A new marker in the diagnosis of coeliac disease

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Celiac Disease

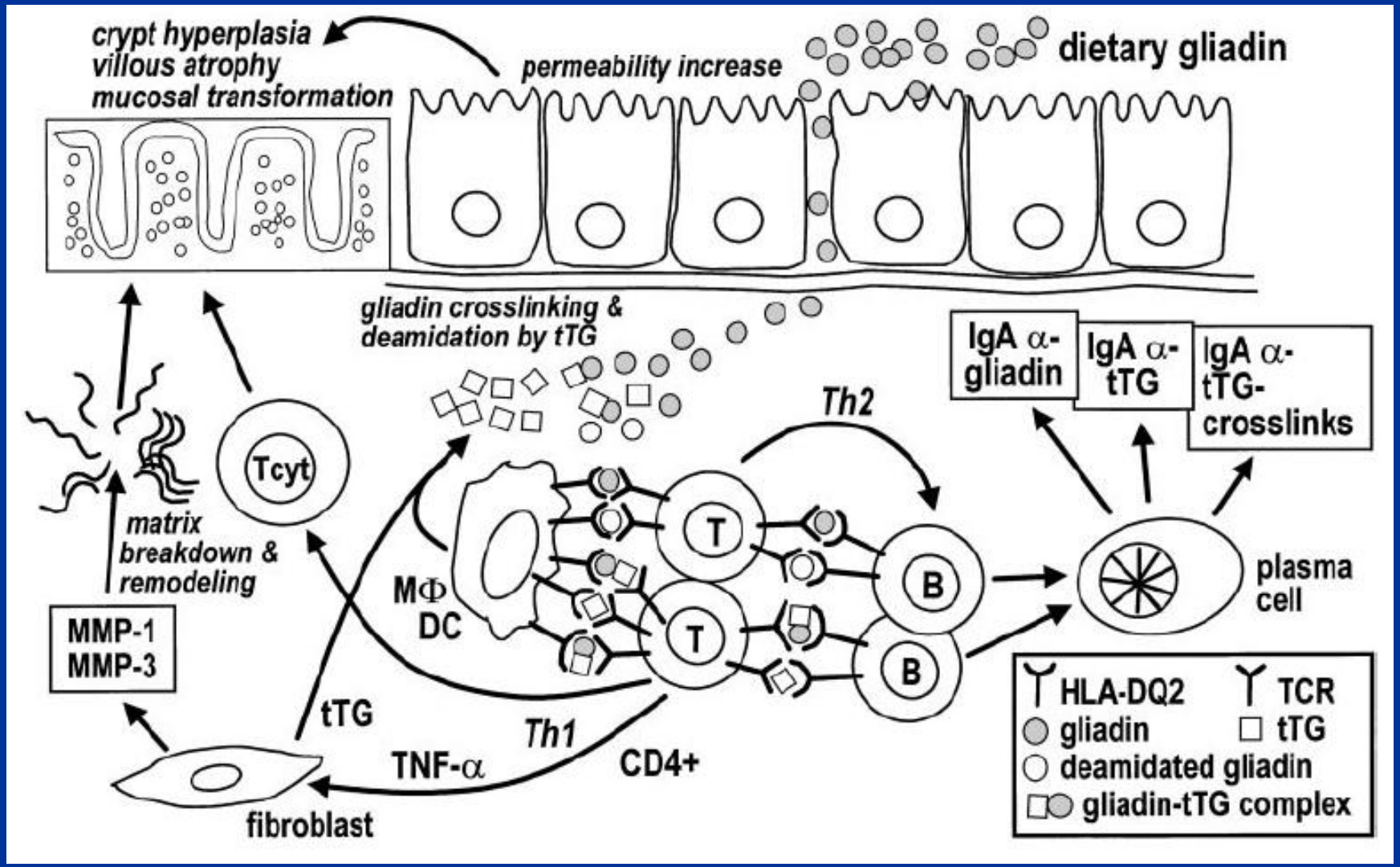
DEFINITION

Celiac disease is a permanent, immuno-mediated, gluten-dependent enteropathy which affects genetically predisposed subjects

