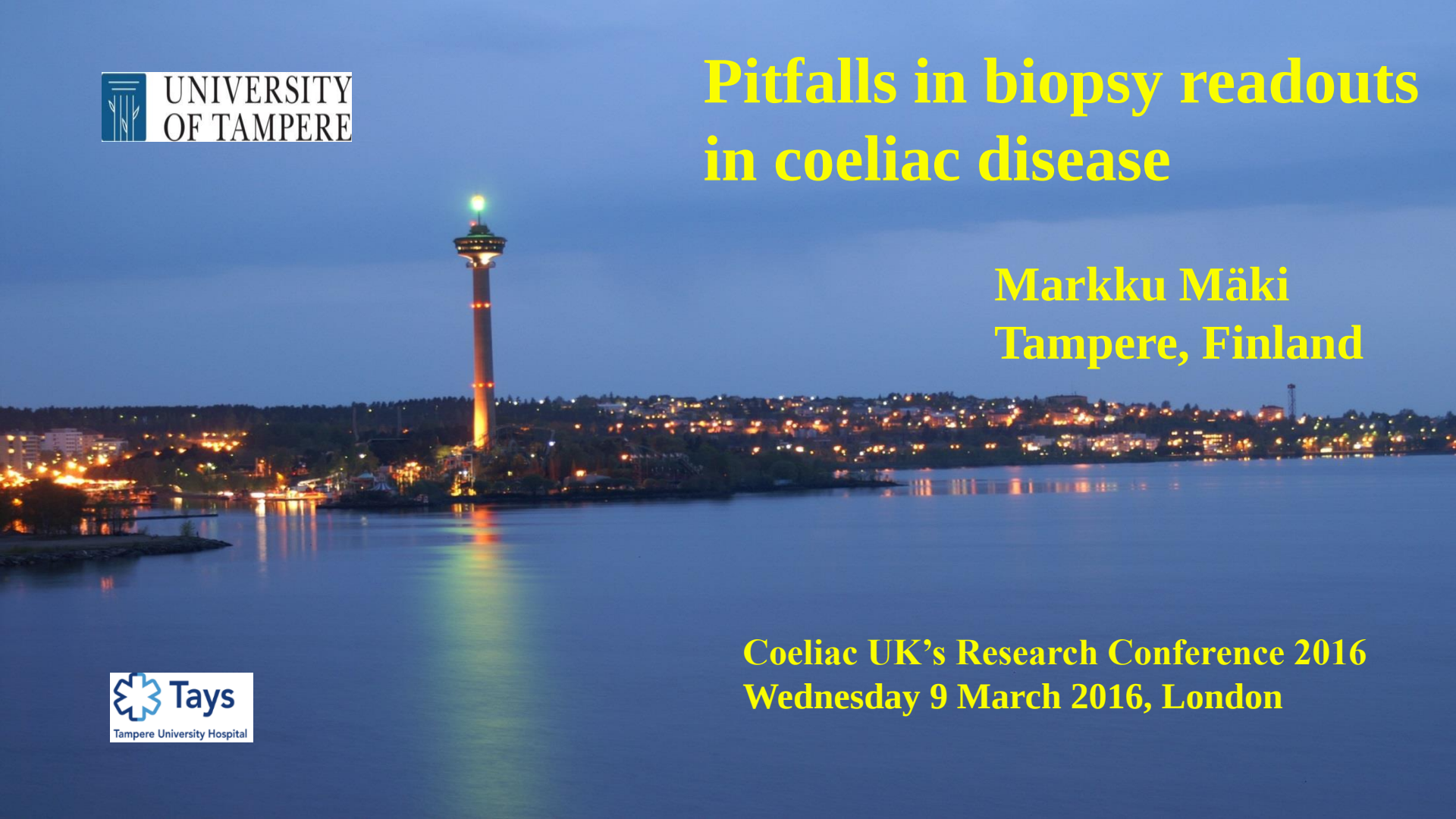


Pitfalls in biopsy readouts in coeliac disease

Markku Mäki
Tampere, Finland

Coeliac UK's Research Conference 2016
Wednesday 9 March 2016, London

Disclosures

Advisory Board Member of

- Finnish Celiac Society
- Alvine Phamaceuticals Inc, USA
- BioLinerx Ltd, Israel
- ImmusanT Inc, USA
- Trivitron Ltd, India
- Abbvie, USA
- Celimmun, USA

Consultations

- Finn Medi Oy, Tampere, Finland
- Glenmark
- GSK Vaccines
- Coeliac Societies (support groups) in Finland and abroad

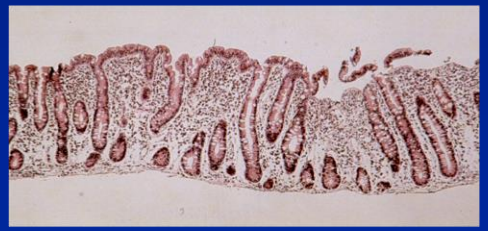
Coeliac disease

- Environmental insult (gluten) and genetic susceptibility (DQ2 90%, DQ8 10%).
- Small intestinal mucosal inflammation ➔ villous atrophy and crypt hyperplasia
- Gastrointestinal symptoms, malabsorption of nutrients and extraintestinal disorders = systemic disease

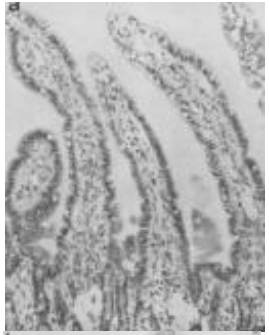
Gluten-containing diet

⇌

Gluten-free diet



Protective mucosal response against environmental insults

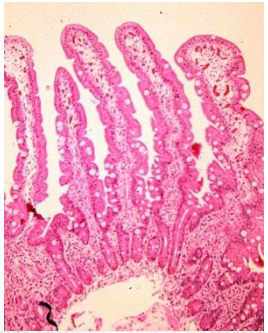


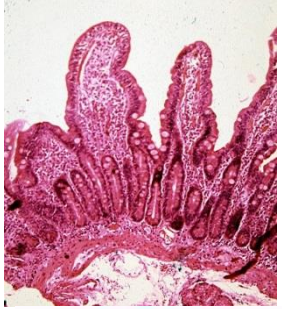
”Get rid of the bug”

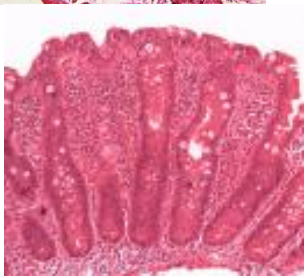
rotavirus **gluten**

Celiac disease
Cow’s milk protein intolerance
Chronic diarrhea
Giardiasis
HIV
Rotavirus

Lindfors et al.
Autoimmunity Reviews, 2010







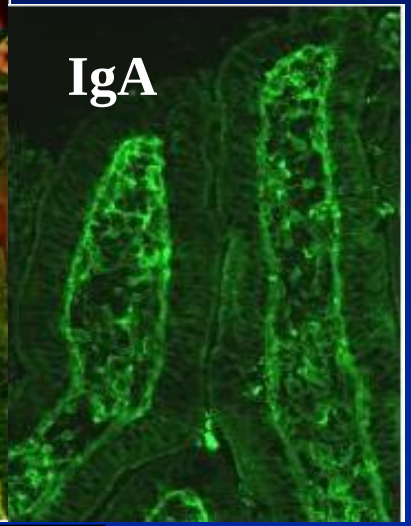
J Clin Invest 1977;60:1402-9

In situTG2-specific IgA-deposits in early developing CD

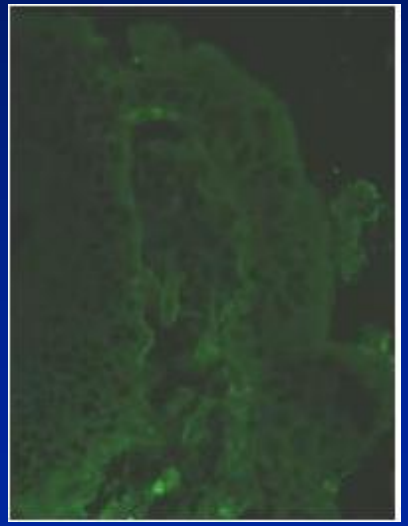
Korponay-Szabo I et al., Gut 2004; 53:641-8

Kaukinen et al., Scand J Gastroenterol 2005;40:564-72.

