A methylated DNA blood test for colorectal cancer screening returns a lower false positivity rate than a faecal immunochemical test in people with benign bleeding conditions.

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EL Symonds, SK Pedersen, JM Osborne, G Gopalsamy, S Gaur, D Murray, A McEvoy, R Baker, SR Cole, LC LaPointe, GP Young

FLINDERS CENTRE for INNOVATION in CANCER

Colorectal cancer screening

- CRC screening with faecal immunochemical test (FIT) shown to downstage cancer[1], reduce mortality and decrease incidence by up to 22%[2]
  - FIT positivity is based on the presence of haemoglobin in faeces

  - Colorectal cancer **treatment**
  - Adenoma **prevention**
  - Inflammatory bowel disease
  - Haemorrhoids
  - Diverticular disease
  - Radiation proctitis

false positive

- In the Australian National Bowel Cancer Screening Program, 24% of positive results = non-neoplastic bleeding conditions

= 5382 patients

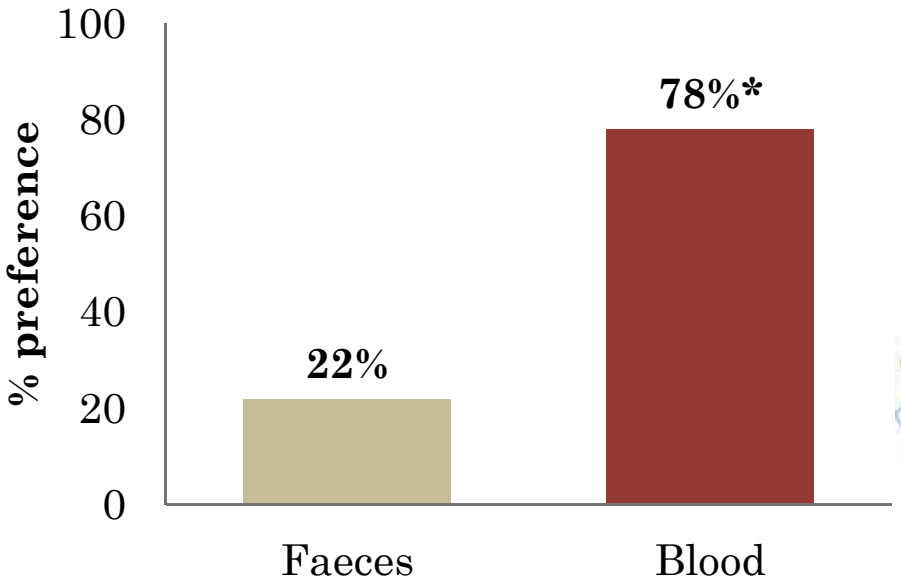
1. Cole *et al* Med J Aust 2013; 198: 327-30
2. Ventura *et al* Dig Liver Dis 2014; 46: 82-6

FLINDERS CENTRE for INNOVATION in CANCER

Colorectal cancer screening preference

- People with these diagnosed benign bleeding conditions can be advised to not use the FIT for bowel cancer screening
- Alternative screening options for these people:
  - Colonoscopy
  - Sigmoidoscopy
  - Blood test

Screening options in USA / UK

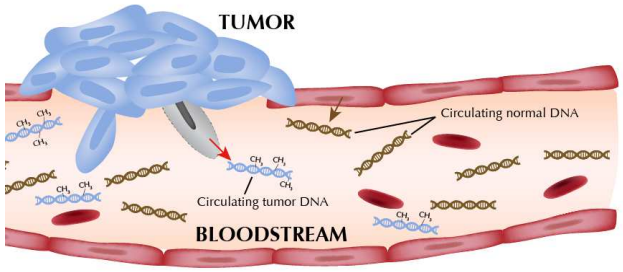


*Osborne *et al* Open J Prev Med 2012; 2:326-31

FLINDERS CENTRE for INNOVATION in CANCER

A blood test for bowel cancer screening

- We have developed an assay that detects the presence of tumour-derived methylated genes (BCAT1 and IKZF1) in plasma



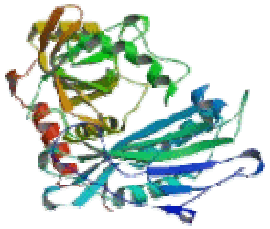
http://healthcare.utah.edu/huntsmancancerinstitute/research/labs/varley/#dna

BLOOD PLASMA OR SERUM SAMPLE

BCAT1

Branched-chain amino acid transaminase 1

- Indirectly inhibits cell growth and promotes apoptosis



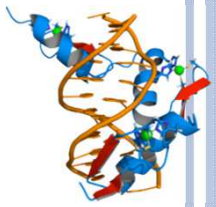
Disrupted expression:
↑ growth (in yeast)

IKZF1

Ikaros family zinc finger 1, DNA binding protein

- Tumour suppressor in colorectal cancer HCT116 cells

Disrupted expression:
↓ DNA repair genes
↑ cell-cycle progression genes
inhibition of apoptosis



For colorectal cancer:
Sensitivity = 65.4%
Specificity = 94.2%

This could be a more appropriate screening test for people with benign bleeding conditions

FLINDERS CENTRE for INNOVATION in CANCER

Aim of the study

To compare positivity rates between FIT and the blood test in people with benign gastrointestinal bleeding conditions.

FLINDERS CENTRE for INNOVATION in CANCER

N = 827 (median age 63y, 54% male)

Faecal immunochemical test → Overt bleeding?

