
بسم الله الرحمن الرحيم

Laboratory Diagnosis of IBD

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Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, relapsing-remitting inflammatory disorder of the gastrointestinal (GI) tract. CD and UC vary in the region of the GI tract they affect; the extent of inflammation and resulting tissue damage; and their associated symptoms. UC tends to exhibit continuous superficial inflammation which spreads from the rectum and is limited to the colon (large intestine), with bloody diarrhoea as the most common symptom. Inflammation associated with CD is transmural (affects all layers of GI tract tissue), patchy (areas of inflammation are interspersed with healthy unaffected tissue) and can present anywhere along the entire GI tract and, in some cases, in the skin and joints. The symptoms of CD are more diverse than for UC, including diarrhoea, abdominal pain and weight loss.

The exact cause of IBD is unclear. There appear to be four main factors which influence the disease: host genetic susceptibility, a dysregulated immune response and impairment of intestinal epithelial barrier function, and environmental factors (Figure 1).

Genetic susceptibility

Genome-wide association studies (GWASs) have identified a number of genetic loci associated with IBD, including genes specific to either CD or UC, and some which are common to both (Figure 2). The risk of developing IBD conferred by variants at each locus is small for all but a few genes (e.g. *IL10RA* and *IL10RB*). It may be the case that IBD only occurs when multiple genetic variations associated with the disease are present. Another possibility is that environmental factors are required to trigger the onset of disease in genetically susceptible individuals.

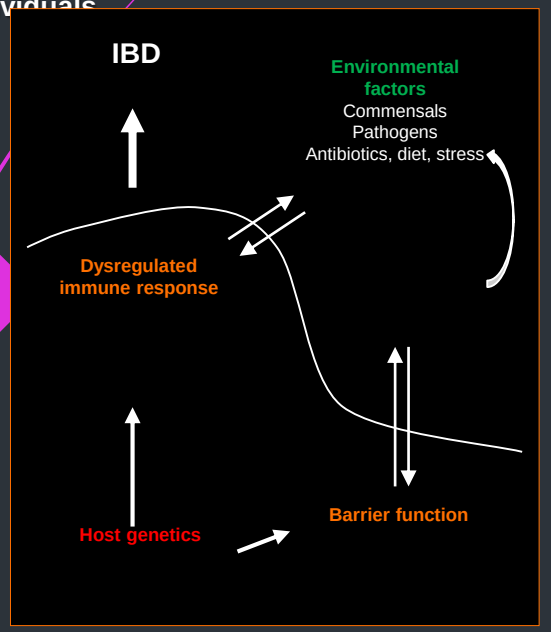

Figure 1. Factors internal and external to the host that influence inflammatory bowel disease (IBD).

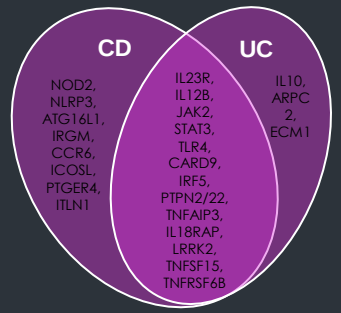

Figure 2. Genes associated with Crohn's disease (CD), ulcerative colitis (UC) or both forms of IBD, from recent genome-wide association studies and their meta-analyses.

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Environmental factors

Environmental factors such as the **commensal microflora**, pathogenic infections and metabolic factors are thought to play a role in the development and perpetuation of IBD. The **intestinal microbiota**, which is dominated by **bacteria**, but also includes **viruses**, **fungi** and **protozoa**, is crucial for development of the host immune system but also appears to be the target of the inflammatory response during IBD. The composition of the intestinal microbiota appears to be altered during disease, although whether this causes, or results from, intestinal inflammation is unclear. The effects of antibiotics, pathogenic infections and diet on IBD might be explained by their impact on the commensal microflora.

Inflammatory Bowel Disease
cont.

