Protein biomarkers in the rectal mucosa: a novel test for colorectal cancer?

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Introduction

Earlier detection of colorectal cancer is a clinical priority, with surgery for early stages (Dukes A and Dukes B) being related to 5 year disease free survival rates >85%. Currently very large numbers of patients are referred to hospital under the 2 week rule system, with a majority of patients being referred for colonoscopy. The 14, 31 and 62 day targets which each of these referrals triggers puts a major strain on services in colorectal clinics and endoscopy departments. However, with a pick-up rate for cancer of 1:20 or less, NHS trusts across the country are investing large amounts of clinical time and money in financially difficult times investigating large numbers of the worried well. With the potential for major complications including torrential haemorrhage and perforation, the vast number of negative colonoscopies is not without risk to patients. The aim of this study was to assess the correlation of protein biomarkers captured from the rectal mucosa in an outpatient setting with the presence or absence of colorectal cancer.

Methods

A case-control study of 20 patients with colorectal cancer and 20 controls was conducted. All patients had been referred to colorectal outpatients with potentially worrying symptoms as defined by a 2WW referral form for suspected colorectal cancer; presence or absence of cancer had been determined by colonoscopy or CT virtual colonoscopy.

A novel sampling device, OriCol™ (Fig 1), was employed to collect samples from the rectal mucosa for biomarker analysis. The device incorporates a nitrile membrane which, following insertion into the unprepared rectum via a standard proctoscope, is inflated to make contact with the rectal mucosa for a period of 10 seconds. Upon deflation and retraction of the membrane, a preservation buffer is added to preserve the sample prior to analysis. Sampling can be performed in an outpatient setting in under 2 minutes and has been shown to be safe and well tolerated in >2500 patients.

The levels of various antibodies, haemoglobin and carcinoembryonic antigen were analysed using conventional ELISA techniques. Statistical analyses of the trial results was performed using the Wilcoxon test for non-parametric comparisons with two-sided P values. The area under the receiver operating characteristic (ROC) curve for distinguishing between the two diagnostic groups, together with its confidence interval, was calculated for each biomarker. Logistic regression analyses were used to investigate the performance of different combinations of biomarkers.

This study was conducted with appropriate research ethics committee approval.

