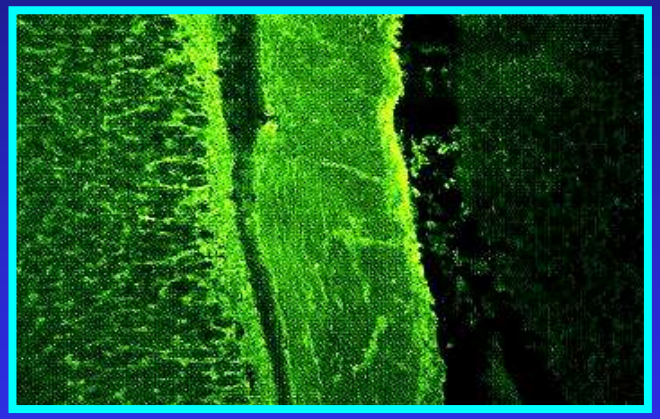
Marqueurs sérologiques de la maladie coeliaque

L.Lutteri
CHU Liège

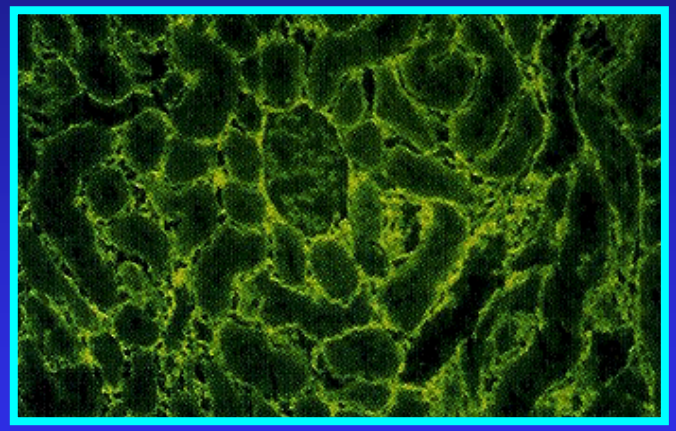
Anti-réticuline (1970)

- IFI sur rein,foie,estomac de rat, R1 (et Rs), IgA

estomac



rein

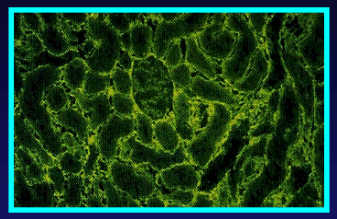


Musc.mucosae + , fibrilles entre cell. épithéliales.

fluo. péritubulaire
fluo. périglomérulaire

+ foie : vaisseaux + , fluo. linéaire autour sinusoïdes

Anti-réticuline R1 IgA

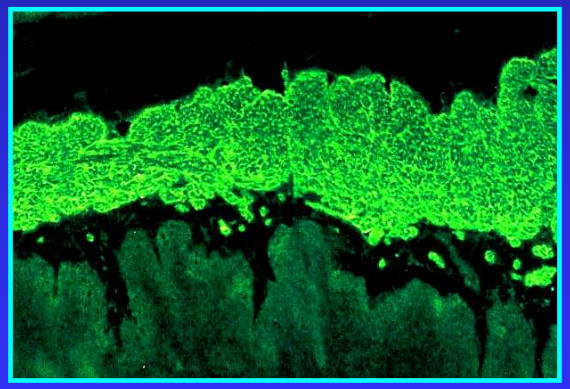


- Sensibilité : 40-60 %
- Spécificité : +/- 100 %

Anti-endomysium (1983, Chorzelski)

Réagissent avec la substance intermyofibrillaire du muscle lisse.

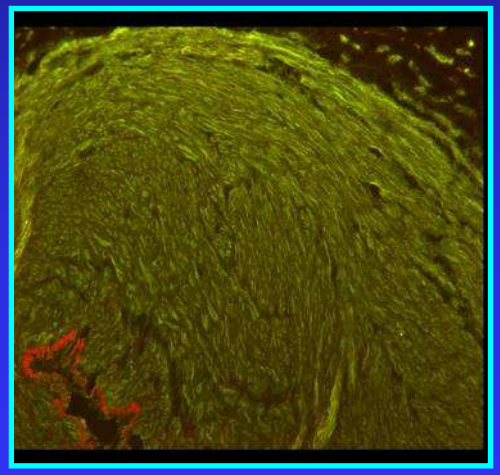
- IFI :
  - œsophage de singe

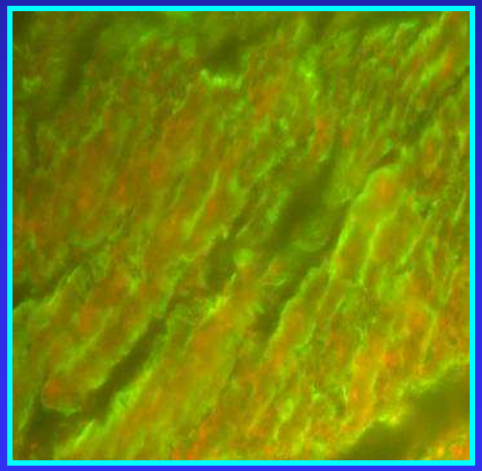


Fluorescence en nid d'abeille de la muscularis mucosae du tiers inférieur de l'œsophage de singe (partie contenant le plus d'antigène « endomysium »)

Anti-endomysium

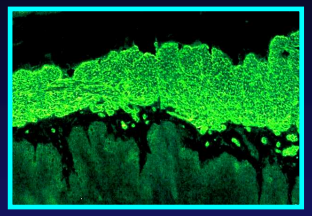
- IFI :
  - cordon ombilical humain (fluorescence de type rayon de miel des couches musculaires des veines du cordon)





A. Ocmant Erasme.

Anti-endomysium



- Recherche des IgG uniquement en cas de déficit en IgA.
- Sensibilité : > 90 %
- Spécificité : > 99 %
- Surveillance régime sans gluten : ↓ après 3 à 12 mois de régime sans gluten.
- Cible antigénique principale : *transglutaminase tissulaire.*

Anti-transglutaminase (1997 : Dieterich)

- Auto-anticorps ⇒ maladie auto-immune.
- 1ère génération : TG de foie de cobaye
  2ème génération : TG tissulaire humaine recombinante ou purifiée (TG 2) .

Anti-transglutaminase

	Anti-Endo	Anti-gptTG	Anti-htTG
CD	24/24	24/24	24/24
Controls	0/183	15/183	6/183
Sensitivity	100 %	100 %	100 %
Specificity	**100 %**	**92 %**	**97 %**

→ Crohn, colites, mal. auto-immunes,...

Carroccio. Clinical Chemistry 48:9; 1546-1550 (2002)

Diagnostic Accuracy of Ten Second-Generation (Human) Tissue Transglutaminase Antibody Assays in Celiac Disease

Britta Van Meensel,[1] Martin Hiele,[2] Ilse Hoffman,[3] Severine Vermeire,[2] Paul Rutgeerts,[2] Karel Geboes,[4] and Xavier Bossuyt[1*]

Clinical Chemistry 50:11 2125–2135 (2004)

. We found a high correlation among the methods from the different manufacturers, but agreement was low, which means that the result of one ELISA method cannot be replaced by the result of another. Thus, anti-tTG titers should not be used interchangeably. The international scientific community and the commercial private sector should undertake efforts to harmonize the assays.

It has previously been suggested that EMA could recognize other endomysial antigens in addition to tTG (*41*, *42*). In our study, however, the assays did not identify samples that were negative for IgA tTG and positive for IgA EMAs. By contrast, the assays detected five samples negative for IgA EMAs and positive for IgA tTG. This indicated that the second-generation tTG assays are more sensitive than the EMA assay.

Anti-transglutaminase

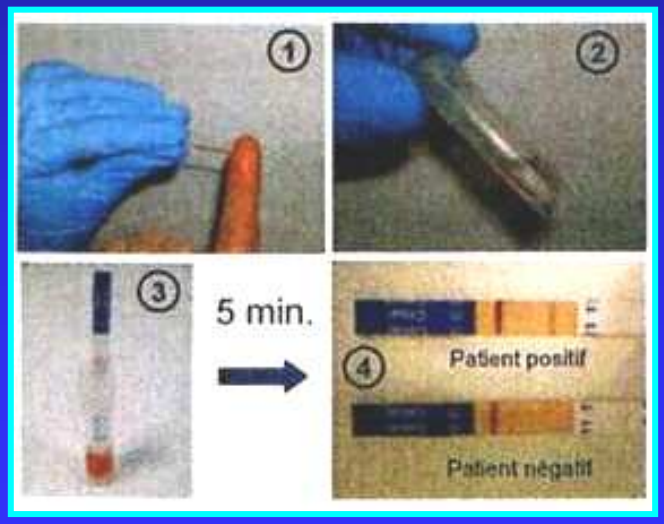
- faux positifs ⇒ anti-endomysium : reconnu comme le test le plus spécifique : à faire comme test de confirmation d'un anti-tTG + avant de faire biopsies.