Dysregulated immune response

IBD is an **immune-mediated disease**, but is **not considered to involve autoimmunity**. The intestine contains an enormous antigenic load derived from the food and microbial flora present. Of the approximately 10^{14} bacteria in the intestines, the majority are harmless commensals which are beneficial to our health in numerous ways such as aiding digestion and preventing the colonisation of pathogenic species. The intestinal immune system is separated from this luminal content by a single epithelial cell layer, and must initiate the appropriate response – tolerance or protective immunity – upon exposure to each antigen. IBD is thought to arise when an inappropriate immune response is mounted against commensal bacteria. GWASs and experimental results have indicated a number of facets to this dysregulation, for example pro-inflammatory pathways driven by IL-23, decreased immune regulatory mechanisms, and defective barrier function of the intestinal epithelium. **Figure 3** shows some key cell populations and mediators thought to be involved in intestinal inflammation. Monoclonal antibody-mediated blockade of the pro-inflammatory cytokine TNFa is very effective at reducing disease in many cases of IBD, highlighting a key role for this molecule in intestinal inflammation. Patients with disease refractory to this treatment, which is only employed after the failure of other therapies such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and immunosuppressants, necessitate the discovery and development of novel therapeutic strategies.

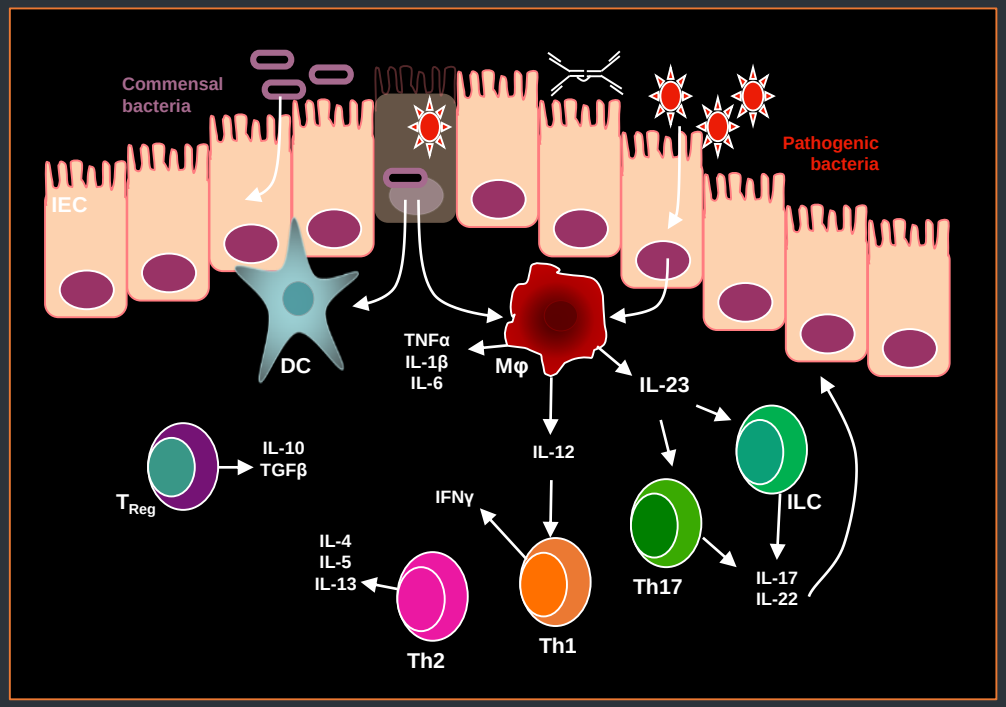

Figure 3. Key cellular populations and mediators in intestinal homeostasis and the pathogenesis of inflammatory bowel disease.
DC, dendritic cell; IEC, intestinal epithelial cell; ILC, innate lymphoid cell; Mφ, macrophage; sIgA, secretory IgA; Th, helper T cell; T_{Reg}, regulatory T cell.

Key references

Maloy and Powrie. 2011. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*.
Kaser, Zeissig and Blumberg. 2010. Inflammatory Bowel Disease. *Annu. Rev. Immunol.*

IBD Mimics: Most Common Conditions Misdiagnosed as IBD

Infectious

Small intestine / Terminal ileum

- Coccidioides
- Yersinia
- Tuberculosis
- Histoplasma
- Salmonella

Colon

- *C. difficile*
- CMV
- Salmonella
- Shigella
- *E. coli*
- Campylobacter
- Aeromonas
- Amebiasis (*E. histolytica*)

Always RULE OUT infection (CMV, *C. diff* esp.) obtain travel & exposures history

Non-Infectious

Granulomas present

- Sarcoidosis, Small vessel vasculitides
- Hermansky-Pudlak syndrome
- CVID (common variable immunodeficiency)

Ulcers in mouth & small/large intestine

- Behcet's disease

Colon inflammation

- Diverticulitis
- SCAD (segmental colitis associated with diverticulosis)
- Drug induced colitis (NSAIDs, immuno-therapy)
- Ischemic colitis
- SRUS (solitary rectal ulcer syndrome)

Non-specific mucosal changes without chronic inflammation

- IBS (irritable bowel syndrome)
- Cancer: Adenocarcinomas, GI lymphomas, others

The unknowns in IBD

- Inflammatory bowel diseases (IBD) comprise a group of long- term conditions , including ulcerative colitis and Crohn's disease.

