Australasian Gastrointestinal Pathology Society
4th Annual Scientific Meeting
27 & 28 October 2018
Clinical Education Centre, Auckland Hospital, New Zealand
www.agps.org.nz

Opportunistic Infections of the Colon in Inflammatory Bowel Disease

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COUNTIES MANUKAU HEALTH

Second European Consensus on prevention, diagnosis and management of infections in IBD on behalf of the European Crohn's and Colitis Organisation (ECCO)

- Treatment of IBD revolutionized over past decade by immunomodulators
- Increased risk of opportunistic infections
- Often difficult to recognize
- Associated with appreciable morbidity and mortality

J.F. Rahier, F. Magrob, C Abreue, et al. Journal of Crohn's and Colitis. 2014; 8: 443–468

"Despite evidence of defective mucosal immunity, there is no proof of a systemic immune defect in patients with IBD in the absence of concomitant immunomodulator therapy." ECCO

- Immunomodulators in IBD:

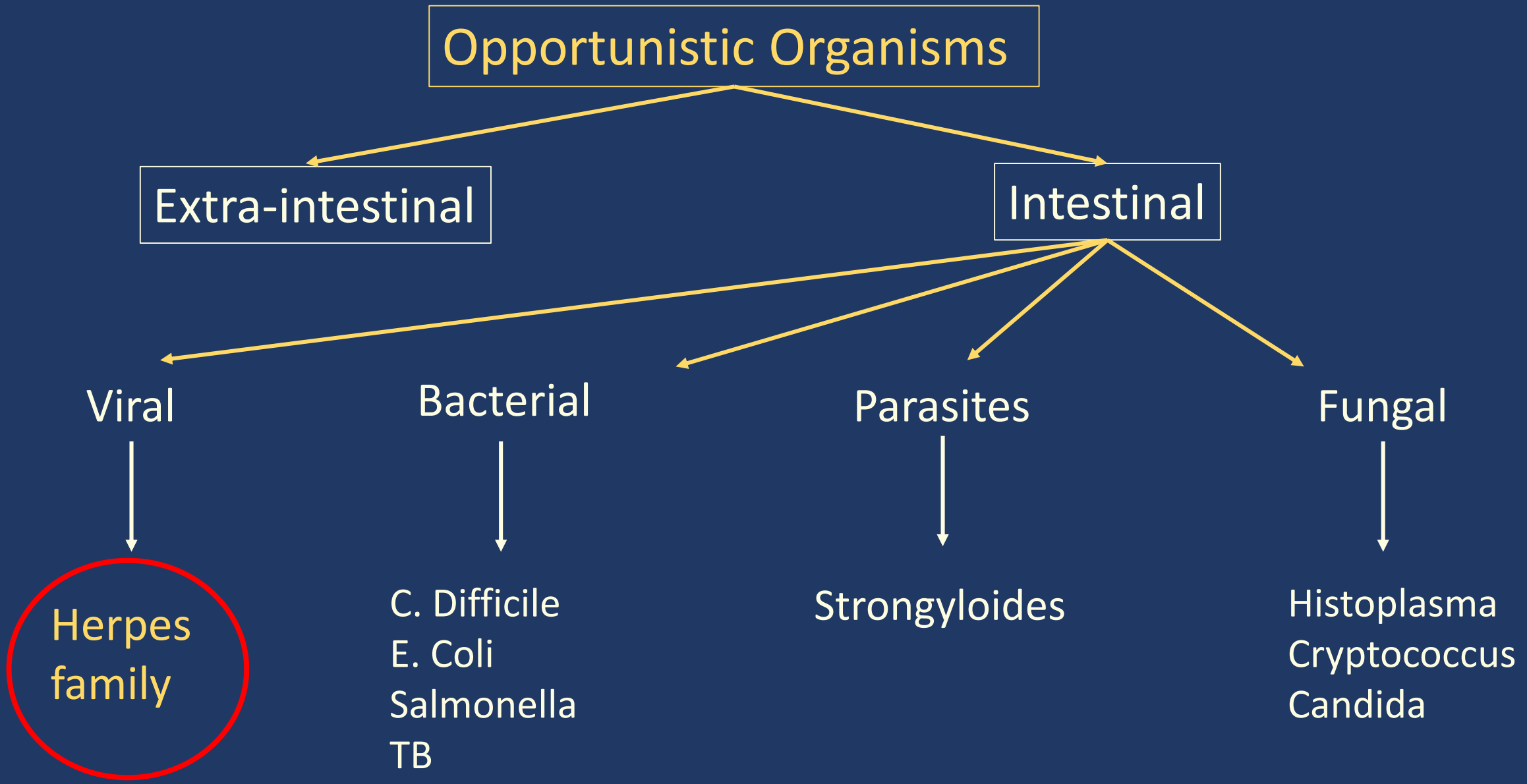
  Corticosteroids (≥20 mg of prednisolone/day for ≥2 weeks), thiopurines, methotrexate, calcineurin inhibitors, anti-TNF agents, other biologics

- Relative risk of opportunistic infection increases with no of drugs:

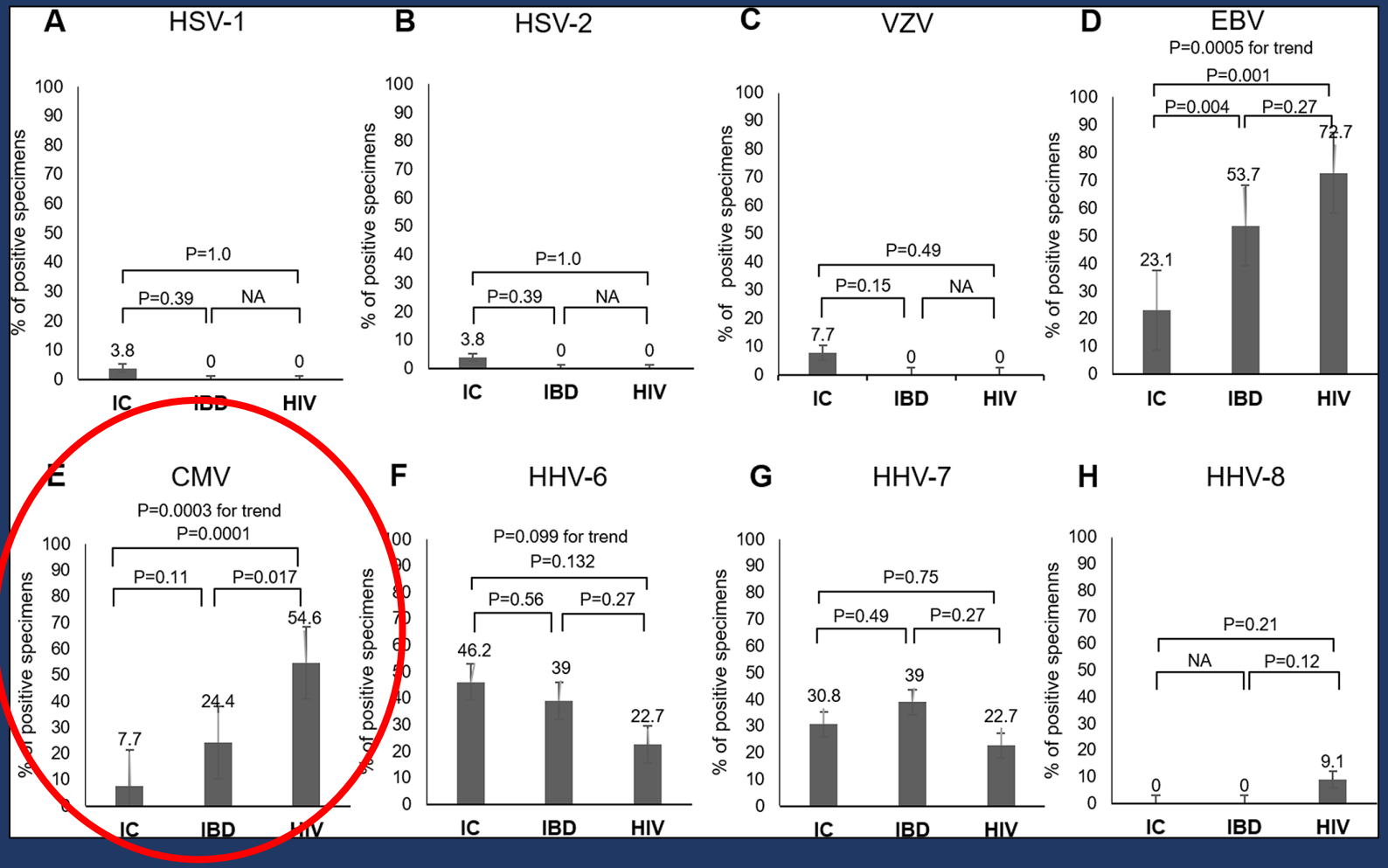
  1x immunomodulator: 3x increased risk (**OR 2.9**, 95% CI 1.5–5.3)

  2+ immunomodulators used concomitantly: increases substantially

  (**OR 14.5**, 95% CI 4.9–43)


Opportunistic Organisms
Extra-intestinal
Intestinal
Viral
Bacterial
Parasites
Fungal
Herpes family
C. Difficile
E. Coli
Salmonella
TB
Etc
Strongyloides
Histoplasma
Cryptococcus
Candida