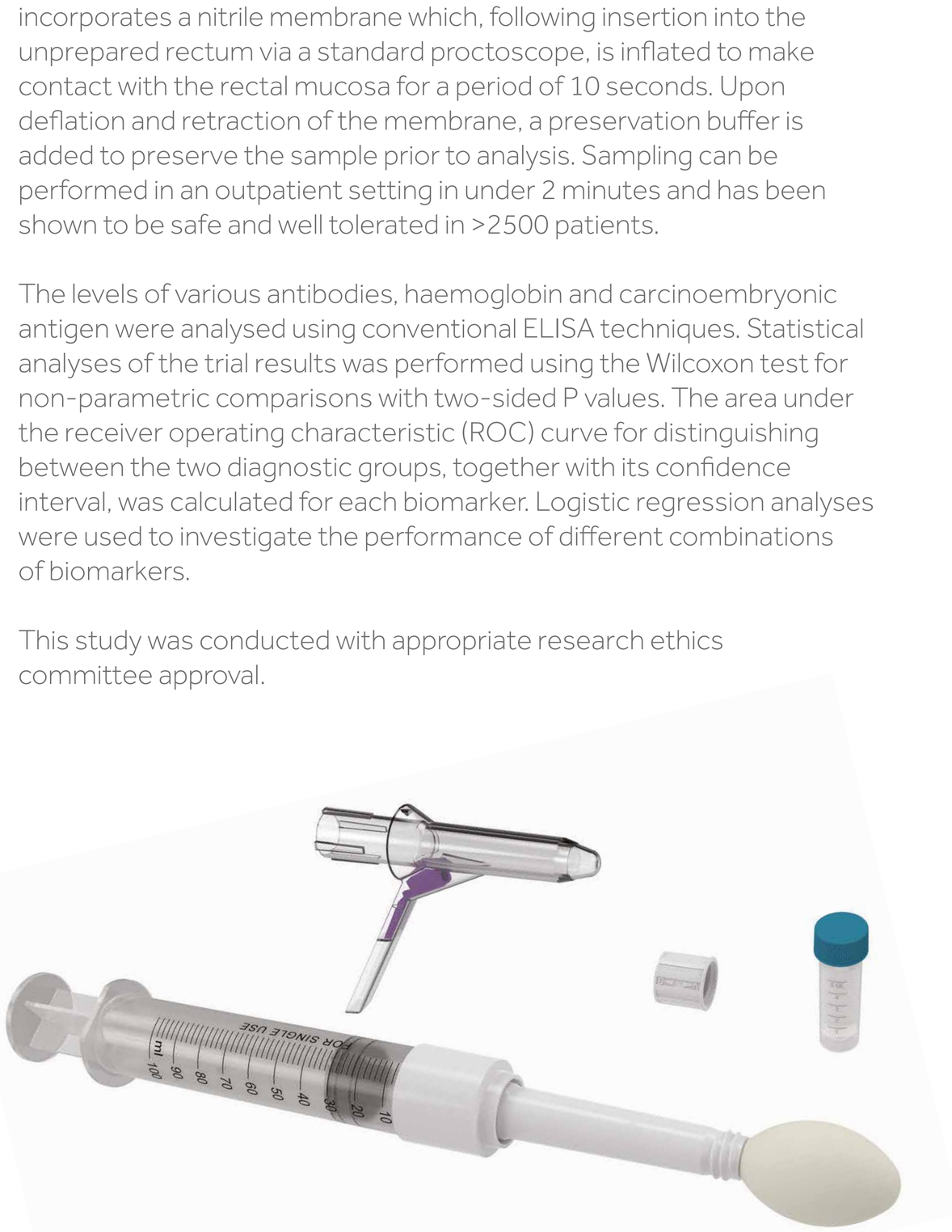

Figure 1. OriCol™ device

Results

Univariate analyses identified five candidate predictive biomarkers for colorectal cancer. The univariate analysis for the five candidate markers is shown in Fig 2. All combinations of two and three predictors were investigated using logistic regression. The best performing combination of biomarkers was Haemoglobin and IgA. The area under the ROC curve for this best linear combination was 0.86 (Fig 3). No triple predictors improved significantly on this pair. For a sensitivity comparable with that offered by colonoscopy (95%) the performance of the Haemoglobin and IgA biomarker combination offers a specificity of 68%, and if we accept a specificity of 30% then sensitivity of 100% is achieved in this pilot study.

Biomarker	P-value (two-sided)	Direction
Anti-Tn antigen (IgG)	0.0265	Positive
Anti-Lewis A (IgG)	0.0006	Positive
Anti-Lewis X (IgG)	0.0167	Positive
Haemoglobin	0.0030	Positive
Total IgA	0.0056	Negative