- IBD can vary a lot from person to person and there are still many unknowns – scientists don't yet have all the puzzle pieces in place to see the full picture of exactly what drives the disease in each individual and why some people respond to treatments and others don't.

- Better understanding the intricacies of IBD is key to ultimately developing treatments that target the underlying causes of this disease.

Inflammatory Bowel Disease
cont.

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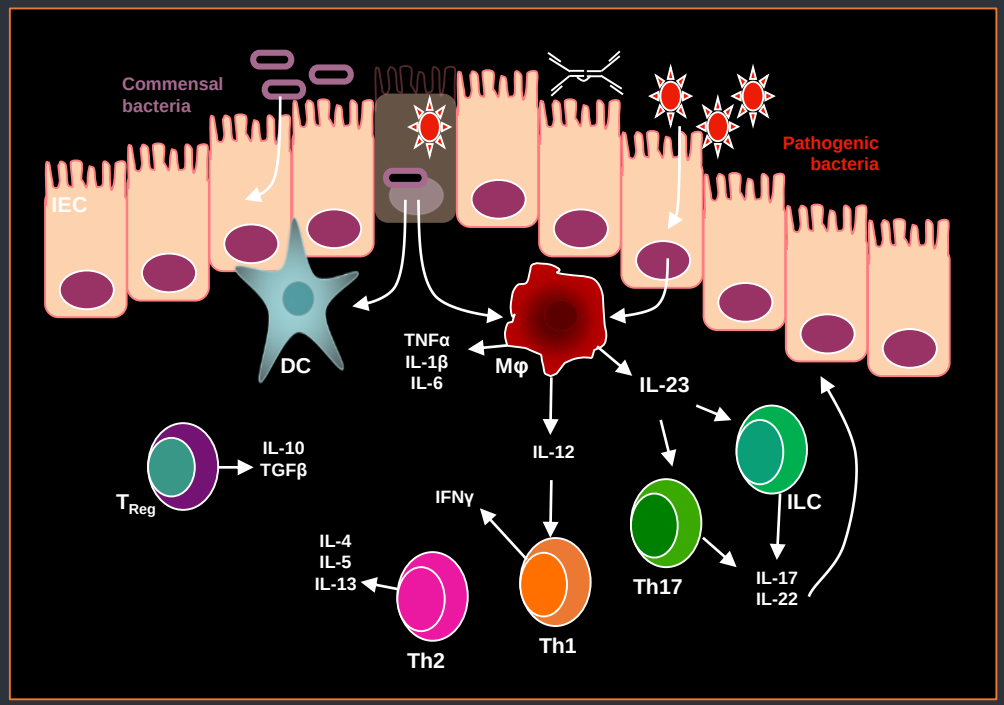

