Risk Stratification in Pediatric Inflammatory Bowel Disease: Are we there yet?

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on behalf of many others

Inflammatory Bowel Diseases

- Disorders characterized by chronic intestinal inflammation from a dysregulated immune response to the enteric microbiome in a genetically predisposed host
- Today we label them as Crohn's disease and ulcerative colitis though we recognize they often have similar clinical features and large genetic homology
- Presenting symptoms range from mild to severe and clinical course is often unpredictable but may range from easily controlled to fulminant disease
- Short of finding a cure, or preventing these disorders, it would greatly aid patient care if we could...

Predict the Future and Match Our Therapy to Anticipated Course: Risk Stratify

What is Risk Stratification?

- A statistical process to determine detectable characteristics associated with an increased chance of experiencing unwanted outcomes.
- By identifying factors before the occurrence of an event, it is possible to develop targeted interventions to mitigate their impact.

What are the unwanted outcomes?

- Continued active gastrointestinal and extraintestinal symptoms
- Growth failure
- Impaired quality of life
- Relentless progression of disease
- Cancer
- Surgery

Progressive Bowel Damage in CD

What you see on the outside does not always indicate what is going on inside

Pariente et al. Inflamm Bowel Dis 2011
Crohn's Disease Progression in Children: Pre-Biologic Era: 1988-2002

Vernier-Massouille et al. Gastroenterology 2008;135:1106

Inflammatory

Stricturing

Penetrating

Cumulative Probability of Colectomy by Disease Activity at Diagnosis

Moderate/Severe (n=98)

Mild (n=73)

Mild vs moderate/severe, P <0.03


EPIMAD Study from Northern France


20% at 5 years

9% (E1), 23% (E2), 29% (E3)

We Need To Do A Better Job Of Understanding Our Patients Before We Treat Them

One approach does not fit all patients

IBD patients may have unique signatures that predict complicated or treatment refractory disease

Exploding Head Syndrome

Exploding head syndrome is a condition that causes the sufferer to occasionally experience a tremendously loud noise as originating from within his or her own head, usually described as the sound of an explosion, roar, waves crashing against rocks, loud voices, or a ringing noise. Sufferers often feel a sense of fear and anxiety after an attack, accompanied by elevated heart rate. There may be a correlation with stress. Can occur at any time.
### The Manhattan Project of Risk Stratification

**Risk Study – CCFA 2008**

- Advanced age
- Male sex
- Low socioeconomic status
- Family history of Crohn’s disease
- Negative parental antibody test
- Smoking

**PROTECT Study – NIDDK 2012**

- Early onset of disease
- Male sex
- Family history of Crohn’s disease
- Positive parental antibody test
- Smoking

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### Predictors of Disabling Crohn’s

**Referred cohort of 1128 CD patients**

- Three factors independently predictive of disabling CD course within 5-year
  - Initial requirement for steroids
    - OR: 3.1 [CI: 2.2 – 4.4]
  - Age at diagnosis below 40
    - OR: 2.1 [CI: 1.3 – 3.6]
  - Perianal disease at diagnosis
    - OR: 1.8 [CI: 1.2 – 2.8]

Beaugerie L et al. Gastroenterology 2006;130:650-6

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### Consensus Predictors of Poor Outcome*

- Deep colonic ulcers on endoscopy
- Persistent severe disease despite adequate induction therapy
- Extensive (pan-enteric) disease
- Marked growth retardation (> -2.5 height Z scores),
- Severe osteoporosis
- Strictureing or penetrating disease (B2 and/or B3 disease behavior) at onset
- Severe perianal disease

*Raucorne et al. J. Crohn’s Colitis 2014;8:1179

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### Prognosis of CD Patients with Severe Endoscopic Ulcerations

- Retrospective cohort
- 102 adult patients with active CD
- Severe endoscopic lesions (SEL) defined as deep ulcerations >10% of mucosal area with at least one colonic segment
- Risk of colectomy associated with SELs
- All that developed penetrating disease had SELs
- This was not QI diagnosis
- These are not pediatric data