(1wk before colonoscopy)

METHODS

- Patients were mailed the screening test to their home address (Eiken OC Sensor faecal immunochemical test)
- One faecal sample was collected and sent to lab via postage paid envelope
- Samples were analysed for haemoglobin concentration

- ≥50ng/mL (10µg haemoglobin /g stool) = **POSITIVE**

N = 827 (median age 63y, 54% male)

Faecal immunochemical test → Overt bleeding?

↓

Blood collected

(pre-colonoscopy)

Blood analysis

- DNA extracted from 4mL plasma
- Bisulphite converted
- Analysed with multiplexed real time PCR for methylated BCAT1 and IKZF1
- Presence of either methylated gene = **POSITIVE**

FLINDERS CENTRE for INNOVATION in CANCER

N = 827 (median age 63y, 54% male)

Faecal immunochemical test → Overt bleeding?

↓

Blood collected

↓

COLONOSCOPY

↓

41% benign bleeding conditions (n = 342)

20% no findings ("normal") (n = 165)

FLINDERS CENTRE for INNOVATION in CANCER

N = 827 (median age 63y, 54% male)

Faecal immunochemical test → Overt bleeding?

↓

Blood collected

↓

COLONOSCOPY

↓

41% benign bleeding conditions (n = 342)

16 reported overt bleeding

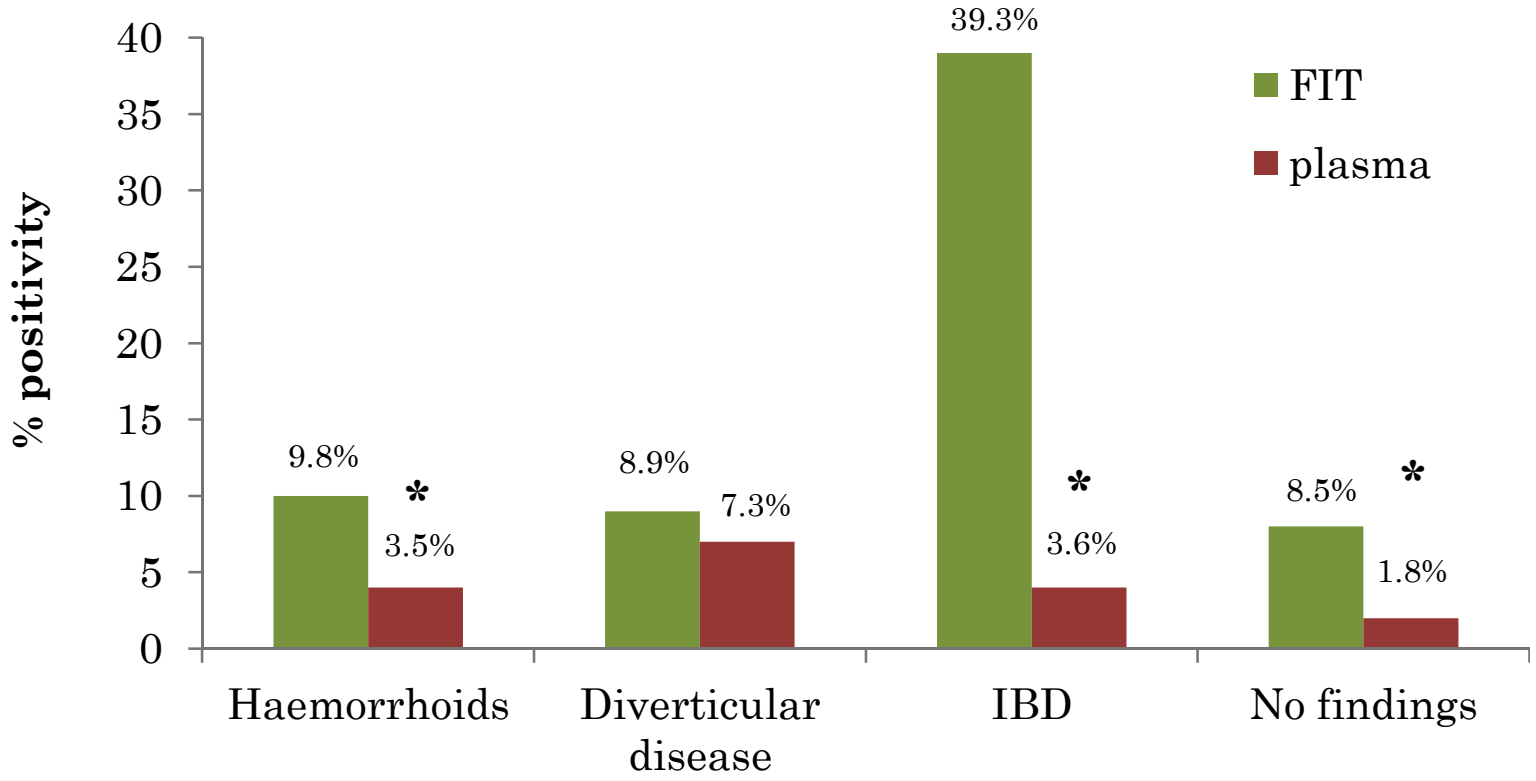
N = 173 haemorrhoids

N = 124 diverticular disease

N = 28 inflammatory bowel disease

FLINDERS CENTRE for INNOVATION in CANCER

Results



Overt bleeding:
6/16 positive FIT (38%)
1/16 positive blood test (6%)

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Summary

- Current screening options for patients with benign bleeding conditions:
  - FIT- risk of false positive
  - Colonoscopy- increase workload
- The false positivity rate was significantly higher with FIT (12%) compared to the blood test (5%).

Conclusions

- For patients with benign bleeding conditions the blood test could provide a more appropriate CRC screening mode
- This will improve screening program specificity
- Result in decreased colonoscopy workload

FLINDERS CENTRE for INNOVATION in CANCER

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