Laboratory Findings in Colorectal Cancers

(from chemistry to molecular tests)

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Epidemiology

- Colorectal cancer is the third most common cancer in the UK, US and Iran.
- 75% of colorectal cancer cases occur in people aged 65 and over.
- Alcohol, inactivity, a diet with a high intake of red and processed meat, family history and age all increase the risk of colorectal cancer.
How long does it take cancer to develop, and what are your chances?

• In most people, colorectal cancers develop slowly over a period of several years.
  – 10 to 20 years

• Chances of developing colorectal cancer sometime in your life:
  – A man has a 1 in 17 chance.
  – A woman has a 1 in 18 chance.
Why does it develop?

- Colorectal cancer usually begins as a non-cancerous (or benign) polyp.
- A polyp
  - is a growth inside the colon or rectum that is not normal.
  - can be several types.
  - is not always cancerous.
When Cancer Forms in a Polyp

• It can eventually grow through the lining and into the wall of the colon or rectum.
• 95 percent of colorectal cancers grow from cancerous polyps and move into the inside layer of the wall of the colon and rectum.
Importance of Screening and Early Detection

• Once a non-cancerous (benign) polyp is removed, it will never have the chance to develop into cancer.
• Regular screenings for colorectal cancer and removal of polyps
  – Reduce a person’s lifetime risk of dying by 80 percent.
• When colorectal cancer is detected early
  – It is highly curable!
Personal History Risk Factors for Colorectal Cancer

- **Personal History of Cancer**
  - If you’ve already been treated for colorectal cancer, you’re at an increased risk for developing it again.

- **Personal History of Polyps**
  - If you have had a polyp removed,
    - You are no longer at risk of that particular polyp developing into cancer.
  - If you have had an adenomatous polyp removed, you are more likely to have other polyps in the future.
    - Adenomatous polyps are groups of polyps with abnormal cells that multiply and may eventually become cancerous.
Having Inflammatory Bowel Disease and Type 2 Diabetes Are Risk Factors

- Inflammatory bowel disease (IBD) includes:
  - Ulcerative colitis and
  - Crohn’s disease

  - The overall increased risk of colorectal cancer for someone with IBD is estimated to be 4-20 times higher than normal.

- Personal history of type 2 diabetes
  - Increases your risk of having colorectal cancer and colorectal polyps by 50 percent
Family History and the Role of Genetics

• You have a higher risk of developing colorectal cancer if:
  – One or more immediate family members were diagnosed with colorectal, uterine, or stomach cancer
  – Immediate family members include:
    • Parent
    • Sibling
    • Child
What if something is found?

- If you have polyps
  - They can be removed before they turn into cancer.
  - Finding and removing adenomatous polyps can decrease colorectal cancers by 60-90 percent.

- If cancer is found
  - It is often curable in its early stages.
What test do I take to get screened?

• There are several tests to screen for colorectal cancer.
• Some tests are used alone, while others are used in combination with other tests.
Fecal Occult Blood Test (FOBT)

- Recommended to be done yearly
- Checks for hidden blood in the stool
  - If blood is found, a colonoscopy will be needed.
- A disadvantage of this test
  - The test is often negative in people who have adenomatous polyps and colorectal cancer.
Flexible Sigmoidoscopy (Flex Sig)

- Recommended every 5 years
- Examines the lining of your rectum and lower part of your colon
- Uses a thin, flexible, lighted tube called a sigmoidoscope
  - It is inserted into your rectum and lower part of your colon.
  - If polyps or lesions are found, a follow-up test is needed.
- Disadvantages:
  - Patient discomfort – but not painful
  - Only looks at lower part of colon, therefore polyps in the upper colon can go undetected.
  - If a polyp is found, it needs to be followed by a colonoscopy to remove the polyp.
Combination FOBT and Flex Sig

- Some experts recommend using both of these tests to increase the chance of finding polyps and cancers.
- It is recommended every 5 years.
Colonoscopy