MAIN CHARACTERISTICS

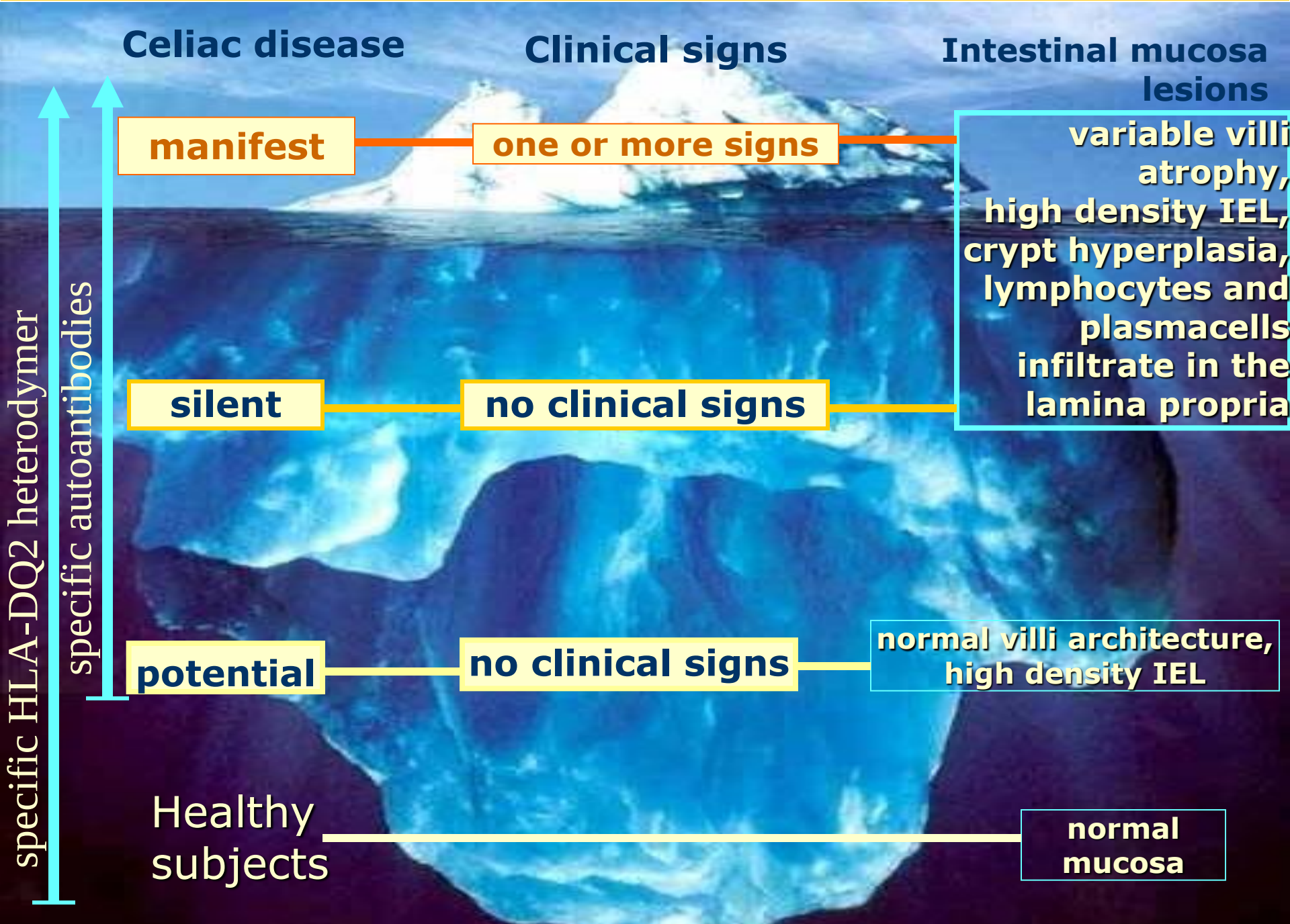
- high clinical and histological variability
- frequent association with autoimmune diseases

Current concepts of celiac disease pathogenesis



Schuppan D. Gastroenterology 2000

Clinical and histological aspects of celiac disease



Celiac Disease Diagnosis: the serologic tests

ADVANTAGES

- **AGA**
  - children < 2 years of age
  - IgA deficiency
  - monitoring dietary compliance
- **AEA**
  - high sensitivity (85-98%)
  - high specificity (98-100%)
- **TGA**
  - easy to perform
  - high sensitivity
  - monitoring dietary compliance ?

LIMITS

- **AGA**
  - poor specificity, poor PPV
- **AEA**
  - children < 2 years of age
  - IgA deficiency
  - monitoring dietary compliance
  - not always easy to interpret
- **TGA**
  - high variability in specificity depending on the kit used

modified after Farrell RG and Kelly CP, NEJM 2002

The "gold standard" of celiac disease diagnosis
the intestinal biopsy

- 1970: 3 biopsies
  - at time of initial presentation
  - on gluten-free diet
  - after a gluten challenge
- 1990: 1 biopsy
  - at time of initial presentation + follow-up of serologic tests and clinical signs
- future: 0 biopsy ?

