Biomarkers in Crohn’s disease/ IBD

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Disclosures

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Overview of workshop

- Introductions
- Definition of a biomarker
- Faecal biomarkers
- Serum biomarkers
- Novel biomarkers in research
- What do you use?
- Role/limitations of these biomarkers
Who are you? (Audience)
Definition of a Biomarker (Biological marker)

- NIH definition:

  *A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*

- Diagnostic
- Staging
- Prognostic
- Predictive/monitoring

Types of biomarkers

- **Diagnostic**
  - Identification of a certain disease in patient with symptoms
  - Eg elevated glucose in patient with diabetes

- **Staging**
  - Measuring disease extent
  - Eg CEA in certain cancers, PSA in prostate cancer

- **Prognostic**
  - Indicates potential favourable or adverse outcomes
  - Eg LDH in lymphoma

- **Predictive/ monitoring**
  - Blood cholesterol concentration for risk of heart disease

Further NIH definitions

- **Clinical endpoint**
  - Used in clinical trials to determine the efficacy or lack-there-of an intervention
  - A clinical characteristic of how a patient feels, functions, or survives
  - The most credible characteristic to determine the effect and safety of a therapeutic intervention

- **Surrogate endpoint**
  - A *biomarker* that is intended to substitute for a clinical endpoint
  - Expected to *predict clinical benefit* based on prior epidemiologic, therapeutic, pathophysiologic, or other scientific evidence

Surrogate endpoint

- Note, not surrogate *marker*
- Surrogate = “to substitute for”
- Essentially a subset of biomarkers
  - Only a few biomarkers will reach status of surrogate endpoint

Why do we need biomarkers in IBD?

- Diagnose IBD/ avoid invasive procedures in functional bowel disease
- Differentiate Crohn’s from UC
- Predict prognosis/ complications
- Monitor effect of therapy (important for clinical trials)
- Predict mucosal healing
- Predict relapse
- Stratify treatment
  - Does early intervention in patient with worse prognosis (based on biomarkers) change long term outcomes
• What biomarkers can you think of?
• What samples can we measure biomarkers in?
• What biomarkers do you use in clinical practice?
Calprotectin

- 36 kilodalton calcium- and zinc-binding protein
- Represents 60% cytosolic proteins in granulocytes
- Stable in faeces in room temperature for up to a week
- Indirect measure of neutrophil infiltration of bowel mucosa
- Correlates with 4-day faecal excretion of $^{111}$indium-labelled white blood cells\(^1\)
- Member of S100 protein family (S100A8)
  - 100% soluble in (NH\(_4\))\(_2\)SO\(_4\) at pH 7

Calprotectin - diagnostic biomarker

- Meta-analysis 20 studies
- n- = 5983 (1210 = IBD)
- Calprotectin > 50mcg/g for diagnosing IBD (vs. FD)
  - Sensitivity 89%
  - Specificity 81%
  - AUC 0.95
- Better for children cf adults
  - Adults sens 71%, spec 80%, AUC 0.94
- Cut-offs of 100mcg/g also used
  - Greater sensitivity 98% - ?implausible

Calprotectin – monitoring disease activity

- For detecting active mucosal disease
  - 11+ studies, variable results
  - Sensitivity 70-100%
  - Specificity 44-100%
  - Depends on cut-off values

- Correlates with *colonic* inflammation better than *ileal* inflammation

- Correlates better with CDEIS than CDAI/CRP
  - Are we treating symptoms (CDAI) or endoscopic activity (deep remission?)??