• Similar to the Flexible Sigmoidoscopy except:
  – It allows the doctor to look at the lining of your rectum and entire colon.
  – Done as an outpatient procedure
  – Done with “conscious sedation”
    • An IV line is inserted to help you remain calm and comfortable. Some patients sleep through the procedure.
    • Not everyone needs sedation.
  – Uses a thin, flexible, lighted tube called a colonoscope
  – It is inserted into your rectum and colon.
  – The doctor can also find and remove polyps and some cancers using the colonoscope.
  – It is recommended every 10 years for:
    • Individuals with no family or personal history of colon cancer and no symptoms.
Colonoscopy (continued) …

- Procedure takes 15–30 minutes.
- May take longer if polyps are removed.
  - Called a polypectomy
  - A wire loop is passed through the scope to cut the polyp from the lining of the colon using an electrical current.
  - Polyps are collected and sent to the lab for evaluation.
Irritable Bowel Syndrome

- Abdominal pain associated with altered stools
  - No organic cause identifiable

- Epidemiology
  - Very common ~10% Western population
  - Up to 50% of visits to gastroenterology

- Diagnosis
  - Diagnostic criteria somewhat helpful
    - Rome III criteria
      - Recurrent Abdo pain for 3 days in the last 3 months
        with 2 of:
        » Improvement with defecation
        » Onset associated with change in stool frequency
        » Onset associated with change in stool form
IBS

• Symptoms include
  – Bloating, flatus, mucous in stool
  – Exacerbated by stress

• Investigation
  – If typical syndrome in young (<50) patient probably nothing.
  – If alarm symptoms:
    • GI bleeding, LOW, Age >50, FHx Abnormal laboratory tests
  – Need to exclude:
    • In young: Coeliac disease and IBD
    • In elderly: Colorectal cancer, Coeliac disease, IBD
Inflammatory Bowel Disease (IBD)

• **Crohn’s Disease**
  – Pathology throughout GI tract
  – Often skip lesions with intervening normal gut
  – Transmural inflammation and fistulous disease

• **Ulcerative Colitis**
  – Localised to the colon and rectum
  – Mucosal inflammation characteristic
  – Usually contiguous disease

• Occasionally difficult to distinguish the two
  – Indeterminate Colitis

• **Epidemiology**
  – Incidence in Western countries up to 14/100,000
  – Prevalence in West up to 240/100,000
IBD

• Aetiology
  – Probable polygenic disease
  – Environmental (gut infection)
  – Immunological

• Symptoms
  – Can be varied depending on site
  – Often: Diarrhea, abdominal pain, PR bleeding, PR mucous, LOW, LOA
  – Nutritional disorders
    • Iron deficiency, Vitamin B12 deficiency, Folate deficiency etc
  – Extraintestinal manifestations
    • Arthritis, Uveitis, Skin changes, Primary Sclerosing Cholangitis
Coeliac Disease

- Immune mediated disease due to allergy to dietary gluten (protein in wheat, rye, barley)
- Pathology
  - Exposure to gluten causes damage to small intestine leading to malabsorption
  - Strong genetic association with HLA-DQ2 HLA-DQ8
  - Environmental factors important
  - Prevalence 1:100
Coeliac Disease

- **Symptoms**
  - GI: diarrhoea, bloating, mouth ulcers, IBS type
  - Anaemia, Osteoporosis, lethargy Chronic fatigue
  - Thyroid disease, Type 1 DM
  - Migraines
  - Infertility
  - Abnormal liver function
  - Arthralgia
  - Asymptomatic
Coeliac Disease

- Diagnosis/Investigation
  - Coeliac serology Tissue Transglutaminase ab
  - Endoscopy with small bowel biopsy
    - Establish diagnosis and assess disease
  - Therapeutic trial
    - Improvement on gluten free diet
    - Important in paediatric setting but helpful for adult

- Therapy
  - Lifelong gluten free diet
  - Dietician important
Calprotectin
(calprotectin stool test)
(Fecal Calprotectin)
What is calprotectin?