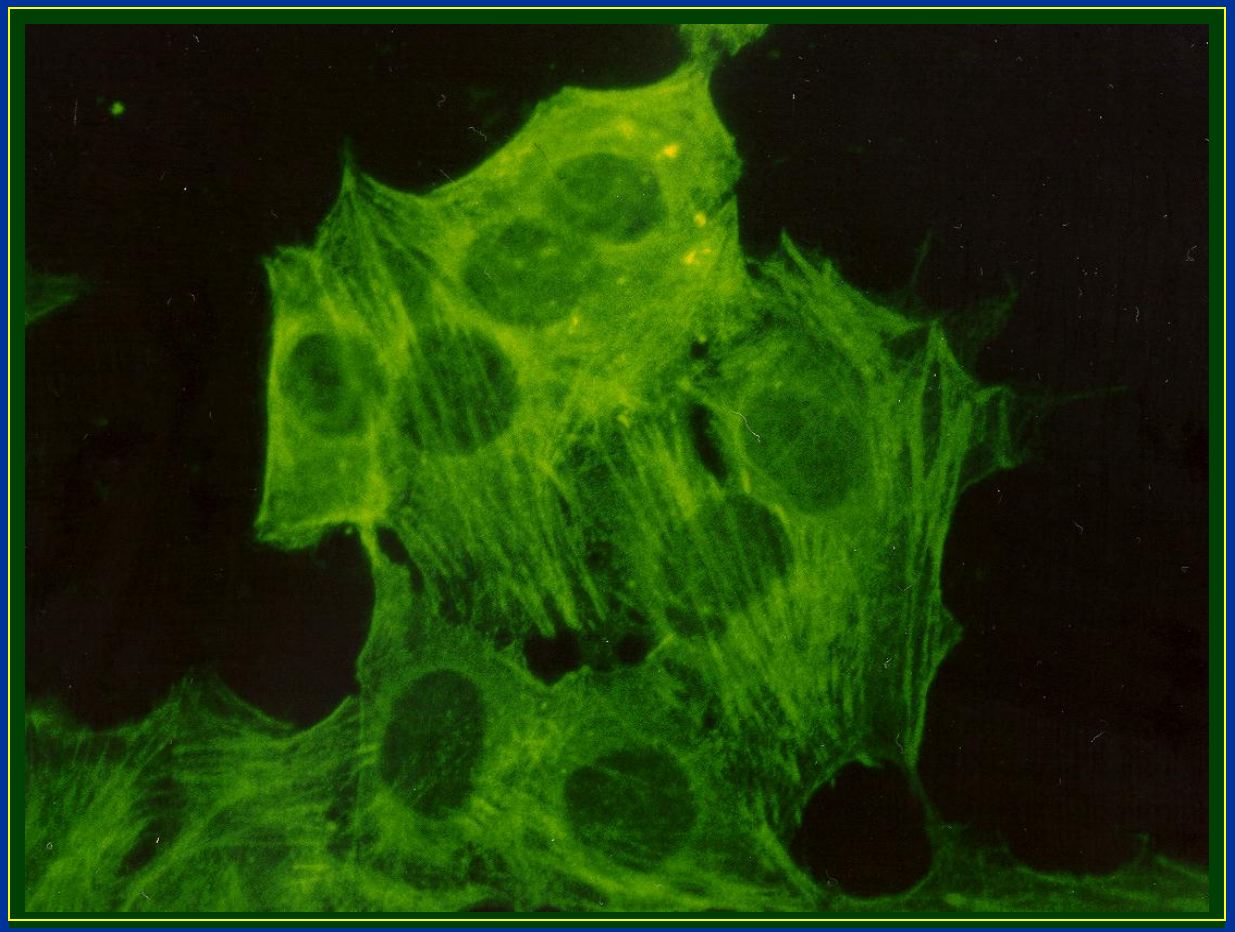
Major diagnostic pitfalls

- lesions are typical but not specific for CD
- patchy lesion distribution
- poor sensitivity of endoscopic markers
- difficulty handling and/or correctly orienting biopsy specimens
- correct biopsy interpretation

IMMUNOREACTION AGAINST THE CYTOSKELETON IN COELIAC DISEASE

(MG Clemente,MP Musu,F Frau,G Brusco,G Sole,GR Corazza,S De Virgiliis. **Gut 2000; 47:520***)*

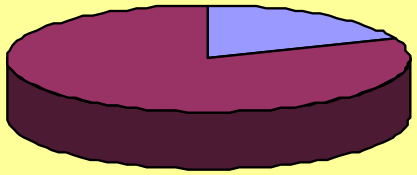
Serum autoantibodies against actin filaments (AAA) were detected for the first time in patients with active celiac disease



Immunofluorescence on laryngeal carcinoma (HEp-2) cells using a celiac patient serum

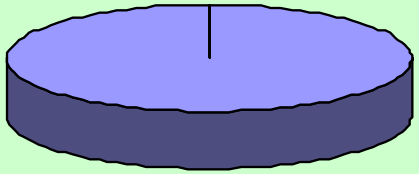
Actin stress fibres appear as long cytoplasmic parallel filaments

Celiacs with subtotal and total IVA



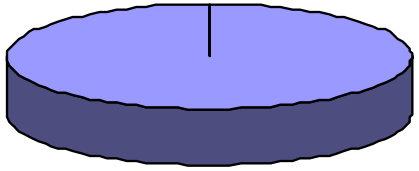
81% of celiac patients with severe intestinal villous atrophy (IVA) tested positive for anti-actin antibodies (AAA).

Celiacs with partial IVA



However, 19% of celiacs with subtotal and total IVA and 100% of those with partial IVA tested AAA negative.

Controls, no IVA



No one of subjects without IVA was positive for AAA.

A high correlation was found between presence of AAA and IVA: Relative Risk 86.1

Clemente et al. Gut 2000

Aims of the Study

- to improve the sensitivity of the IgA-AAA test by substituting laryngeal carcinoma (HEp-2) cells with intestinal epithelial cells (IEC-6) as substrate

- to conduct a double-blind multicenter study, which started in 1999 and involved 9 Italian Gastroenterology Center (6 of which paediatric) as well as the Mucosal Biology Research Center in Baltimore, Maryland, USA

Enterocyte actin autoantibody detection: a new diagnostic tool in celiac disease diagnosis. Results of a multicenter study.

M.G. Clemente[1], M.P. Musu[1], R. Troncone[2], U. Volta[3] , M.Congia[1], C. Ciacci[4], E. Neri[5], T. Not[5], G.Maggiore[6], P. Strisciuglio[7], G.R. Corazza[8], G Gasbarrini[9], L. Cicotto[1], G. Sole[1], A. Fasano[10], S. De Virgiliis[1]

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10. Mucosal Biology Research Center, University of Maryland School of Medicine, Baltimore

American Journal of Gastroenterology 2004, in press

Patient groups enrolled

PROSPECTIVE STUDY

223 subjects at risk for CD that tested positive for AEA and/or TGA:

- 104 adults (age range 18-70)
- 119 children (range < 1 - 17)

After evaluation for IgA-AAA, patients were biopsied to confirm the diagnosis of CD