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### Antibody Sum and Disease Behavior

- **P trend < 0.0001**
- **P trend < 0.0001**

- * Odds Ratio
- N=199
- N=262
- N=194
- N=57

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### Anti-microbial Serologic Signature

**ASCA, anti-omp C, anti-Cbir1**
**NOD2/CARD15**

- NOD2 gene associated with an intracellular protein in the proinflammatory nuclear factor kappa B (NFkB) pathway, involved with a receptor for bacterial products
- This "defect" in the innate immune system may allow intracellular bacteria to escape the first-line defense of the immune system, thereby leading to an enhanced adaptive response
- NOD2 acts as a negative regulator following bacterial stimulation of the cell surface receptor toll-like receptor-2, and CD-associated mutations result in a loss of this "brake" on the immune response leading to elevated NFkB
- NOD2 mutations may lead to a reduction in the production of alpha-defensins (small antibacterial proteins) by Paneth cells located in the small bowel
- NOD2 may have some role in autophagy, a process that, among other things, deals with intracellular "debris," including bacterial products

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**But,**

- Only 1/3 of patients with Crohn’s disease have polymorphisms of NOD2/CARD15
- Having 2 mutations is much worse than having one mutation for RR of surgery
- Virtually no Japanese patients with Crohn’s disease have polymorphisms of NOD2/CARD15
- Is it the gene or is it ileal location?
**What About Other Genes?**

- **IL-23** is a cytokine that acts as a proinflammatory mediator of autoimmune and chronic inflammatory diseases.
- In association with **IL-12**, it is part of the T-helper 17 cell axis.
- The **IL-12B** gene, alternatively known as natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, or p40, encodes the p40 subunit of IL-12B (ligand) and IL-23R (receptor), both of which are dimeric proteins.
- The **IL-12B** gene contains two major sites, both of which appear susceptible to variation and at which different alleles are associated with variable levels of gene expression.

- **IL-12**
- **IL-23**
- **IL-12B**
- **p40**
- **IL-23R**

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**Kaplan–Meier cumulative survival plots for time to first stricture**

Rs1363670 is gene linked to the **IL12B LOCUS**

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**But what about our patients**

**We need pediatric specific data**

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**CCFA Sponsored Clinical Research Network: PRO-KIDS RISK Study**

1100 children with Crohn’s at diagnosis between 2008-2012
Follow-up to 2017

- **Study:**
  - DNA
  - Fecal microbiome
  - Immune reactivity to bacteria, food, infections etc
  - Environmental Exposures

  3 years

  160 – 200 patients with complication / surgery

**RISK Study: Ileal biopsy at the initial endoscopy**

- Age, gender and ethnicity were not statistically different.
- CD groups - perianal involvement, BMI, PCDAI were not statistically different.

- DNA >> 16s (Broad, Illumina MiSeq )
- RNA >> mRNA-seq (CCHMC, single end 50bp, Illumina HiSeq 2000)
RISK Study: Using Next Gen Sequencing to Classify the Intestinal Microbiome and Genome at Diagnosis

• Processed ~ 5300 intestinal biopsies from 950 CD, UC, IBDU patients and non-IBD controls
• DNA yield: 10,500 (8,468, 12,670) ng
• RNA yield: 11,490 (9,351, 13,640) ng
• RNA quality sufficient for PCR or RNASeq in >95%
• Microbiome: 1000 ng DNA
• RNASeq: 1000 ng RNA