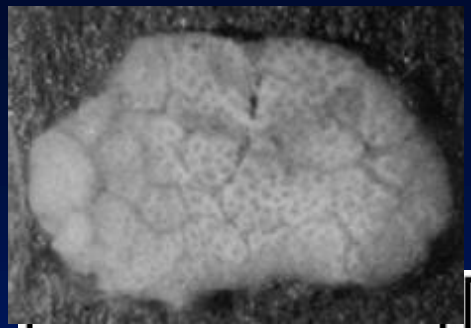
Before

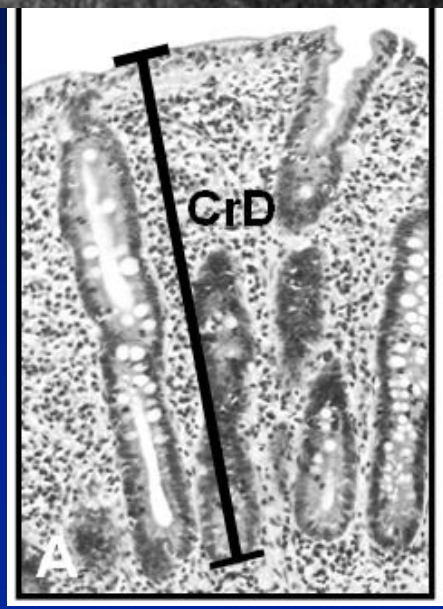


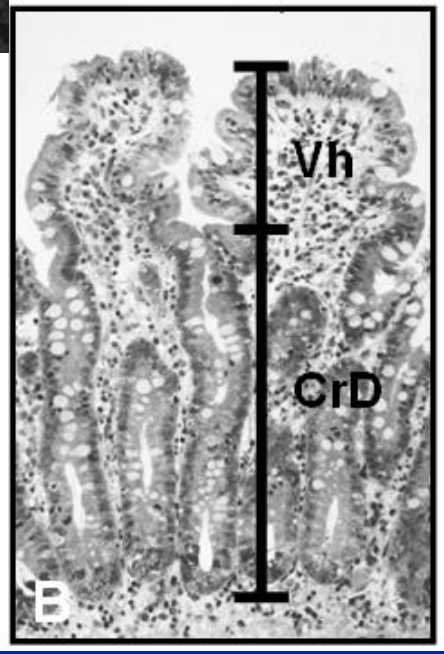
After GFD

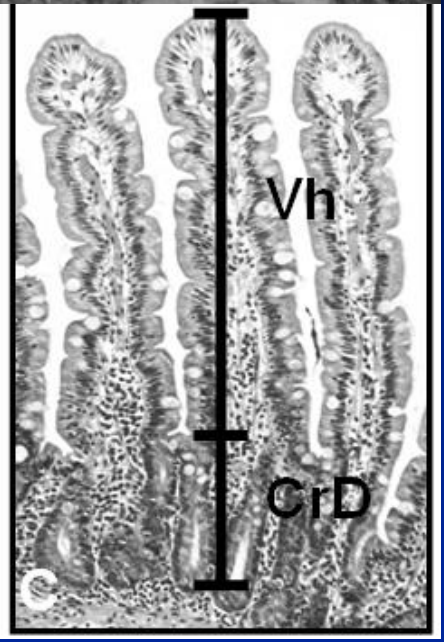


Mucosal repair









Rubin et al., 1960

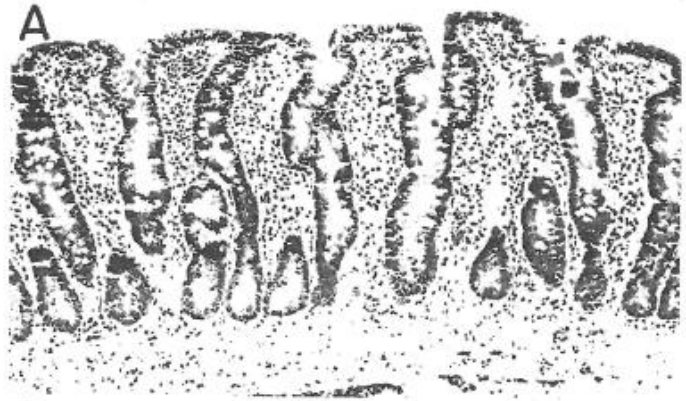


FIG. 13. Variation in severity within the same patient (× 100): *A*, moderate; *B*, severe.

Thurlbeck et al., 1960

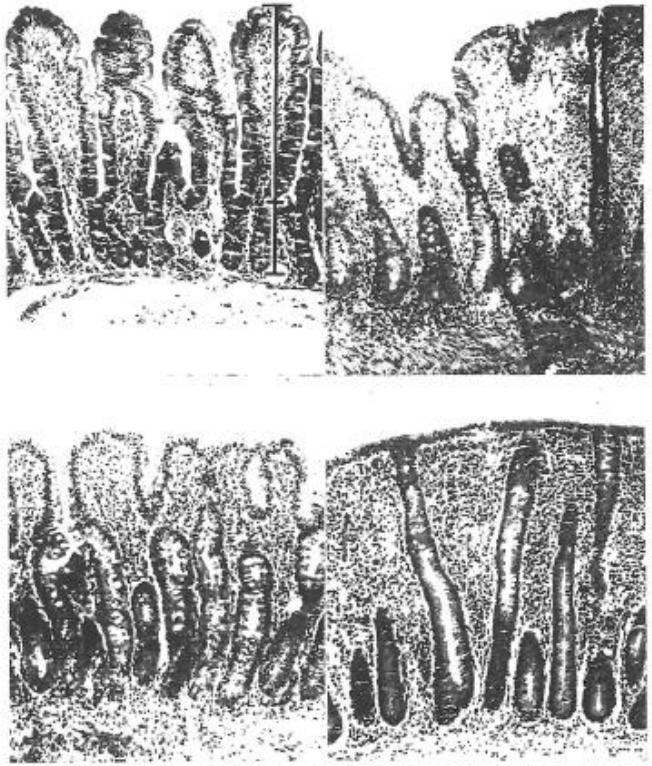


FIG. 1 (upper left). Case 4. Normal jejunal epithelium. The height of the villus and depth of the crypt are compared. Hematoxylin and eosin. × 100.

FIG. 2 (upper right). Case 8. Focal area of regeneration resembling sprue in otherwise normal biopsy specimen of jejunum. Hematoxylin and eosin. × 100.

FIG. 3 (lower left). Case 12. Moderate changes in sprue. Note that the villi are short, the crypts elongated, and the thickness of the mucosa approximately normal. Hematoxylin and eosin. × 100.

FIG. 4 (lower right). Case 20. Advanced changes in sprue, with no recognizable villi and conspicuous elongation of crypts. Hematoxylin and eosin. × 100.

Coeliac Disease Study Group

Risdon et al. Gut 1974: Only those biopsies were considered in which the plane of sectioning was prependicular to the luminal surface, as judges by the fact that the crypts of Lieberkuhn were cut longitudinally and not in cross section (fig1)

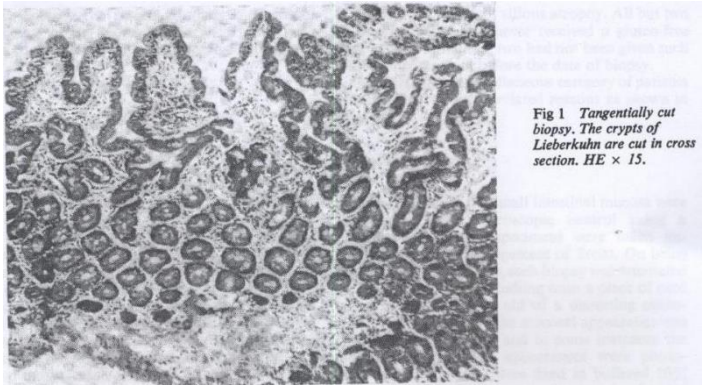
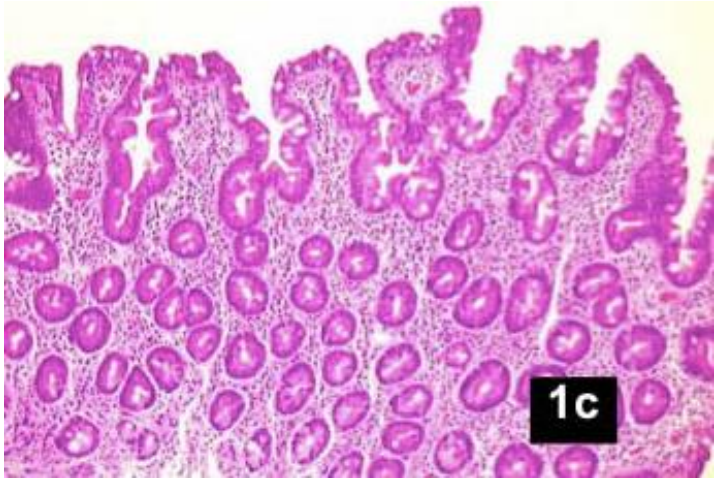


Fig 1 *Tangentially cut biopsy. The crypts of Lieberkuhn are cut in cross section. HE × 15.*

Biopsy validation 2009
Figure 1. Photographs of the small intestine: classifcation of histopathology.