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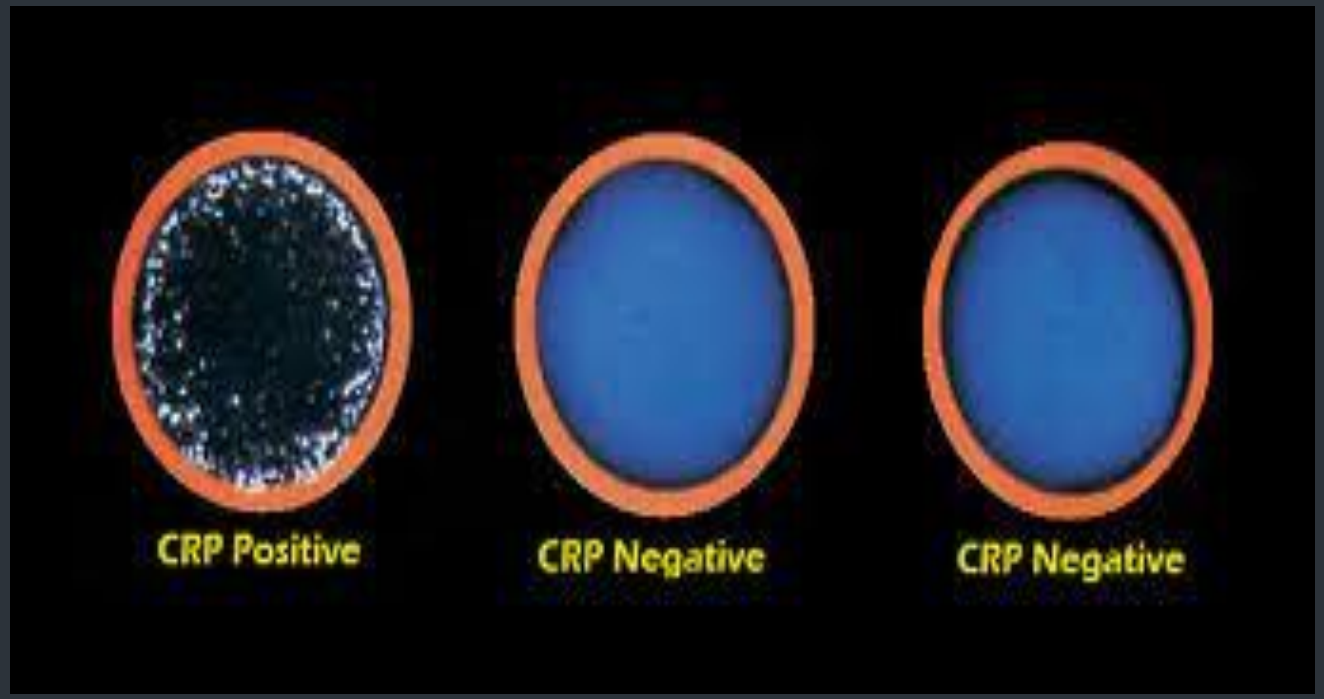
ESR

CRP

- In CD, serum concentrations of C-reactive protein (CRP) correlate well with disease activity and with other markers of inflammation as the CD activity Increased CRP (45 mg/L) in patients with IBD predicts with a high certainty the need for colectomy.

CRP Agglutination test



Elisa test plate

Automation

CBC

NLR

The neutrophil/lymphocyte ratio (NLR) has been gaining increasing attention across many fields of medicine within the past five years. Currently, there are 2,230 publications about this in PubMed, mostly within the past few years.

Neutrophil-to-Lymphocyte Ratio

The NLR is simply the number of neutrophils divided by the number of lymphocytes. Under physiologic stress, the number of neutrophils increases, while the number of lymphocytes decreases. The NLR *combines* both of these changes, making it more sensitive than either alone:

NLR

Endogenous cortisol and catecholamines may be major drivers of the NLR. Increased levels of cortisol are known to increase the neutrophil count while simultaneously decreasing the lymphocyte count.[1] Likewise, endogenous catecholamines (e.g. epinephrine) may cause leukocytosis and lymphopenia.[2] Cytokines and other hormones are also likely to be involved.

Thus, NLR is *not* solely an indication of infection or inflammation. *Any* cause of physiologic stress may increase the NLR (e.g. hypovolemic shock).

NLR increases rapidly following acute physiologic stress (<6 hours).[3] This prompt response time may make NLR a better reflection of acute stress than labs which are more sluggish to respond (e.g. white blood cell count or bandemia).[4]


COVID Rescue
Corticosteroids
COVID
COVID
Corticosteroid use advised
High Risk of Mortality
NLR>6.11
Warning
NLR≤6.11
Low Risk of Mortality
Use of Corticosteroids is not advised

Fecal Lactoferrin, Stool Lactoferrin

To detect inflammation in the intestines; to help identify active inflammatory bowel disease (IBD); to distinguish between IBD and non-inflammatory bowel conditions; to monitor IBD activity

Lactoferrin

Calprotectin, fecal

- in vitro diagnostic to aid in the diagnosis of inflammatory bowel disease (IBD), Crohn's disease, and ulcerative colitis, and to differentiate IBD from irritable bowel syndrome (IBS) in conjunction with other clinical and laboratory findings.