Herpes family: Colonic mucosal ulcers - PCR positivity rate



IC = immunocompetent, IBD = Inflammatory Bowel Disease

Shimada 2017

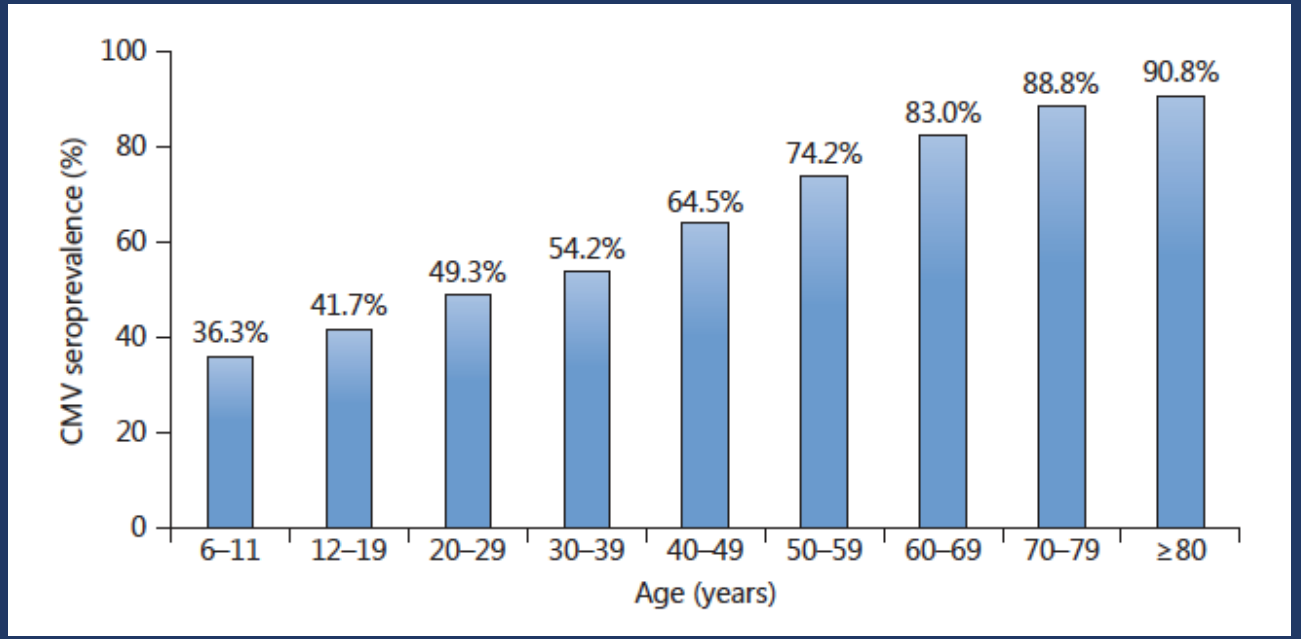
CMV – Epidemiology and Life Cycle

- Ubiquitous
- Transmitted in body fluid
- Prevalence: Australia:

  seropositivity 1-59 years = 57%

  (National serosurvey 2006)

Lancini 2014

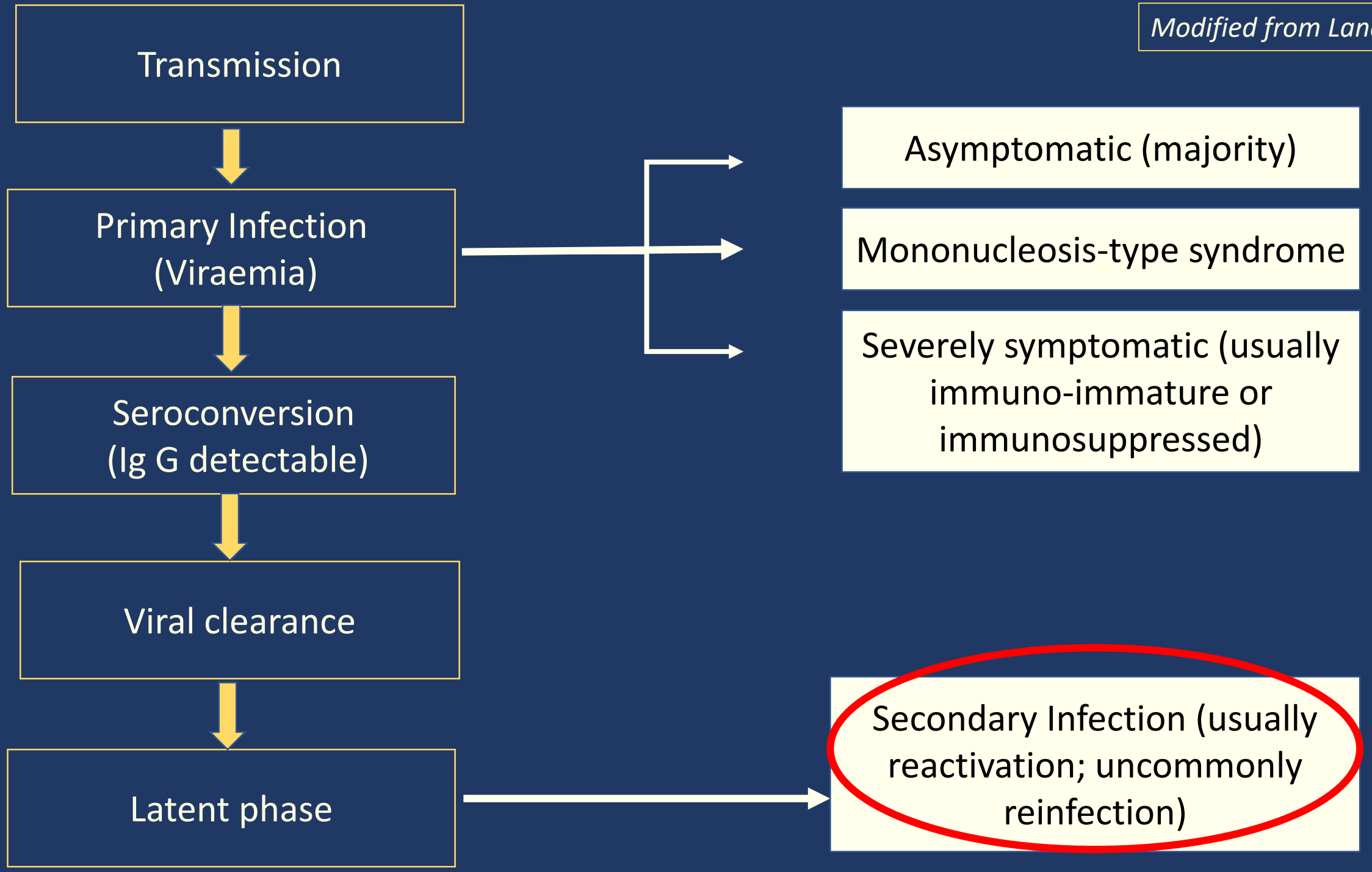


CMV seropositivity general population USA 1988-1994

Published in the third National Health and Nutrition Examination Survey (1988–1994). Cited in Nakase 2016

Virus remains latent for life with potential to reactivate

Modified from Lancini 2014



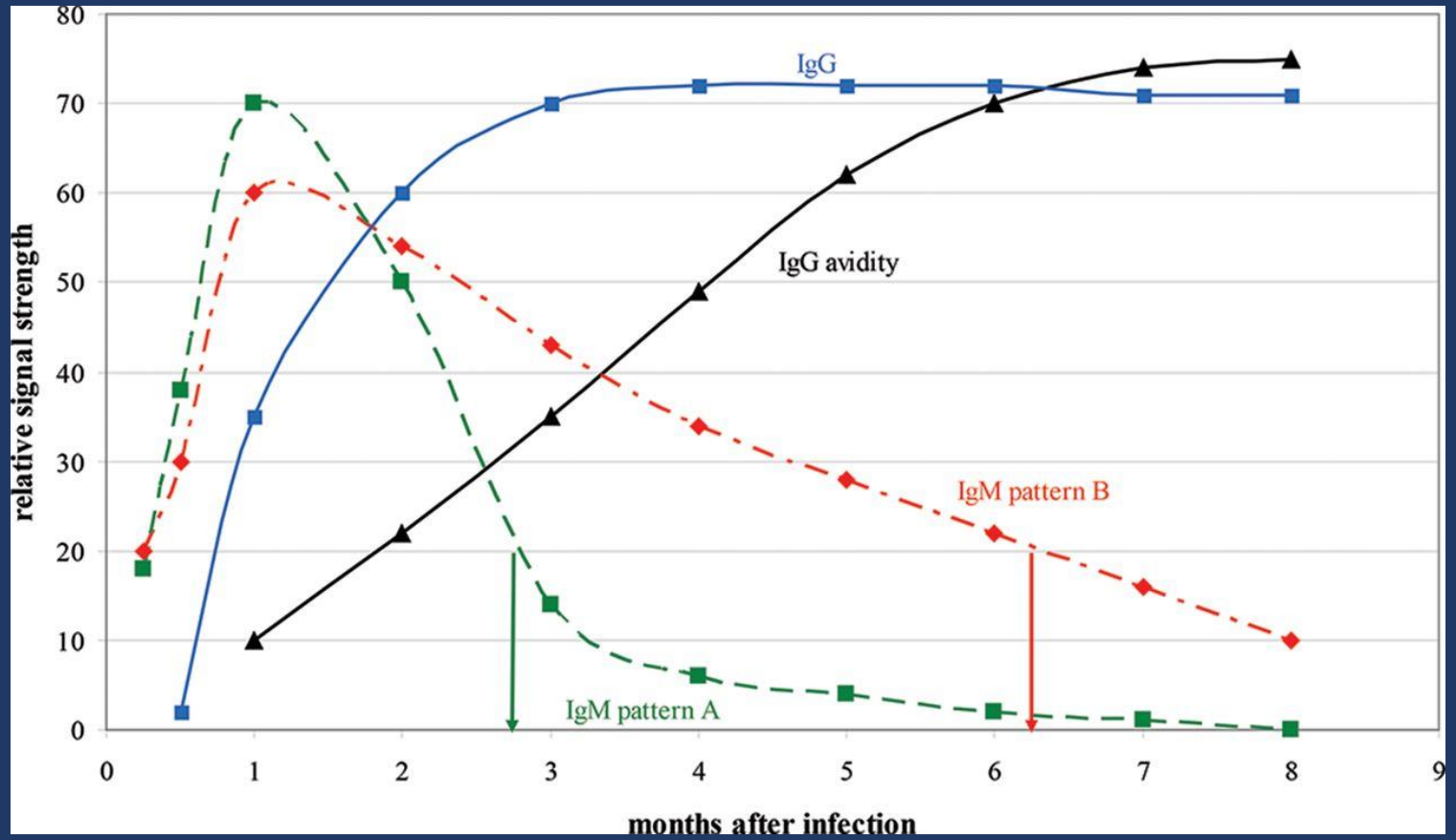
Transmission

Primary Infection
(Viraemia; IgM detectable)

Seroconversion
(Ig G detectable)