- Calprotectin is a calcium- and zinc-binding protein having many different functions in the cell.
- It is a major part of the cytoplasm of neutrophil granulocytes, monocytes and epithelial cells, and makes up to 60% of the soluble ingredients of neutrophil granulocytes.¹
Why does calprotectin occur in the stool?

- In inflammation, the leukocytes migrate through the intestinal wall, leading to increased calprotectin level in the stool.
- The concentration of calprotectin correlates with the number of granulocytes in the intestinal lumen, and thus with the level of intestinal inflammation.¹
What is the disease association of calprotectin?

- Calprotectin in stool specifically indicates intestinal inflammation and is elevated in inflammatory bowel diseases (IBD).
- The most frequent IBD are Crohn’s disease and ulcerative colitis.
What is the use of detecting fecal calprotectin?

• Fecal calprotectin is able to provide early diagnostic guidance by differentiating between inflammatory bowel diseases (IBD, such as Crohn’s disease or ulcerative colitis) and non-inflammatory functional bowel diseases, e.g. irritable bowel syndrome (IBS).

• As first line test a negative result can rule out an inflammatory process while a positive result may prioritize endoscopy in the diagnostic path.

• Fecal calprotectin is an efficient marker for therapeutic effectiveness and mucosal healing since its level correlates well with endoscopic and histological findings in inflammatory bowel diseases.
What is the advantage of fecal calprotectin compared to other inflammation markers?

• Fecal calprotectin outperforms markers for general inflammation, such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR).\textsuperscript{6}

• The ROC analysis below shows that calprotectin is both more sensitive for Crohn‘s disease and more specific than CRP and ESR.
Why Calprotectin? ▶ Early diagnostic guidance!

- An internal study using samples from 132 patients with IBD and 59 samples from patients with IBS and other functional bowel disorders shows that EliA Calprotectin has very high predictive values, providing early diagnostic guidance for the physician.

<table>
<thead>
<tr>
<th></th>
<th>EliA Calprotectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity in %</td>
<td>97.7</td>
</tr>
<tr>
<td>Specificity in %</td>
<td>89.8</td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>0.96</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>0.95</td>
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</tbody>
</table>
Colorectal cancer- genetic syndromes

• FAP (Familial Adenomatous polyposis)
  – Autosomal dominant
  – Colonic Polyposis (classical >1000 polyps; attenuated >20-100 polyps)
  – Extracolonic manifestations
    • Gastric Cancer
    • Duodenal and ampullary cancer
    • Desmoid disease
    • Osteoma (commonly of mandible)
    • Skin lesions

• Lynch Syndrome
  – Autosomal dominant
  – Most common hereditary Syn
  – Extracolonic cancers
    • Endometrial cancer
    • Gastric cancer
    • Ovarian Cancer
    • Uro-epithelial cancer
    • Skin lesions (Muir-Torre)

• Genetic testing
  – Appropriate referral to genetic service
  – Screen FHx
  – Pregentic tests
  – Genetic test
    • APC gene
    • MMR genes (MLH1, MSH2, MSH6, PMS2)
Colorectal cancer - clinical

• Symptoms
  – Often none
  – Altered bowel habit, PR bleeding, LOW, Malaise, Iron deficiency anaemia

• Investigation
  – Colonoscopy
  – Barium Enema/CT colography

• Management
  – Part of MDT (Surgeon, Oncologist, Radiologist, Pathologist, Nurse, Stoma
  – Resection
  – Surveillance
# Common genetic tests in CRC

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Genes</th>
<th>Mutations</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal Cancer</td>
<td>1 / 25</td>
<td>1 / 1.000</td>
<td>MLH1, MSH2, MSH6, APC, MUTYH</td>
<td>Mutations in 19 exons&lt;br&gt;Mutations in 16 exons&lt;br&gt;Mutations in 10 exons&lt;br&gt;Mutations in 15 exons&lt;br&gt;Mutations in 16 exons</td>
<td>Complicated / expensive</td>
</tr>
</tbody>
</table>
Colorectal Cancer Genetic Tests