Elisa test plate

RA/1000

ANTIBODIES & AUTOANTIBODIES

Anti-Saccharomyces cerevisiae Antibodies Increased concentrations of antibodies to the baker's and brewer's yeast Saccharomyces cerevisiae (ASCAs) are found in patients with CD (36). Both IgG and IgA antibodies are formed. They have been demonstrated in 60%–70% of patients with CD, 10%–15% of patients with UC, and 0%–5% of control individuals

AUTOANTIBODIES

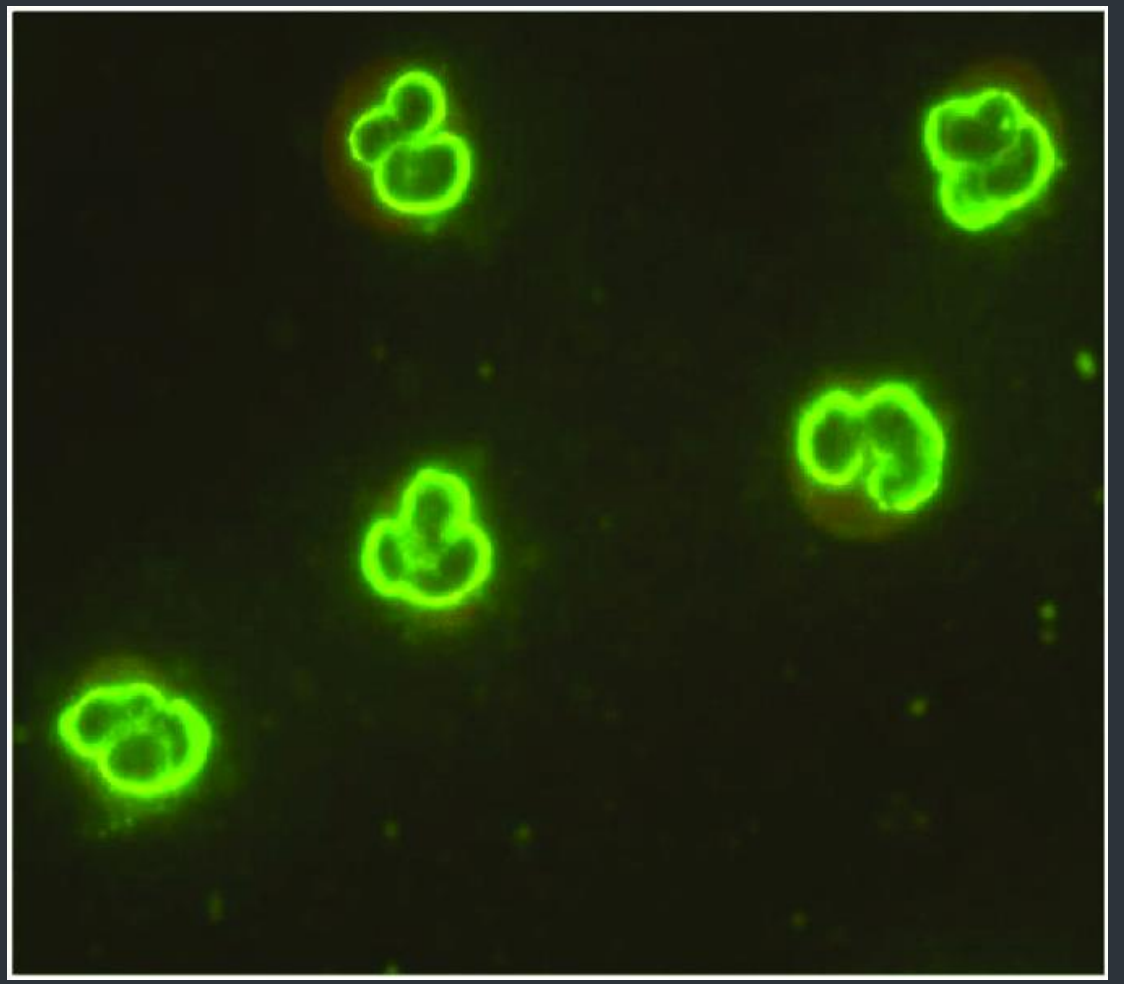
ANCAs have also been reported in patients with chronic inflammatory disorders, such as UC (60%– 80%) (8, 9), primary sclerosing cholangitis (88%) (10), autoimmune hepatitis (81%) (11), and to a lesser extent, CD (5%–25%)