Viral clearance

Immune response – both cellular and humoral

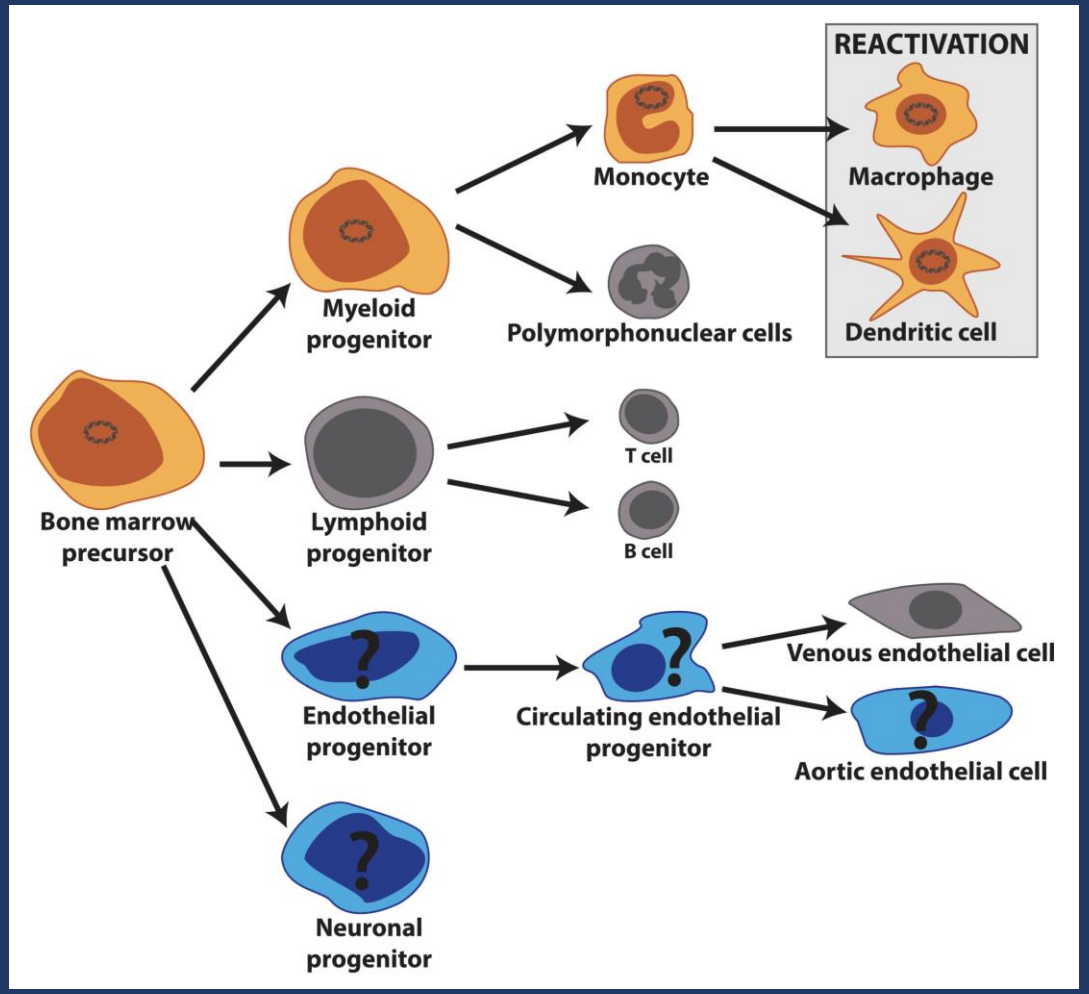


Low avidity = more recent infection
High avidity = longer time from infection

Prince 2014

Latent phase

- Cellular immunity controls viral replication. Incomplete clearance = latency
- DNA in episomal form (non-integrated) in host cell nucleus
- Minimal viral expression and no viral particle production
- Present in low numbers in small proportion of mononuclear cells (frequency of 0.004 to 0.01% on ISH)



Human cytomegalovirus natural latency in cell lineages

Dupont 2016

Reactivation

- Colon common site
- Often asymptomatic and self limiting
- Triggered by immunosuppression and inflammation
- Risk correlates with degree of immunosuppression
- Some suggest reactivation in immunocompetent hosts under-recognised

CMV Infection ≠ CMV Disease

Isolation of the CMV virus or

detection of viral proteins or

nucleic acid in any body fluid or

tissue specimen

Combination of

(1) Clinical symptoms,

(2) Endoscopic mucosal lesions

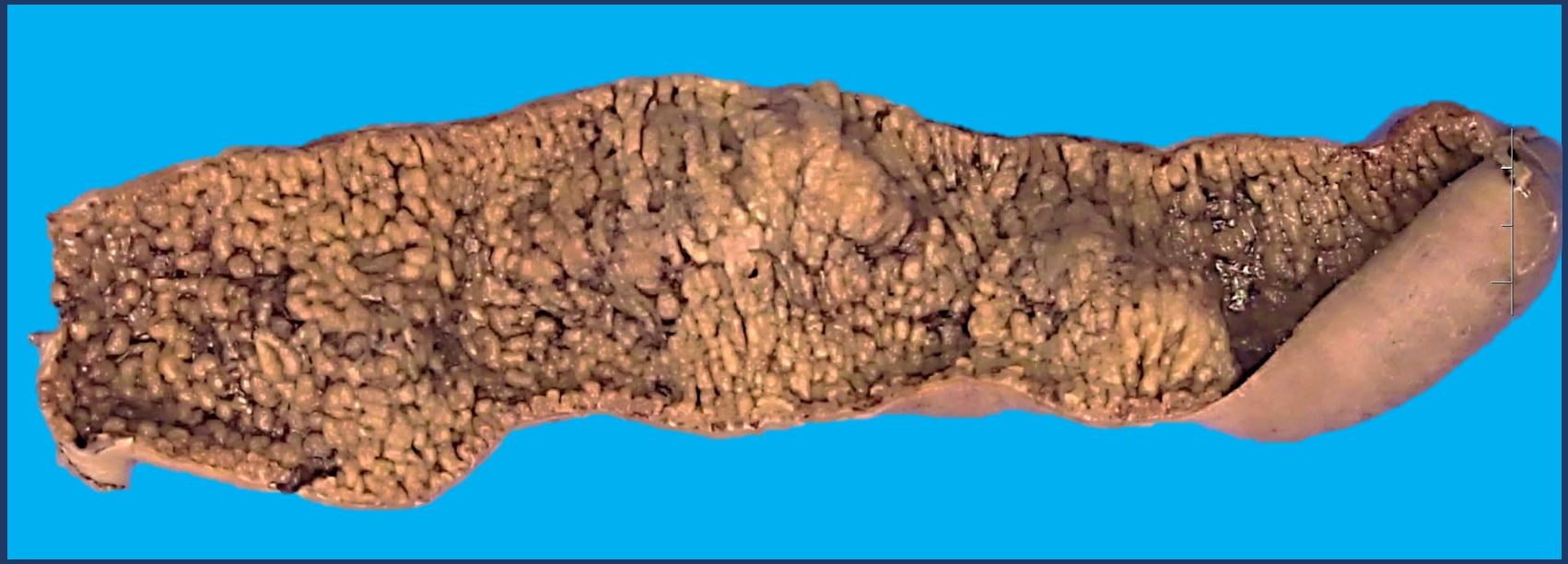
(3) Demonstration of CMV infection in GIT

