Using clinical research data to evaluate a blood-based biomarker panel for the detection of colorectal cancer

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HEALTH AND BIOSECURITY  
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Background

• Worldwide, Colorectal Cancer (CRC) is 3rd most common form of cancer and 2nd leading cause of cancer death

• Immunological Faecal Occult Blood Test (FOBT) is only widely-used non-invasive screening test in Australia
  • However, has lower sensitivity & specificity than colonoscopy.

• Research shows: a robust, blood-based diagnostic assay would increase screening participation and compliance¹,²

What is the diagnostic challenge?

Highly curable and preventable:

• 90% of colorectal cancers can be cured surgically if detected early
• Overall 5 year survival is 69%
• Preventable in 75% of CRC cases by lifestyle changes: diet and exercise

Dukes Stage | Diagnosis % | Survival %
---|---|---
A | 9 | 90
B | 27 | 70
C | 49 | 44
D | 15 | 5


Colorectal Cancer Staging

• **Stage 0 (Carcinoma in Situ)**
  
  Abnormal cells found in the mucosa (innermost layer)

• **Stage I**

  Cancer has formed in the mucosa of the colon wall & spread to submucosa (layer of tissue under the mucosa)
Colorectal Cancer Staging (cont)

- **Stage II**
  - Stage IIA: Cancer has spread through the muscle layer of the bowel wall to the serosa (outermost layer) of the bowel wall.
  - Stage IIB: Cancer has spread through the serosa (outermost layer) of the bowel wall but has not spread to nearby organs.
  - Stage IIC: Cancer has spread through the serosa (outermost layer) of the bowel wall to nearby organs.
Colorectal Cancer Staging (cont)

• **Stage III**

  - **Stage IIIA**: Cancer has spread through mucosa to submucosa and may have spread to muscle layer
  - **Stage IIIB**: Cancer has spread through muscle layer to serosa or to nearby organs
  - **Stage IIIC**: Cancer has spread to the serosa but not to nearby organs
Colorectal Cancer Staging (cont)

• Stage IV
The TNM staging system

- **T** describes **tumour size** and how far it has grown into – and through the colon wall – on a scale of **T0** to **T4**.

- **N** describes **lymph node involvement** - from **N0**: no lymph nodes affected, to **N2**: 4 or more lymph nodes affected.

- **M** describes whether **metastases** (secondary tumours) are present. **M0** indicates no evidence of cancer having spread, while **M1** indicates evidence.
Why: National Challenge

Impact of disease burden:

- Colorectal cancer (CRC) has been estimated to cost Australia more than $2B annually.
- Australia has the highest incidence rates of CRC globally & second highest cause of cancer related death: estimated 16,682 new cases in 2017 and 4,114 deaths.
- Disease of affluence with Western societies having the highest rates: opportunity for simple diet and lifestyle interventions and early detection

http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx?cancer=colorectal
Major limitations with current screening for Colorectal Cancer

National Bowel Cancer Screening Program (began in 2006) using faecal occult blood testing (FOBT) reduces CRC mortality by **15%-25%**, cost-effective and leads to earlier diagnosis.

- Overall *compliance* is **low** (37% participation rate in 2013-2014)
- *High risk* populations (family history, polyp surveillance), only 30% participate
- Only **7%** were FOBT positive.
  - Of these, **1 in 32 confirmed** CRC by follow-up diagnostic assessment
- **Low sensitivity** for adenomas and early stage CRC
- FOBT **unstable** above **27°C**
- All positive diagnosis require colonoscopy: **invasive, costly & risky**

Diagnostic Blood Test for Screening of Colorectal Cancer

• Cross-sectional research study conducted across 2 sites in Adelaide

• Objectives:
  “To assess the efficacy of a blood-based screening test to diagnose colorectal cancer at an early stage when chance of cure > 95%”

• Patient:
  • Volunteered Demographics, Family History, Medical History
  • Consented for blood collection
  • Undertook FOBT and Colonoscopy procedures

• Devised a blood-based biomarker test comprising a panel of three protein-based biomarkers
Workflow for cohort selection

Patients recruited at 2 sites

*RAH, LMH*

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**Rolling Recruitment Model**

1. **Scheduled Colonoscopy**
   - Patients recruited into trial by Clinical Nurse

2. **Participants attend pre-admission clinic. Bloods sampled; FIT provided to patients.**

3. **FIT completed at home prior to Colonoscopy; returned to Pathology lab for analysis**

4. **Patients attend for colonoscopy after Bowel Prep (performed at home)**

5. **Clinical data (cancers; histology etc) collated by nurse and entered into study database**

**Questionnaire:** Preference for blood vs stool test (compliance)

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**ELISA Assay of patient samples using the 9-biomarker panel & statistical analysis**
Cohort Recruitment in Study