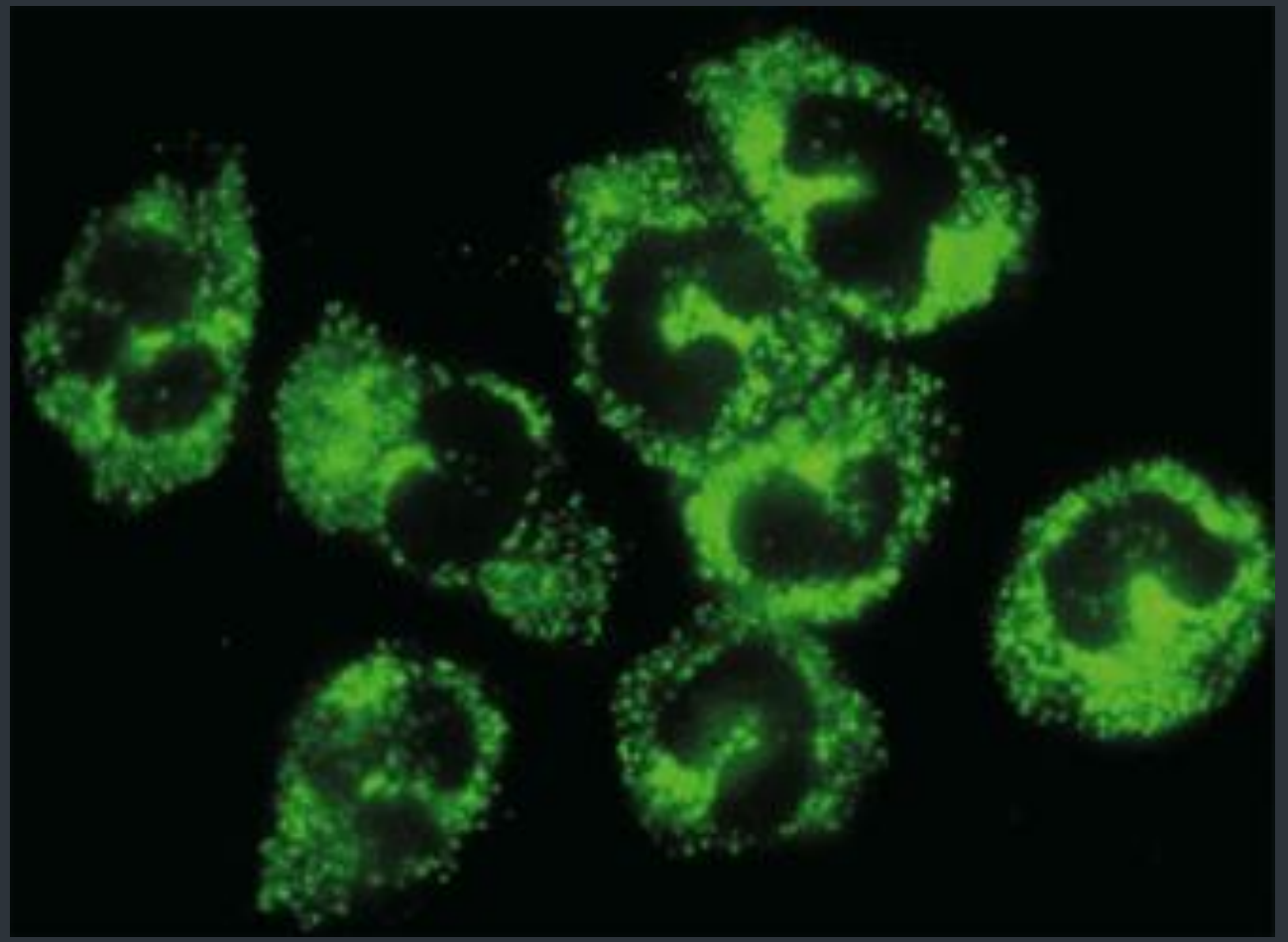
Autoantibodies to Neutrophils: ANCAs and Atypical P-ANCAs

- ANCAs have also been reported in patients with chronic inflammatory disorders, such as UC (60%– 80%) (8, 9), primary sclerosing cholangitis (88%) (10), autoimmune hepatitis (81%) (11), and to a lesser extent, CD (5%–25%). In these disorders, a (atypical) P-ANCA staining pattern is usually found. The antigen is not myeloperoxidase. The atypical P-ANCA is characterized by a broad inhomogeneous rim-like staining of the nuclear periphery (12).