CMV Disease requires
evidence of end organ damage

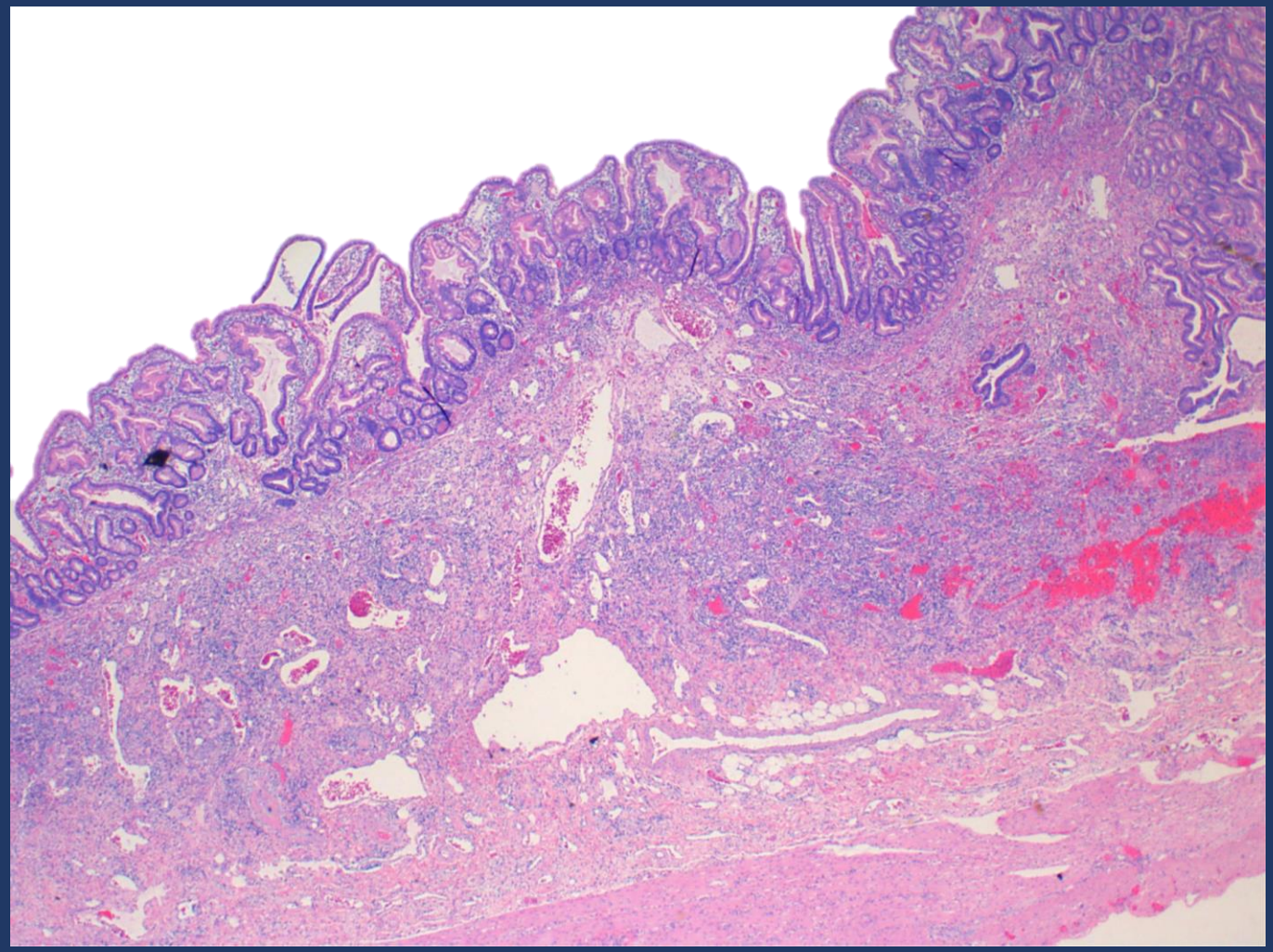
Ljungman 2002

Case presentation

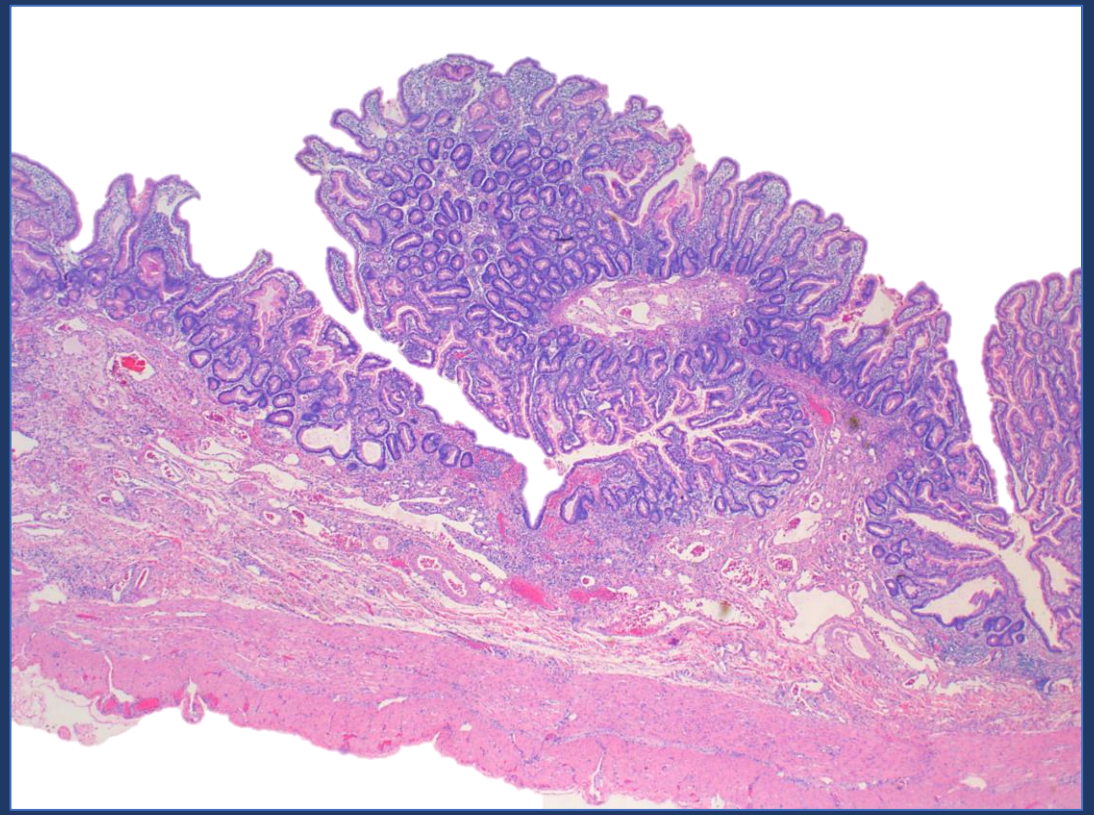
- Young woman with autoimmune disease
- On several immunomodulators with azathioprine added in months prior to presentation
- Developed abdominal symptoms over several months
- Post admission experienced significant rectal bleed requiring resuscitation
- Surgery - bowel grossly unremarkable
- On table endoscopy – markedly abnormal segment of small bowel - resected



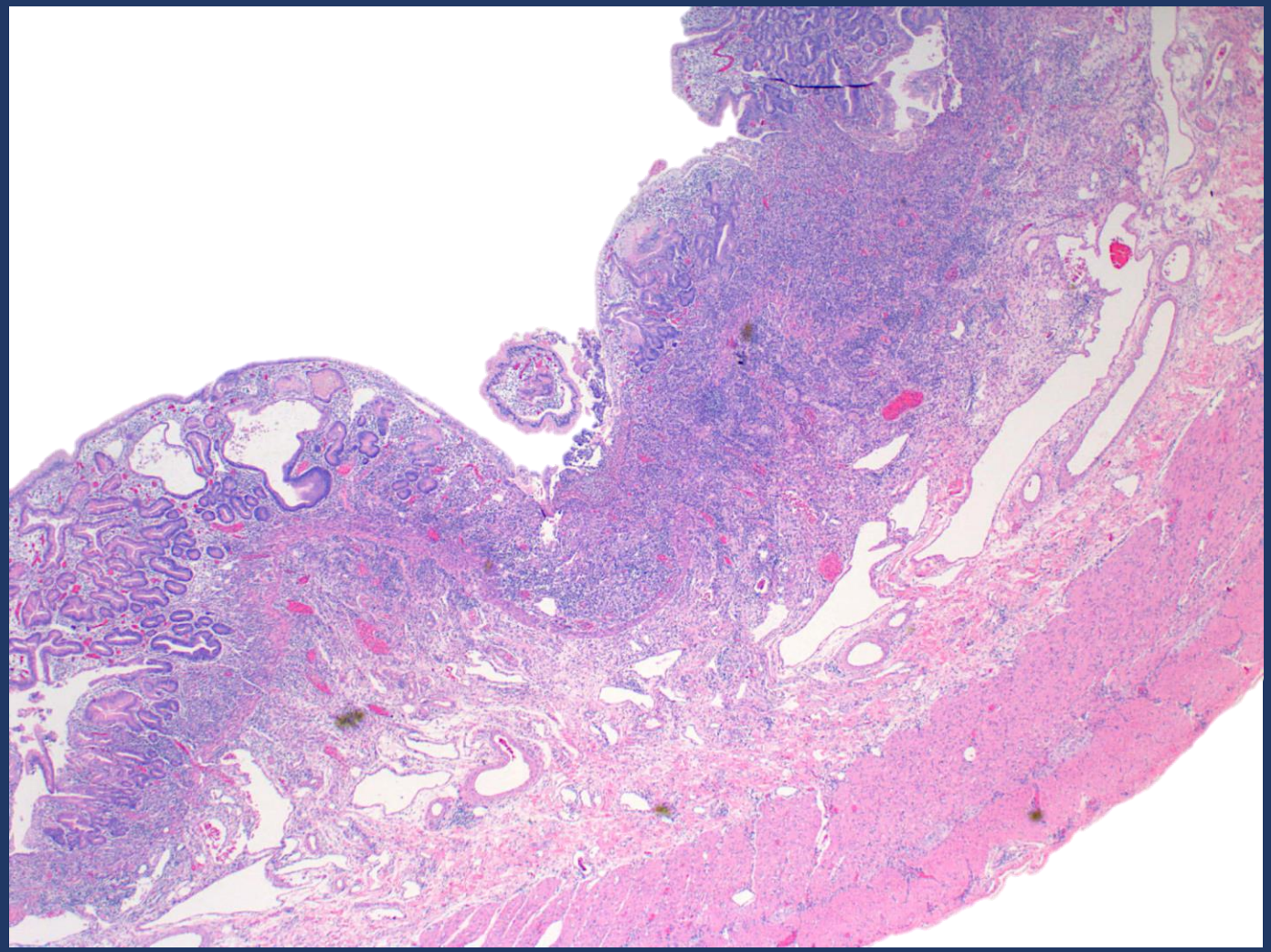
Jejunum, 260mm
Polypoid mucosa along
entire length of specimen