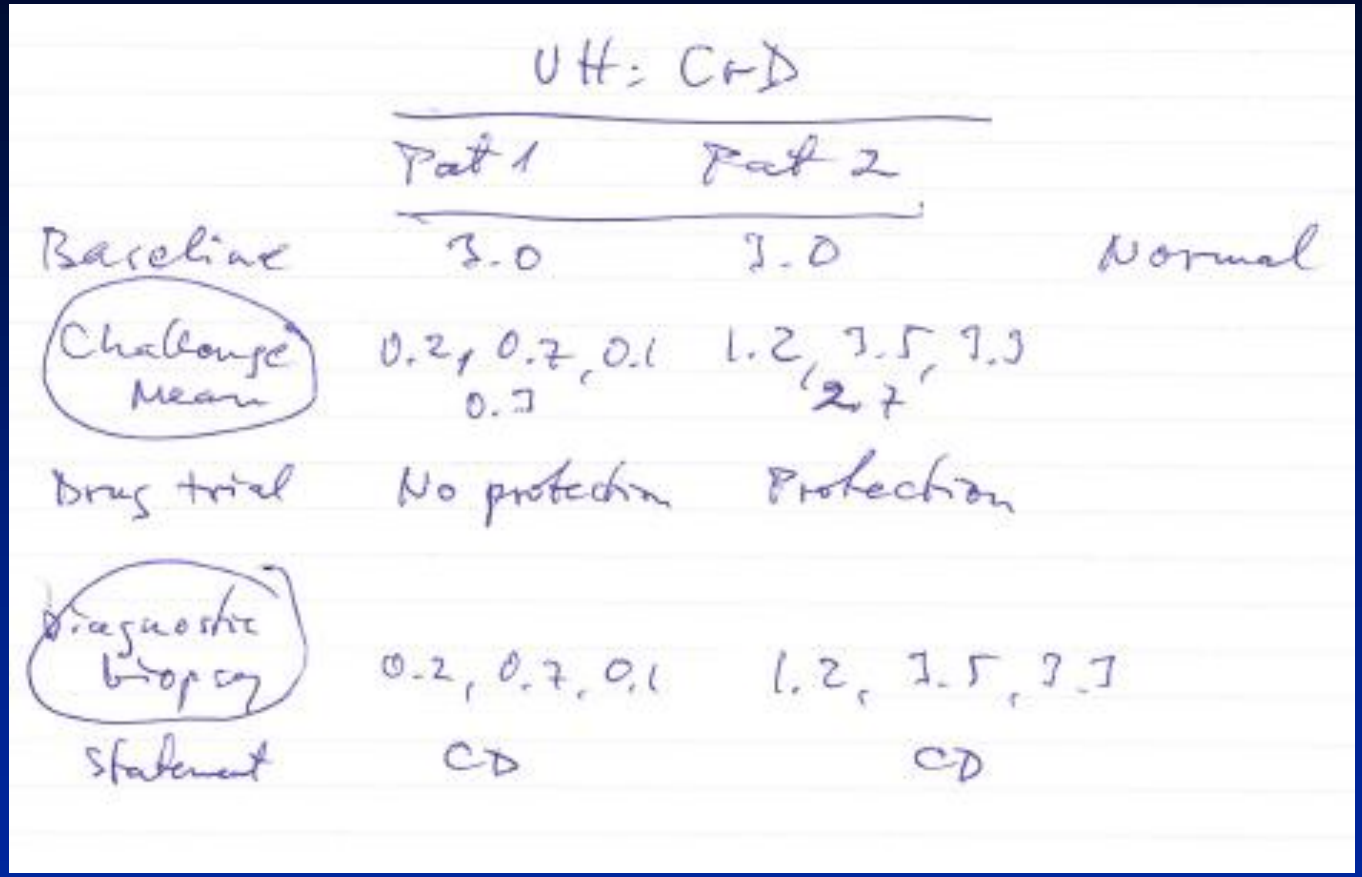


Villous height (VH) and crypt depth (CrD)
CD3-positive IELs/100 epithelial cells

- Morphometry for morphology and inflammation separately
- SOPs
- Readouts by trained and accredited evaluators
- Formal quantification and validation of readouts performed

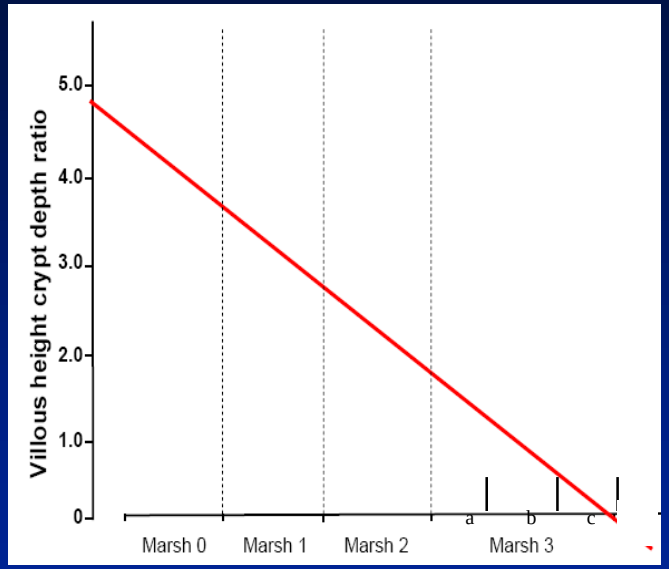
- Pitfall in coeliac disease diagnostics: Readers must learn to identify proper biopsy orientation, the correct cutting, where and when VH:CrD is allowed to be measured.

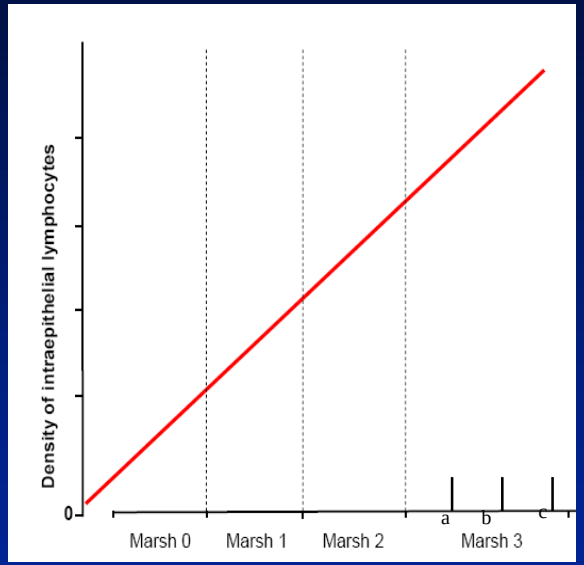
- In case of non-optimal orientation, the reader instructs the technician as to block rotation, reorientation and recutting, restainings, followed by rereading, and again, if needed (guarantee of quality).


UH: CrD
Pat 1
Pat 2
Baseline
3.0
3.0
Normal
Challenge Mean
0.2, 0.7, 0.1
0.3
1.2, 3.5, 3.3
2.7
Drug trial
No protection
Protection
Diagnostic biopsy
0.2, 0.7, 0.1
1.2, 3.5, 3.3
Statement
CD
CD

Marsh-Oberhuber classification 2000

Grouped classification: Morphology and inflammation in one, how to measure significance??





Morphometry: continuous parametric variables instead of grouped classes.
Separate evaluation of morphology (VH:CrD) and inflammation (IEL densities)

Significant morphometrical changes even within one Marsh class

How to measure biopsy specimens?
Reproducibility of Marsh classes

Mubarak et al. Reproducibility of the histological diagnosis of coeliac disease. Scand J Gastroenterol 2011 (The Netherlands)

Interobserver variability for the different Marsh classifications as moderate, Kappa value 0.486

Corazza et al. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clin Gastroenterol Hepatol 2007 (Italy)

Interobserver variability for the Marsh-Oberhuber classification was fair, Kappa value 0.35

Arguelles-Grande et al. Variability in mall bowel histopahology reporting between different pathology practice settings: impact on the diagnosis of coeliac disease. J Clin Pathol 2012 (USA)

Within different Marsh score categories, agreement was poor (Kappa <0.0316) for score 1 and 2 and fair or moderate for scores 3a and 3b.