1. KRAS Gene, Mutations within codon 12 and 13
2. BRAF Mutation (T1799A) Analysis by PCR and Sequencing, colon tumor
3. EGFR Mutation Analysis
4. PI3K Mutation Analysis
5. Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen
6. MLH1 Mutation Screen
7. MSH6 Mutation Screen
8. MLH1/MSH2 Mutation Screen
9. MSH2 Mutation Screen
10. EPCAM Deletion Analysis
11. APC Comprehensive for Familial Adenomatous Polyposis
12. MUTYH (MYH) Sequence
13. PMS2 Comprehensive Sequence & Deletion Duplication Analysis
**Useful For:**

Prognostic markers for cancer patients treated with epidermal growth factor receptor-targeted therapies: Cetuximab (Erbitux) or Panitumumab (Vectibix)
1. KRAS Gene, Mutations within codon 12 and 13

Patients are selected for EGFR-targeted therapy in combination with standard chemotherapy.

Method Name:
Polymerase Chain Reaction (PCR) Analysis followed by sequencing

Reporting Name
EGFR Gene, Mutation Analysis, Tumor

Specimen Type
Varies

Specimen Required
Pathology report must accompany specimen in order for testing to be performed.
Preferred:
Specimen Type: Tissue

Cautions
Not all patients that have wild-type KRAS respond to epidermal growth factor receptor (EGFR)-targeted therapies.
Rare polymorphisms exist that could lead to false-negative or false-positive results.
**Useful For**

As an adjunct to HNPCC/17073 Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen, when colon tumor demonstrates microsatellite instability (MSI-H) and loss of MLH1 protein expression, to help distinguish a somatic versus germline event prior to performing expensive germline testing.

As an adjunct to negative MLH1 germline testing in cases where colon tumor demonstrates MSI-H and loss of MLH1 protein expression.

**Note:** Our lab preferred screening test (MLBRF/87931 MLH1 Hypermethylation and BRAF Mutation Analyses) includes both MLH1 promoter hypermethylation and BRAF V600E testing. Please note that test can only be performed on colon tumors.

**Method Description**

Polymerase Chain Reaction (PCR) Analysis and PCR Sequencing

**Specimen Type**

Varies

**Specimen Required**

Pathology report must accompany specimen in order for testing to be performed.

**Specimen Type:** Tissue block or slide

**Aliases**

V600E
Useful For:
Identifying cancers that may respond to epidermal growth factor receptor-tyrosine kinase inhibitor therapies

Clinical Information:
EGFR is a growth factor receptor that is activated by the binding of specific ligands, resulting in activation of the RAS/MAPK pathway. Activation of this pathway induces a signaling cascade ultimately leading to cell proliferation. Dysregulation of the RAS/MAPK pathway is a key factor in tumor progression for many solid tumors. Targeted therapies directed to tumors harboring activating mutations within the EGFR tyrosine kinase domain (exons 18-21) have demonstrated some success in treating a subset of patients with cancers by preventing ATP-binding at the active site.
**Method Name**: Polymerase Chain Reaction (PCR) Analysis followed by sequencing

**Reporting Name**
EGFR Gene, Mutation Analysis, Tumor

**Specimen Type**
Varies

**Specimen Required**
Pathology report must accompany specimen in order for testing to be performed.

**Preferred:**
Specimen Type: Tissue
Container/Tube: Tissue block

**Collection Instructions:** Submit a formalin-fixed, paraffin-embedded tissue block.

**Acceptable:**
Specimen Type: Tissue
Container/Tube: Slides
Specimen Volume: 1 stained and 5 unstained
Collection Instructions: Submit 1 slide stained with hematoxylin-and-eosin and 5 unstained, nonbaked slides with 10-micron thick sections of the tumor tissue.