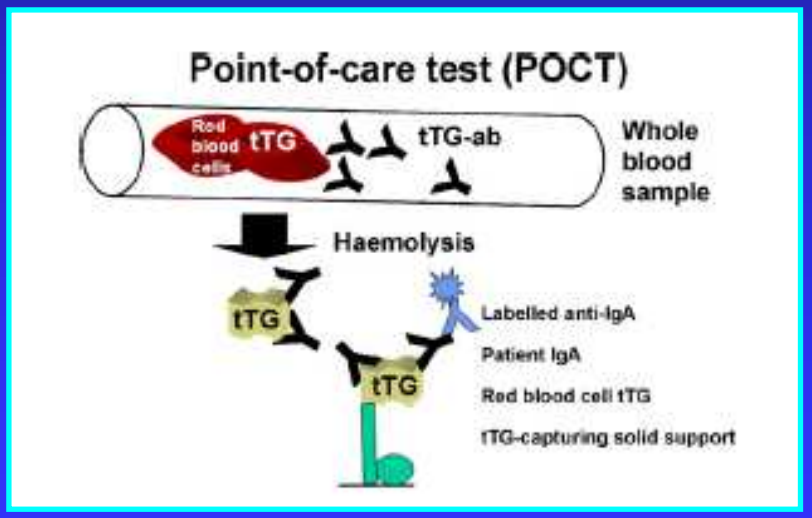
- Anti-tTG : + sensible
- Anti-endomysium : + spécifique

Anti-transglutaminase

TG de GR humains :

BIOCARD CELIAC DISEASE Test.





Anti-gliadines

- Contre la fraction alcoolo-soluble du gluten $\rightarrow$ α-gliadine

- <u>Méthode de détection</u> :
  - *IFI* : rein de rat + gliadine (se fixe sur fibres de réticuline) (Interférence des anti-réticuline !)
  - *ELISA* (pas de standard : en unités arbitraires) ou *dot* (pas quantitatif !)

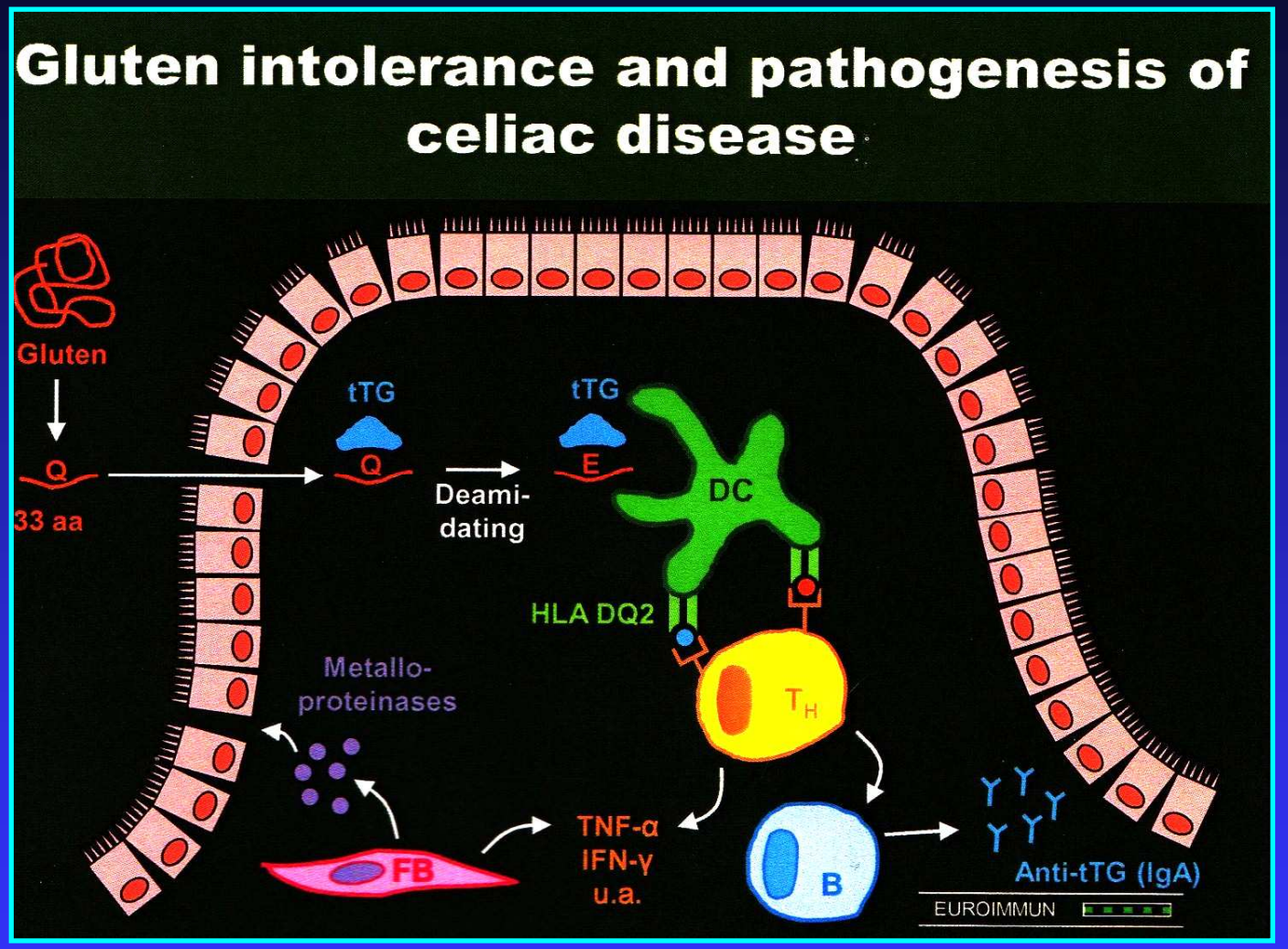
Anti-gliadines

- Sensibilité IgA : 65-100 %
  IgG : 68-91 %
- Spécificité IgA : 50-95 %
  IgG : 42-95 %

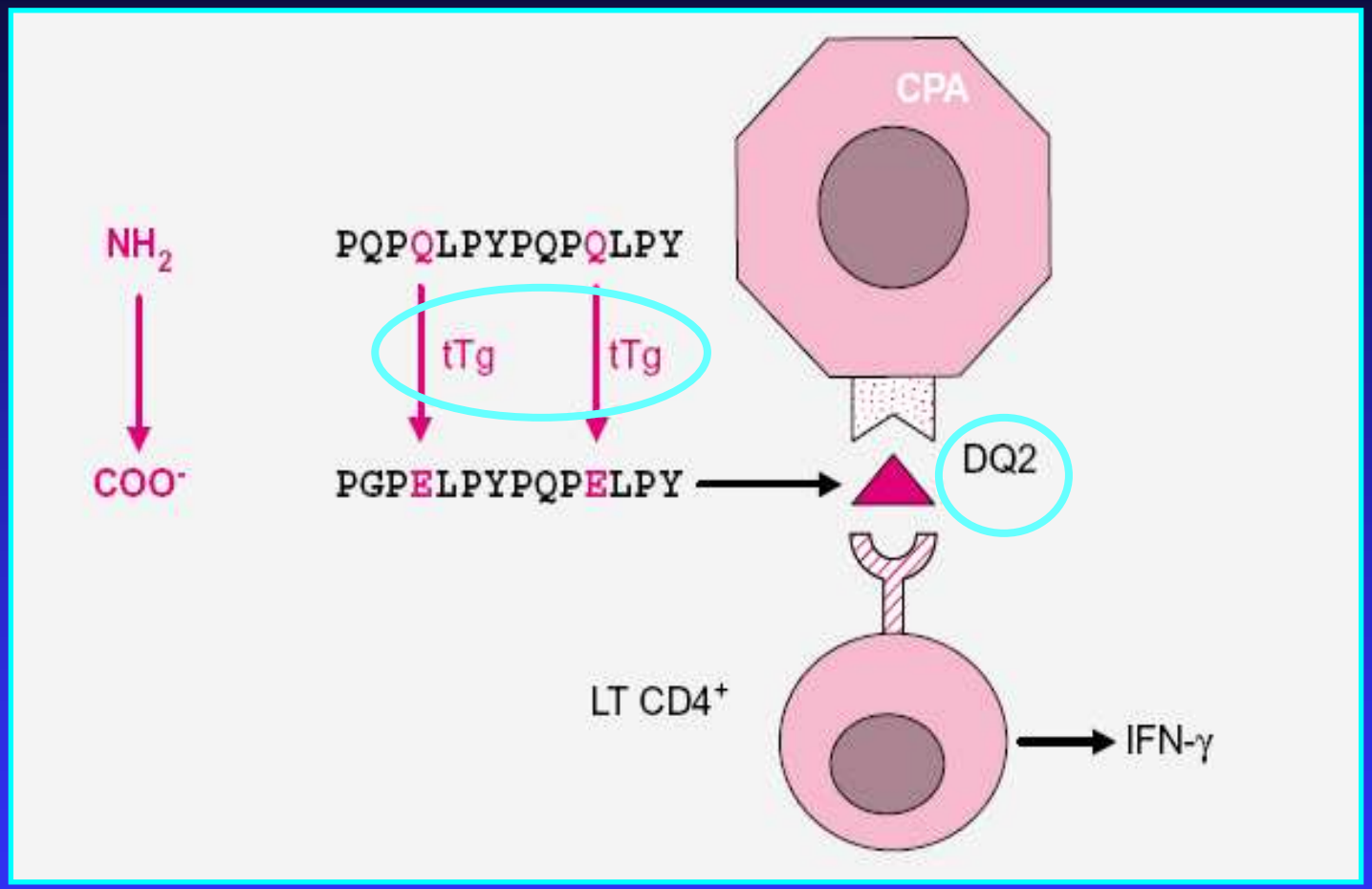
⇒ Sensibilité et spécificité basses

retrouvés dans PR, néphropathies à IgA, maladies chroniques du foie ou autres maladies inflammatoires de l'intestin.