Diagnostic Value of Autoantibodies to Neutrophils (Atypical P-ANCAs) and ASCAs in IBD

- The combination of atypical P-ANCAs and ASCAs may be useful in the differential diagnosis of UC and CD in patients with IBD. A survey of studies (35, 40 – 42, 46) that combined the 2 markers to distinguish UC from CD is given in Table 5. The CD-associated pattern was ASCA/P-ANCA, whereas the UC-associated pattern was ASCA/P-ANCA. The combined evaluation of ANCAs and ASCAs had a higher specificity (90% in most studies and 80% in all studies) to differentiate CD from UC than the separate use of either ANCAs or ASCAs. The increased specificity, however, was associated with decreased sensitivity.





The combined use of atypical P-ANCA and ASCA test results substantially affects pretest–posttest probability in distinguishing UC from CD in patients with IBD. The P-ANCA/ASCA combination is specific for UC, whereas the ASCA/P-ANCA combination is specific for CD. This may be of help in patients in whom distinction between CD or UC is not obvious with the classic diagnostic tools (patient history, radiologic examination, endoscopy, and biopsy).

In addition, the presence of ASCAs is stable over time and is independent of CD activity and duration (49, 52). ASCA titers most often remain stable after treatment (52). Hence, serial measurement of ANCA and ASCA titers in IBD is not useful for follow-up of disease activity and prediction of relapses

Adverse Effects: Treatments for Moderate-Severe IBD

Medication	Possible Adverse Effects
Thiopurines	Bone marrow suppression, hepatotoxicity, pancreatitis, pneumonitis, GI upset, rash, alopecia, fever, arthralgia, lymphoproliferative disorders, myeloid neoplasias, hepatosplenic T-cell lymphoma (young males), non-melanoma skin cancer, hemophagocytic lymphohistiocytosis (after EBV or CMV infection)
Methotrexate	Bone marrow suppression, alopecia, hepatic fibrosis, hypersensitivity pneumonitis, increased risk of infection
Anti-Tumor Necrosis Factor Agents	Increased risk of infection, infusion or injection site reactions, dermatologic and neurologic manifestations, melanoma, lymphoma
Vedolizumab	Upper respiratory tract infections, infusion related reaction
Natalizumab	Headache, rash, nausea, increased risk of infection, infusion related reaction, arthralgia, progressive multifocal leukoencephalopathy (in those with +JC virus antibody)
Ustekinumab	Injection site reaction, cold symptoms, headache, fatigue, increased risk of infection
Tofacitinib	Herpes Zoster, lipid abnormalities, venothromboembolism (specifically pulmonary embolism)

Prior to Starting Medications

Medication	Testing Prior to Starting	Recommended Monitoring
Mesalamines	Consider baseline renal function test	Annual renal function monitoring
Corticosteroids		Document plan for long-term therapy, consider ophthalmology exam, DEXA.
Thiopurines	TPMT enzyme activity, CBC and liver function	Routine CBC and liver function while on therapy
Methotrexate	CBC, liver and renal function	Routine CBC, liver and renal function monitoring while on therapy
Anti-Tumor Necrosis Factor Agents	TB screening prior to start, check Hepatitis B panel, CBC and liver function	Assess for TB exposure annually while on therapy; CBC and liver function routinely while on therapy
Vedolizumab	CBC and liver function	CBC and liver function periodically while on therapy
Natalizumab	Enrollment in CD Touch® Prescribing Program	Assess for signs/symptoms suggestive of PML, routine CBC and liver function testing, JC virus antibody testing every 6 months, per CD Touch® Prescribing Program
Ustekinumab	TB screening prior to start, check Hepatitis B panel, CBC and liver function	Assess for TB exposure annually while on therapy; CBC and liver function routinely while on therapy
Tofacitinab	CBC, liver, fasting lipid panel and TB	Assess for TB exposure annually while on therapy, routine CBC and liver function monitoring while on therapy; repeat fasting lipid panel 4-8 weeks after start of therapy.

1. Adapted from: Crohn's & Colitis Foundation; Diagnosing and Monitoring IBD Brochure. 2. Farray FA et al. Am J Gastro 2017;112(2):241-258. 3. Tysabri [Medication Guide/Package Insert]. Cambridge, MA: Biogen Inc. Revised August 2019.
4. Vedolizumab [Medication Guide/Package Insert]. Deerfield, IL: Takeda Pharmaceuticals, Inc; May 2014
5. Ustekinumab [Medication Guide/Package Insert]. Janssen Biotech, Inc. September 2016. 6. Tofacitinib [Medication Guide/Package Insert]. NY, NY: Pfizer; Revised December 2019.