**Interpretation:**
An interpretive report will be provided.

**Cautions**
A negative (wild type) result does not rule out the presence of a mutation that may be present but below the limits of detection for this assay (approximately 10%).
A negative (wild type) result does not rule out the presence of other activating mutations in the EGFR gene.
The predictive value of epidermal growth factor receptor (EGFR) testing applies to EGFR-TKI therapies, not to other therapeutic agents.
Not all patients that have activating EGFR mutations detected by this assay respond to EGFR-TKI therapies.
Rare polymorphisms exist that could lead to false-negative or false-positive results.
Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen

Useful For

Identification of individuals at high risk for having hereditary nonpolyposis colon cancer (HNPCC)/Lynch syndrome
Useful For:
Determining whether absence of *hMLH1* (evidence of defective mismatch repair), demonstrated by immunohistochemistry on tumor tissue, is associated with a germline mutation in the affected individual. Establishing a diagnosis of mismatch repair-related hereditary nonpolyposis colorectal cancer (Lynch syndrome).
**Method Description:**
Polymerase Chain Reaction (PCR)/DNA Sequencing Analysis

**Reporting Name:**
MLH1 Mutation Screen

**Specimen Type**

Varies

**Specimen Required**
Specimen must arrive within 96 hours of draw.

**Container/Tube:**
**Preferred:** Lavender top (EDTA) or yellow top (ACD)
**Acceptable:** Any anticoagulant

**Specimen Volume:** 3 mL

**Collection Instructions:**
1. Invert several times to mix blood or Bone Marrow.
2. Send specimen in original tube.
**Useful For:**
Testing for the presence of a mutation in all 10 exons of the \( hMSH6 \) gene

Determining whether absence of MSH6 protein in tumor tissue, as demonstrated by immunohistochemistry (evidence of defective mismatch repair [MMR]), is associated with a germline mutation in the affected individual

Establishing a diagnosis of MMR-related hereditary nonpolyposis colorectal cancer (Lynch syndrome)

Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:

- 40% associated with a mutation in \( hMSH2 \)
- 40% associated with a mutation in \( hMLH1 \)
- 10% associated with a mutation in \( hMSH6 \)
- 10% other (unknown)
Method Description:
Polymerase Chain Reaction (PCR)/DNA Sequencing Analysis

Reporting Name:
MSH6 Mutation Screen, B

Specimen Type
Varies

Specimen Required
Specimen must arrive within 96 hours of draw.

Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood or Bone Marrow.
2. Send specimen in original tube.

Interpretation:
An interpretive report will include specimen information, pedigree (when appropriate), assay information, and whether or not results are consistent with a diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC), or indicate a risk to develop HNPCC.
If a mutation has been identified in an affected family member, the absence of it in another family member strongly indicates that the individual is not at risk for having HNPCC.
**8MLH1/MSH2 Mutation Screen**

**Useful For:**
An alternative approach to establishing a diagnosis of mismatch repair-related hereditary nonpolyposis colorectal cancer (HNPCC) (Lynch syndrome)
Detection of mutations in *hMLH1* and *hMSH2*, the 2 most commonly affected genes in HNPCC, when microsatellite instability and immunohistochemistry tumor testing is not possible and when a diagnosis of HNPCC is suspected
Method Description:
Polymerase Chain Reaction (PCR)/DNA Sequencing Analysis

Reporting Name:
MLH1 Mutation Screen

Specimen Type
Varies

Specimen Required
Specimen must arrive within 96 hours of draw.

Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood or Bone Marrow.
2. Send specimen in original tube.

Interpretation:
An interpretive report will include specimen information, pedigree (when appropriate), assay information, and whether results are consistent with a diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC), or indicate a risk for developing HNPCC.

If a mutation has been identified in an affected family member, the absence of it in another family member strongly indicates that the individual is not at risk for having HNPCC.
Useful For:
Determining whether absence of \textit{hMSH2} (evidence of defective mismatch repair [MMR]), demonstrated by immunohistochemistry on tumor tissue, is associated with a germline mutation in the affected individual.