→ utilisés uniquement pour suivi du régime sans gluten (et chez jeunes enfants)


Gluten intolerance and pathogenesis of celiac disease
Gluten
Q
33 aa
tTG
Q
Deami-
dating
tTG
E
DC
HLA DQ2
Metallo-
proteinases
TH
TNF-α
IFN-γ
u.a.
FB
B
Anti-tTG (IgA)
EUROIMMUN

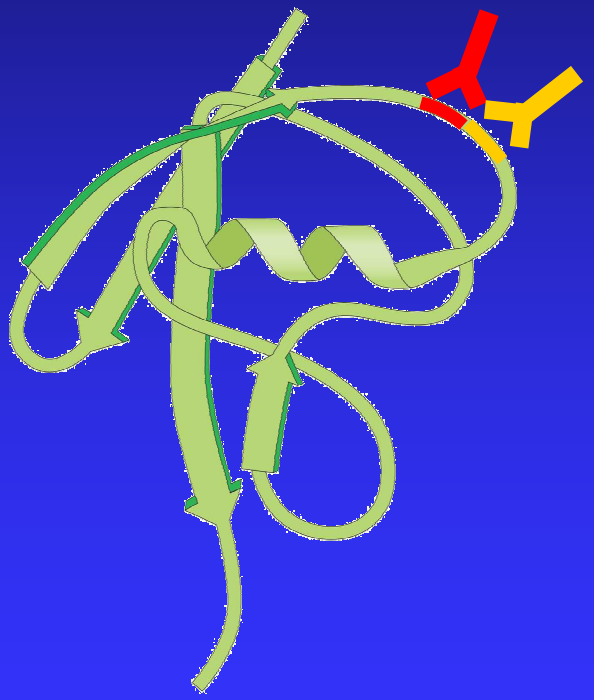
+ IL-15


CPA
NH2
PQPQLPYPQPQLPY
tTg
tTg
COO-
PGPELPYPQPELPY
DQ2
LT CD4+
IFN-γ

Antigenic epitopes of gliadin

Disease controls

CD patients



From EUROIMMUN

Serologic Assay Based on Gliadin-Related Nonapeptides as a Highly Sensitive and Specific Diagnostic Aid in Celiac Disease

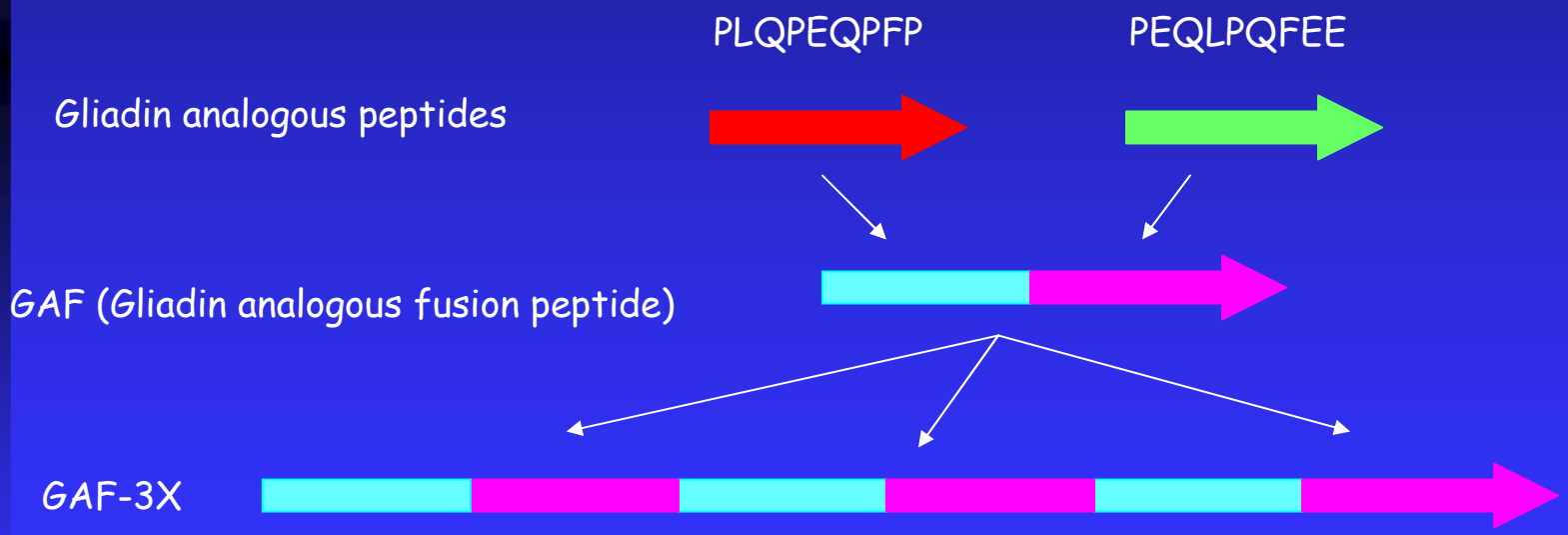
Elke Schwertz,[1] Franka Kahlenberg,[1] Ulrich Sack,[2] Thomas Richter,[3] Martin Stern,[4] Karsten Conrad,[5] Klaus-Peter Zimmer,[6] and Thomas Mothes[1*]

Clinical Chemistry 50:12
2370–2375 (2004)

Conclusion: The peptide antibody assay has higher diagnostic accuracy than AGAs for distinguishing patients with CD from controls, and has diagnostic accuracy similar to that of AtTGAs.

Anti-Gliadin (GAF-3X) ELISA

- Gliadin-Analogous Fusion peptide (GAF), containing 3 repetitive sequences.



Study

- 137 samples from biopsy-proven CD patients.
- 115 biopsy-proven disease controls.
- 626 samples from patients with collagenosis.
- 400 samples from healthy blood donors.

Sensitivity - Specificity

137 biopsy-proven CD patients and 115 biopsy-proven disease controls

ELISA	Sensitivity	Specificity
Anti-Gliadin IgA	68.6 %	90.7 %
Anti-Gliadin IgG	91.2 %	78.7 %
Anti-Gliadin IgA (GAF-3X)	83.2 %	96 %
Anti-Gliadin IgG (GAF-3X)	85.4 %	98.7 %
Anti-tTG (IgA)	97.8 %	96 %
Anti-tTG (IgG)	32.1 %	100 %

Specificity

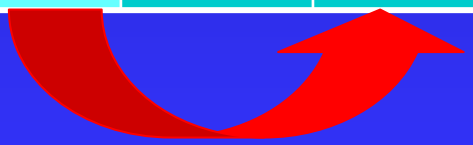
626 patients with collagenosis

Samples	n	AGA A	AGA G	GAF A	GAF G	TTG A	TTG G
RA	200	15.5%	9.5%	3%	0.5%	0.5%	0%
SS	200	14.5%	5.5%	4.5%	2%	1.5%	0%
SLE	100	23%	21%	3%	7%	0%	0%
PSS	126	14.3%	4%	4.8%	2.4%	3.2%	0.8%
SPECIFICITY	626	83.9%	91.1%	96.2%	97.6%	98.7%	99.8%

Specificity

626 patients with collagenosis

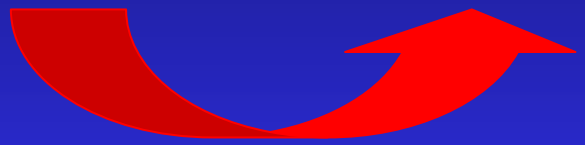
Samples	n	AGA A	AGA G	GAF A	GAF G	TTG A	TTG G
RA	200	15.5%	9.5%	3%	0.5%	0.5%	0%
SS	200	14.5%	5.5%	4.5%	2%	1.5%	0%
SLE	100	23%	21%	3%	7%	0%	0%
PSS	126	14.3%	4%	4.8%	2.4%	3.2%	0.8%
SPECIFICITY	626	83.9%	91.1%	96.2%	97.6%	98.7%	99.8%



Specificity

400 healthy blood donors

Samples	n	AGA A	AGA G	AGA II A	AGA II G
Blood Donors	400	3.3 %	22 %	2.3 %	2.0 %



Sensitivity

	Sensitivity at a specificity of 95 %
AGA A	56 %
AGA G	31 %
GAF 3X A	83 %
GAF 3X G	94 %
tTG A	96 %
tTG G	63 %

Sensitivity

	Sensitivity at a specificity of 95 %
AGA A	56 %
AGA G	31 %
GAF 3X A	83 %
GAF 3X G	94 %
tTG A	96 %
tTG G	63 %

Etude au CHU de Liège

Gliadines Natives Binding Site UI/ml		Gliadines Natives Chorus AU/ml		Gliadines Natives Biorad U/ml		Gliadines Déamidées Euroimmun U/ml		Gliadines Déamidées Binding Site U/ml		Gliadines Déamidées Inova U/ml	
neg<5	neg <10	neg<12	pos>18	neg<15		neg<25		neg<10		neg<20	
IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG
<5	27.6			<15	23,8	<25	<25	<10	<10	<20	<20
<5	47,1		61,9	<15	28,6	<25	<25	<10	<10	<20	<20
	17.4		59,3	<15	21,0	<25	<25	<10	10,46	<20	<20
	77.8		43,2	<15	27,3	<25	<25	<10	<10	<20	<20
<5	30.0		46	<15	27,7	<25	<25	<10	<10	<20	<20
	16.8		33,4	<15	20,0	<25	<25	<10	<10	<20	<20
<5	56.9			<15	<15	<25	<25	<10	<10	<20	<20
<5	15,2			<15	15,9	<25	<25	<10	<10	<20	<20
<5	16.3			<15	15,8	<25	<25	<10	<10	<20	<20
<5	12.1		29,8	<15	15,6	<25	<25	<10	<10	<20	<20
<5	16.4		22,6	<15	17,4	<25	<25	<10	<10	<20	<20
		38,5	33,7	18,9	21,1	45	<25	<10	<10		
<5	11,4			<15	15,5	<25	<25	<10	<10		
	24,3		55,6	<15	27,5	<25	<25	<10	<10	<20	<20
<5	14.4		44,4	<15	26,7	<25	<25	<10	16,2	<20	<20
	15.5		30,3	<15	21,2	<25	<25	<10	<10	<20	<20
<5	90.9		73,8	<15	32,4	<25	<25	<10	<10	<20	<20
<5	12.7		59,3	20,0	18,5	<25	<25	<10	<10	28	<20
<5	18,4		31,6	<15	18,3	<25	<25	<10	<10	23	<20
<5	21		44,2	<15	18,9	<25	<25	<10	<10	<20	<20
<5	13.2		27,4	<15	18,2	<25	<25	<10	<10	<20	<20
<5	15.9			<15	20,684	<25	<25	<10	<10	<20	<20

