A Blood-Based Biomarker for screening for Colorectal Cancer

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Greetings from the National University of Singapore!
Outline

- Limitation with current FIT Performance
  - Take-up rate- despite long history of use in population screening, FIT has a low uptake rate.

- New Tests on the horizon: The Future is a blood test
  - Data on blood miRNA test
  - Patient preference blood test vs stool test.
  - Cancer detection = sensitivity x % takeup.

- Conclusion
FIT is recommended by national screening guidelines worldwide

- Out of 51 countries reviewed, 31 have organised CRC screening programmes, 26 include FIT, 9 gFOBT, while Poland adopted OC.

<table>
<thead>
<tr>
<th>Country</th>
<th>ASRi</th>
<th>ASRm</th>
<th>Region(s)</th>
<th>Programme type</th>
<th>Status of organised programme</th>
<th>Type of test</th>
<th>Definition positive test</th>
<th>Starting year</th>
<th>Age range (years)</th>
<th>Screening interval (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armenia</td>
<td>19.3</td>
<td>11.1</td>
<td></td>
<td>Unknown</td>
<td>Rollout ongoing</td>
<td>FIT</td>
<td></td>
<td></td>
<td>50−74</td>
<td>60</td>
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<tr>
<td>Australia</td>
<td>38</td>
<td>9</td>
<td>All</td>
<td>Organised</td>
<td>Rollout ongoing</td>
<td>OC</td>
<td></td>
<td></td>
<td>50−74</td>
<td>60</td>
</tr>
<tr>
<td>Brunei</td>
<td>25</td>
<td>12</td>
<td></td>
<td>Opportunistic</td>
<td></td>
<td>FIT</td>
<td></td>
<td></td>
<td>50−74</td>
<td>60</td>
</tr>
<tr>
<td>China</td>
<td>14.2</td>
<td>7.4</td>
<td>Hong Kong Several including Shanghai and Hangzhou regions</td>
<td>Organised Pilot</td>
<td>gFOBT/OC</td>
<td></td>
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<td>2003</td>
<td>50+</td>
<td>40−74</td>
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<tr>
<td>Israel</td>
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<td>11.1</td>
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<td>FIT</td>
<td></td>
<td>1990</td>
<td>50−74</td>
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<tr>
<td>Japan</td>
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<td>11.9</td>
<td>All</td>
<td>Organised</td>
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<td>FIT</td>
<td></td>
<td>1992</td>
<td>40−69</td>
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<td>15.5</td>
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<td>FIT</td>
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<td>50+</td>
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<td></td>
<td></td>
<td></td>
<td>50+</td>
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<tr>
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<td>10.7</td>
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<td></td>
<td>FIT</td>
<td></td>
<td>2004</td>
<td>50+</td>
<td>12</td>
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<tr>
<td>Korea, South</td>
<td>45</td>
<td>12</td>
<td>All</td>
<td>Unknown</td>
<td>No organised screening</td>
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<td></td>
<td></td>
<td>50+</td>
<td>12</td>
</tr>
<tr>
<td>Malaysia</td>
<td>18.3</td>
<td>9.4</td>
<td></td>
<td>No organised screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50+</td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>37</td>
<td>15</td>
<td>Waitemata</td>
<td>Organised</td>
<td>Pilot</td>
<td>FIT</td>
<td>15 μg Hb/g</td>
<td>2011</td>
<td>50−74</td>
<td>12</td>
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<tr>
<td>Singapore</td>
<td>33.7</td>
<td>11.8</td>
<td>All</td>
<td>Organised</td>
<td>Rollout ongoing</td>
<td>FIT</td>
<td></td>
<td>2004</td>
<td>50−74</td>
<td>12</td>
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<tr>
<td>Taiwan</td>
<td>All</td>
<td></td>
<td></td>
<td>Organised</td>
<td>Pilot</td>
<td>FIT</td>
<td></td>
<td>2004</td>
<td>50−74</td>
<td>12</td>
</tr>
<tr>
<td>Thailand</td>
<td>12.4</td>
<td>7.3</td>
<td>Lampang Province</td>
<td>Organised</td>
<td>Pilot</td>
<td>FIT</td>
<td>200 ng/mL</td>
<td>2011</td>
<td>50−65</td>
<td></td>
</tr>
</tbody>
</table>

Poor Take-up Rate for Stool Tests

Compliance to FIT/gFOBT screening is low in most programmes

<table>
<thead>
<tr>
<th>Country</th>
<th>Start of program</th>
<th>Test type</th>
<th>Uptake rate</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>1992</td>
<td>FIT</td>
<td>M: 41.4%/F: 34.5%</td>
<td>51 million</td>
</tr>
<tr>
<td>Taiwan</td>
<td>2004</td>
<td>FIT</td>
<td>21.4%</td>
<td>1.2 million</td>
</tr>
<tr>
<td>Australia</td>
<td>2006</td>
<td>FIT</td>
<td>37%</td>
<td>2.2 million</td>
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<tr>
<td>Croatia</td>
<td>2007</td>
<td>gFOBT</td>
<td>19.9%</td>
<td>1.1 million</td>
</tr>
<tr>
<td>Ontario, Canada</td>
<td>2008</td>
<td>gFOBT</td>
<td>29.8%</td>
<td>2.6 million</td>
</tr>
<tr>
<td>France</td>
<td>2009</td>
<td>gFOBT</td>
<td>34.3%</td>
<td>9.7 million</td>
</tr>
</tbody>
</table>