RETROSPECTIVE STUDY

84 celiac children were analysed retrospectively for the presence of IgA-AAA

Control groups enrolled

BIOPSIED CONTROLS

78 biopsied controls

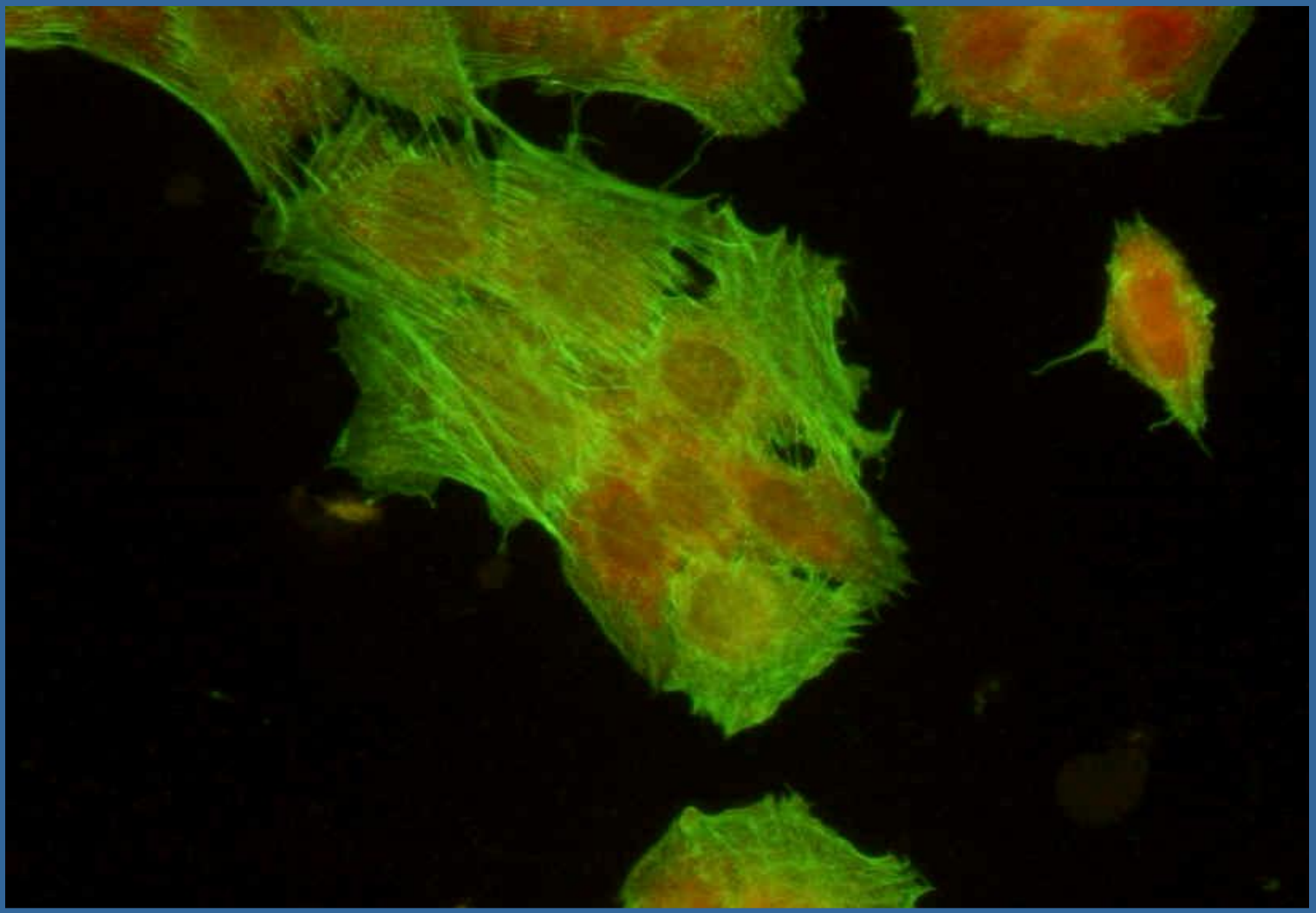
- 8 irritable bowel syndrome
- 14 Crohn's disease
- 8 ulcerative colitis
- 3 autoimmune enteropathy
- 4 unspecific colites
- 5 non-ulcerative dyspesia syndr.
- 6 gastro-esophageal reflux
- 14 cow's milk and food allergy
- 2 hypertransaminasemia
- 7 small bowel bacterial overgrowth

… and failure-to-thrive, iron deficiency anemia, giardiasis, Sjogren's syndrome, antiphospholipid syndrome, pericarditis, leukemia

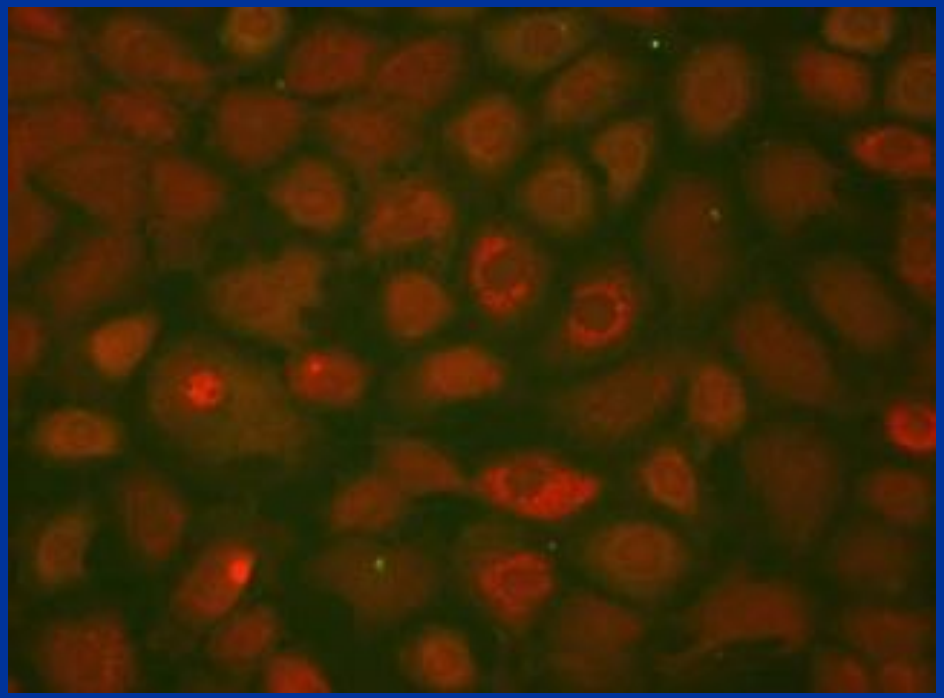
NON-BIOPSIED CONTROLS

2000 consecutively collected serum samples from subjects in whom CD was excluded on the basis of negative serologic tests