RISK Study: Gene prioritizing for further analysis

<table>
<thead>
<tr>
<th>Top 5 up-regulated genes</th>
<th>IC3D-1 FC</th>
<th>IC3D-2 FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUOX2</td>
<td>43.6</td>
<td>50.2</td>
</tr>
<tr>
<td>MMP3</td>
<td>29.5</td>
<td>24.2</td>
</tr>
<tr>
<td>IQK9</td>
<td>29.4</td>
<td>35.0</td>
</tr>
<tr>
<td>IL8</td>
<td>23.5</td>
<td>26.0</td>
</tr>
<tr>
<td>DUOX2</td>
<td>14.3</td>
<td>24.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Top 5 down-regulated genes</th>
<th>IC3D-1 FC</th>
<th>IC3D-2 FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOA1</td>
<td>-8.6</td>
<td>-10.7</td>
</tr>
<tr>
<td>NAT8</td>
<td>-8.8</td>
<td>-9.8</td>
</tr>
<tr>
<td>AGXT2</td>
<td>-8.8</td>
<td>-9.5</td>
</tr>
<tr>
<td>CUBN</td>
<td>-9.0</td>
<td>-9.4</td>
</tr>
<tr>
<td>FAM151A</td>
<td>-10.4</td>
<td>-8.9</td>
</tr>
</tbody>
</table>

FC = fold change


RISK Study: A Core CD Ileal Gene Expression Signature Contains DUOX2 and APOA1 Co-expression Signatures

RISK Study: Microbial Profiling

• Ileal Firmicutes and Proteobacteria taxa abundance are associated with the APOA1 and DUOX2 gene coexpression signatures and clinical outcomes.
• 70 significant associations between gene expression and microbial taxa and 34 significant associations between clinical parameters and microbial taxa


So?

• Gene expression, microbial profiling, and clinical data used to model outcome compared to clinical data alone
• Neither age at dx or clinical disease activity by PCDAI predicted 6 month SSFR
• Higher APOA1 expression and and certain microbial taxa including Blautia (worse) and Veillonella (better) were prognostic factors

A multi-omic model is superior in predicting surgery and steroid free remission in comparison to clinical factors alone.

<table>
<thead>
<tr>
<th>The relative goodness of fit of the models, P &lt;0.0043</th>
</tr>
</thead>
<tbody>
<tr>
<td>C statistics (AUC)</td>
</tr>
<tr>
<td>Clinical variables only</td>
</tr>
<tr>
<td>Clinical, expression and microbial</td>
</tr>
</tbody>
</table>

Multiple regression analysis including clinical, gene expression, and microbial variables.

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age≥10 vs. &lt;10</td>
<td>0.8864</td>
<td>0.944</td>
<td>0.438,2.075</td>
</tr>
<tr>
<td>Deep DU vs. no DU</td>
<td>0.6244</td>
<td>0.771</td>
<td>0.272,2.388</td>
</tr>
<tr>
<td>PCDAI&lt;30</td>
<td>0.0002</td>
<td>4.713</td>
<td>1.701,13.067</td>
</tr>
<tr>
<td>Anti-TNF therapy</td>
<td>0.0020</td>
<td>5.181</td>
<td>1.828,14.706</td>
</tr>
<tr>
<td>APOA1 expression level &gt; 80th percentile</td>
<td>0.0173</td>
<td>5.181</td>
<td>1.828,14.706</td>
</tr>
<tr>
<td>Blautia abundant (≥70th percentile) vs non-abundant</td>
<td>0.0028</td>
<td>0.231</td>
<td>0.089,0.604</td>
</tr>
</tbody>
</table>

Microbiome was profiled in 800 RISK subjects enrolled at 28 pediatric centers in US/CAN 500 cases + 300 controls

RISK Study: The Microbiome shifts in pediatric Crohn’s disease: Decreased diversity, losses and gains

Determining the key players of microbial dysbiosis in new-onset pediatric CD

- Taxa identified as significant biomarkers for disease, including members of the Pasteurellaceae, Veillonellaceae, Neisseriaceae, and Fusobacteriaceae.
- Antibiotic exposure amplifies the microbial dysbiosis, by further loss of Bacteroides, Clostridiales, and Erysipelotrichaceae, and increase in Fusobacteriaceae and Enterobacteriaceae
- Hypothesis: Does your flora help determine your outcome? Can manipulation of the bacterial flora change the outcome?
Is it or is it not out of our hands? By the time we see someone is it too late?

THOUGHTS ON PREDESTINATION

Is Outcome Determined At the Time of Diagnosis or Does Timing and Specificity of Treatment Matter? (Does it matter what your genes or bugs are?)