45% is acceptable
65% is recommended

European guidelines for quality assurance in colorectal cancer screening and diagnosis. Endoscopy 2012; 44(S 03): SE49-SE64

Barriers

1. Financial difficulty
2. Limited service accessibility
3. Discomfort / physical harm
4. Embarrassment
5. Gender difference

Wong et al. Perceived Obstacles of Colorectal Cancer Screening and Their Associated Factors among 10,078 Chinese Participants. PLoS ONE 2013, 8: e70209


## Other Non-Invasive Screening Tests

<table>
<thead>
<tr>
<th>Modality</th>
<th>Stool</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>gFOBT</td>
<td>FIT</td>
<td>Methylated DNA</td>
</tr>
<tr>
<td>FIT</td>
<td>Multi-target DNA</td>
<td>miRNA</td>
</tr>
<tr>
<td>Multi-target DNA</td>
<td>Nucleosome</td>
<td></td>
</tr>
</tbody>
</table>

### Multitarget stool DNA (FIT+DNA)

- Higher sensitivity for CRC than FIT (92.3% vs 73.8%), but lower specificity (84.4% vs 93.4%)

### Methylated SEPT9 DNA in plasma for detection of CRC

- **N=1,516**
- Sensitivity for CRC: 48.2%
- Specificity for CRC: 91.5%
- Sensitivity for CRC stages I–IV: 35.0%, 63.0%, 46.0% and 77.4%


miRNA: Master Regulator of Gene Expression

DNA: The Blueprint
mRNA: The Messenger
Protein: The Workhorse

Typical gene
Direct protein assembly

Average: 1400 nt

22 nt miRNA - The Master Regulator
Involved in many aspects of cancer

Why is miRNA suited for cancer diagnostics?

1. Active Regulator of cancer progression
2. Highly Accessible and Stable in Biofluids (blood, urine, saliva…)
3. Low Sample Requirement
4. Adaptable to existing clinical instruments and workflow (qPCR based)
miRNA Discovery workflow

- **Multiplex RT**
- **qPCR**
- **Data Analysis**
- **Candidate Biomarker Panels**

**Absolute quantification of miRNA**
- Synthetic miRNA Standards: chemically synthesized RNA template based on known human miRNA sequence
- One template per miRNA
- Control and normalize individual assay efficiency
- Enable absolute quantification of miRNA copy numbers, by comparing signal from clinical sample to standard curve
miRNAs: Dedicated Hi-throughput Lab

- Dedicated miRNA profiling facility with high throughput
- Standardized, automated workflow
- Accredited laboratory
miRNA panel for colorectal cancer

243 candidate miRNAs (miRBase annotated high confidence miRNA)

Pilot Study Cohort (Proof of Concept)
Singapore Population N=137
- Control N=74
- CRC N=63

Discovery Cohort 1
Singapore Population
- Control
- Non-Adv Adenoma
- Adv Adenoma
- CRC (Stages 1-4)

Discovery Cohort 2
Caucasian Population
- Control
- CRC (Stages 1-2)

Validation I
Case-control
Singapore Population
- Control
- Non-Adv Adenoma
- Adv Adenoma
- CRC (Stages 1-4)

AA/CRC Biomarker Panel

Validation II
Prospective cohort
Singaporean Population N >4000

Preliminary results of a multi-candidate miRNA panel show AUC > 0.90 for CRC vs controls

UNPUBLISHED DATA
miR-92 as plasma marker for CRC screening

Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening

E K O Ng,1 W W S Chong,1,2 H Jin,1 E K Y Lam,1 V Y Shin,1,2 J Yu,1,2 T C W Poon,1,2 S S M Ng,1,3 J J Y Sung1,2

- miRNA profiling in plasma and tissue for marker discovery (n=10)
- Marker selection and validation in plasma (n=45) and tissue pre-post surgery (n=10)
- Marker validation (n=180) in plasma from CRC, GC, IBD and healthy controls

- 90 CRC vs 50 healthy controls
- Sensitivity 89%
- Specificity 70%
- AUC 0.885
- Plasma miR-92 levels in controls, GC and IBD patients are comparable

People’s preference to give blood over stool sample

- **Australia**: 78% of 393 people surveyed preferred to provide a blood sample over stool sample (p<0.001).


- **Singapore**: 78.2% of 109 people surveyed preferred a blood test over a stool test, assuming equal cost and performance (p<0.001)

  KG Yeoh, unpublished data
Effectiveness = Sensitivity x Compliance x Access

Effectiveness of FIT:
- Sensitivity: 79%
- Compliance: 20-41%
- Access: 24%

Effectiveness of Blood miRNA test:
- Sensitivity: 80%
- Compliance: 78%
- Access: 62%


Factors affecting effectiveness:
- Cost
- Capacity
- Geographic distance
- Time away from work
- Noncoverage by payer
Conclusions

• FIT is the current standard and evidence-based screening test.

• Despite long history of use in population screening, stool tests have a low uptake rate.

• Promising new technologies eg blood-based miRNA or nucleosome tests. Validation studies are in progress. More data needed.

• People prefer blood tests!
The best screening test is the test that gets done.
(Effectiveness = Sensitivity x Compliance)

• The future modality for CRC screening could well be a blood test based on miRNA.