reported a previous history of bowel polyps; 17 (1.7%) had had a previous diagnosis of bowel cancer and 232 participants (23.2%) reported a family history of bowel cancer.

Outcome of clinical investigations. Overall, 47 cases of neoplasia (30 colorectal adenocarcinomas, one other cancer of the colon/rectum and 16 high-risk polyps) were identified as a result of investigations undertaken at or subsequent to, the clinic appointment, representing 4.7% of the study population. Other findings included 52 low-risk polyps (5.2%), 22 cancers at sites other than

Table 1. Patient demographic characteristics

Demographic characteristics	Number (%); interquartile range (IQR)
Total number of patients	1002 (100.0)
Age	
Median = 57 years	Range = 19–92 years (IQR: 43–68)
Gender	
Male	488 (48.7)
Female	514 (51.3)
Ethnic group[a]	
White	891 (88.9)
Non-white	108 (10.8)
Unknown	3 (0.3)
BMI[b]	
Median (n = 774; BMI: 26.0)	Range = 10.8–53.3 (IQR: 23.4–29.4)
Still menstruating (females only)	
Yes	160 (31.1)
No	353 (68.7)
Unknown	1 (0.2)
Mode of referral[c]	
Urgent	249 (24.9)
Routine	682 (68.1)
Unknown	71 (7.0)

[a]Ethnic group derived from patient-reported ethnicity.
[b]BMI = body mass index, derived from patient-reported height and weight.
[c]Mode of referral (urgent or routine) was derived from patient medical records.

the colon/rectum (2.2%) and 45 patients with inflammatory conditions of the colon/rectum (e.g., diverticulitis or inflammatory bowel disease (IBD); 4.5%). No abnormality was found after investigation for 225 participants (22.5%).

Serum MMP9 results. The median serum MMP9 concentration was 380.3 ng ml^{-1} (range: 99.9–1112.8; IQR: 236.4–578.8) in the neoplasia group, and 357.5 ng ml^{-1} (range: 52.5–1963.2; IQR: 221.3–560.1) in the non-neoplasia group, although this difference was not statistically significant (Mann–Whitney U: 21637; $P = 0.678$) (Table 3). In the univariate analyses undertaken to compare median MMP9 values between groups for each potential predictive factor (not tabulated), ethnicity was statistically significant (median MMP9 for the 'white' ethnic group = 369.2 *vs* 306.9 for the 'non-white' group; $P \leqslant 0.001$), although it is likely that this finding is an artefact of the large difference in the number of individuals in each group (891 white *vs* 108 non-white patients). Median MMP9 concentrations were also significantly higher in patients reporting IBD than in those without it ($P = 0.004$). No other factors were statistically associated with median MMP9 concentrations.

Factors associated with the presence of colorectal neoplasia. Univariate analysis demonstrated that increasing age, male gender, the absence of anal pain, diabetes and blood in stools were significantly associated with the presence of neoplasia (Table 3). Other significant univariate predictors of neoplasia included being referred urgently (OR: 2.75; 95% CI: 1.46–5.16), having had previous bowel polyps (OR: 4.26; 95% CI: 2.16–8.40) and having had previous bowel cancer (OR: 12.66; 95% CI: 4.48–35.71). Although we may expect that higher MMP9 concentrations would have a stronger association with neoplasia than lower concentrations, when categorised into quintiles, only MMP9 quintile two demonstrated a statistically significant association (OR: 2.92; 95% CI: 1.03–8.27), with a P-value of 0.044.

Entering all potential predictive sociodemographic and symptom-related factors into a backwards elimination stepwise logistic regression model, along with potential confounding factors such as recent injuries and co-morbidities (which could affect observed MMP9 concentrations) resulted in a final list of factors predictive of neoplasia in the study population (Table 4). The first model, in which MMP9 was forcibly retained, shows that gender, age, referral type, absence of tiredness, blood in stools, previous bowel polyps, previous bowel cancer and having an MMP9 measurement in quintile two were all statistically significant independent predictors of neoplasia. The pseudo R^2 (i.e., the proportion of variance between factors explained by the model) was 0.181.