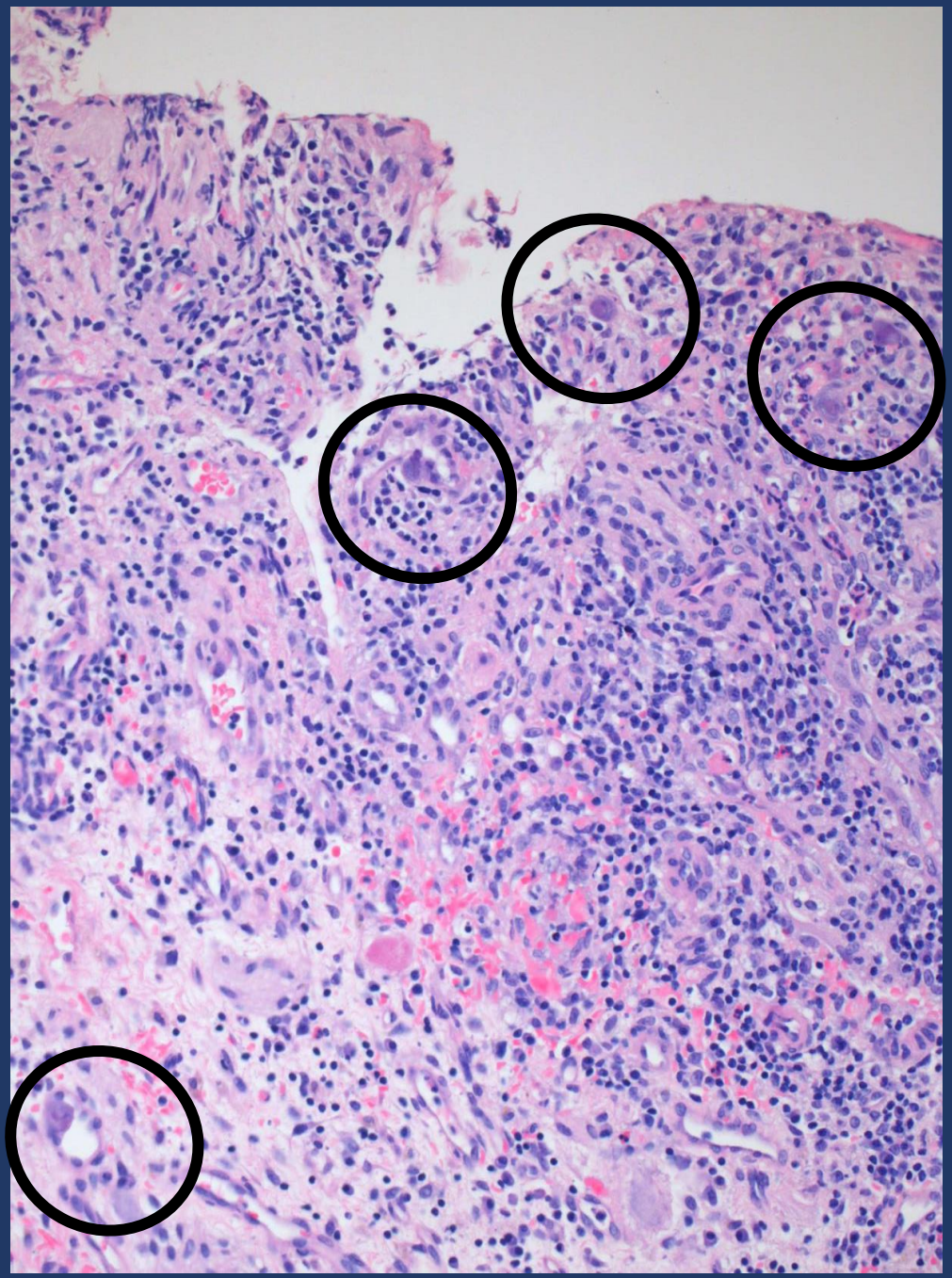


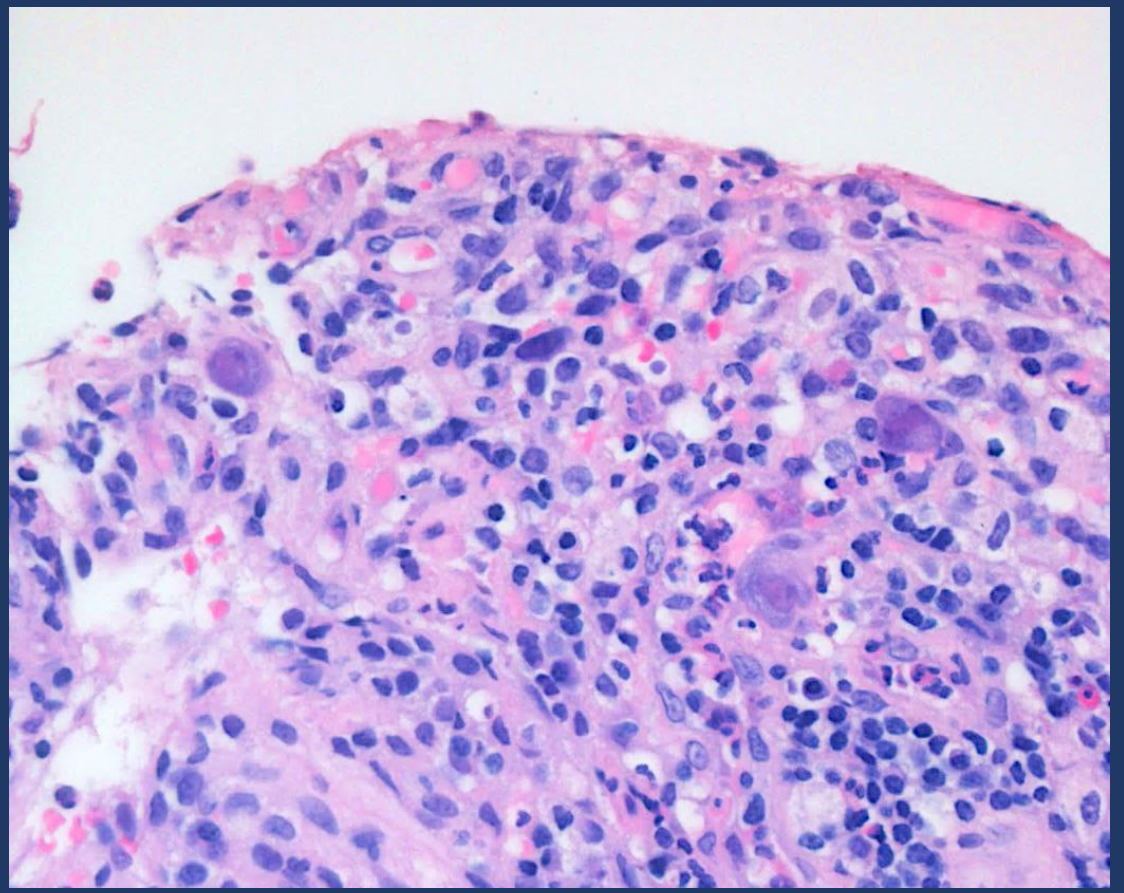
Inflamed and oedematous mucosa with pseudopolyps



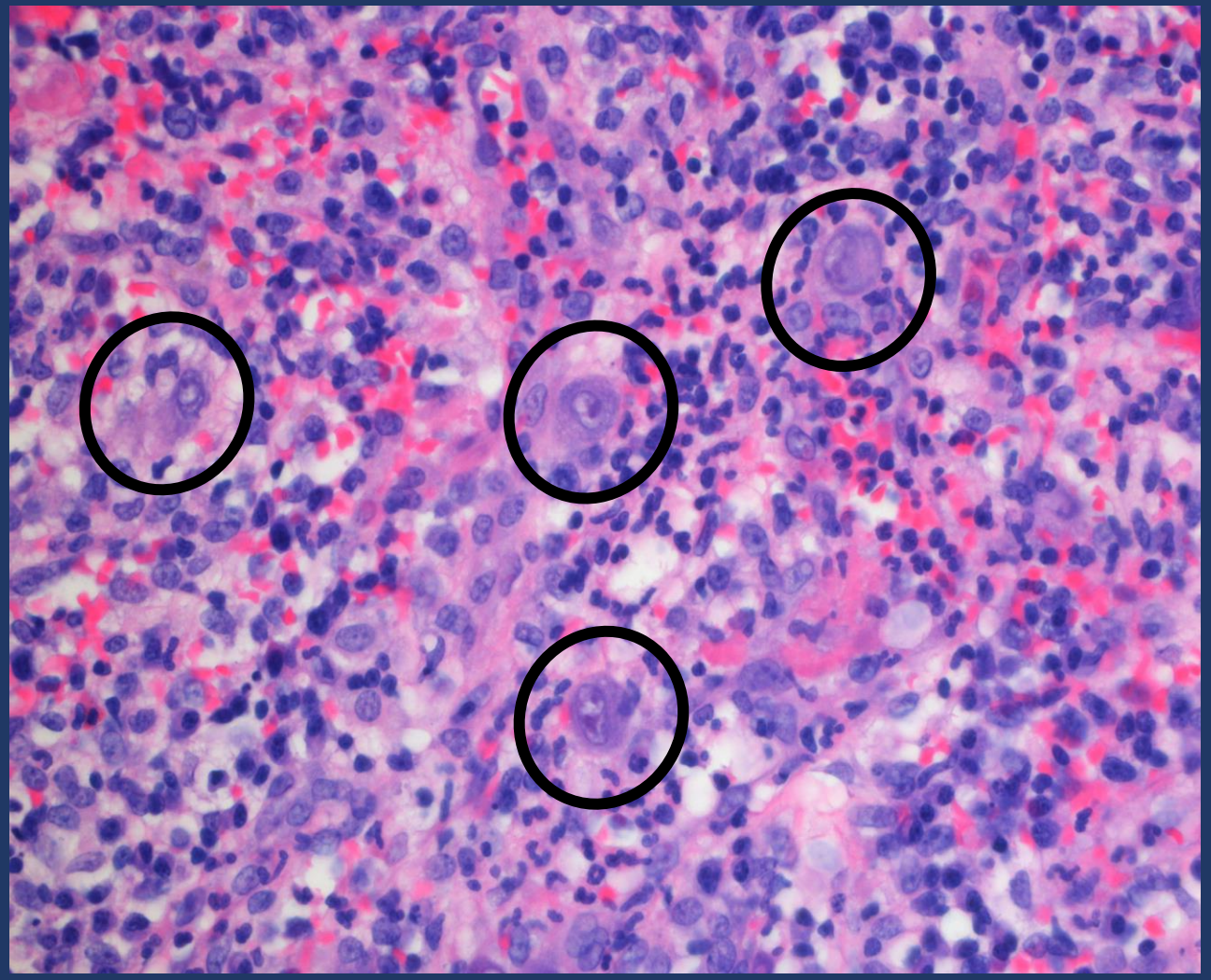
- Multiple broad shallow ulcers
- No deep fissuring ulcers
- No transmural lymphoid aggregates
- No granulomas