Picarelli et al. Weaknesses of histological analysis in celiac disease diagnosis: new possible scenarios. Scand J Gastroenterol 2014 (Italy)

Our study stresses the limits of histological interpretation due to the lack of uniformity in the use of Marsh-Oberhuber classification

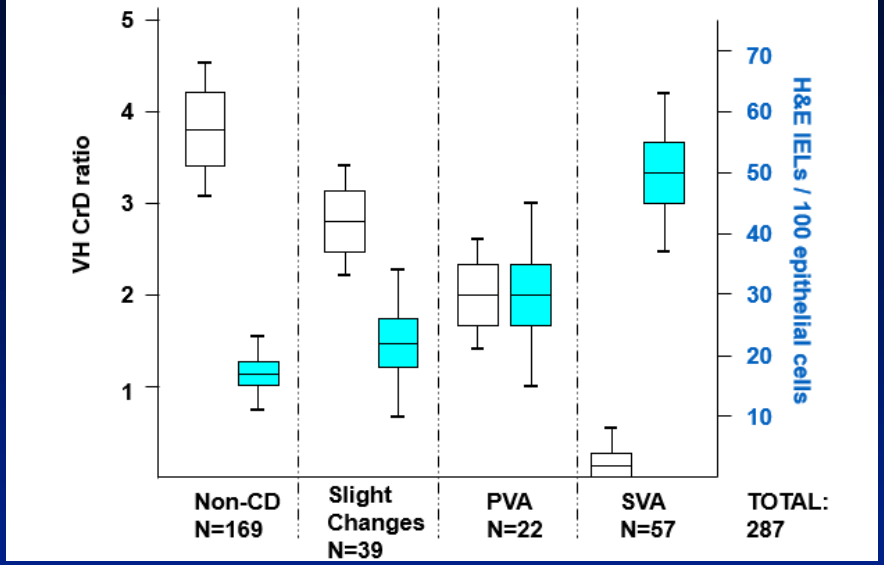
Comparison Histopathology in ProCeDE

Local vs central – Marsh staging

Local histology Marsh Staging	Reference pathology				
	Marsh 0	Marsh 1	Marsh 2	Marsh 3A-C	Total
Marsh 0	**28**	20	7	8	63
Marsh 1	7	**10**	6	9	32
Marsh 2	1	3	**1**	14	19
Marsh 3A-3C	11	13	14	**545**	583
No staging available	13	3	0	**18**	34
Total	60	49	28	594	731

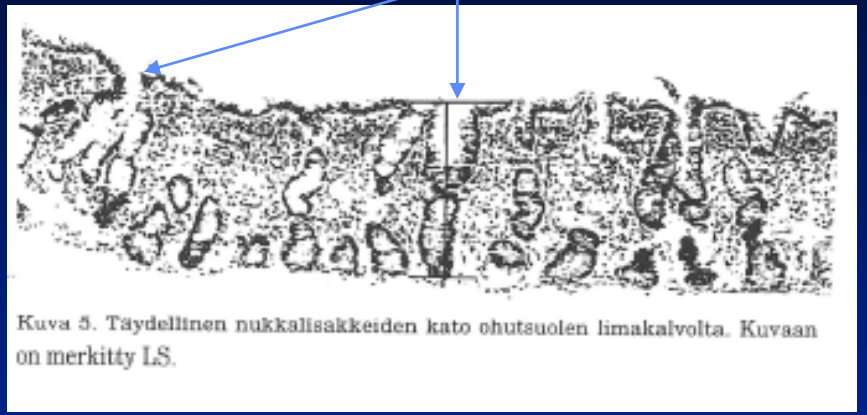
(Werkstetter, Koletzko et al , unpublished)

Grouped classification vs. morphometry
Randomly selected Celiac Research Laboatory stored jejunal biopsies from 1976-1986, Hartikainen M., Advanced studies, University of Tampere, 1994



Leading morphometry paper
Kuitunen et al., JPGN 1982

"basins"



Kuva 5. Täydellinen nukkalisäkkeiden kato ohutsuolen limakalvolta. Kuvaan on merkitty LS.

Gastroenterology and Hepatology From Bed to Bench.
©2015 RIGLD, Research Institute for Gastroenterology and Liver Diseases

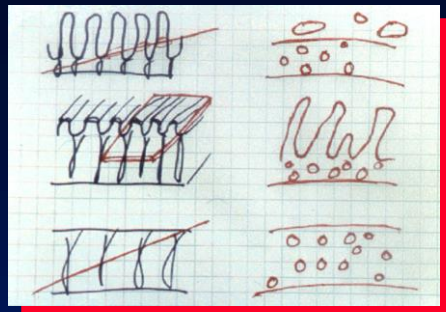
REVIEW ARTICLE

Gastroenterol Hepatol Bed Bench 2015;8(2):99-109).

Mucosal histopathology in celiac disease: a rebuttal of Oberhuber's sub-division of Marsh III

Michael N Marsh[1, 2], Matt W Johnson[1], Kamran Rostami[3]

Our data revealed on the surfaces of flat (Marsh III) mucosae, large open "basins", surrounded by raised collars - the latter, when viewed in histological section, being easily misconstrued as "villi".



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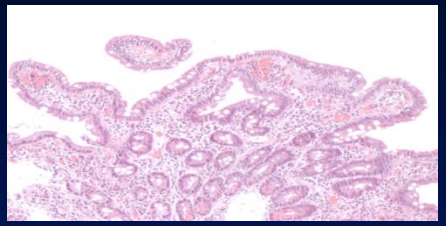
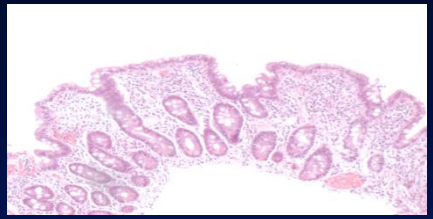
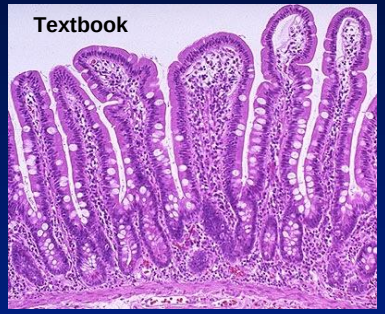
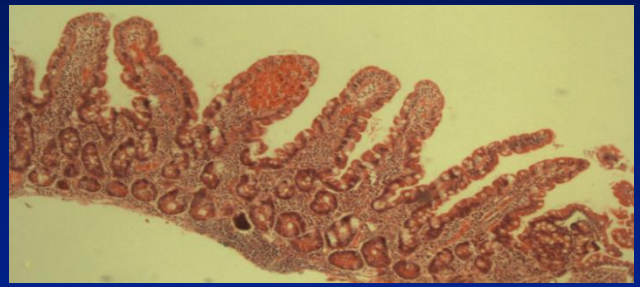


Reoriented, recut

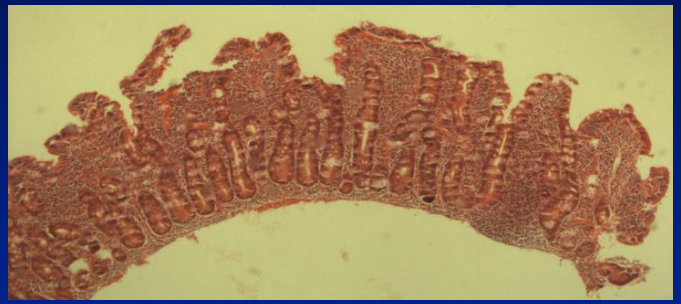




No readout allowed



Reoriented, recut



OPEN ACCESS Freely available online PLOS ONE

Validation of Morphometric Analyses of Small-Intestinal Biopsy Readouts in Celiac Disease

Juha Taavela[1], Outi Koskinen[1], Heini Huhtala[2], Marja-Leena Lähdeaho[1], Alina Popp[1,3], Kaija Laurila[1], Pekka Collin[4], Katri Kaukinen[4,5,6], Kalle Kurppa[1], Markku Mäki[1]*

PLoS ONE 8(10): e76163. 2013

• Marsh 0-I	Marsh IIIc
• Marsh I	Marsh IIIb
• Marsh 0-I	Marsh IIIc
• Marsh 0-I	Marsh IIIb
• Marsh I	Marsh IIIb