The second logistic regression model, in which MMP9 quintile was allowed to 'drop out' of the model when its statistical significance became >0.2, showed the same final list of independent predictive factors for neoplasia as the first. The P-values and OR for all factors retained in the model remained similar to their values in the first model, as did the pseudo R^2 for the model without MMP9 ($R^2 = 0.161$).

DISCUSSION

This study investigated the association between serum MMP9 concentrations and colorectal neoplasia in a group of symptomatic patients referred from primary care to a secondary care colorectal clinic. In contrast to other work that has demonstrated a significant association between elevated serum MMP9 concentration and neoplasia in a group of urgently referred patients (Hurst *et al*, 2007), and a study in which serum MMP9 was found to be associated with neoplasia in a cohort of patients recruited from primary care on the basis of self-reported colorectal symptoms (Wilson *et al*, 2012), the present study did not find an association between serum MMP9 levels and neoplasia. This was despite the inclusion of MMP9 concentration as both a continuous and a categorical variable to enable the potential predictive value of MMP9 to be modelled more accurately.

The lack of an association observed here corroborates the findings of a recently published study which found that blood-based biomarkers did not appear to be a viable alternative to existing screening and diagnostic tests used in detecting colorectal neoplasia (Tao *et al*, 2012). Similarly, Mroczko *et al* (2010) showed only a weak association between MMP9 and CRC in a study of 75 patients. Studies that have demonstrated an association between MMP9 and CRC have typically included healthy control groups (Biasi *et al*, 2012). Our study measured MMP9 levels in a group of patients in which MMP9 measurements would be useful as a screening test to detect significant colorectal pathology, rather than in patients where the diagnosis was already known.

Previous research has drawn attention to the need for robust sampling, handling and processing protocols when undertaking research using blood biomarkers (Jung, 2008). Strengths of this study include duplicate determination of serum MMP9 levels and dual data entry to ensure reliability of results. All sample processing was undertaken in the same laboratory and by the same technician. The use of serum MMP9 estimation has been criticised for showing increased MMP9 levels compared with plasma estimation (Jung, 2008), and the use of citrate plasma has been cited as the gold standard for estimating circulating MMP9 (Makowski and Ramsby, 2003). The use of clot activators in blood-sampling tubes for serum estimation has been shown to result in a 15-fold increase in serum MMP9 levels compared with that of