Comparaison faux positifs
Euroimmun/Binding Site (/Inova)

Gliadines Déamidées Euroimmun UI/ml neg<25		Gliadines Déamidées Binding Site U/ml neg<10		Gliadines Déamidées Inova U/ml neg<20		Anti-tTG	Anti-endomysium
IgA	**IgG**	**IgA**	**IgG**	**IgA**	**IgG**	**IgA**	**IgA**
79	<25	<10	<10	<20	<20	Négatif	Négatif
<25	34	12	<10			Négatif	Négatif
<25	31	<10	11			Négatif	Négatif
67	<25	<10	<10			Négatif	Négatif
<25	<25	<10	10,5	<20	<20	Négatif	NF
63	<25	15	<10			Négatif	Négatif
<25	89	<10	<10	21	<20	Négatif	Négatif
>200	51	<10	<10	<20	32	Négatif	Négatif
<25	94	<10	<10	<20	48	Négatif	Négatif
<25	108	<10	37			Négatif	Négatif
45	<25	<10	<10			Négatif	Négatif
68	27	<10	<10			Négatif	Négatif
<25	45	<10	16			Négatif	Négatif
<25	43	<10	<10	<20	27	Négatif	Négatif
<25	<25	<10	16	<20	<20	Négatif	NF
<25	47	<10	12			Négatif	Négatif
<25	<25	<10	36	<20	<20	Négatif	Négatif
183	<25	<10	20			Négatif	Négatif
<25	94	<10	78			Négatif	NF
<25	<25	<10	13			Négatif	NF
48	<25	<10	<10			Négatif	Négatif
82	<25	<10	<10			Négatif	NF
Faux positifs	**9**	**11**	**2**	**10**			

Usefulness of Antibodies to Deamidated Gliadin Peptides in Celiac Disease Diagnosis and Follow-up

Umberto Volta · Alessandro Granito · Erica Fiorini · Claudia Parisi · Maria Piscaglia · Georgios Pappas · Paolo Muratori · Francesco B. Bianchi

Dig Dis Sci (2008) 53:1582–1588

Table 1 Prevalence of serological tests in CD (before and after GFD) and in disease controls

	IgA DGP-AGA	IgG DGP-AGA	IgA AGA	IgG AGA	IgA Anti-tTG	IgA EmA
Celiac disease (CD)						
Untreated CD 128 cases	107 (83.6%)	108 (84.4%)	93 (72.6%)	94 (73.4%)	124 (96.8%)	120 (93.7%)
Treated CD 53 cases	18 (33.9%)	13 (24.5%)	13 (24.5%)	19 (35.8%)	19 (35.8%)	21 (39.6)
Disease controls (DC)						
Malabsorption 43 cases	6 (14%)	0	9 (20.9%)	17 (39.5%)	2 (4.6%)	0
PBC 25 cases	1 (4%)	1 (4%)	2 (8%)	7 (28%)	3 (12%)	0
AIH 20 cases	2 (10%)	0	3 (15%)	3 (15%)	2 (10%)	0
Connective tissue disorders 46 cases	4 (8.7%)	1 (2.1%)	4 (8.7%)	4 (8.7%)	5 (10.8%)	0
Total DC 134 cases	13 (9.7%)	2 (1.5%)	18 (13.4%)	31 (23.1%)	12 (9%)	0

DGP-AGA, antibodies to deamidated gliadin peptides; AGA, antigliadin antibodies; anti-tTG, antibodies to tissue transglutaminase; EmA, antiendomysial antibodies; CD, celiac disease; GFD, gluten-free-diet; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis

Sévérité de la pathologie

Marsh Score	n=	Actin IgA	Gliadin II (DGP) IgA	Gliadin II (DGP) IgG	Celiac DGP Screen	h-tTG IgA	h-tTG IgG	h-tTG/DGP Screen
M1	22	0%	19%	10%	10%	19%	0%	26%
M2	7	0%	22%	22%	22%	11%	0%	33%
All M3 (M3a,b,c)	61	39%	75%	62%	70%	72%	3%	80%
M3-TVA (M3c) only	40	53%	95%	83%	90%	98%	5%	100%

D'après poster Inova (GL Norman)

Dermatite herpétiforme

- Intolérance au gluten mais à expression essentiellement cutanée. Souvent associée à la maladie coeliaque.
- Sérums de patients DH réagissent avec une forte avidité avec TG3 (transglutaminase épidermique) et TG2.

n = 18	tTG IgA	AGA II IgA	AGA II IgG
Sensitivity	72 %	78 %	78 %

Déficit en IgA

Testing for IgG class antibodies in celiac disease patients with selective IgA deficiency. A comparison of the diagnostic accuracy of 9 IgG anti-tissue transglutaminase, 1 IgG anti-gliadin and 1 IgG anti-deaminated gliadin peptide antibody assays

Danilo Villalta [a,*], Maria Grazia Alessio [b], Marilina Tampoia [c], Elio Tonutti [d], Ignazio Brusca [a], Marcello Bagnasco [f], Giampaola Pesce [f], Sergio Stella [a], Nicola Bizzaro [g]

Clinica Chimica Acta 382 (2007) 95–99

- IgA endomysium, tTG, ~~Gliadines~~
- IgG gliadine : peu spécifique et peu sensible.
- IgG endomysium : spécifique mais IFI…
- IgG tTG (rHu) : ….

Déficit en IgA : anti-tTG IgG

Table 1
Number and percentage of IgG anti-tTG positivities in celiac disease (CD) and in the control population (SIgAD without CD: patients with selective IgA immunodeficiency affected by gastrointestinal disease other than celiac disease; CLD: chronic liver diseases; HS: healthy subjects) obtained with 9 different ELISA methods

Method	SIgAD and CD	SIgAD without CD	CLD	HS
	No. (%)	No. (%)	No. (%)	No. (%)
Euroimmun	16/20 (80)	0/9 (0)	0/54 (0)	0/49 (0)
Orgentec	19/20 (95)	0/9 (0)	6/54 (11.1)	1/49 (2)
Immco	19/20 (95)	2/9 (22.2)	4/54 (7.4)	4/49 (8.1)
Phadia	19/20 (95)	0/9 (0)	0/54 (0)	0/49 (0)
D-tek	15/20 (75)	1/9 (10.1)	1/54 (1.8)	1/49 (2)
Aesku	17/20 (85)	1/9 (10.1)	2/54 (3.7)	1/49 (2)
Generic assays	19/20 (95)	6/9 (66.6)	3/54 (5.5)	2/49 (4.1)
Inova	15/20 (75)	0/9 (0)	1/54 (1.8)	0/49 (0)
Radim	19/20 (95)	0/9 (0)	13/54 (24.1)	0/49 (0)

tTG + gliadines →

↗Sensibilité ...???
↘ Spécificité !!!

← Sensibilité

Spécificité →

Déficit en IgA

	Sensibilité	Spécificité
IgG AGA	40 %	87 %
IgG DGP	80 %	98 %

Déficit en IgA

	IgG	
Patient	Anti-tTG	AGA II
1	Neg	Pos
2	Pos	Pos
3	Pos	Pos
4	Pos	Pos
5	Pos	Pos

Enfants de moins de 2 ans

	tTG	Sensibilité < 2 ans	Sensibilité
Tonutti, 2003	Foie de cobaye	87,8 %	96,7 % (2-14 ans)
Fabiani, 2001	Foie de cobaye	69 %	91,5 % (tous)
Agardh, 2003	Recom. humaine	63,1 %	93,7 % (2-15 ans)

Enfants de moins de 2 ans

- AC anti-gliadine présents avant AC anti-endomysium/ anti-tTG.

↓

mauvaise spécificité

↓

gliadines déamidées.

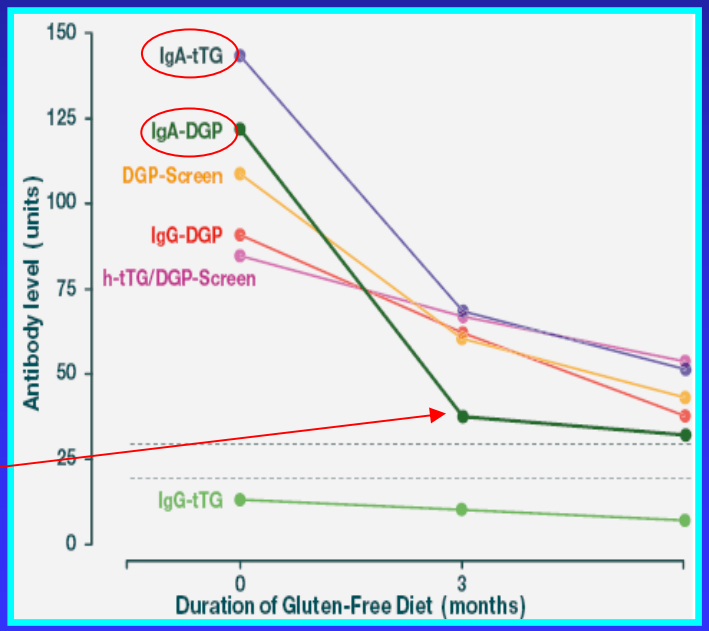
Régime sans gluten

- Tous les AC disparaissent +/- vite sous régime sans gluten et réapparaissent lors de la réintroduction de gluten.