IgA-AAA detection in celiac patients: fluorescence microscopy image of colchicine-treated intestinal epithelial cells (IEC-6)



82.5% of celiac patients were positive for IgA-AAA (titer range 1:5 to > 1:1280)



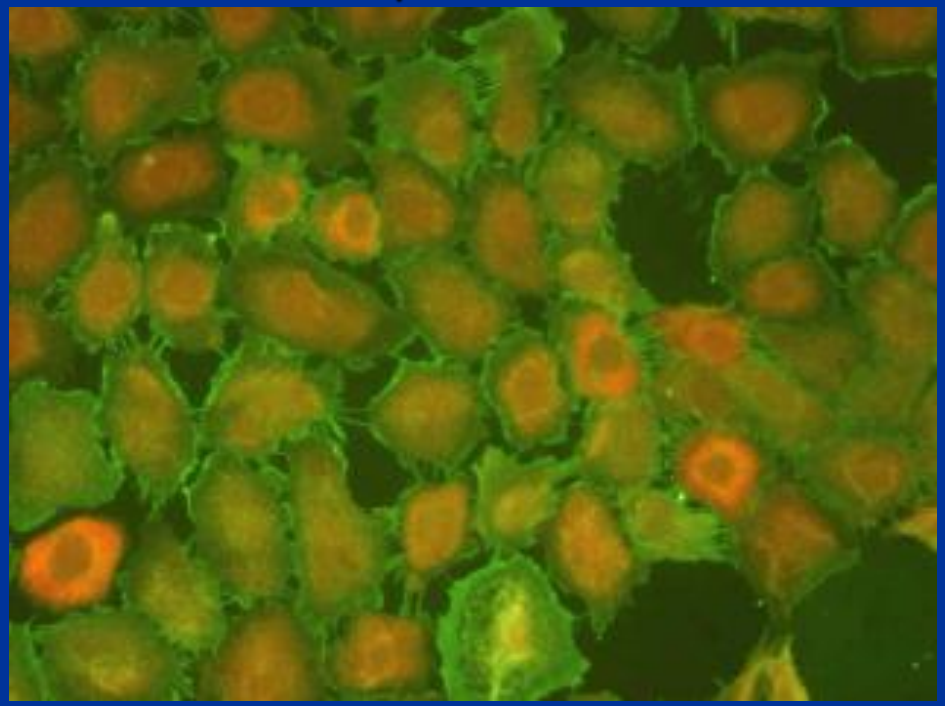
No fluorescent signal was detected using the monoclonal anti-tissue transglutaminase antibody

Am J Gastroenterol 2004, in press

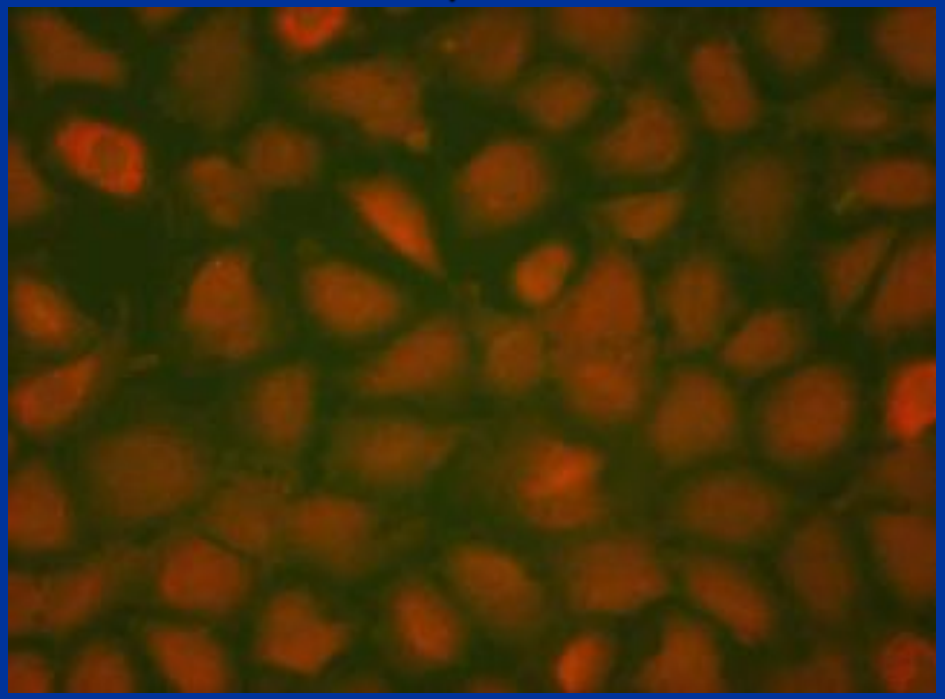
Absorption experiments

Sera from IgA-AAA positive celiac patients were incubated overnight under continuous shaking with bovine muscle actin.