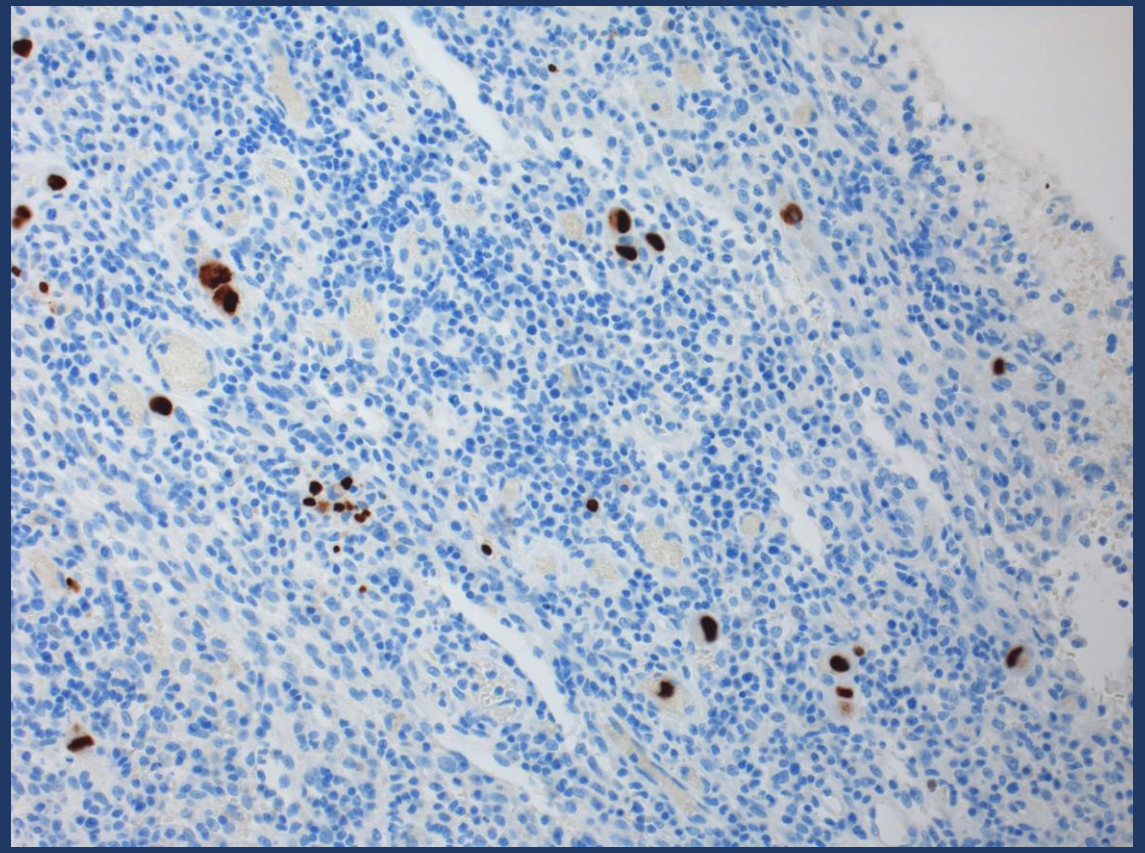




Multiple large atypical cells in ulcer bed

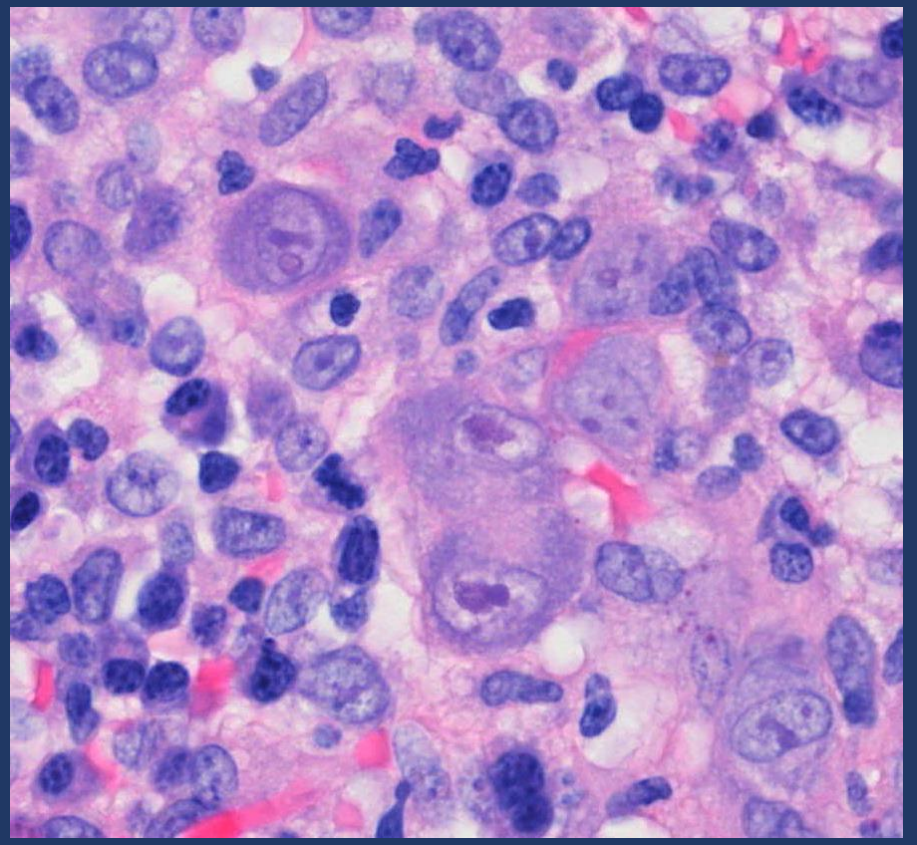


Numerous CMV positive cells on immuno-histochemistry



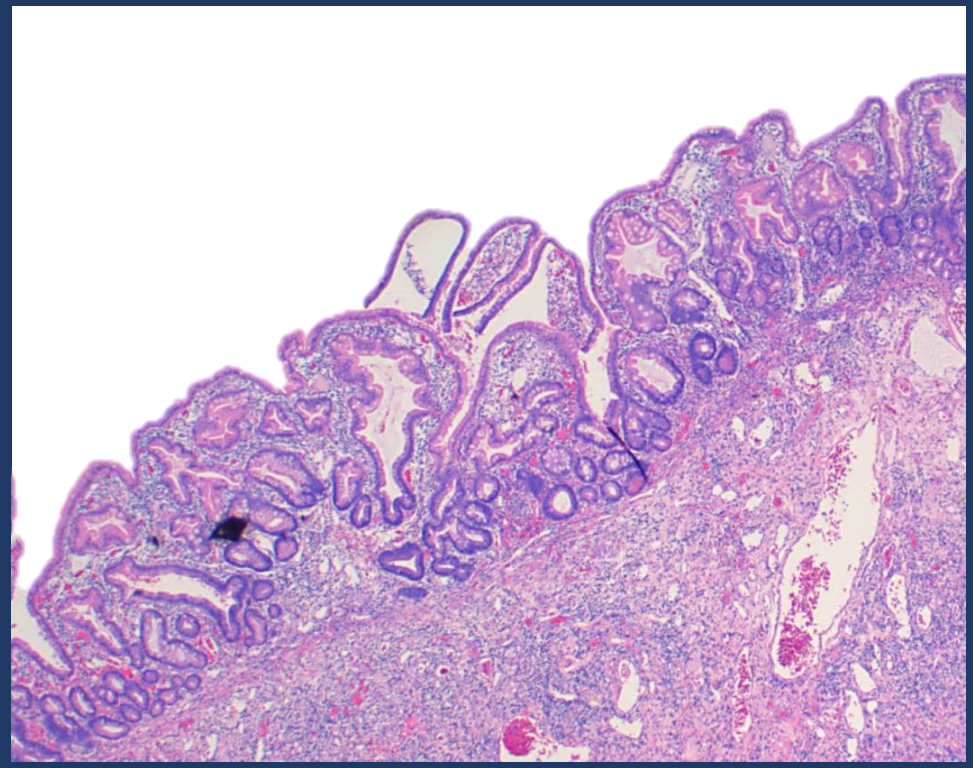
Diagnosis: CMV Enteritis

- IV valgancyclovir then oral valgancyclovir for 3/12
- Viral load decreased (6946 IU/ml to “not detected” at 2/12)
- Symptoms resolved
- Remains well at follow up (10/12)
- No evidence of IBD



CMV Enteritis/Colitis - a clinical and histological mimic

- Clinically and endoscopically mimics IBD and other infections
- Overlap in histological features:
  - Crypt architectural distortion
  - Basal plasmacytosis
  - Active inflammation –incl deep fissuring ulcers
  - Pseudopolyps
  - Vasculitis of submucosal vessels, ischaemia
  - Necrosis



What is the Role of CMV in IBD?

Whether or not CMV promotes inflammation in IBD is an on-going controversy with multiple studies over 5 decades

Cytomegalic Inclusion Disease and Ulcerative Colitis.
Report of a Case in a Young Adult.
Robin D Powell, Nancy E Warner, Robert S Levine, Joseph B Kirsner. Am J Med 1961: 30:2: 334-340

Direct causative role of CMV in IBD never confirmed and considered unlikely

Prevalence of reactivated CMV in blood and tissue more common in IBD patients (20x more in UC patients than controls on PCR GIT tissue)

Is the presence of reactivated CMV a consequence of the IBD-related inflammation (and a surrogate marker of severe disease)?
ie: Innocent bystander

or

Does CMV exacerbate inflammation and contribute to adverse outcome in infected patients (and therefore requires treatment)?
ie: Active pathogen

Dimitroulia 2006

The Literature – Clarity or Confusion?

- Many studies, small sizes
- Multiple different definitions. *(e.g.: Terms "CMV infection" and "CMV disease" used interchangeably?*
- Variation in tests
- Different sensitivities, no consistent viral load cut off….
- Heterogeneous patient populations
- No validated definition of outcome (clinical response or relapse)