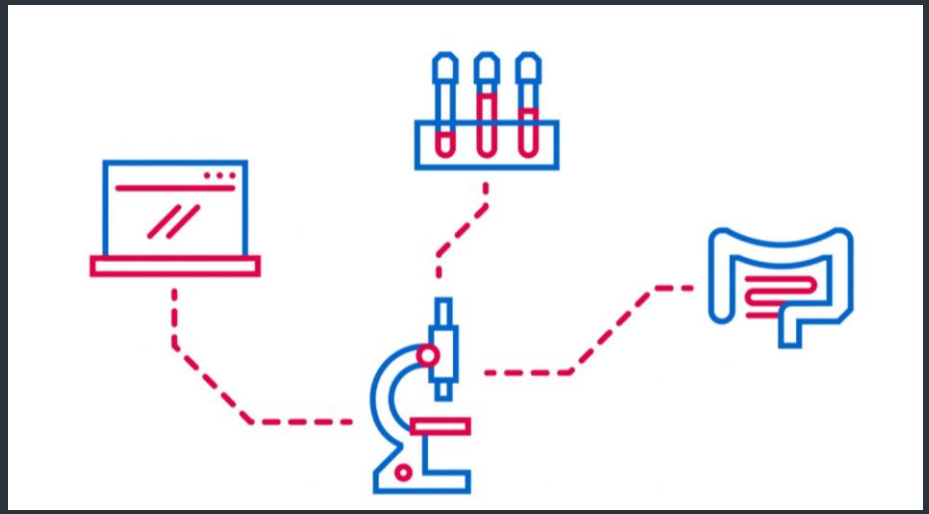

TPMT
Activity
NORMAL
INTERMEDIATE
LOW
MINIMAL
MODERATE
HIGH
Toxicity
Risk

TPMT Testing The simplest is the most effective

- Detection of individuals with low thiopurine methyltransferase (TPMT) activity who are at risk for excessive myelosuppression or severe hematopoietic toxicity when taking thiopurine drugs.

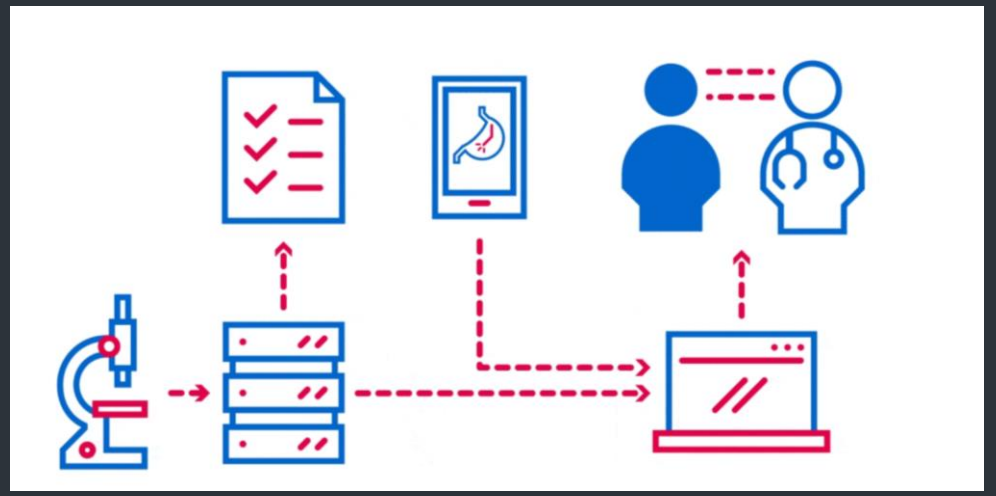
today

- Three common tools used to diagnose inflammatory bowel diseases (IBD) are blood tests, stool tests and endoscopy/colonoscopy. These tests help exclude other causes of the patient's symptoms and confirm the IBD subtype.
- Individuals are then risk-assessed as having mild, moderate or severe ulcerative colitis or Crohn's disease.



Ideal future

- Data collection
- In an ideal future with personalised healthcare, large quantities of data will be integrated from multiple sources, including molecular information, clinical data, stool samples, images from colonoscopy and real world data such as patient-reported outcomes and data derived from digital technology.
- Advanced analytics
- With modern technology, machine learning and ‘artificial intelligence’, the data will be assessed in order to receive a detailed view of the disease. We would be able to identify previously-unrecognised patterns of the disease, to better understand which patient will benefit from which treatment.



Personalised Med.

"Here's my DNA sequence."


GOD
BLESS
YOU
BIGSTOCK