OPEN ACCESS Freely available online

PLOS ONE

Validation of Morphometric Analyses of Small-Intestinal Biopsy Readouts in Celiac Disease

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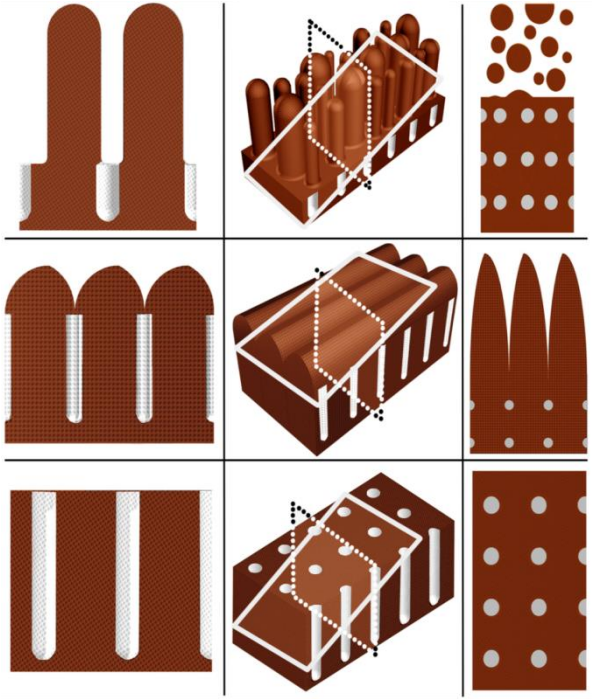
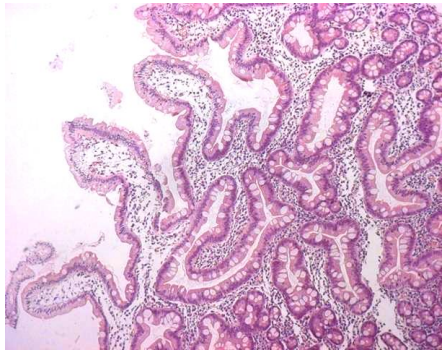
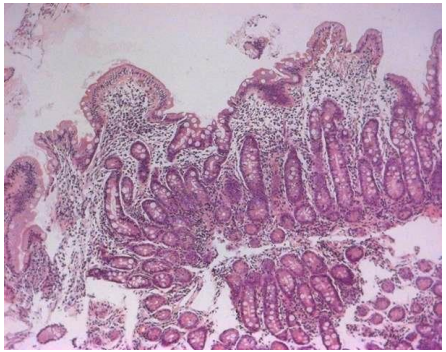


The effect of tangential sectioning has been 3D-modeled.

Be aware !!!! Wrong cutting of diseased celiac mucosa might show ”villus-like artefacts” and crypts cut only in cross sections

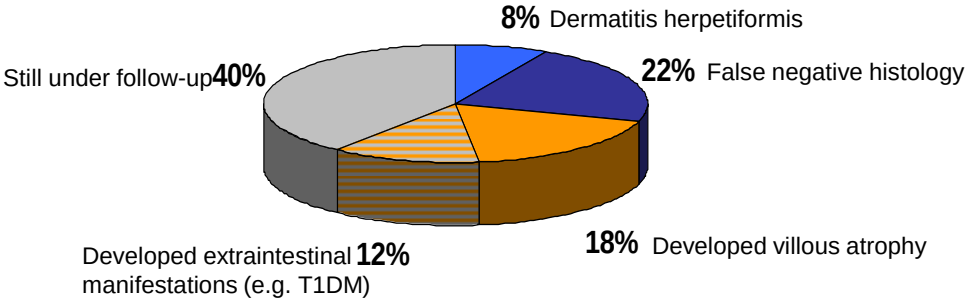
Seropositive patients without villous atrophy n=242

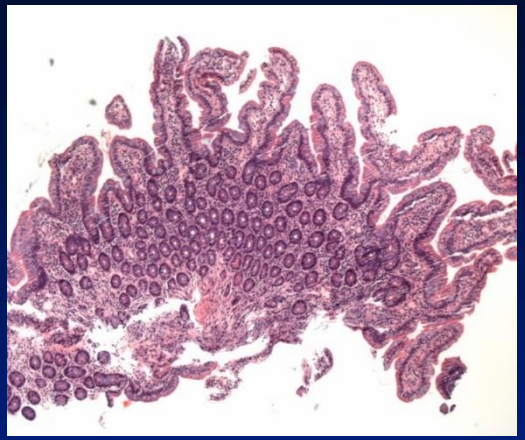




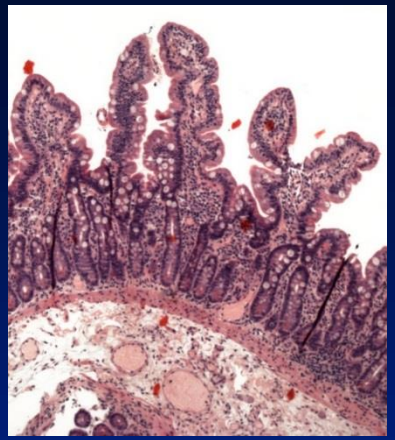
58 (22%)