- Slightly better in UC than in Crohn’s
Calprotectin – monitoring for mucosal healing

- Handful small studies
- Small sample sizes
- All support positive correlation
- Differing optimal cut-off points
- Only modest sensitivity and specificity for CD recurrence post ileocolonic resection
  - Due to minor ileal inflammation not generating large enough amounts of calprotectin
Calprotectin – predicting disease relapse

- Patients with quiescent IBD
- Increased FC concentration predict relapse in several studies
- Use cut-offs of 100-150mcg/g
- Better in Ulcerative Colitis than Crohn’s disease
  - Sensitivity 81%
  - Specificity 90%
- Negative predictive value in Crohn’s only 43%
Other stool biomarkers

- Lactoferrin
  - Iron-binding protein in neutrophil granules and serum
  - Similar performance to calprotectin

- 100A12
  - Another S100 protein
  - Better in children than in adults
High sensitivity C-reactive Protein – as diagnostic biomarker

- Acute phase protein
- Studies from 1980s onwards suggesting it as diagnostic biomarker in Crohn’s disease
- 2002 study 224 patients
  - IBD vs. FD
  - hsCRP >2.3mg/L
  - Sensitivity 100%
  - Specificity 67%
  - But rigid sigmoidoscopy as dx

hsCRP as biomarker for active disease

- Using endoscopy as gold standard (Crohn’s):
  - Sensitivity 4-68%
  - Specificity 58-75%
  - Most use cut-off 8 mcg/L

- Some Crohn’s patient never have elevated CRP

- CRP performs better in Crohn’s than UC

- BUT clinical symptoms and raised CRP – better positive predictive value
CRP in Acute Severe Colitis

- Prognostic biomarker
- Patients admitted with ASC (Truelove and Witt’s)
- Day 3 IV corticosteroids
  - >8 stools/day or
  - 3-8 stools/day and CRP >45
- Give rescue therapy

Other serum biomarkers

- **P-ANCA** - more common in UC and Crohn’s pancolitis

- **ASCA** – anti-Saccharomyces cerevisiae antibodies
  - Identifies Crohn’s with high specificity (96-100%) but low sensitivity (50%)
  - assoc with need for surgery (retrospective, ASCA + increases over time)

- **Meta-analysis 60 studies**
  - ASCA +ve/ p-ANCA –ve for diagnosis CD
    - Sensitivity 55%, specificity 93%
    - Slightly better for children (sensitivity 63%)

- **Lower response to infliximab in CD patients who are p-ANCA positive**

Other serum biomarkers

- **ALCA** – against laminaribioside (sugar glycan on surface of microorganism)
- **ACCA** – against chitobioside
  - ALCA and ACCA associated with Crohn’s disease
- **OmpC** – against E Coli outer membrane protein
- **I2** – Ab to Pseudomonas fluoroscens- associated sequence
  - ASCA, Omp C, and I2 are associated with various features of complicated disease (fibrostenosis, penetrating, surgery)
- **CBir1** – against flagellin
  - Assoc with SB disease, penetrating phenotype, fibrostenosis
- **ESR** – more commonly elevated in Crohn’s cf UC
Mucosal calprotectin in UC

- 97 patients with UC
- Simple clinical colitis activity index
- Histology from sigmoidoscopy
- Immunohistochemistry for S100A8/9 (calprotectin) performed on colon biopsy tissue (and 20 controls)
- Follow up median 51 months
  - Steroid use
  - Hospitalisation
  - Colectomy

Mucosal calprotectin in UC

- Mean IMC 2.7 cells/HPF in patients in remission (IQR 0.5-6.8)
- IMC > 38 cells/HPF in patients with active disease (p<0.001)

- IMC < 10/HPF associated with
  - 88% 1 year steroid free remission
  - 63% 5 year steroid free remission

CD8+ T cell transcription signatures

- 35 patients with CD
- 32 patients with UC

Treatment naive

- CD4 and CD8 T cells positively selected from PBMC
- Whole-genome transcriptional analyses
- Patients managed using step-up conventional strategy
- Identified 2 CD8 transcription signatures which separated IBD patients into 2 groups:
  - IBD 1
  - IBD 2

High clinical risk = 2 or more of following:
• Age < 40
• Required steroids at diagnosis
• Perianal disease

Conclusion

- Scarcity biomarkers in clinical practice currently
  - Faecal calprotectin
  - High sensitivity CRP

- Multiple biomarkers in research
  - Serum, stool, mucosal

- Whether identification of biomarkers to predict prognosis/disease response causing therapy augmentation will alter course of disease remains to be seen