Table 2. Patient clinical characteristics

Characteristic ($n=1002$)	Yes (%)	No (%)	Missing (%)	Median duration (IQR)
Patient-reported symptoms, duration and median duration in weeks[a]				
Abdominal pain	433 (43.2)	563 (56.2)	6 (0.6)	12 (4–14+)
Weight loss	142 (14.2)	846 (84.4)	14 (1.4)	14 (6–14+)
Tiredness	510 (50.9)	483 (48.2)	9 (0.9)	14 (8–14+)
Blood in stools	341 (34.0)	642 (64.1)	19 (1.9)	8 (2.5–14+)
Rectal bleeding	484 (48.3)	502 (50.1)	16 (1.6)	12 (3–14+)
Bowel habit – harder stools	340 (33.9)	639 (63.8)	23 (2.3)	13 (6–14+)
Bowel habit – looser stools	505 (50.4)	478 (47.7)	19 (1.9)	12 (6–14+)
Open bowels less frequently	231 (23.1)	745 (74.4)	26 (2.6)	12 (5–14+)
Open bowels more frequently	450 (44.9)	539 (53.8)	13 (1.3)	12 (6–14+)
Anal pain	546 (54.5)	447 (44.6)	9 (0.9)	14 (8–14+)
Medical conditions (self-reported)				
Angina	119 (11.9)	871 (87.0)	12 (1.2)	—
High blood pressure	293 (29.2)	695 (69.4)	14 (1.4)	—
Diabetes	73 (7.3)	914 (91.2)	15 (1.5)	—
Asthma	165 (16.5)	826 (82.4)	11 (1.1)	—
Inflammatory bowel disease	47 (4.7)	938 (93.6)	17 (1.7)	—
Arthritis	321 (32.0)	659 (65.8)	22 (2.2)	—
Muscular problems	144 (14.4)	843 (84.1)	15 (1.5)	—
Epilepsy	20 (2.0)	968 (96.6)	14 (1.4)	—
Other medical conditions	379 (37.8)	585 (58.4)	38 (3.8)	—
Injuries <3 months previously	90 (9.0)	901 (89.9)	11 (1.1)	—
Previous bowel polyps	92 (9.2)	888 (88.6)	22 (2.2)	—
Previous bowel cancer	17 (1.7)	973 (97.1)	12 (1.2)	—
Family history of bowel cancer	232 (23.2)	745 (74.4)	25 (2.5)	—
Haematology results	**Number (%)**	**Median**	**Range (IQR)**	
MMP9 (crude) ng ml^{-1}	1002 (100.0)	357.5	52.5–1963.2 (221.3–560.1)	
Outcome of clinical investigations		**Number (%)**		
Adenocarcinoma of colon/rectum		30 (3.0)		
Cancer not specified of colon/rectum		1 (0.1)		
High-risk polyp		16 (1.6)		
Total 'neoplasia' group		47 (4.7)		
Cancer at site other than colon/rectum[b]		22 (2.2)		
Low-risk polyp		52 (5.2)		
Intraepithelial neoplasia at other site		2 (0.2)		
Inflammatory benign condition of colon/rectum		45 (4.5)		
Inflammatory benign condition at other site		12 (1.2)		
Other benign condition of colon/rectum		266 (26.5)		
Other benign condition at other site		331 (33.0)		
No abnormality		225 (22.5)		
Total 'non-neoplasia' group		955 (95.3)		

Abbreviations: MMP = matrix metalloproteinase; IQR = interquartile range.
[a]Symptom durations were coded in weeks; any duration longer than 13 weeks was recorded as 14+.
[b]Cancer of the prostate ($n=7$), breast ($n=2$), endometrium ($n=2$), lymphoma ($n=2$), pancreas ($n=1$), tongue ($n=1$), stomach ($n=1$), connective tissue ($n=1$), lung ($n=1$), skin ($n=1$), oesophagus ($n=1$), bladder ($n=1$) and disseminated cancer with unknown primary site ($n=1$).

citrate plasma (Jung *et al*, 2005). However, the blood-sampling tubes used in this study did not contain any clot activators. The time between phlebotomy and centrifugation is also associated with elevated serum MMP9 levels in samples left at room temperature before centrifugation (Gerlach *et al*, 2007). In our study, samples were kept on ice until delivery to the processing laboratory, and our data showed no correlation between time to centrifugation and serum MMP9 levels (Pearson correlation, $r=-0.010$; $P=0.801$).

The regression modelling undertaken in this study did not demonstrate that serum MMP9 levels have viable potential in predicting significant colorectal pathology. The model in which MMP9 quintile was forcibly retained as a predictive variable did not show an improvement in the predictive value of the other variables retained in the final iteration of the model over and above that demonstrated by these variables in the model where MMP9 was removed. Despite this, it is reassuring to note that one of the strongest predictive factors for patients in the neoplasia group was the mode of referral, with those referred through the urgent pathway by their general practitioner being nearly three times more likely to be diagnosed with colorectal malignancy than those referred routinely.

An accurate biomarker for colorectal neoplasia would have potential as an alternative or secondary screening method to the

Table 3. Factors considered predictive of neoplasia

Variable	Number	Neoplasia Median (IQR)	Non-neoplasia Median (IQR)	*U*	*P*-value
Age	1002	66 (54–76)	57 (43–68)	15127	**0.01**
BMI	774	26 (24–29)	26 (23–29)	21395	0.587
MMP9	1002	380 (236–579)	357 (221–560)	21637	0.678