Before absorption, dilution 1:500

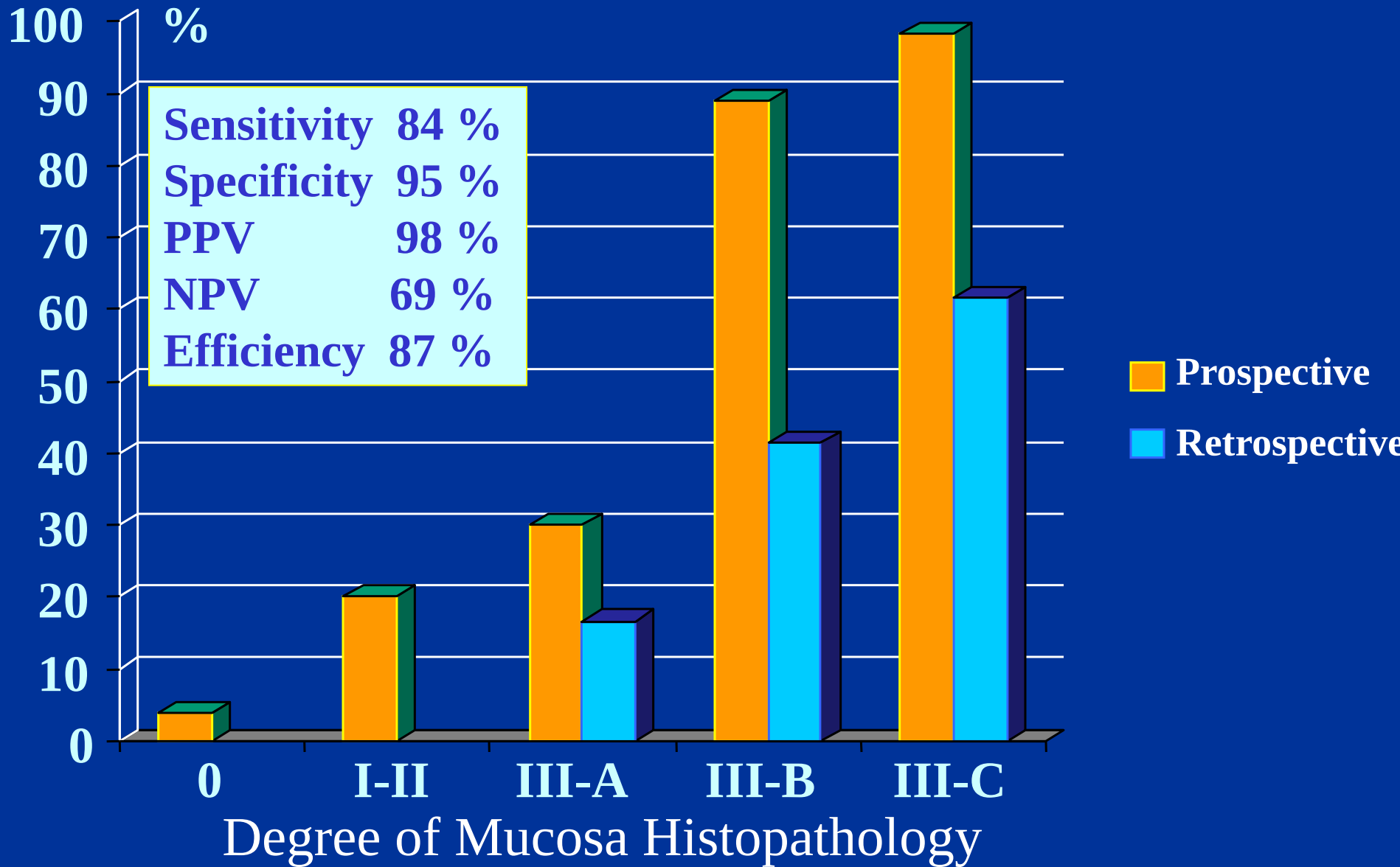


After absorption with actin

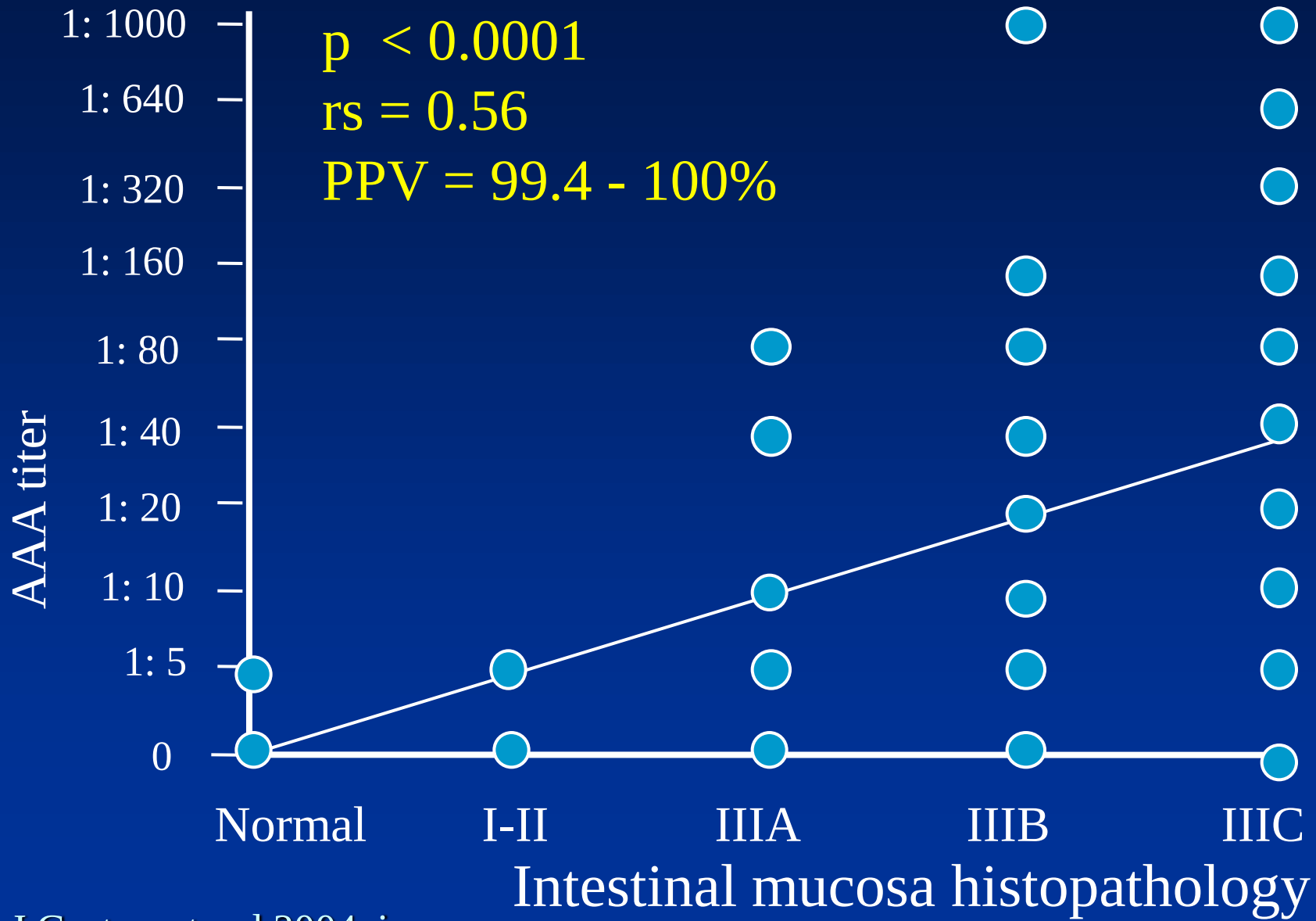


Am J Gastroenterol 2004, in press

Percent of IgA-AAA positive celiac patients and biopsied controls subdivided according to the degree of mucosa histopathology in the prospective and retrospective studies