Establishing a diagnosis of MMR-related hereditary nonpolyposis colorectal cancer (Lynch syndrome)
Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:
- 40\% associated with a mutation in \textit{hMSH2}
- 40\% associated with a mutation in \textit{hMLH1}
- 10\% associated with a mutation in \textit{hMSH6}

- 10\% other (unknown)
Method Description:
Polymerase Chain Reaction (PCR)/DNA Sequencing Analysis

Reporting Name:
MSH2 Mutation Screen, B

Specimen Type
Varies

Specimen Required
Specimen must arrive within 96 hours of draw.

Container/Tube:

Preferred: Lavender top (EDTA) or yellow top (ACD)

Acceptable: Any anticoagulant

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood or Bone Marrow.
2. Send specimen in original tube.
Techniques Used in Molecular Investigation
Techniques for Mutation Detection

- Nucleotide Mutation:
  - Nonsense mutation
  - Insertion/deletion
  - Missense mutation
  - Splice site mutation
  - Silent mutation
- Trinucleotide Repeat Expansion
- Microdeletion/Microduplication
- Chromosomal aneuploidy or rearrangement
- Uniparental disomy
- Genomic instability
- Epigenetic alteration

**PCR, RFLP, Mutation Scanning, Sequencing**

**PCR, Southern blot**

**MLPA, FISH, array**

**Karyotyping, FISH, array**
Laboratory Genetics in the Clinic
Molecular Pathology in the Clinic

- Diagnosis of genetic conditions
- Carrier testing
- Predictive testing
- Prenatal diagnosis
- Treatment management
Gene Mutations in Cancer

• Oncogenes
  • Genes that promote cell survival or cell proliferation when activated
    » Ie. KRAS, EGFR
  • May include novel fusion genes
    » Ie. BCR-ABL

• Tumour Suppressor Genes
  • Genes that inhibit cell proliferation or inappropriate cell survival when active
  • Contribute to tumourgenesis through gene inactivation
    » Ie. TP53, BRCA1, RB1
Hereditary Cancer Predisposition Syndromes

- BRCA1 and BRCA2 – associated breast/ovarian cancer
- Hereditary Non-Polyposis Colon Cancer
  - MLH1
  - MSH2
  - PMS2
  - MSH6
- Familial Adematous Polyposis
  - APC
- Neurofibromatosis
  - NF1
- Genomic Instability Syndromes
Molecular Investigation of Tumour Cells

• Requires sensitive assays to detect small population of cells with mutation

• Limited to type of tissue available:
  • Formalin Fixed Paraffin Embedded Tissue
    – Often only tissue available
    – DNA is cross-linked to other cellular material by fixation process
    – Often degraded/fragmented during extraction process
  • Frozen tissue preferable

• May require quick turn around times to allow modulation of therapy accordingly
Hereditary versus De Novo Cancer in Colorectal Cancer

Chromosome Instability pathway

- Sporadic
- FAP
- MUTYH

- frequently arise in distal colon
- no microsatellite instability
- chromosome aneuploidy
- arise from adenomatous polyps
- Poorer prognosis compared to MSI-H group

Mutator pathway

- MSI – Lynch
- MSI - Sporadic

- frequently arise in proximal colon
- microsatellite instability
- may have BRAF mutation in sporadic cases
- arise from serrated polyps
- Longer survival ; lower freq of metastasis
Lynch Syndrome – MMR mutation

- MLH1 and MSH2
  - Account for 90% of mutations (50 and 40% respectively)
- MSH6
  - 7-10% of mutation
- PMS2
  - <5% of mutation
- EPCAM
  - 1-3% of mutation
Molecular Genetics as a Diagnostic Tool

- Molecular diagnosis of hereditary disease
- Differentiate between similar neoplasias in some instances
- Confirmation of a clonal process
- Identification of biomarkers for disease progression
- Monitoring of minimal residual disease
- Treatment management
“Normal human genomes are all alike, but every cancer genome is abnormal in its own way”

-Leo Tolstoy
Thank you

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