- Initially no without villous atrophy n=189 followed



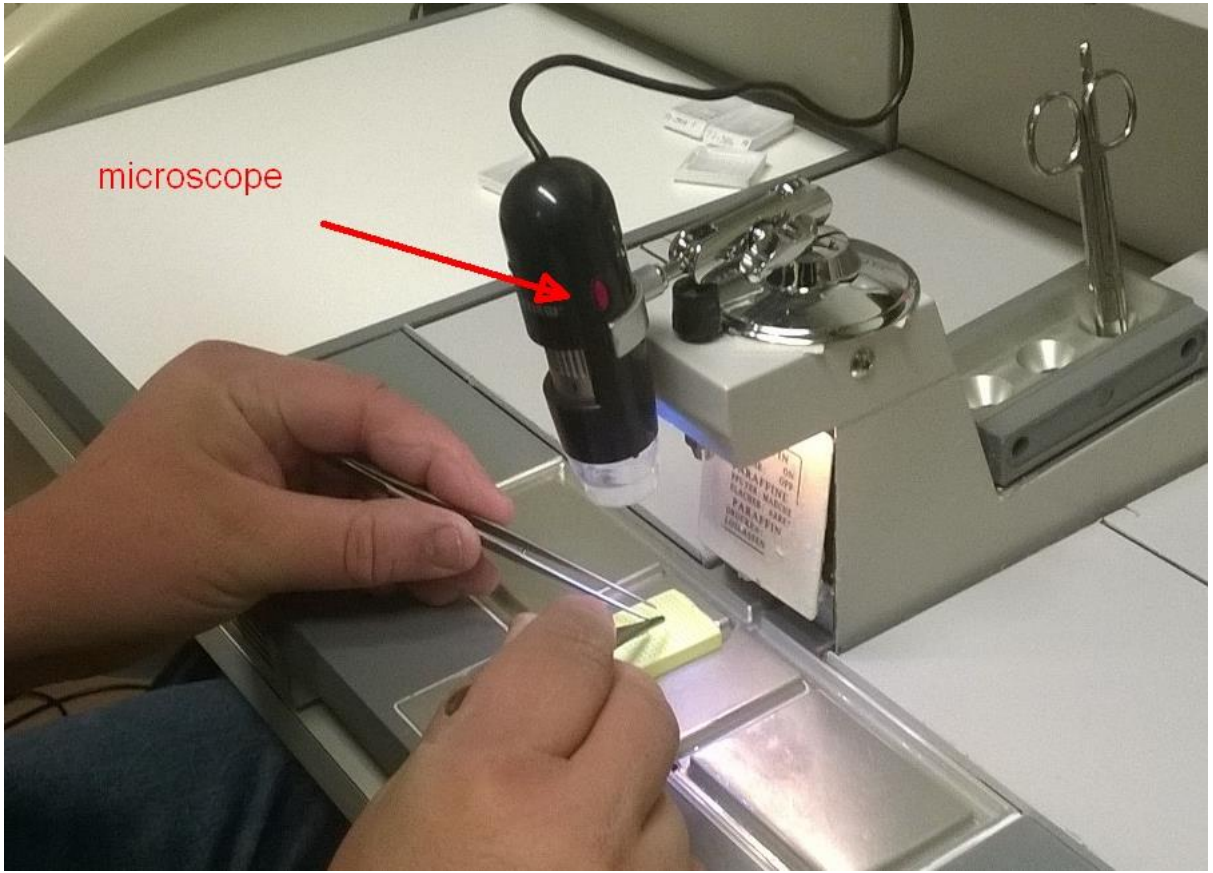


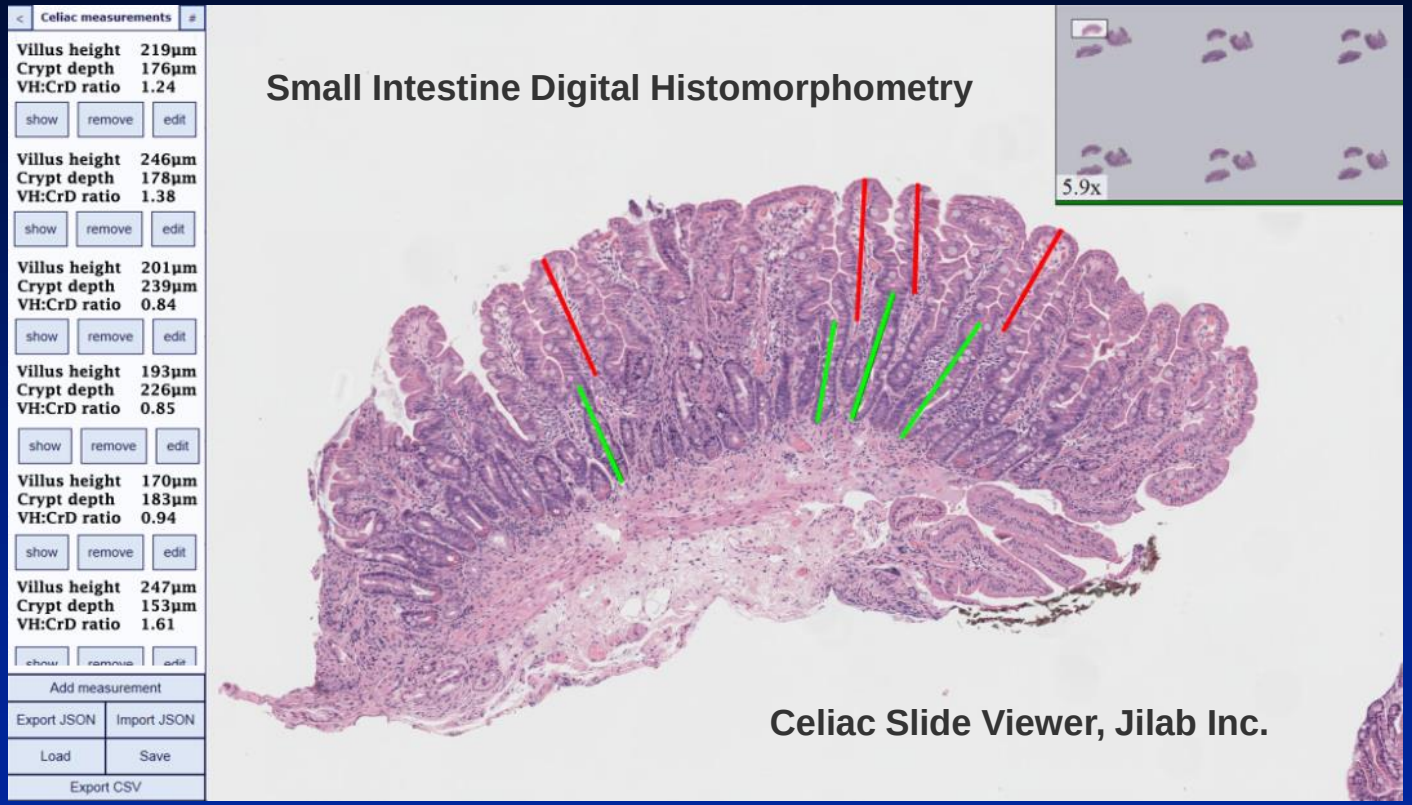
Initial evaluation: Marsh 1 lesion
Morphometry: Tangential cutting,
VH:CrD not measurable,
Depths of crypts not identifiable
Marsh class cannot be stated
Hallmark of wrong cutting:
cross sections of crypts
CD3 +ve IELs: 79 cells/100 ECs



Recutting of same biopsy block
Now better cutting, hallmark
longtudinally cut crypts, allowed
to be evaluated and measured
Marsh class 2 lesion
Morpohometry:
mean VH:CrD 2.0,
CD3+ve IELs: 76 cells/100 ECs

Microscope-assisted biopsy embedding

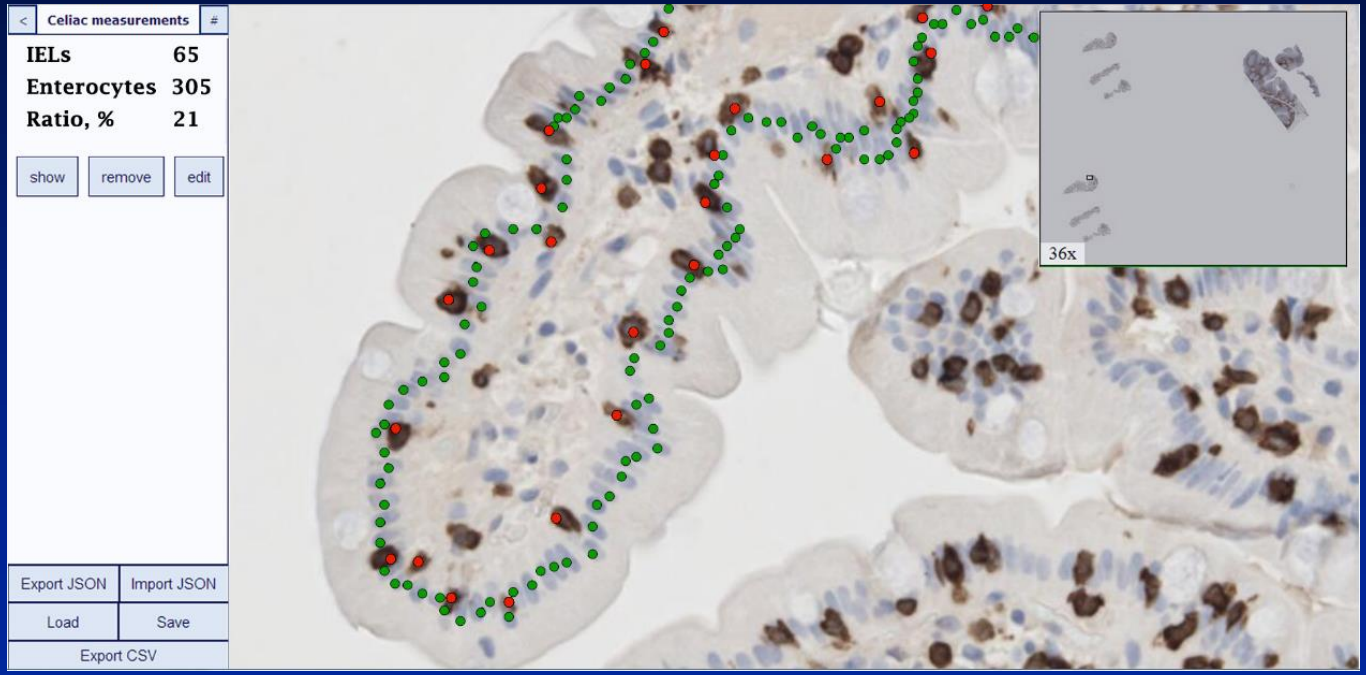



Celiac measurements
Villus height 219µm
Crypt depth 176µm
VH:CrD ratio 1.24
show remove edit
Villus height 246µm
Crypt depth 178µm
VH:CrD ratio 1.38
show remove edit
Villus height 201µm
Crypt depth 239µm
VH:CrD ratio 0.84
show remove edit
Villus height 193µm
Crypt depth 226µm
VH:CrD ratio 0.85
show remove edit
Villus height 170µm
Crypt depth 183µm
VH:CrD ratio 0.94
show remove edit
Villus height 247µm
Crypt depth 153µm
VH:CrD ratio 1.61
Add measurement
Export JSON
Import JSON
Load
Save
Export CSV
Small Intestine Digital Histomorphometry
5.9x
Celiac Slide Viewer, Jilab Inc.

Small Intestine Digital Histomorphometry

CD3 +ve IELs per 100 epithelial cells

Celiac Slide Viewer, Jilab Inc.



Marsh classification vs. quantitative morphometry

Converting morphometric measurements (villus height, VH, crypt depth, CrD and their ratio, VH:CrD, and the density of CD3+ve intraepithelial lymphocytes, IELs, to Marsh-Oberhuber (2000) celiac disease diagnostic classes.