Figure 2. Univariate analysis of 5 candidate biomarkers

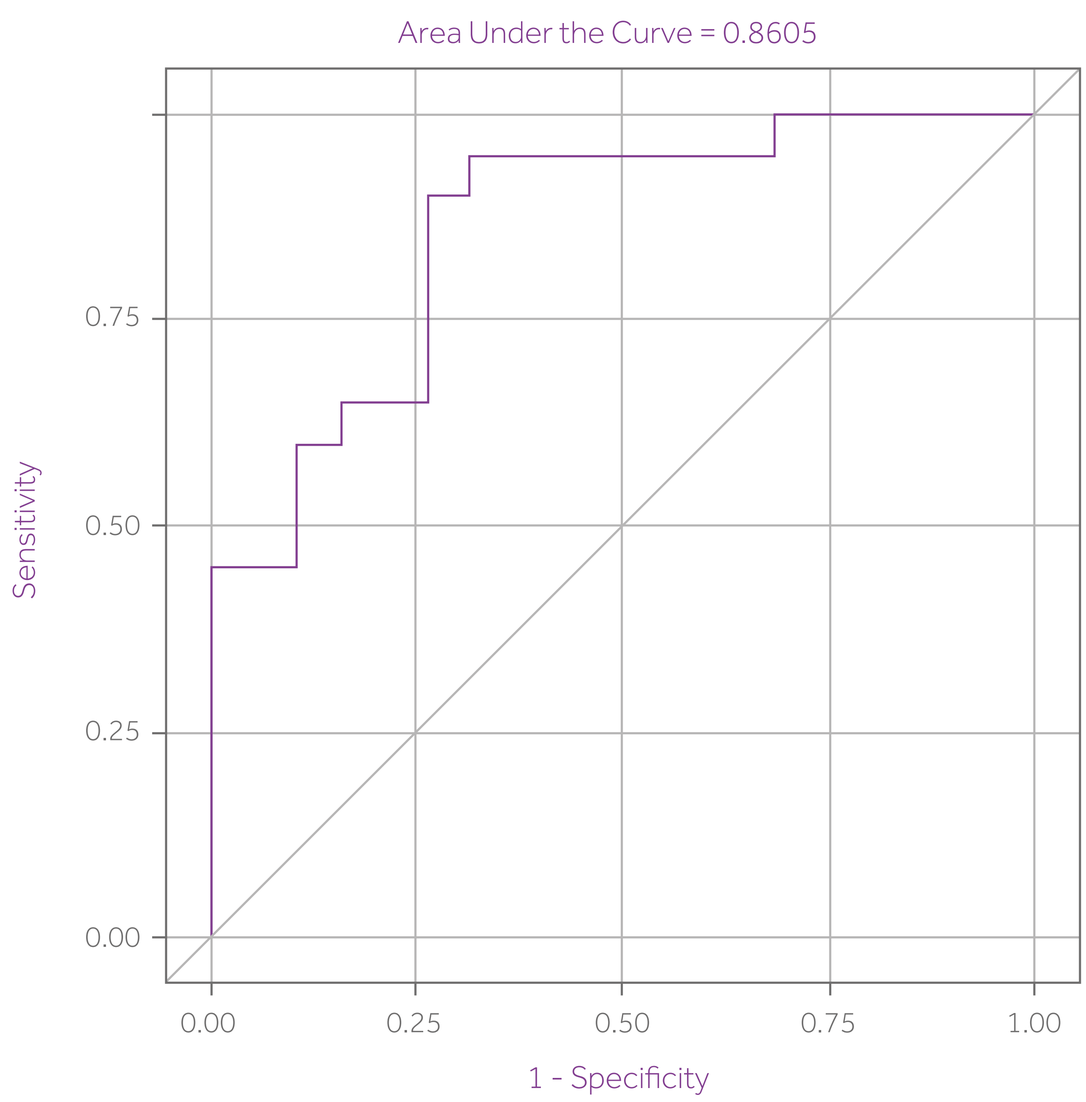

Figure 3. ROC curve for best linear combination of Total IgA and Haemoglobin

Conclusion

We suggest that ELISA analysis of protein biomarkers collected with the OriCol™ device offers a potentially useful and cost-effective pre-colonoscopy screening tool in patients referred under the 2 week rule criteria. Data from this pilot study suggests that a sensitivity of ≥95% can be achieved whilst massively reducing the number of patients requiring urgent colonoscopy to exclude a diagnosis of cancer. Patients not referred for colonoscopy following biomarker analysis using the OriCol™ device could safely be treated on a benign disease pathway which may or may not include endoluminal investigation, but on a timescale which is less demanding of clinical resources.