- Anti-gliadines natives
  - Sous régime sans gluten, IgA disparaissent; ils réapparaissent moins de 48 heures après réintroduction du gluten dans l'alimentation.
  - IgG peuvent parfois persister plus longtemps.

Régime sans gluten

Les anticorps anti-gliadines déamidées IgA augmentent et diminuent plus rapidement que les anti-tTG. Ils sont donc meilleurs pour le suivi du régime sans gluten.



Chute la plus significative
après 3 mois de régime sans gluten.

Agardh, 2007

Régime sans gluten

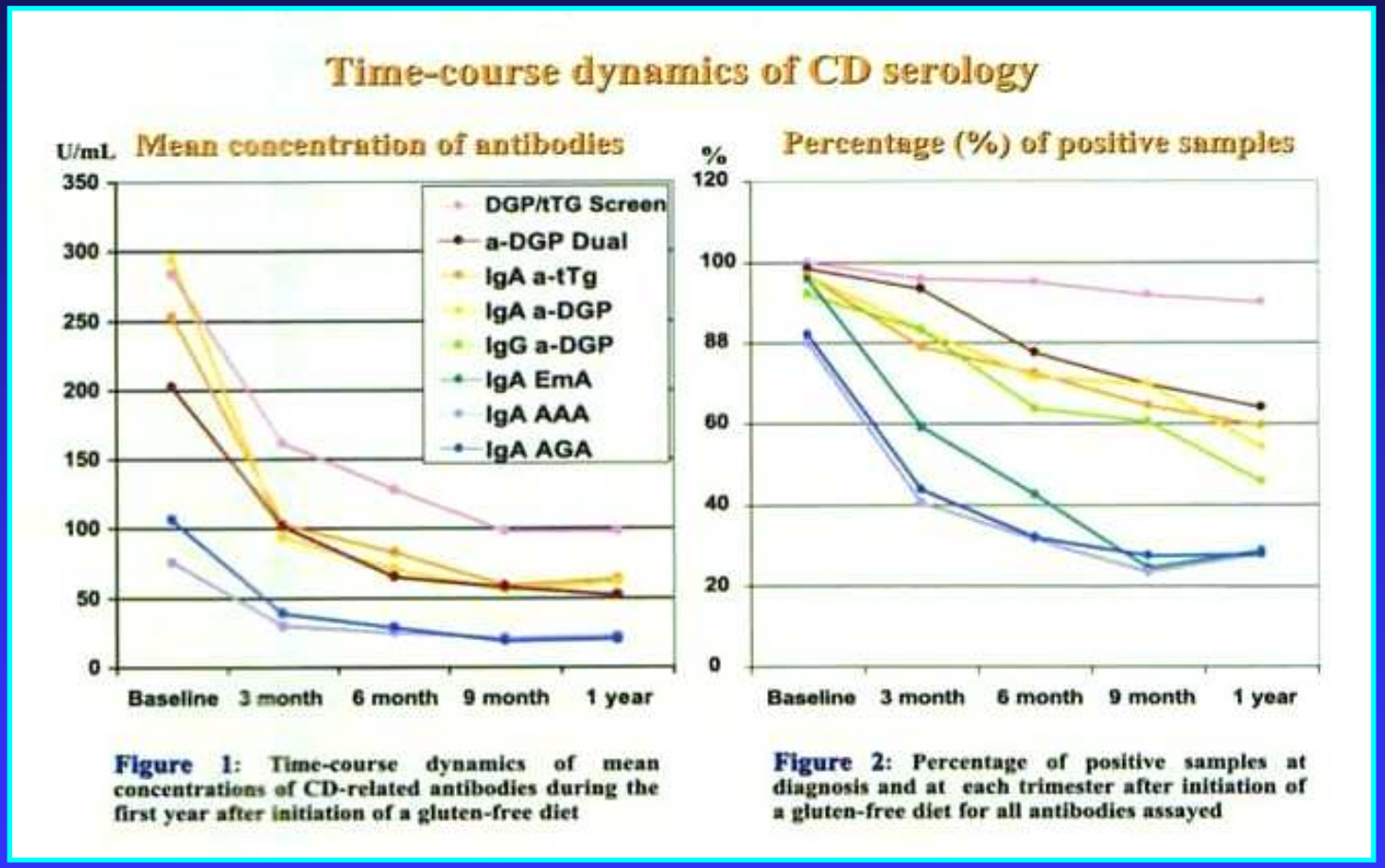


Figure 1: Time-course dynamics of mean concentrations of CD-related antibodies during the first year after initiation of a gluten-free diet

Figure 2: Percentage of positive samples at diagnosis and at each trimester after initiation of a gluten-free diet for all antibodies assayed

Table 3 Persistence of DGP-AGA (IgA and/or IgG) in relation to compliance with the gluten-free diet (GFD) and improvement of duodenal histology

53 CD patients after one-year-GFD	Improved histology (38 cases)		Not improved histology (15 cases)	
Compliance	DGP-AGA+	DGP-AGA–	DGP-AGA+	DGP-AGA–
Strict 39/53	5	29	3	2
Low 14/53	3	1	7	3

DGP-AGA in pts with low compliance vs. DGP-AGA in pts with strict compliance: $P < 0.005$. DGP-AGA in pts with no improvement of duodenal histology vs. DGP-AGA in pts with improved histology: $P < 0.005$. DGP-AGA: antibodies to deamidated gliadin peptides

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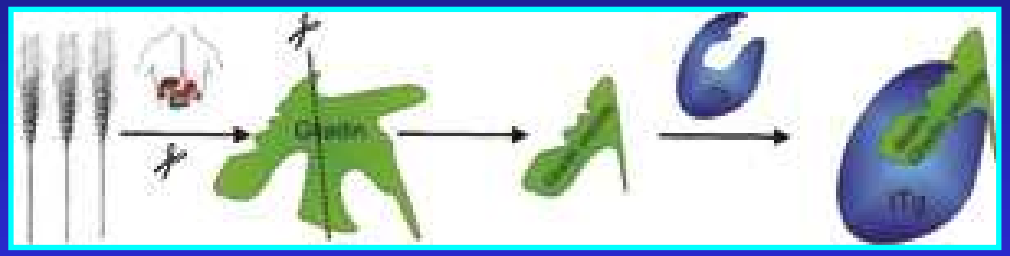
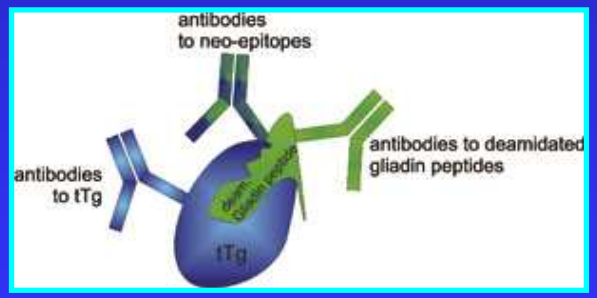
Anti- « transglutaminase »

- Néo-épitope : tTG + gliadine





Sensibilité : 82.9 %
Spécificité : 92.3 %

Aeskulisa Celicheck New Generation IgA + IgG

Reeves
Eur J Gastroenterol Hepatol
2006

Etude avec le kit de Biorad

- MC, tTG néoépitope + : n = 4
  - tTG IgA +, Endo IgA +, DGP IgA et IgG +
- MC, tTG néoépitope - : n = 2
  - tTG IgA +, Endo IgA +, DGP IgG +, DGP IgA -
  - tTG IgA +, Endo IgA +, DGP IgG et IgA -
- DH, tTG néoépitope + : n =1
  - tTG IgA -, DGP IgA +, DGP IgG -
- Pas MC, tTG néoépitope + : Faux positifs
  - N = 1 : tTG IgA -, DGP IgA et IgG - : RCUH
  - N = 3 : tTG IgA -, DGP IgA et/ou IgG + : Biopsies -

Screening tTG/DGP IgA et IgG INOVA

Combination testing for antibodies in the diagnosis of coeliac disease: comparison of multiplex immunoassay and ELISA methods

S. RASHTAK*, M. W. ETTORE†, H. A. HOMBURGER† & J. A. MURRAY*

Aliment Pharmacol Ther **28**, 805–813

	Sensitivity (%)	Specificity (%)
Test	ELISA†	ELISA†
DGP IgA	75.0*	95.2
DGP IgG	65.2	98.4
TTG IgA	79.3*	98.4
TTG IgG	28.3	96.8

→ Plus d'intérêt en cas de déficit en IgA qu'anti-tTG IgG !!