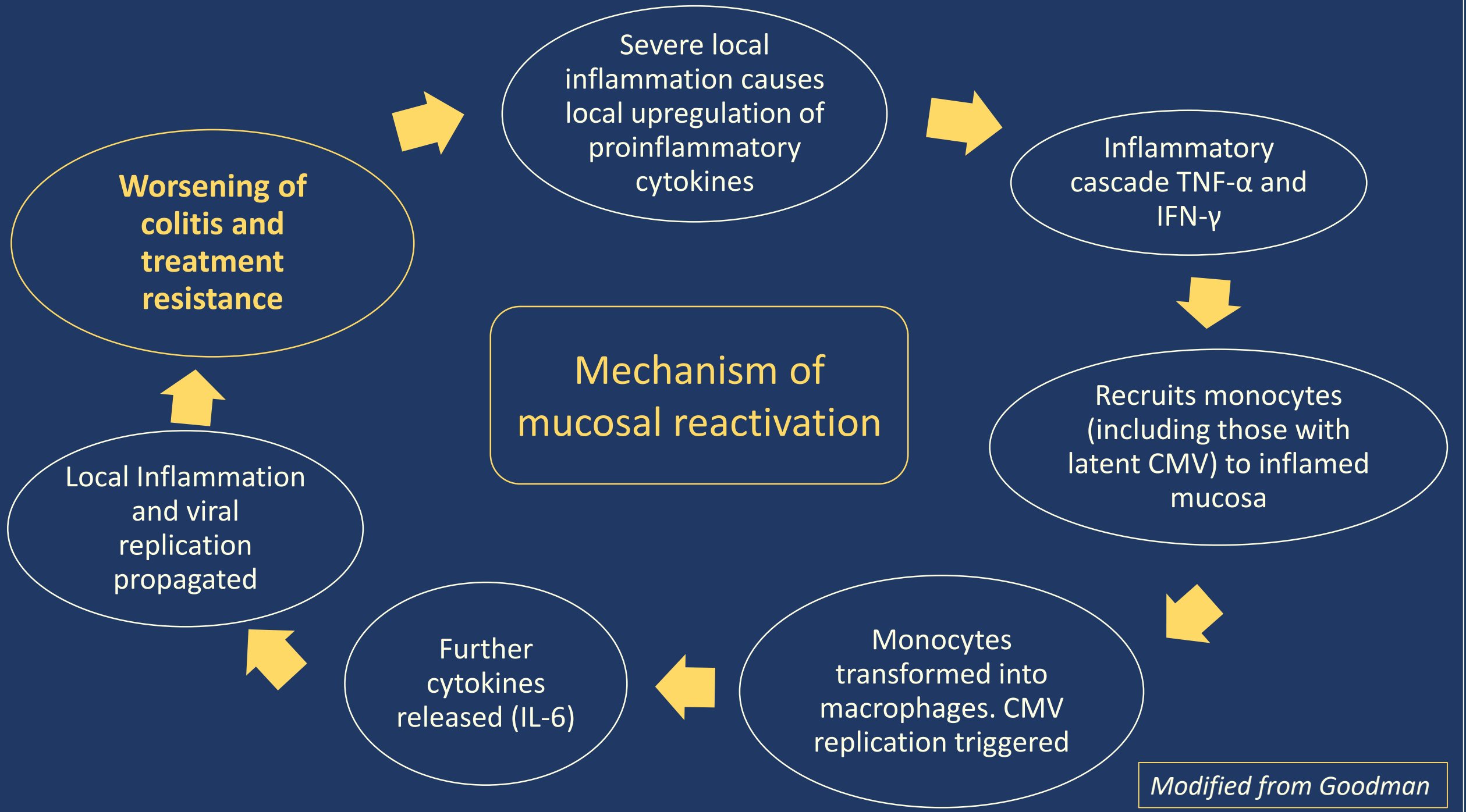
No single gold standard exists for (clinically relevant) CMV infection in IBD

However, the collective evidence suggests…….

CMV-positive IBD patients have worse outcome than CMV-negative patients

- Increased risk of colectomy
- Risk of hospitalisation
- Increased duration of hospitalisation
- Increased mortality

ECCO: *"CMV colitis mimicking an acute exacerbation of ulcerative colitis (UC) or Crohn's disease (CD) is associated with a poor outcome and a higher colectomy rate."*


Mechanism of mucosal reactivation
Severe local inflammation causes local upregulation of proinflammatory cytokines
Inflammatory cascade TNF-α and IFN-γ
Recruits monocytes (including those with latent CMV) to inflamed mucosa
Monocytes transformed into macrophages. CMV replication triggered
Further cytokines released (IL-6)
Local Inflammation and viral replication propagated
Worsening of colitis and treatment resistance
Modified from Goodman

CMV detection rates vary according to severity of colitis

Detection of CMV negligible in normal mucosa, inactive or mild to moderate colitis

↓

Even in the presence of immunomodulators

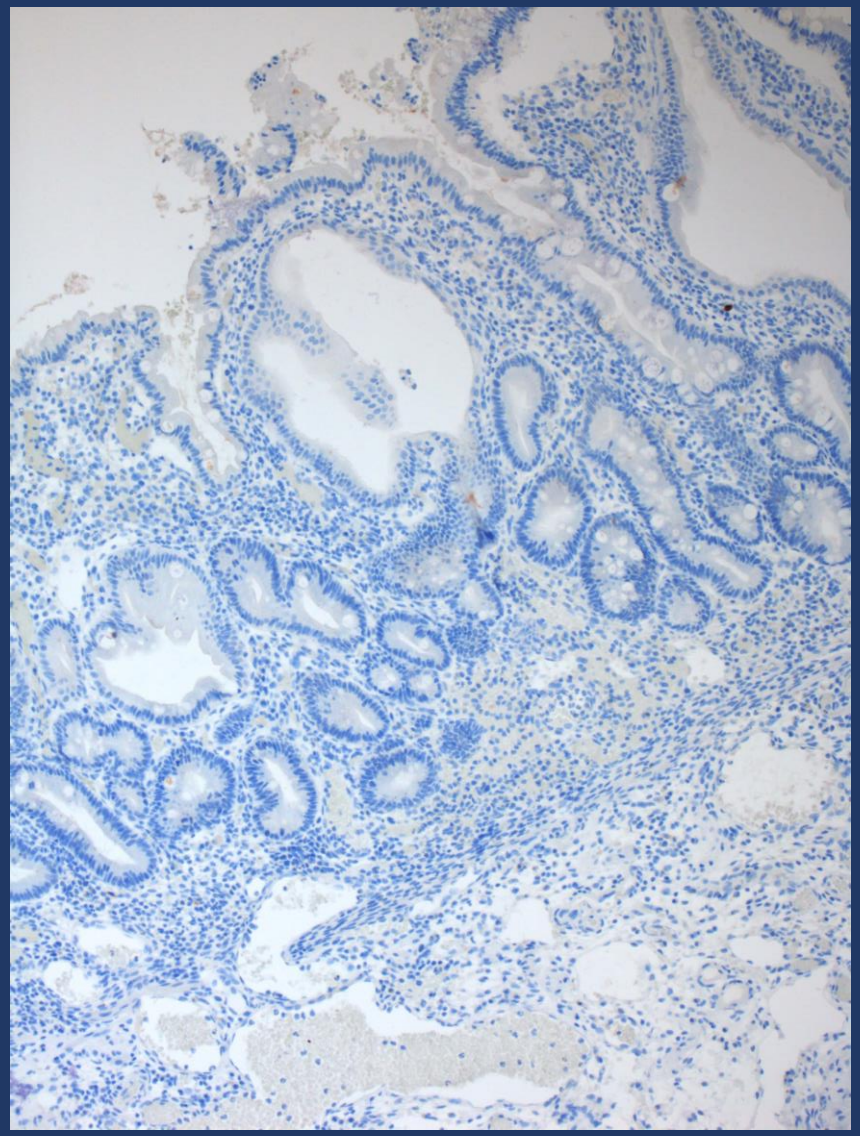
Reactivated CMV detected in about 30% of severe and/or steroid-refractory UC

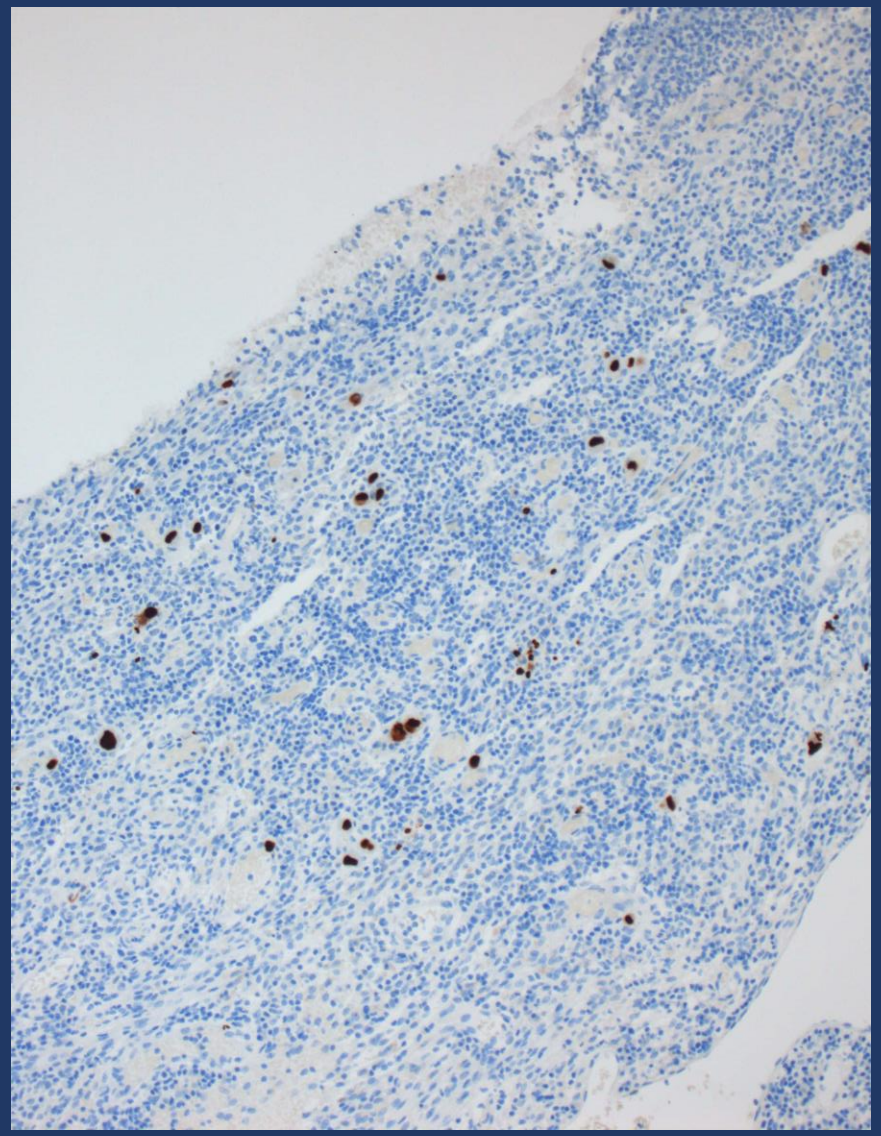
↓

Even in the absence of immunomodulators

Domenech 2008; Kojima 2006; Roblin 2011; Zidar 2015

Viral load (IHC): Mildly-moderately inflamed mucosa vs ulcerated mucosa





UC vs Crohns (CD)

- Seroprevalence similar to general population in both UC and CD
- Multiple studies report that CMV **reactivation** more common in UC than CD (but remains controversial)
- Estimated 10-fold increase in CMV **colitis** in UC compared to CD
- Difference attributed to differential cytokine profiles

UC:

TNF-α production prominent – stimulates CMV reactivation

Crohns:

T helper cell 1-mediated (IFN-γ) pathway more prominent - suppresses CMV reactivation

McCurdy 2015; Nakase 2010

4 questions.....