Marsh class	Type 0	Type 1	Type 2	Type 3a	Type 3b	Type 3c
				CD	CD	CD
IELs*	<25	>25	>25	>25	>25	>25
Crypts	Normal	Normal	Hypertrophic	Hypertrophic	Hypertrophic	Hypertrophic
Villi	Normal	Normal	Normal	Mild atrophy	Marked atrophy	Absent
VH:CrD	≥2.5	≥2.5	2.0-2.4	1.2-1.9	0.6-1.1	0-0.5

*Numbers are given as CD3 +ve IELs per 100 epithelial cells

Mäki et al. manuscript

ORIGINAL CONTRIBUTIONS

▲ Top

PEDIATRICS

A Prospective Study on the Usefulness of Duodenal Bulb Biopsies in Celiac Disease Diagnosis in Children: Urging Caution

Juha Taavela MD, Alina Popp MD, Ilma Rita Korponay-Szabo MD, Adina Ene MD, Martine Vornanen MD, Päivi Saavalainen PhD, Marja-Leena Lähdeaho MD, Tarja Ruuska MD, Kaija Laurila MSc, Alexandru Parvan MD, Ioana Anca MD, Kalle Kurppa MD and Markku Mäki MD

Am J Gastroenterol 111: 124-133; advance online publication, January 5, 2016; doi:10.1038/ajg.2015.387

Abstract | Full Text | PDF

Subject Category: Pediatrics

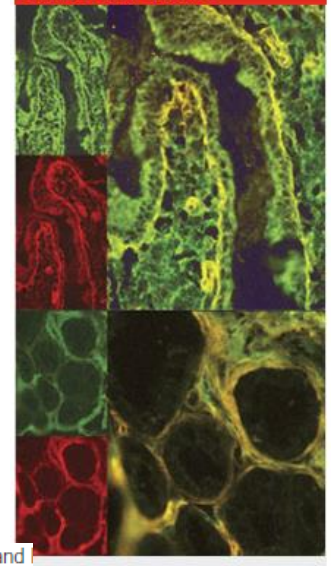
Juha Taavela, MD[1,8], Alina Popp, MD[1,2,8],

[8]These authors contributed equally to this work;

[1]Tampere Centre for Child Health Research, School of Medicine, University of Tampere and Tampere University Hospital, Tampere, Finland;

[2]University of Medicine and Pharmacy "Carol Davila" and Institute for Mother and Child Care, Bucharest, Romania;

Image of the month



A Prospective Study on the Usefulness of Duodenal Bulb Biopsies in Celiac Disease Diagnosis in Children: Urging Caution (From Juha Taavela, Alina Popp, Ilma Rita Korponay-Szabo, Adina Ene, Martine Vornanen, Päivi Saavalainen, Marja-Leena Lähdeaho, Tarja Ruuska, Kaija Laurila, Alexandru Parvan, Ioana Anca, Kalle Kurppa and Markku Mäki, see pages 124-133.)

www.medscape.com

Duodenal Bulb Biopsies for Celiac Diagnosis Often Unreadable, Misleading

By Anne Harding | January 14, 2016

REUTERS HEALTH INFORMATION

NEW YORK (Reuters Health) - Caution should be used in both acquiring and interpreting duodenal bulb biopsies for the diagnosis of celiac disease in children, according to a new prospective study.

Table 3. Coeliac disease patients. Villous heights to crypt depth ratio (VH:CrD) and density of immunohistochemically stained intraepithelial lymphocytes (IELs) and transglutaminase 2 targeted IgA deposits in **the bulb biopsy specimens** in 22 children with a biopsy-proven celiac disease.

Case number	VH (µm)	CrD (µm)	VH:CrD	CD3+ IELs cells/mm	γδ+ IELs cells/mm	IgA-deposits
1	ND*	ND*	ND*	76	22.9	+++
2	13	490	0.03	94	32.3	+++
3	ND*	ND*	ND*	76	39.3	+++
4	27	463	0.06	93	18.7	+++
5	ND*	ND*	ND*	ND†	ND†	ND†
6	23	450	0.05	78	39.5	+++
7	ND*	ND*	ND*	63	8.4	++
8	17	383	0.04	90	47.3	+++
9	ND*	ND*	ND*	106	38.2	+++
10	175	395	0.44	47	15.3	+++
11	63	353	0.18	103	25.5	+++
12	ND*	ND*	ND*	95	16.1	+++
13	ND*	ND*	ND*	47	21.0	+++
14	ND*	ND*	ND*	32	10.5	+++
15	ND*	ND*	ND*	69	23.1	+++
16	ND*	ND*	ND*	ND†	ND†	ND†
17	ND*	ND*	ND*	ND‡	ND ‡	+++‡
18	10	595	0.02	107	50.6	+++
19	135	475	0.28	32	18.3	+++
20	267	327	0.81	44	31.8	++
21	140	533	0.26	74	36.8	+++
22	13	243	0.05	30	8.8	++

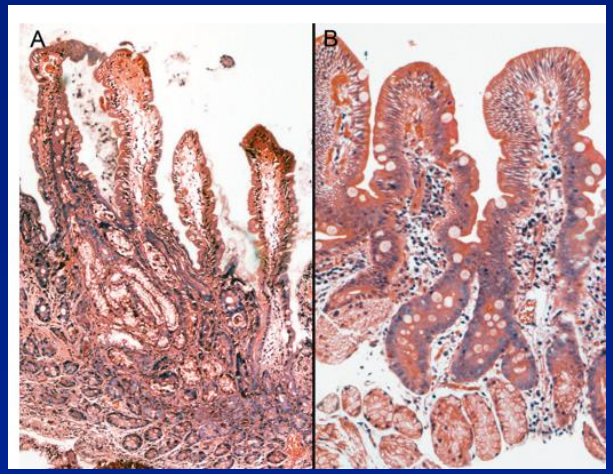
* Due to small and fragmented biopsies reliable measurements were impossible even after re-cuttings
† Frozen sample was not available from bulb
‡ The frozen sample had no epithelium and therefore IEL were not measurable but IgA-deposits were

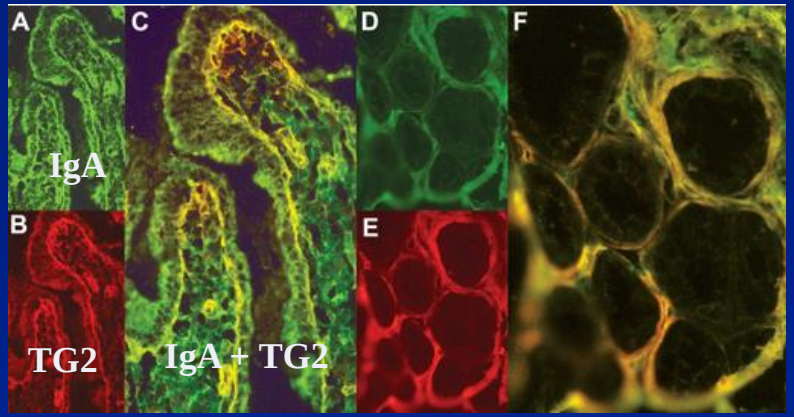
Table 4. Non-coeliac disease controls. Villous heights to crypt depth ratio (VH:CrD) and density of immunohistochemically stained intraepithelial lymphocytes (IELs) and transglutaminase 2 targeted IgA deposits in the **bulb biopsy specimens** in 22 children without evidence of celiac disease