N=1191 Participants with signed consent and questionnaires

N=774 Participants required to undertake FOBT

N=573 Lyell McEwin Hospital

N=618 Royal Adelaide Hospital

N=296

N=522 Participants who completed FOBT, blood collection and colonoscopy

N=478

N=187

N=111 RAH Participants with a Normal diagnosis

N=124 RAH Participants with Polyps

N=300 LMH

N=98 LMH

N=314 N=203

N=314 Participants with a Normal diagnosis

N=164 Participants having positive FOBT

N=66 RAH

N=92 Dropout

N=92 LMH Dropouts

N=296

N=187

N=478

N=522

N=335

N=187

N=111 RAH

N=124 RAH

N=300 LMH

N=98 LMH

N=314

N=203

N=164

N=66
Workflow

Screening

Obtain serum sample

Measure protein biomarkers (ELISA)

Experimentation and molecular assay data

Sensitivity/specificity for individual markers

Statistical modelling for biomarker combination

Clinical data and cohort selection

Statistical analysis

Evaluate test performance against FOBT

Determine accuracy of test for disease detection

Threshold for positive detection

$log(x) = \beta_0 + \beta_1C_{W1} + \beta_2C_{W2} + \beta_3C_{W3}$
## Cohort for panel evaluation

<table>
<thead>
<tr>
<th></th>
<th>Colonoscopy Negative (controls)</th>
<th>Colorectal cancer</th>
<th>Other diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>177</td>
<td>233</td>
<td>129</td>
</tr>
<tr>
<td>AJCC stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>unknown stage</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Non advanced adenoma</td>
<td></td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>Advanced precursor adenoma</td>
<td></td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>Other GI diagnosis</td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>
Panel Evaluation process

• A panel of protein-based biomarkers was identified
  • Insulin like growth factor binding protein 2 (IGFBP2)
  • Dickkopf-3 (DKK3);
  • Pyruvate kinase M2 (PKM2)

• Biomarkers were selected using a forward stepwise variable selection and Bayesian information criterion (BIC) penalty to prevent over-fitting.

• This process of variable selection and estimation of coefficients was performed in a training data set and then to a test data set.

• The model was then applied to both cohorts to identify the best performing panels that cross validated.
Results

At 95% specificity, the three biomarker panel identified can detect CRC at all stages of disease and has better performance than reported for iFOBT \(^1\).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFOBT (^1)</td>
<td>5.4 – 62.6%</td>
<td>98.5% - 94.3%</td>
</tr>
<tr>
<td>3 biomarker panel</td>
<td>73%</td>
<td>95%</td>
</tr>
</tbody>
</table>


Current study: We are currently directly evaluating the accuracy and performance of our biomarker panel against the iFOBT results.
Conclusion

- 3-panel biomarker *performs better* than FOBT
  - At all stages of colorectal cancer
  - Particularly at the early stages
  - FOBT:
    - 5.4% sensitivity at 98.5% specificity;
    - 62.6% sensitivity at 94.3% specificity
  - 3-panel biomarker: 73% sensitivity at 95% specificity

- The 3-panel biomarkers offer a *non-invasive* way of detection

- Work in Progress
  - Doing a comparison between the biomarker panel and FOBT in same cohort

- Future Work
  - Looking at adenomas (129 participants from slide 15)
Collaborators

Clinical collaborations
• Prof Andrew Ruszkiewicz (SA Pathology)
• Mr James Moore (Royal Adelaide Hospital)
• Dr Michelle Thomas (Royal Adelaide Hospital)
• Emma Berton/Deborah Krinas (Royal Adelaide Hospital)
• Prof Rajvinder Singh (Lyell McEwen)
• Amanda Ovenden
• Peter Gibbs (WEHI/RMH)
• Paul McMurrick (Cabrini)
• Jeanne Tie (WEHI/RMH)

Academic collaborations
• Prof Tony Burgess and Prof Richard Simpson (LICR/WEHI), Prof Ed Nice (Monash University)

CSIRO
• Adelaide:
  • Cosgrove Lab: Kim Fung, Kathy Surinya, Ilka Priebe, Jean Wei, (Gemma Brierley, Leanne Purins, Damien Belobradjic, Celine Pompeia)
  • Ingrid Flight, Julie Syrette & Ian Zajac
• North Ryde: Trevor Lockett, Charles Lindall (BD)
• Parkville: Tim Adams, Lesley Pearce, John Bentley.
• AEHRC: Hugo Leroux, (Simon McBride)
• Data61: Mike Buckley, (Bruce Tabor, Rob Dunne, Aloe Patak, Ian Saunders)
Questions ...

... Thank you

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Study set-up

Clinical data

Cohort selection

Experimentation

Molecular assay data

Data export

Report generation

Statistical modelling and analysis

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