OU

Test	Sensitivity (%) ELISA	Specificity (%) ELISA
DGP IgA or IgG	78.3	94.4
TTG IgA or IgG	80.4	96.0
DGP IgA or TTG IgA	83.0	95.2
DGP IgG or TTG IgA	82.6	97.6
DGP IgA or IgG or TTG IgA	83.7	94.4
DGP IgA or IgG or TTG IgA or IgG	83.7	91.9

ET

Test	Sensitivity (%) ELISA	Specificity (%) ELISA
DGP IgA and IgG	61.4	99.2
TTG IgA and IgG	25.3	99.2
DGP IgA and TTG IgA	71.3	98.4
DGP IgG and TTG IgA	60.9	99.2
DGP IgA and IgG and TTG IgA	58.6	99.2
DGP IgA and IgG and TTG IgA and IgG	20.7	99.2

DGP IgG / tTG IgA

Combinaison la plus intéressante d'autant plus car détection des déficits en IgA !

Anti-Actine IgA

Tableau I / Fréquence des IgA anti-actine dans les maladies cœliaques non traitées (adultes et enfants).

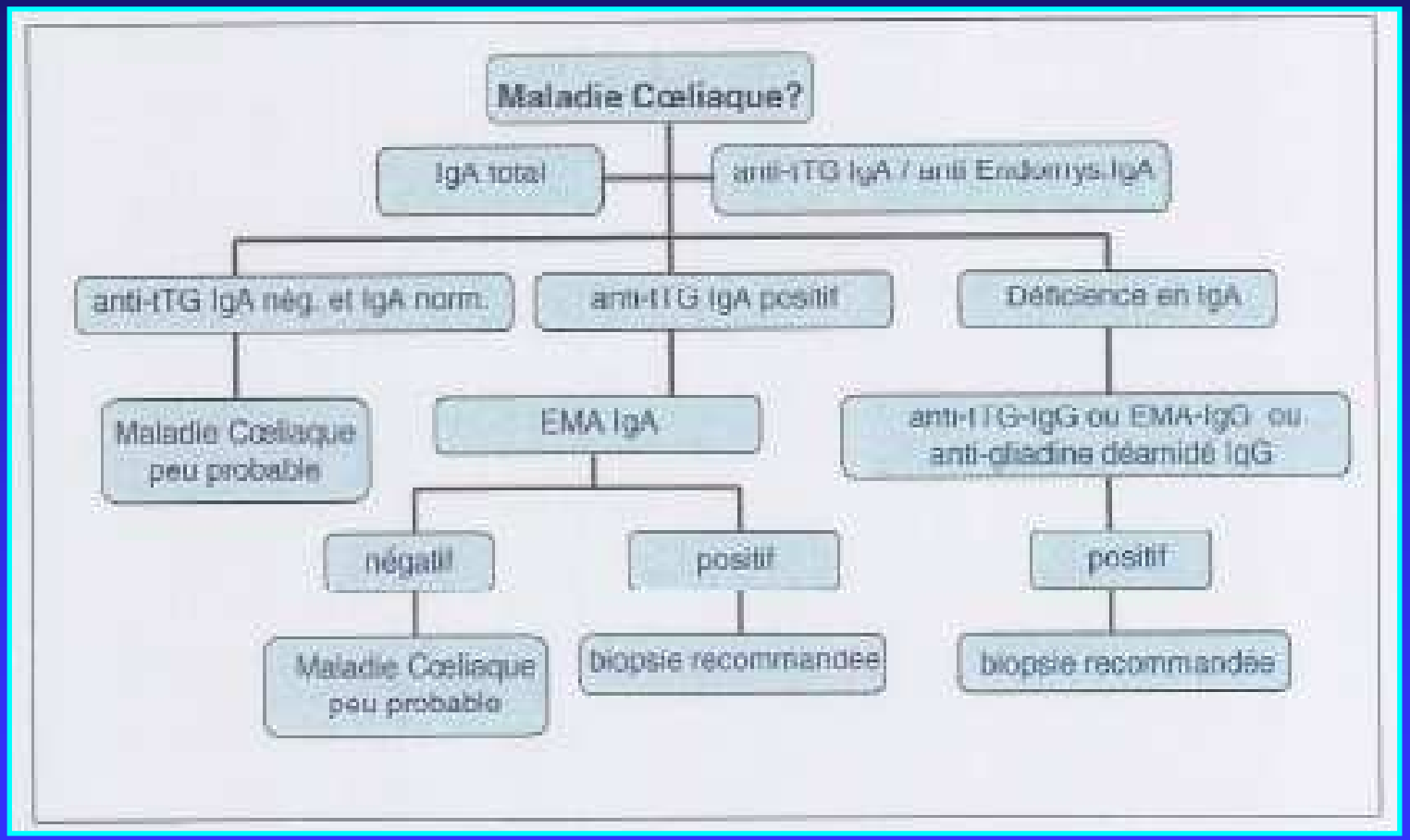
Substrat antigénique	HEp-2 (IFI)	Foie, rein, estomac (IFI)	Actine G (ELISA)	Actine F (ELISA)
Clemente (2000)	71 %	49 %	30,5 %	
Granito (2004)	25 %	4,9 %		
Carrocio (2005)	93 %			86 %

4ème colloque du GEAI

-Corrélation avec titre d'AC et sévérité de l'atrophie : atrophie totale : toujours +
atrophie partielle : rare

-Disparition sous régime sans gluten.

Algorithme pour le diagnostic de la maladie coeliaque



Jeudis de Fleurus 18.12.08

Marqueurs sérologiques des MICI

Serological Diagnostic Tests

- Antibodies against Saccharomyces cerevisae : ASCA
- ANCA
- Antibodies against exocrine pancreas.
- Antibodies anti-glycan.
- Antibodies against microbial epitopes.

ASCA

- Against carbohydrate epitopes in phosphopeptidomannan, a glycoprotein of the cell wall of the yeast *Saccharomyces cerevisae*.
- Antibodies or autoantibodies ?

ASCA : Prevalence

- Crohn's disease : 40-60 %
- Ulcerative colitis : 5-15 %
- Healthy blood donors : 0-5 %
- AIH, coeliac disease,...

ASCA : PPV

- Crohn's disease : 54-92 %

Comparaison de cinq techniques de détection des anticorps anti-*Saccharomyces cerevisiae* (ASCA) dans le sérum pour le diagnostic de la maladie de Crohn

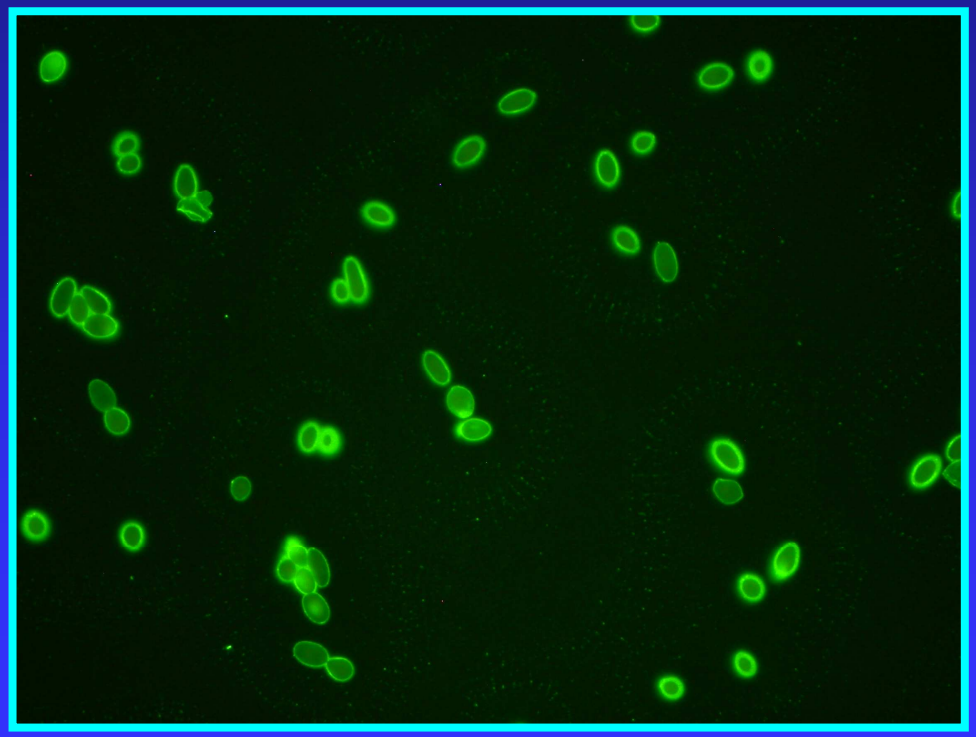
C. Le Goff
J.P. Chapelle
L. Lutteri

Ann Biol Clin 2007 ; 65 (6) : 601-8

N = 139,
CD= 37, UC = 37, inflammatory diseases = 35, healthy subjects = 30

ASCA

- Standard method :
  Indirect Immunofluorescence.