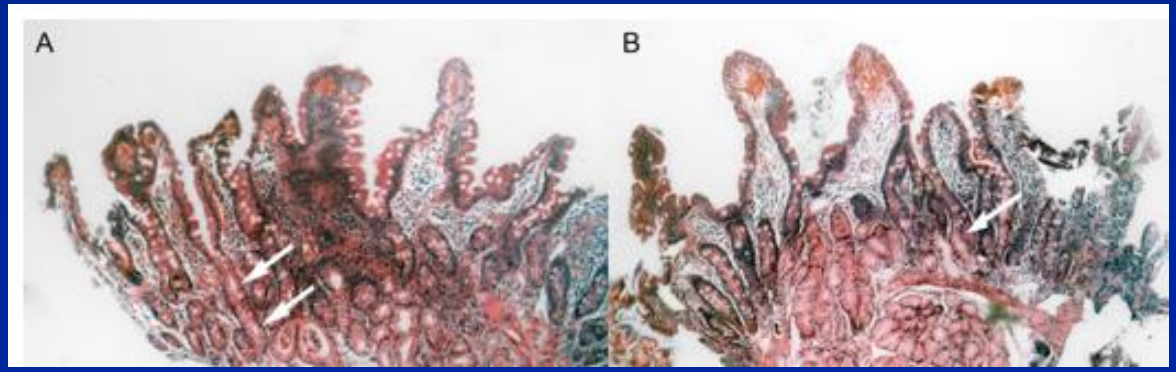
Case number	VH (µm)	CrD (µm)	VH:CrD	CD3+ IELs cells/mm	γδ+ IELs cells/mm	IgA-deposits	Final diagnosis
1	ND*	ND*	ND*	20	2.0	0	Irritable bowel syndrome
2	403	295	1.4	22	6.1	0	Irritable bowel syndrome
3	275	360	0.8	13	1.4	0	Recurrent abdominal pain
4	310	220	1.4	22	2.2	0	Iron deficiency anemia
5	308	193	1.6	39	0.7	0	Irritable bowel syndrome
6	ND*	ND*	ND*	28	7.0	0	Eosinophilic gastroententeritis
7	353	373	0.9	24	4.2	0	H. Pylori
8	ND*	ND*	ND*	40	3.6	0	Cow's milk allergy
9	333	320	1.0	43	0.7	0	Giardia lamblia
10	445	170	2.6	11	3.5	0	Gastroesophageal reflux
11	ND*	ND*	ND*	20	1.9	0	Cow's milk allergy
12	ND*	ND*	ND*	24	4.9	0	Postenteric syndrome
13	408	238	1.7	30	8.5	0	Giardia lamblia, H. Pylori
14	420	187	2.3	ND†	ND†	ND†	Giardia lamblia
15	ND*	ND*	ND*	ND†	ND†	ND†	H. Pylori negative chronic gastritis
16	ND*	ND*	ND*	ND†	ND†	ND†	Ascaris lumbricoides
17	30	263	0.1	70	4.9	0	Entamoeba histolytica
18	ND*	ND*	ND*	ND†	ND†	ND†	Intestinal lymphangiectasia‡
19	183	307	0.6	22	0.7	0	Giardia lamblia
20	263	213	1.2	58	3.7	0	H. Pylori negative chronic gastritis
21	493	227	2.2	38	5.5	0	H. Pylori negative chronic gastritis
22	ND*	ND*	ND*	ND†	ND†	ND†	Postenteritic syndrome

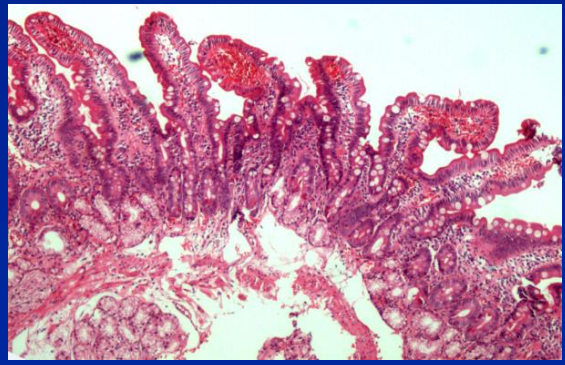
* Due to small and fragmented biopsies reliable measurements were impossible even after re-cuttings
† No sample from the anatomical bulb.
‡ with protein losing enteropathy

Duodenal bulb biopsies, urging caution









TaavelaPopp et al. Am J Gastroenterol, 2016

Advertising Tampere Celiac Disease Symposium

Maki Celiac Disease Tampere Prize
April 17, 2015.
Rector Kaija Holli and
Prof. Detlef Schuppan.

SAVE THE DATE !

Tampere Celiac Disease Symposium
Measuring Treatment Outcome

November 25, 2016, Tampere Hall, Tampere, Finland

Abstract **deadline** for short oral and poster presentations September 15, 2016.

Announcing the **2nd Maki Celiac Disease Tampere Prize,** € 15 000.

Requests for nominations will open in March 2016.

The symposium is held in parallel with the **Gluten-free Life Expo 2016 ,** organised by the Finnish Celiac Society, Nov 25-26, 2016

THANK YOU !

Table 1. Coeliac disease patients. Demographic and clinical data and serum autoantibody titres at diagnosis and human leucocyte antigen (HLA) DQ2 and DQ8 status in 22 children with a biopsy-proven celiac disease.

Case Number	Age, years	Sex	Main symptom or sign	Family history	EmA titre	TG2-ab U/ml	HLA
1	6.8	F	Abdominal pain	-	1000	>100.0	DQ2.5
2	12.2	F	Poor growth	+	1000	>100.0	DQ2.5
3	10.8	M	Arthralgia	-	500	>100.0	DQ2.5
4	2.6	M	Abdominal pain	+	500	>100.0	DQ2.5
5	2.7	F	Asymptomatic*	-	4000	>100.0	DQ2.5/DQ8
6	4.8	F	Poor growth	+	200	>100.0	DQ2.5/DQ8
7	13.3	F	Abdominal pain	+	200	8.9	DQ8
8	4.4	M	Abdominal pain	-	2000	>100.0	DQ2.5
9	10.7	F	Abdominal pain	+	4000	>100.0	DQ8
10	13.8	F	Abdominal pain	+	200	46.0	DQ2.5
11	5.0	F	Abdominal pain	-	1000	>100.0	DQ2.5
12	4.1	F	Anemia	-	4000	>100.0	DQ2.5
13	2.5	M	Abdominal pain	-	200	>100.0	DQ2.5/DQ2.2
14	2.6	F	Asymptomatic*	+	50	3.4	DQ2
15	3.1	F	Malabsorption	-	500	>100.0	DQ2.5
16	2.6	M	Asymptomatic*	-	8000	>100.0	DQ2.5
17	17.6	F	Dermatitis herpetiformis	+	1000	>100.0	DQ2.5/DQ2.2
18	5.0	M	Abdominal pain	-	2000	>100.0	DQ2.5/DQ2.2
19	13.3	F	Abdominal pain	+	100	19.0	DQ2.5
20	14.8	F	Anemia	+	4000	>100.0	DQ2.5
21	5.3	F	Anemia	+	2000	>100.0	DQ2.5
22	5.7	F	Abdominal pain	-	50	>100.0	DQ2.5/DQ8

EmA, endomysial antibody; TG2, transglutaminase 2 antibody
* Screen-detected from at-risk group

Table 2. Non-coeliac disease control patients. Demographic and clinical data and serum autoantibody titres at diagnosis and human leucocyte antigen (HLA) DQ2 and DQ8 status in 22 children without evidence of celiac disease

Case Number	Age, years	Sex	Main symptom or sign	Family history	EmA titre	TG2-ab U/ml	HLA
1	3.4	F	Recurrent abdominal pain	-	<5	<5.0	DQ2.5
2	8.0	F	Recurrent abdominal pain	+	<5	<5.0	DQ2.5
3	9.4	M	Recurrent abdominal pain*	-	<5	<5.0	DQ2.5
4	3.8	F	Iron deficiency anemia*	-	<5	<5.0	DQ2.5
5	9.1	M	Chronic diarrhea	-	<5	<5.0	DQ2.5
6	4.1	F	Recurrent abdominal pain	-	<5	<5.0	DQ2.2
7	4.1	F	Dyspepsia	-	<5	<5.0	DQ2.5
8	1.6	M	Chronic diarrhea	-	<5	<5.0	Non-DQ2/DQ8
9	5.9	M	Recurrent abdominal pain	-	<5	<5.0	DQ8
10	4.8	F	Recurrent abdominal pain	-	<5	<5.0	DQ2.5
11	5.4	M	Abdominal pain	-	<5	<5.0	Non-DQ2/DQ8
12	4.3	M	Diarrhea	-	<5	<5.0	Not available
13	5.3	M	Abdominal pain	-	<5	<5.0	Not available
14	15.2	F	Abdominal pain	-	<5	<5.0	Not available
15	14.6	M	Dyspepsia	-	<5	<5.0	DQ2.5 and DQ8
16	10.5	M	Low weight	-	<5	<5.0	DQ2.5
17	14.4	M	Encopresis	-	<5	<5.0	DQ2.5
18	4.1	F	Constipation	-	<5	<5.0	DQ8 homozygote
19	5.1	F	Malabsorption	-	<5	<5.0	Not available
20	6.2	F	Recurrent abdominal pain	-	<5	<5.0	Non-DQ2/DQ8
21	8.4	F	Recurrent abdominal pain	-	<5	<5.0	DQ2 and DQ7
22	3.1	M	Chronic diarrhea	-	<5	<5.0	Not available

EmA, endomysium antibody; TG2, transglutaminase 2 antibody