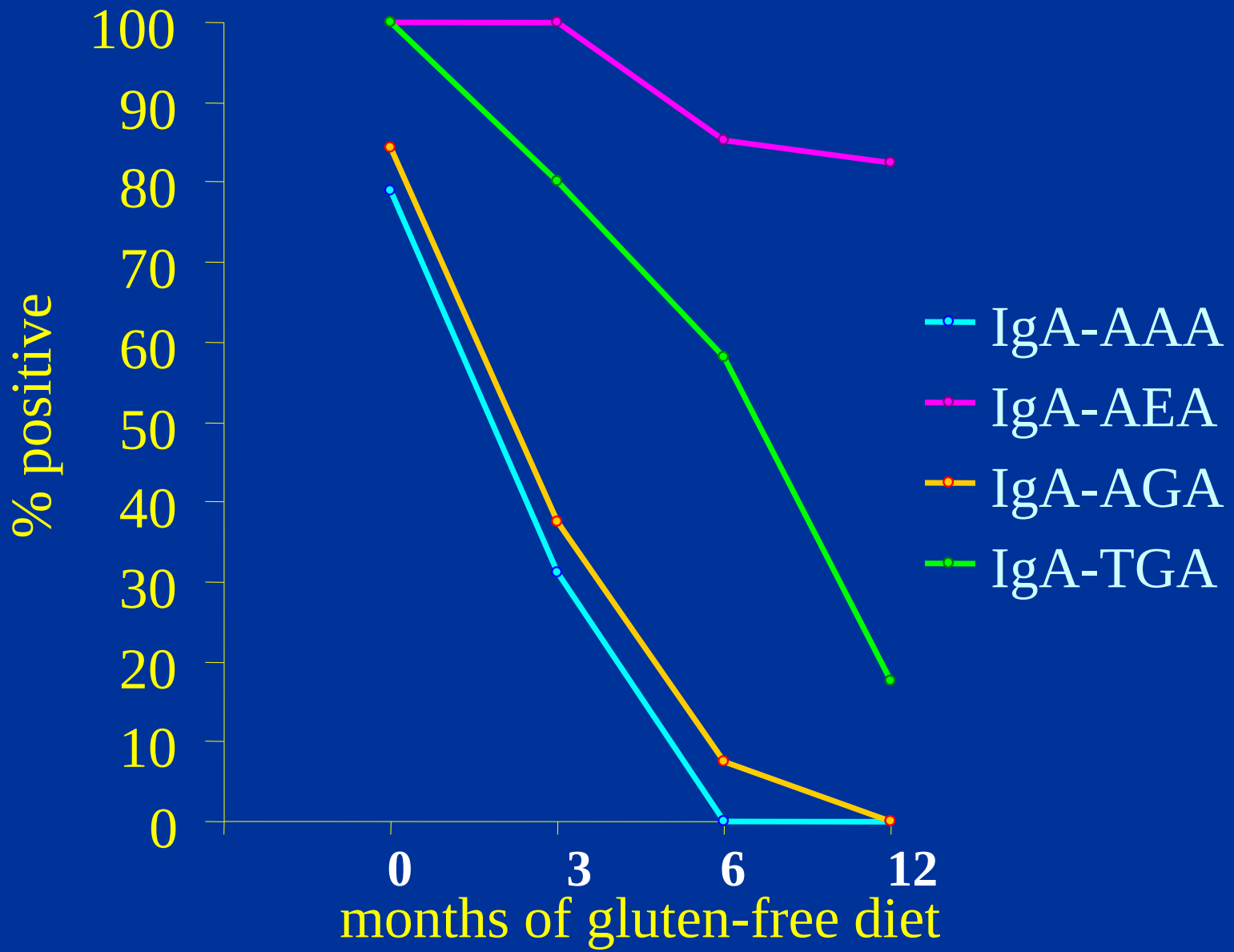


Correlation between IgA-AAA serum titer and intestinal mucosa morphology (bivariate scattergram with regression)



Am J Gastroenterol 2004, in press

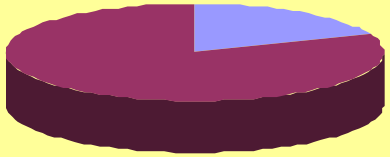
Percent of serologic test positives at the diagnosis and during the 1st year of gluten-free diet