IgA : 1/100
IgG : 1/1000

ASCA by IF in CD

	Sensitivity	Specificity	PPV
ASCA IgA	81 %	93 %	84 %
ASCA IgG	54 %	100 %	84 %
ASCA IgA or IgG	81 %	92 %	77 %
ASCA IgA and IgG	54 %	100%	94 %

ASCA ELISA :

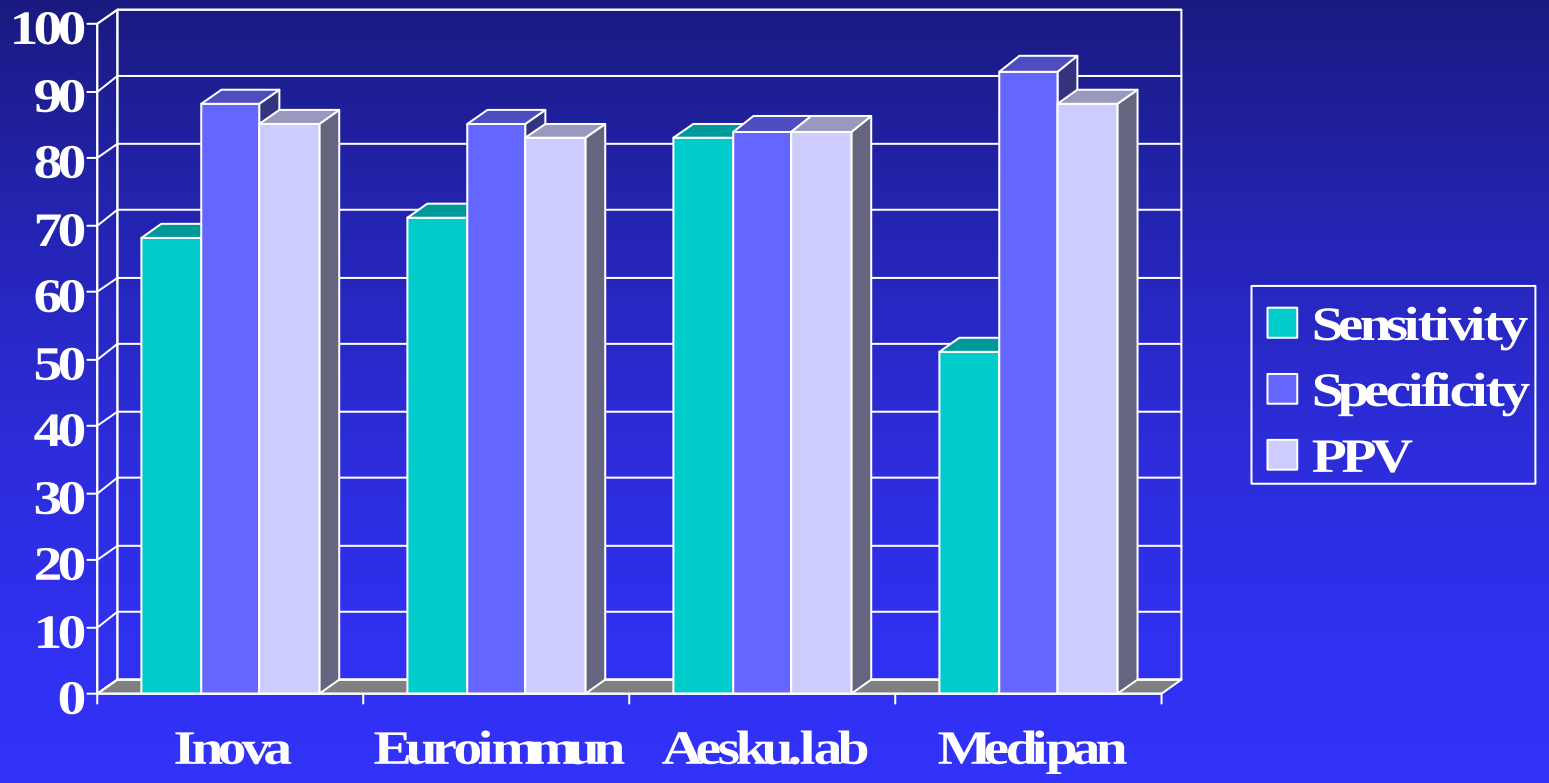
Inova, Euroimmun, Aesku.lab, Medipan.

	Sensitivity	Specificity	PPV
ASCA IgA	41-59 %	92-95 %	88-89%
ASCA IgG	41-75 %	88-96 %	85-91 %
ASCA IgA or IgG	51-83 %	84-93 %	83-88 %
ASCA IgA and IgG	30-53 %	97-98 %	94-95 %

Klebl. Int. J. Colorectal. Dis. 2004.

ASCA ELISA :

Inova, Euroimmun, Aesku.lab, Medipan
IgA or IgG.



Klebl. Int. J. Colorectal. Dis. 2004.

ASCA : Dots

n=24 : 18 CD, 2 UC, 2 IC, 2 HP.

IgA or IgG	IF	DOTS	ELISA
Sensitivity	72 %	33 %	44 - 94 %
Specificity	67 %	100 %	67 %

ASCA

- ASCA (presence or high titer) IgA and/or IgG have been linked with
  - more severe disease course (high rate of complications)
  - small bowel involvement (with or without colonic disease) : more typical than pure colonic disease.
  - earlier onset of disease
  - ASCA IgA + in children : higher risk for relapses.

ASCA

- Presence of ASCA is stable over time, does not change with drug treatment or surgical removal of the affected colon.

- No correlation between titre and disease activity, duration and behaviour or medical treatment.

Vasiliauskas. Gastroenterology 1996;
Vermeire. IBD 2001;
Koutroubakis. Am J Gastoenterol 2001.

Predictive maker

- ASCA may predict development of IBD years before the disease is clinically diagnosed.
- ASCA were detected in 31 % of patients before the clinical diagnosis of CD.

Israeli. Gut. 2005

Anti-Saccharomyces cerevisiae and perinuclear anti-neutrophil cytoplasmic antibodies in coeliac disease before and after gluten-free diet

A. GRANITO*, D. ZAULI*, P. MURATORI*, L. MURATORI*, A. GRASSI*, R. BORTOLOTTI*, N. PETROLINI*, L. VERONESI*, P. GIONCHETTI†, F. B. BIANCHI* & U. VOLTA*

*Departments of *Internal Medicine, Cardioangiology, Hepatology and †Internal Medicine, Alma Mater Studiorum, University of Bologna, Policlinico Sant'Orsola-Malpighi, Italy*

Aliment Pharmacol Ther 2005; 21: 881–887.

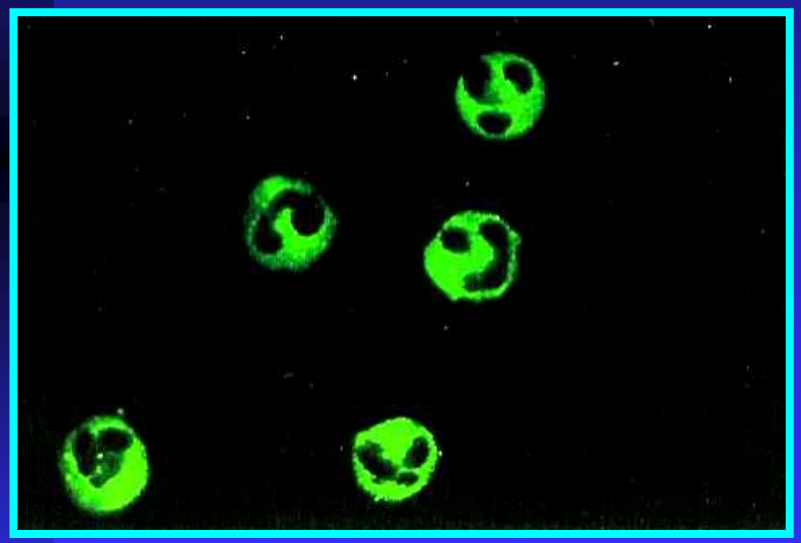
- 59 % of coeliac patients had IgA and/or IgG ASCA.
- After gluten-free diet, 93% lost IgA ASCA whereas 83% maintained IgG ASCA reactivity.

ANCA

- Anti-Neutrophil Cytoplasmic Antibodies
- Diagnostic of different types of vasculitides.
- Standard method :
  - Indirect Immunofluorescence :
  - Ethanol-fixed leucocytes slides

