When ancillary tests lead you astray
– GI tract –

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Diagnostic confusion

Ancillary tests: positive or negative?
Ancillary tests in the GI tract:

Besides Helicobacter staining and PAS there is not much room for ancillary tests in the routine GI-tract

But e.g.

CMV testing in IBD prior to immunomodulation (biologicals!)

Lymphoma proof: clonality?
H69788-19
1971 born, male, known ulcerative colitis since childhood. Colon biopsies.
Now, moderately active phase. Request for CMV immunohistochemistry

H47638-19
1972 born, female, ulcerative colitis known for 1 year. Colon biopsies.
Now, slightly active phase. Request for CMV immunohistochemistry

H33611-19
Request for CMV immunohistochemistry

H40459-19
1968 born, male, ulcerative colitis known since childhood. Colon biopsies.
Now, markedly active phase. Request for CMV Immunohistochemistry.

H78220-19
1966 born, male, ulcerative proctitis known since childhood. Rectum biopsies,
Now, moderately active phase. Request for CMV immunohistochemistry.
H69788-19
Immunohistochemistry: negative for CMV (weak cytoplasmatic)

PCR: 33,7 ng/µl DNA : 71 CMV copies / 100.000 cells
H47638-19

Immunohistochemistry: questionable positive for CMV (strong but not localizable well)

PCR: 42.5 ng/µl DNA : 138 CMV copies / 100,000 cells
H33611-19

Immunohistochemistry: numerous unspecific signals (weak, mostly cytoplasmatic)

PCR: 39.7 ng/µl DNA : 2593 CMV copies / 100,000 cells
H40459-19
Immunohistochemistry: Suspicious signals (strong nuclear but cytoplasmatic as well a bit)

PCR: 77,8 ng/µl DNA : 757 CMV copies / 100.000 cells
Immunohistochemistry: positive for CMV (nuclear but cytoplasmatic as well a bit)

PCR: 23.4 ng/µl DNA: negative for CMV
H99403-19
1938 born, male. Gastric biopsies with markedly chronic, moderately active Helicobacter infection with lymphoid follicles, positive reaction of CD20 close to hyperplastic lymphoid follicles. Positive reaction or reactive T-lymphocytes against CD5. Request for clonality analysis.
Clonal?
Results of the B-cell clonality analysis

- **Method:** IdentiClone IGH/IGK Kit from Invivoscribe: PCR targets in the

![Diagram of PCR targets]

Results of the B-cell clonality analysis

Electropherogramms show fluorescence-labeled PCR products of rearranged IGH genes of FR1/2 and 3

- IGH FR1: clearly polyclonal pattern with a characteristic gaussian size distribution
Results of the B-cell clonality analysis

Electropherograms show fluorescence-labeled PCR products of rearranged IGH genes of FR1/2 and 3

- IGH FR3: polyclonal pattern with a gaussian distribution

![Electropherogram for IGH FR3 showing bands in the range of 90-170 bp](image-url)
Results of the B-cell clonality analysis

Electropherograms show fluorescence-labeled PCR products of rearranged IGH genes of FR1/2 and 3

- IGH FR2: polyclonal background with one single peak (265 bp) => clonality?

IGH FR2:

265 bp

IGH FR2:

230 - 295 bp
Negative controls (IGH FR1, 2 and 3) => were all ok

IGH FR1:

IGH FR2:

IGH FR3:

size ladder
Results of the B-cell clonality analysis

**Method:** IdentiClone IGH/IGK Kit from Invivoscribe: PCR targets in the immunoglobulin Ig kappa genes (IGK):

IGK A:

In particular, combined application of IGH and IGK tubes can detect virtually all clonal B-cell proliferations, even in B-cell malignancies with high levels of somatic mutations.

**Dongen et. al. 2003:** Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936, Leukemia volume 17, pages 2257–2317.c
Results of the B-cell clonality analysis

Method: IdentiClone IGH/IGK Kit from Invivoscribe: PCR targets in the immunoglobulin Ig kappa genes (IGK):

- IGKA: clearly polyclonal pattern
- IGKB: clearly polyclonal pattern
Result

• Marked chronic, moderately active Helicobacter pylori gastritis with lymphoid follicles, mild intestinal metaplasia and mild atrophy. Period!

• Don’t do if no clear hypothesis! Careful analysis! Morphological correlate! Neg clonality is lots more difficult in cases of clear lymphoma! Ask your heamatology Pathologist!
Clonality Gastritis in Children

- IGH clonality in 62 cases with Helicobacter and 17 Helicobacter-negative
- 47% (29/62) cases had at least 1 lymphoepithelial lesion (modified Wotherspoon score of 3 to 5)
- 24% (4/17) of the Helicobacter-negative cases (P=0.5)
- IGH monoclonality in 27% (8/30) of evaluable cases.
- No gastric lymphoma within follow-up.
- 4% of cases could be considered MALT lymphoma in an adult
- caution with lymphoma in a pediatric population
- unclear if these monoclonal lymphoid proliferations require close follow-up.

Clonality Gastritis in Adults

- 15% show monoclonality by IgH PCR
- No lymphoma in 58 months
- LEL correlate with germinal centers but not with monoclonality
- All scores for chronicity and activity
- No clinical significances

Conclusion

CMV: strong nuclear signals
(exclude weak signals, cytoplasmatic signals, eosinophils etc...)

Lymphoma: always have a morphological correlate! No tests with no clear hypothesis!
Ancillary tests always with clear question!