Variable	Category	Number	*n* (Row %)	*n* (Row %)	OR (95% CI); *P*-value
Gender	Male	488	30 (6.1)	458 (93.9)	**1.91 (1.04–3.52); 0.034**
	Female	514	17 (3.3)	497 (96.7)	
Ethnicity	White	891	43 (4.8)	848 (95.2)	1.31 (0.46–3.70); 0.604
	Non-white	108	4 (3.7)	104 (96.3)	
Referral type	Urgent	249	20 (8.0)	229 (92.0)	**2.75 (1.46–5.16); 0.001**
	Routine	682	21 (3.0)	661 (97.0)	
Still menstruating	Yes	160	2 (1.3)	158 (98.7)	0.29 (0.06–1.26); 0.098
	No	353	15 (4.2)	338 (95.8)	
Abdominal pain	Yes	433	18 (4.2)	415 (95.8)	0.80 (0.44–1.46); 0.464
	No	563	29 (5.2)	534 (94.8)	
Weight loss	Yes	142	8 (5.6)	134 (94.4)	1.24 (0.56–2.70); 0.596
	No	846	39 (4.6)	807 (95.4)	
Tiredness	Yes	510	19 (3.7)	491 (96.3)	0.63 (0.35–1.14); 0.127
	No	483	28 (5.8)	455 (94.2)	
Blood in stools	Yes	341	24 (7.0)	317 (93.0)	**2.04 (1.13–3.66); 0.016**
	No	642	23 (3.6)	619 (96.4)	
Rectal bleeding	Yes	484	22 (4.5)	462 (95.5)	0.95 (0.52–1.72); 0.861
	No	502	24 (4.8)	478 (95.2)	
Bowel habit – harder stools	Yes	340	14 (4.1)	326 (95.9)	0.79 (0.42–1.49); 0.467
	No	639	33 (5.2)	606 (94.8)	
Bowel habit – looser stools	Yes	505	21 (4.2)	484 (95.8)	0.75 (0.42–1.36); 0.348
	No	478	26 (5.4)	452 (94.6)	
Open bowels less often	Yes	231	11 (4.8)	220 (95.2)	0.98 (0.49–1.97); 0.965
	No	745	36 (4.8)	709 (95.2)	
Open bowels more often	Yes	450	19 (4.2)	431 (95.8)	0.80 (0.44–1.46); 0.475
	No	539	28 (5.2)	511 (94.8)	
Anal pain	Yes	546	18 (3.3)	528 (96.7)	**0.49 (0.27–0.90); 0.018**
	No	447	29 (6.5)	418 (93.5)	
Angina	Yes	119	7 (5.9)	112 (94.1)	1.30 (0.57–2.97); 0.536
	No	871	40 (4.6)	831 (95.4)	
High blood pressure	Yes	293	19 (6.5)	274 (93.5)	1.65 (0.91–3.01); 0.101
	No	695	28 (4.0)	667 (96.0)	
Diabetes	Yes	73	8 (11.0)	65 (89.0)	**2.76 (1.24–6.13); 0.010**
	No	914	39 (4.3)	875 (95.7)	
Asthma	Yes	165	5 (3.0)	160 (97.0)	0.58 (0.23–1.50); 0.262
	No	826	42 (5.1)	784 (94.9)	
IBD	Yes	47	2 (4.3)	45 (95.7)	0.90 (0.21–3.85); 0.890
	No	938	44 (4.7)	894 (95.3)	
Arthritis	Yes	321	11 (3.4)	310 (96.6)	0.63 (0.32–1.26); 0.194
	No	659	35 (5.3)	624 (94.7)	
Muscular problems	Yes	144	5 (3.5)	139 (96.5)	0.69 (0.27–1.76); 0.434
	No	843	42 (5.0)	801 (95.0)	

Table 3. (Continued)