ANCA

cANCA

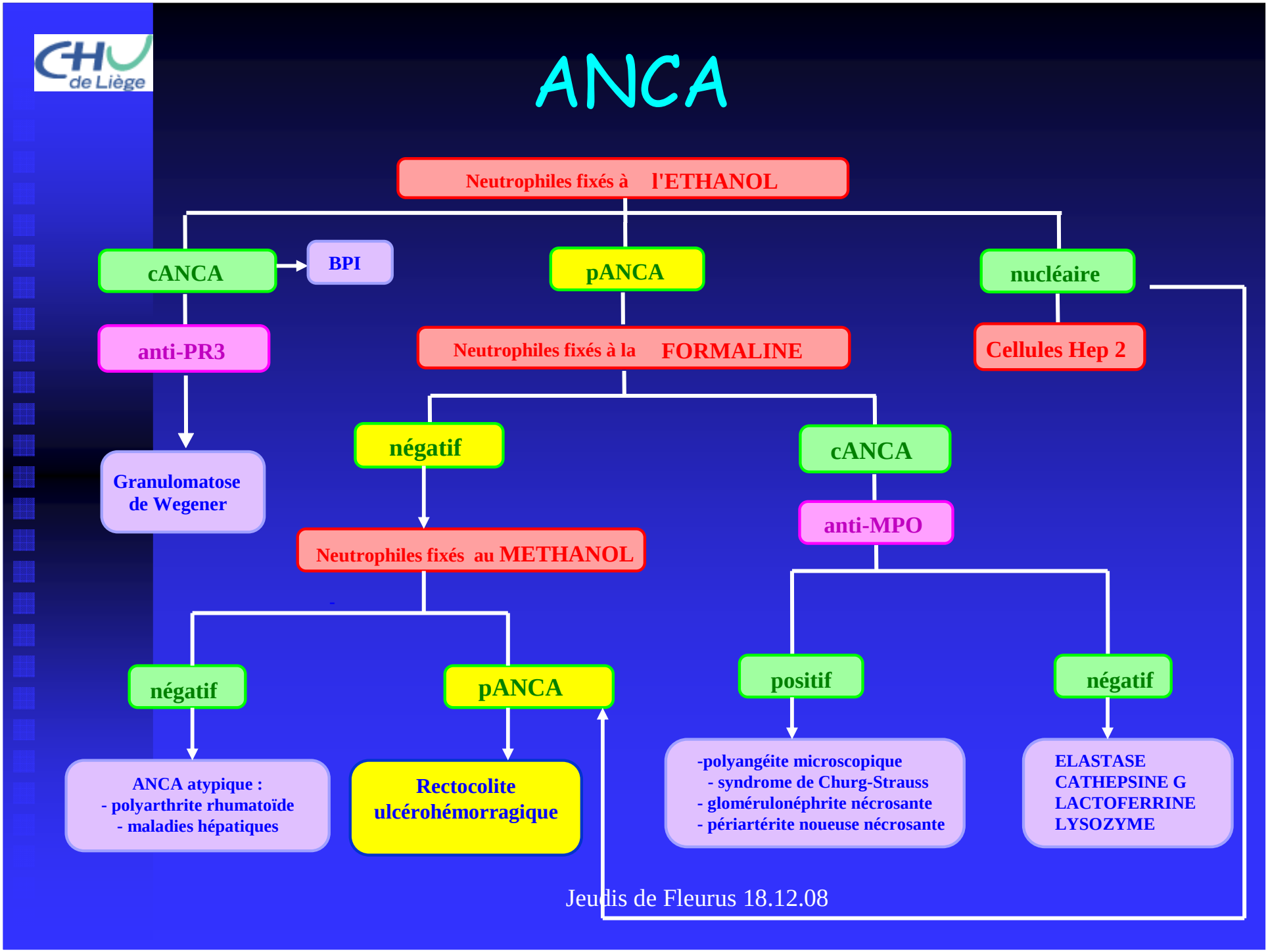


Granular cytoplasmic fluorescence accentuated between the nuclear lobes

pANCA

Fine homogeneous rim-like staining of the perinuclear cytoplasm.

ANCA


Neutrophiles fixés à l'ETHANOL
cANCA
BPI
pANCA
nucléaire
anti-PR3
Neutrophiles fixés à la FORMALINE
Cellules Hep 2
négatif
cANCA
Granulomatose de Wegener
Neutrophiles fixés au METHANOL
anti-MPO
négatif
pANCA
positif
négatif
ANCA atypique :
- polyarthrite rhumatoïde
- maladies hépatiques
Rectocolite ulcérohémorragique
-polyangéite microscopique
- syndrome de Churg-Strauss
- glomérulonéphrite nécrosante
- périartérite noueuse nécrosante
ELASTASE
CATHEPSINE G
LACTOFERRINE
LYSOZYME

Target antigen : ?

- xANCA or NANA
  (nuclear associated neutrophil antibodies) :
  Ag condensed on the inner side of the nuclear membrane of the neutrophil.

pANCA : Prevalence

- Ulcerative colitis : 40-85 %
- Crohn : 4-25 %

pANCA : PPV

- Ulcerative colitis : 70 %

pANCA +

- Ulcerative colitis :
  - more resistant to treatment
  - more aggressive disease
  - requiring surgery early

- Crohn ≡ subgroup

  « ulcerative colitis-like » phenotype:

  left-sided colitis, generally good therapeutical response, uncomplicated disease course, later age of onset.

pANCA

- No correlation between ANCA titres and disease activity (>< vasculitides)

Roozendaal. QJM, 1999

- ANCA titer remains unchanged after colectomy.

Reumaux. IBD 2000

ASCA / ANCA

%	UC		CD		
	Sens	Spe	Sens	Spe	PPV
pANCA	55,3	88,5			
ASCA			53,3	89,2	
pANCA + ASCA -	51,3	94,3			75-93
pANCA - ASCA +			55	93	77-96

Reese. Am J Gas, 2006

Update on Anti-*Saccharomyces cerevisiae* antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: Results of a multicenter study

S Desplat-Jégo, C Johanet, A Escande, J Goetz, N Fabien, N Olsson, E Ballot, J Sarles, JJ Baudon , JC Grimaud, M Veyrac, P Chamouard, RL Humbel

World J Gastroenterol 2007 April 28; 13(16): 2312-2318

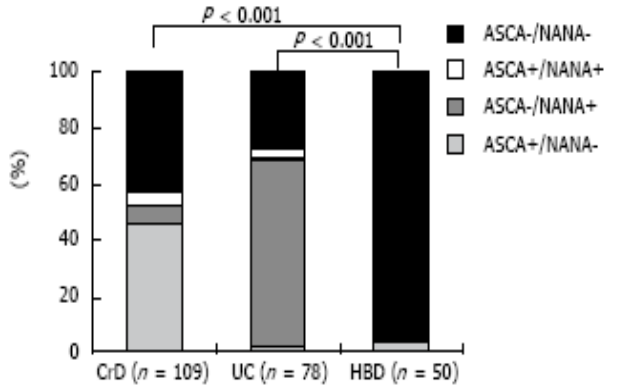


Figure 2 Detection of ASCA and NANA in CrD and UC patient groups and HBD. ASCA: anti-*Saccharomyces cerevisiae* antibodies; NANA: anti-Nuclear Associated Neutrophil Antibodies; CrD: Crohn's disease; UC: ulcerative colitis; HBD: healthy blood donors.

GASTROENTEROLOGY 2002;122:1242–1247

The Value of Serologic Markers in Indeterminate Colitis: A Prospective Follow-up Study

SOFIE JOOSSENS,* WALTER REINISCH,† SÉVERINE VERMEIRE,* BOUALEM SENDID,§ DANIEL POULAIN,§ MARC PEETERS,* KAREL GEBOES,‖ XAVIER BOSSUYT,¶ PEGGY VANDEWALLE,§ GEORG OBERHUBER,# HARALD VOGELSANG,† PAUL RUTGEERTS,* and JEAN–FRÉDÉRIC COLOMBEL**

*Gastroenterology Unit, ‖Departments of Pathology and ¶Laboratory Medicine, Immunology, U.Z. Gasthuisberg, Leuven, Belgium; †Clinic of Internal Medicine IV, Department of Gastroenterology and Hepatology, #Clinic of Pathology, University of Vienna, Vienna, Austria; and §Laboratory of Parasitology-Mycology and **Gastroenterology Unit, CHRU Lille, France

Tableau IV. – Résultats ASCA/ANCA parmi les malades atteints de colite indéterminée ou ayant initialement une colite indéterminée qui a évolué vers une maladie de Crohn (MC) ou une recto-colite hémorragique (RCH).

Results of ASCA and pANCA in patients with indeterminate colitis and patients with new definitive diagnosis of Crohn's disease or ulcerative colitis.

	n n (%)	MC n (%)	RCH n (%)	Colite indéterminée
ASCA+/ANCA–	26 (26,8)	8 (30,8)	2 (7,7)	16 (61,5)
ASCA–/ANCA+	20 (20,6)	4 (20)	7 (35)	9 (45)
ASCA+/ANCA+	4 (4,1)	2 (50)	1 (25)	1 (25)
ASCA–/ANCA–	47 (48,5)	3 (6,4)	4 (8,5)	40 (85,1)
Total	97 (100)	17 (17,5)	14 (14,4)	66 (68,1)

Table 4. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of the Combination of ASCA and pANCA

	Diagnosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ASCA+/pANCA−	CD	8/12 (66.7)	7/9 (77.8)	8/10 (80)	7/11 (63.6)
ASCA−/pANCA+	UC	7/9 (77.8)	8/12 (66.7)	7/11 (63.6)	8/10 (80)

PPV, positive predictive value; NPV, negative predictive value.

Joossens.
Gastroenterology. 2002

Anti- exocrine pancreas

- Prevalence :
  - Crohn : 31 %
  - Ulcerative colitis : 2 %
- IFI on primate pancreas, IgA and IgG, 1/10
- Ag ?
- *No correlation* between titre and disease activity, duration and behaviour or medical treatment or extra-intestinal manifestations.
- Distinct CD subgroup ?

AB against microbial epitopes.

- Anti-OmpC :
  - antibodies against the outer-membrane porin C protein of *Escherichia coli*
  - IgA: 38-55 % of CD, 2-11 % of UC, 2-5% non-IBD subjects
  - Identification of 5-15% of ASCA – CD patients.
  - More aggressive course of disease and higher risk for surgical interventions.

AB against microbial epitopes.

- Anti-I2 :
  - antibodies against a *Pseudomonas fluorescens*-associated structure
  - IgA : 54% of CD, 10 % of UC, 19% other enteric inflammatory diseases, 5% healthy subjects.
  - Increased risk for complications in adult CD patients.

- Anti-CBir :
  - antibodies against the CBir1 flagellin (gram-)
  - 52 % of CD, 6% of UC, 14% of other inflammatory intestinal diseases, 8% control subjects.
  - Associated with ileal involvement in adult CD patients

Anti-glycan antibodies

- Anti-laminaribioside carbohydrate AB (ALCA)
- Anti-chitobioside carbohydrate AB (ACCA)
- Anti-mannobioside carbohydrate AB (AMCA)

- *Sensibility* : 38% (ALCA), 36 % (ACCA), 28% (AMCA)
- *Specificity* : 90 %, at least 2 AB : 99 %
- 44 % of ASCA - CD : anti-glycan +
- In CD, higher levels of AB (ALCA) associated with small intestine disease.

Dotan, Gastroenterology, 2006

ASCA / pANCA

- Lack of standardization → large interlaboratory variation.
  - Identification of antigens (ANCA)
  - Harmonization of ASCA assays :
    - Cut-offs
    - Ag