AAA on HEp-2 cells
Gut 2000

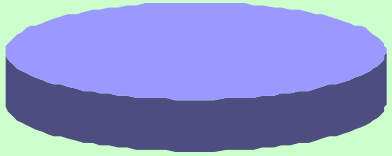
Celiacs with subtotal and total IVA

19% negative



81% positive

Celiacs with partial IVA



100% negative

Controls, no IVA

100% negative

AAA on IEC-6 cells
Am J Gastroenterol 2004

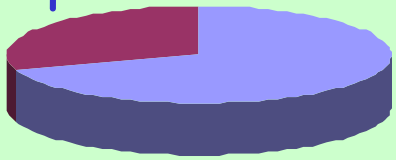
Celiacs with subtotal and total IVA

5% negative

95% positive

Celiacs with partial IVA

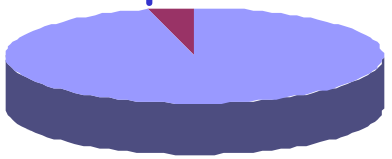
30% positive



70% negative

Controls, no IVA

4% positive



96% negative

Which subjects will benefit from this new test ?

Any AEA and/or TGA positive patient whether:

1 - the biopsy is difficult to interpret:

- patchy distribution of intestinal villous atrophy not picked up by the biopsy
- artifact damage of specimens not adequately handled

2 - the biopsy represents a life-threatening risk:

- any contraindication to procedures
- pregnant women with a history of multiple pregnancy losses
- subjects with coagulation disorders.

Am J Gastroenterol 2004, in press

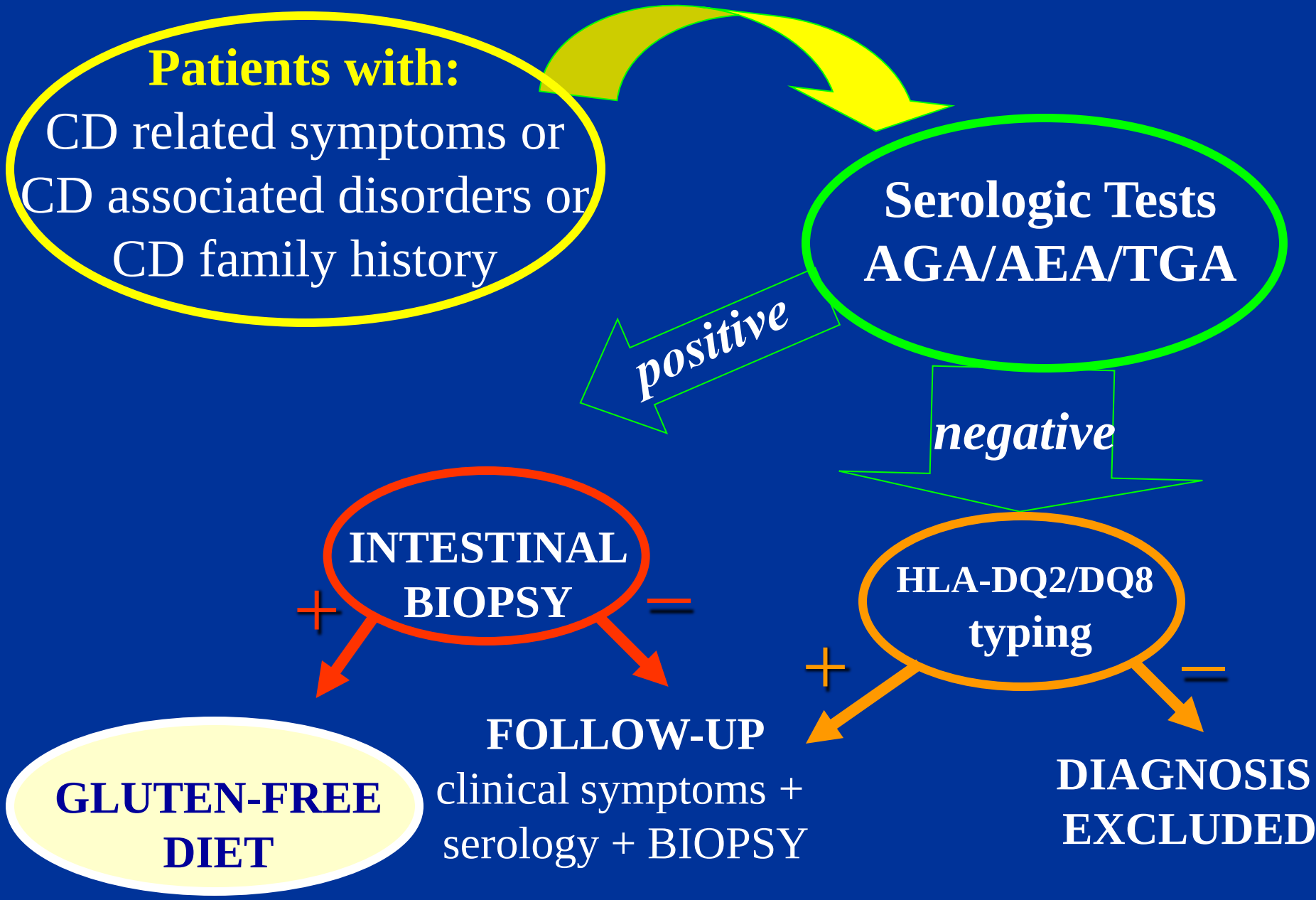
Conclusions

- The new method of IgA-AAA detection is highly indicative of advanced intestinal mucosa lesions in celiac patients

- While serologic detection of AEA and/or TGA is indicative of gluten-induced immune reactions, serologic AAA detection is a sign that the gluten-induced immune reactions have already caused advanced intestinal mucosa lesions

- International multicenter studies and Consensus Conferences will establish whether intestinal biopsy is still necessary in AEA/TGA positive subjects who are also AAA positive to establish a correct diagnosis

Current practical approach to CD diagnosis



New flow chart proposed to CD diagnosis