For Pathologists:

How do we diagnose CMV reactivation accurately in colonic tissue of IBD patients?

For Clinicians:

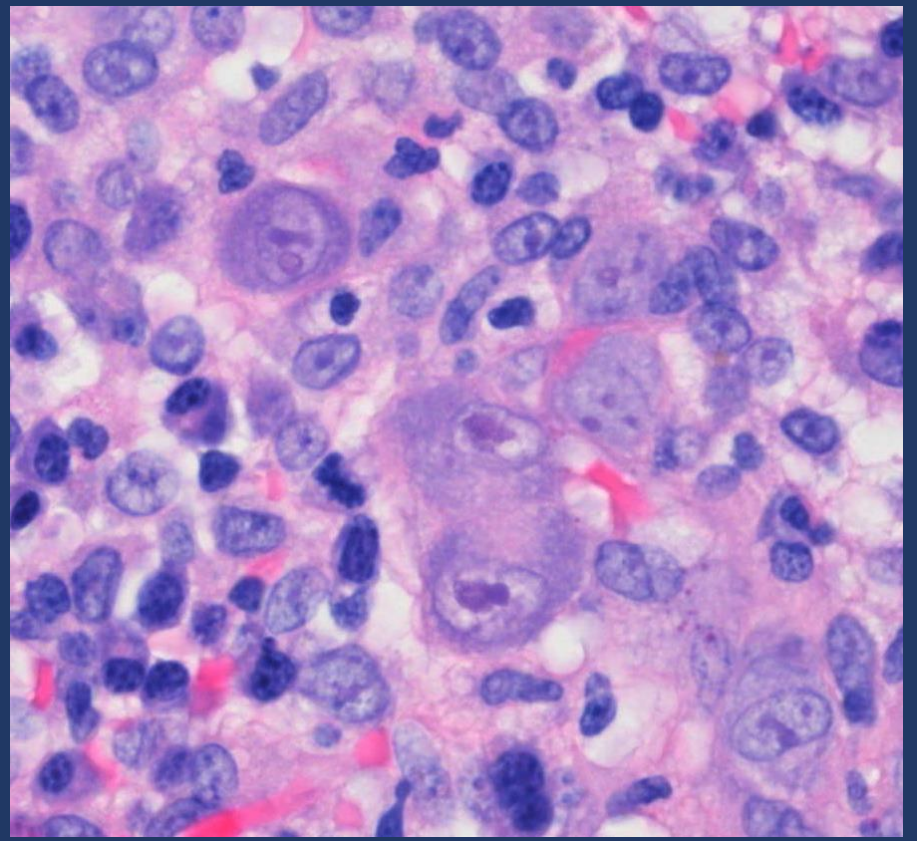
1) How does colonic CMV infection impact evolution of IBD?
2) How do we identify patients at risk of an unfavorable outcome?
3) Does antiviral therapy improve the long-term outcome of IBD (given that it is a toxic medication and many patients resolve spontaneously)?

Test	Characteristics	Sensitivity	Specificity
Serology	Detects previous infection and identifies “at-risk” patients	98-100%	96-99%
Antigenic assay	Detects viral protein pp65 in leucocytes. Does not distinguish latent and active infection. Superseded by PCR	60-100%	83-100%
Histology H&E	Sensitivity depends on site and no of biopsies	10-87%	92-100%
Histological IHC	Gold standard Improves detection	Up to 93%	92-100%
CMV DNA PCR	Rapid Clinical meaning of positivity is unclear - infection or disease? Need cut-off of viral load to determine relevance	92-96.7%	93-98.7%
Culture (Blood, tissue, stool)	Takes 2-4 weeks for results	45-75%	89-100%

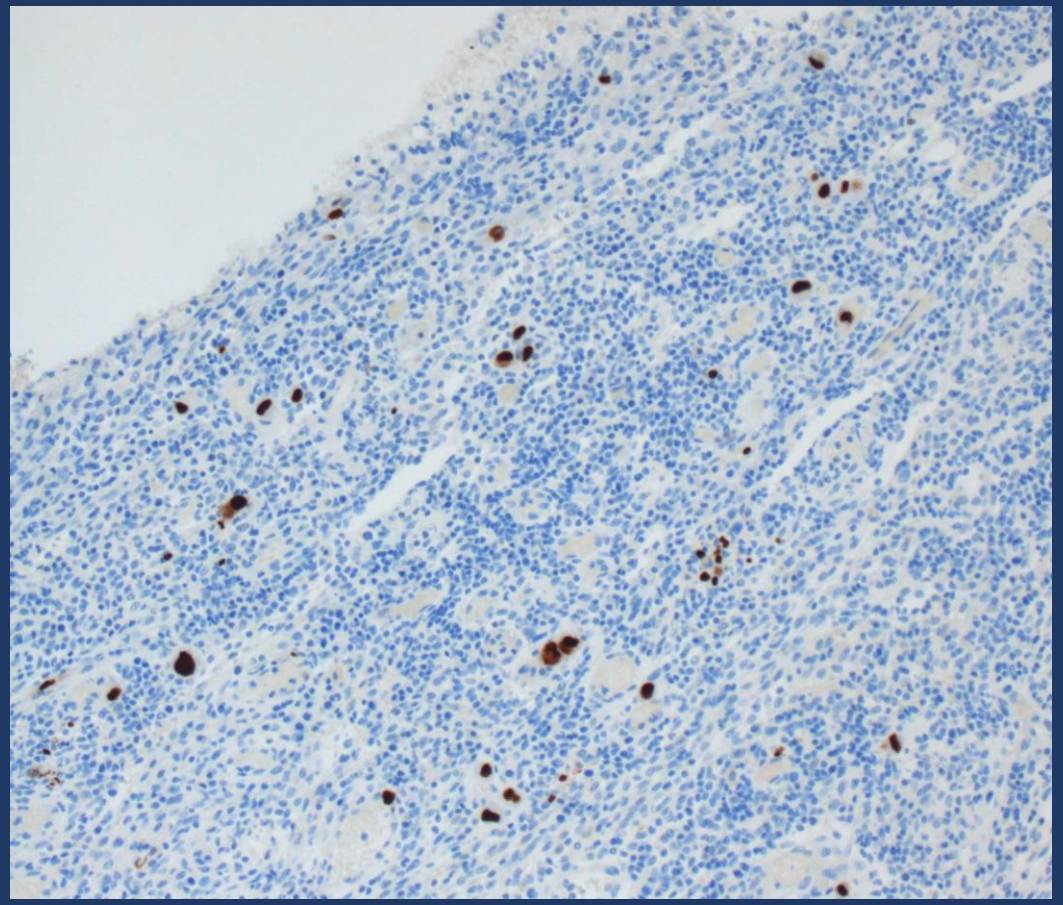
H&E

- Typical CMV-infected cells
  - Large with cytomegalic inclusion bodies with a halo (“owl’s eye” appearance)
  - represent active CMV replicating nucleoprotein cores
  - similar inclusion body in cytoplasm
- No inclusions seen in up to 38% of patients with gastrointestinal CMV disease

“Cyto” = cell
“Megalo” = large



CMV IHC



- IHC improves detection
- Targets viral proteins expressed during replication (ie: detects active CMV)
  - Nuclear expression = antigens expressed during early and intermediate replication
  - Cytoplasmic expression = antigens expressed during late stages of replication

PCR

Pros:

- Rapid and flexible (blood, tissue or stool)
- Very sensitive - detects CMV at an earlier stage of replication than IHC
- Can quantify viral load
- CMV viral load in blood largely correlates with risk of symptomatic CMV disease (Transplantation literature)

Cons:

- Blood PCR does not confirm tissue infection and tissue confirmation required
- Main criticism of colonic tissue PCR
  - Is overly sensitive
  - Postulated to detect latent and mild reactivation of CMV of no clinical relevance

ECCO: Consensus Statement

In patients with acute steroid-resistant colitis, CMV should be excluded, preferably by tissue PCR or immunohistochemistry, before increasing immunomodulator therapy [EL3].

- Sensitivity: PCR >>>IHC in most studies (*Detection using PCR = 60% vs IHC = 6%, Yoshino et al)*
- Viral load to identify clinically relevant CMV: Not yet established
- Cost: Tissue IHC ($40) vs PCR ($210)

What viral load is significant?

Biopsy IHC:

No consensus on significant cut off levels

- Low density: suggested 1-3 CMV + cells/slide
- High density: suggested >2-5 CMV + cells/slide

Biopsy PCR:

CMV DNA load > 250 copies/mg **in tissue** predictive of adverse outcome (treatment resistance, increased hospitalisation)

Beswick 2016; Liao 2016; Roblin 2011

*"…..the recent data suggests that **using IHC positivity** (the more inclusion bodies found per biopsy fragment, the greater diagnostic likelihood of CMV disease) **is preferable** as this appears to **best correlate with CMV disease,** and/or **at least a high probability** of CMV pathogenicity, thus providing the **most discriminative guidance** as to whether to instigate antiviral therapy."*

Tissue IHC should be supported by CMV PCR on blood

(1) Appears to correlate with colitis

(2) Enables viral quantification and where high, supports treatment

(3) Can be used to assess response to treatment

(4) But a minority of patients will have CMV colitis with negative blood PCR

Beswick 2016

So how should we pathologists report our findings?

- High index of suspicion - severe activity/refractory to treatment
- CMV IHC on biopsies with severe activity/ulceration (not normal/mild colitis)
- Document the viral load (organisms/slide)

DIAGNOSTIC SUMMARY

BIOPSY SIGMOID COLON:

1) ***SEVERE CHRONIC COLITIS WITH SEVERE ACTIVITY***
2) ***CMV DETECTED BY IMMUNOHISTOCHEMISTRY***

The rest is up to the clinician……