Increased Effectiveness of Early Therapy With Anti-Tumor Necrosis Factor-α vs an Immunosuppressor in Children With Crohn’s Disease

Walters, Kim et al. RISK Study. Gastroenterology 2014;146:383

2008-2012: 552 children Newly Diagnosed with Crohn’s Disease Enrolled in CCFA RISK Study

Walters, Kim et al. RISK Study Gastroenterology 2014;146:383

Table 2: Characteristics of Patients at Diagnosis of Pseudopyloric Stenosis

Walters, Kim et al. RISK Study Gastroenterology 2014;146:383

2008-2012: 552 children Newly Diagnosed with Crohn’s Disease Enrolled in CCFA RISK Study

Walters, Kim et al. RISK Study Gastroenterology 2014;146:383

12 Month Outcomes For The Three Early Therapy Approaches: PCDAI≤10 Without Resection

Walters, Kim et al. RISK Study Gastroenterology 2014;146:383
### Relationship of Early Therapy to CRP at 12 months*

<table>
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<th>Early Treatment</th>
<th>Elevated CRP at 12 months given elevated CRP at baseline</th>
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</thead>
<tbody>
<tr>
<td>Anti-TNFα only</td>
<td>24%</td>
</tr>
<tr>
<td>IM only</td>
<td>44%</td>
</tr>
<tr>
<td>No early immunotherapy</td>
<td>53%</td>
</tr>
</tbody>
</table>

81% of all patients had an elevated CRP at baseline, no difference between treatment groups ($p=0.007$).

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### Growth Parameters of Study Triads at Diagnosis

#### Early Therapy

<table>
<thead>
<tr>
<th>Early Therapy</th>
<th>Height $z$-score</th>
<th>Weight $z$-score</th>
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<tr>
<td>Anti-TNFα only</td>
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<td>-0.81 (1.3)</td>
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**Difference between groups**: P=0.6 P=0.8 P=0.8

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### Growth Parameters of Study Triads at Diagnosis/One Year

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**Walters, Kim et al. RISK Study. Gastroenterology 2014;146:383**

### Table 3: Additional Therapies Beyond 3 Months for Study Triads

<table>
<thead>
<tr>
<th>Therapy type</th>
<th>Started between 3 and 6 mo</th>
<th>Started between 6 and 12 mo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early immunosuppression</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early immunosuppression + methotrexol</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + methotrexol + azathioprine</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine + MM</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine + MM + IBD</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine + MM + IBD + IL-6</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine + MM + IBD + IL-6 + TNF</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Early immunosuppression + biologic DM + methotrexol + azathioprine + MM + IBD + IL-6 + TNF + anti-TNF</td>
<td>0</td>
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**Walters, Kim et al. RISK Study. Gastroenterology 2014;146:383**

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* $p=0.002$, anti-TNFα group only
So,

- In clinically similar populations of children with moderately to severely active Crohn’s disease, early (<3 mon) therapy with anti-TNFα was superior to early IM or no early immunotherapy despite later addition of those agents.
- But there was no particular clinical or laboratory characteristic that helped predict response or non-response to an initial therapeutic decision.
- We need to better define further characteristics of patients, such as genetics, serology, microbiome, gene expression that help predict outcome.

But... THINK about your patients

- Are there currently known risk factors for doing poorly?
- Am I using therapy that is unlikely to change the history of the disease?
- Am I using current therapies properly?
- What is the safety profile of therapies I am using?
- What is the risk of undertreated disease?
Acknowledgements

- Children and families who have participated in RISK and PROTECT
- Research coordinators, laboratory technicians, administrative personnel
- Crohn’s and Colitis Foundation of America
- NIH (NIDDK): Steve James, Jose Serrano, Frank Hamilton, Dana Anderson

Antibiotics Associated with Increased Risk of New-Onset Crohn’s Disease But Not Ulcerative Colitis: A meta-analysis

![Diagram showing potential outcomes of microbiota and intestinal conditions](image)

Ungaro et al. Am J Gastroenterol 2014