Variable	Category	Number	Neoplasia n (row %)	Non-neoplasia n (row %)	OR (95% CI); *P*-value
Epilepsy	Yes	20	0 (0.0)	20 (100.0)	OR could not be calculated
	No	968	47 (4.9)	921 (95.1)	
Other medical conditions	Yes	379	19 (5.0)	360 (95.0)	1.18 (0.64–2.18); 0.591
	No	585	25 (4.3)	560 (95.7)	
Injuries <3 months before	Yes	90	2 (2.2)	88 (97.8)	0.44 (0.11–1.86); 0.44
	No	901	44 (4.9)	857 (95.1)	
Previous bowel polyps	Yes	92	13 (14.1)	79 (85.9)	**4.26 (2.16–8.40); <0.001**
	No	888	33 (3.7)	855 (96.3)	
Previous bowel cancer	Yes	17	6 (35.3)	11 (64.7)	**12.66 (4.48–35.71); <0.001**
	No	973	40 (4.1)	933 (95.9)	
Family history bowel cancer	Yes	232	9 (3.9)	223 (96.1)	0.77 (0.37–1.63); 0.496
	No	745	37 (5.0)	708 (95.0)	
MMP9 quintile	1 (Lower)	200	5 (2.5)	195 (97.5)	Reference
	2	201	14 (7.0)	187 (93.0)	**2.92 (1.03–8.27); 0.044**
	3	200	7 (3.5)	193 (96.5)	1.42 (0.44–4.53); 0.560
	4	201	12 (6.0)	189 (94.0)	2.48 (0.86–7.16); 0.094
	5 (Higher)	200	9 (4.5)	191 (95.5)	1.84 (0.61–5.58); 0.283

Abbreviations: BMI = body mass index; MMP = matrix metalloproteinase; OR = odds ratio; IBD = inflammatory bowel disease; IQR = interquartile range. Statistically significant associations are highlighted in bold text.

Table 4. Binary logistic regression models for probability of neoplasia

Variable	*β*	*P*-value	Odds ratio (95% CI)
MMP9 included in model			
Intercept	−6.579	<0.001	
Gender	0.731	0.046	2.08 (1.01–4.26)
Age	0.030	0.012	1.03 (1.01–1.05)
Mode of referral	1.000	0.005	2.70 (1.35–5.42)
Tiredness	−0.827	0.025	0.44 (0.21–0.90)
Blood in stools	1.196	0.001	3.31 (1.65–6.62)
Anal pain	−0.501	0.161	0.61 (0.30–1.22)
Previous bowel polyps	1.050	0.027	2.86 (1.13–7.25)
Previous bowel cancer	2.009	0.007	7.45 (1.72–32.36)
MMP9 quintile			
2	1.234	0.046	3.44 (1.02–11.57)
3	0.433	0.521	1.54 (0.41–5.79)
4	1.216	0.057	3.37 (0.96–11.81)
5	0.659	0.329	1.91 (0.52–7.06)
Pseudo $R^2 = 0.181$			
MMP9 NOT included in model			
Intercept	−5.764	<0.001	
Gender	0.722	0.046	2.06 (1.01–4.19)
Age	0.028	0.016	1.03 (1.01–1.05)
Mode of referral	1.042	0.003	2.84 (1.43–5.63)
Tiredness	−0.769	0.033	0.46 (0.23–0.94)
Blood in stools	1.242	<0.001	3.46 (1.75–6.85)
Anal pain	−0.423	0.231	0.66 (0.33–1.31)
Previous bowel polyps	1.032	0.028	2.81 (1.12–7.05)
Previous bowel cancer	1.786	0.014	5.97 (1.43–24.91)
Pseudo $R^2 = 0.161$			

Abbreviations: CI = confidence interval; MMP = matrix metalloproteinase.

currently used screening modalities, which have relatively low patient acceptability, or as an aid to general practitioners in determining the most appropriate referral pathway for symptomatic patients. Although previous studies have found that serum MMP9 estimation may have some predictive value in indicating patients with colorectal neoplasia (Hurst *et al*, 2007; Wilson *et al*, 2012), these studies did not observe a large effect. The current study, which was undertaken on a large number of patients referred to secondary care for colorectal investigations, showed no clear association between MMP9 and colorectal neoplasia. Matrix metalloproteinase 9, therefore, appears to have little value as a tool to aid referral decisions in the symptomatic population.

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