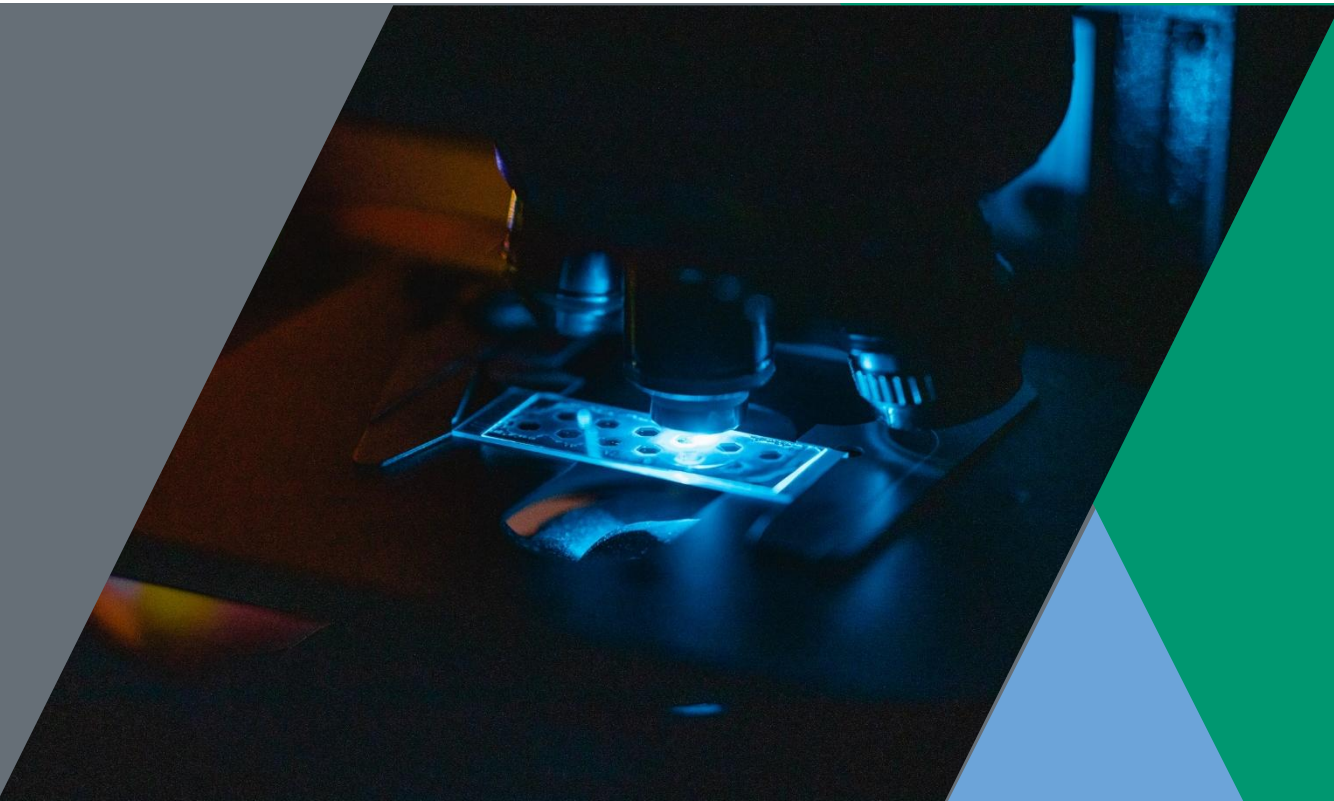


Clinical Validity of Anti-Proteinase 3 Antibodies in Patients with Inflammatory Bowel Disease



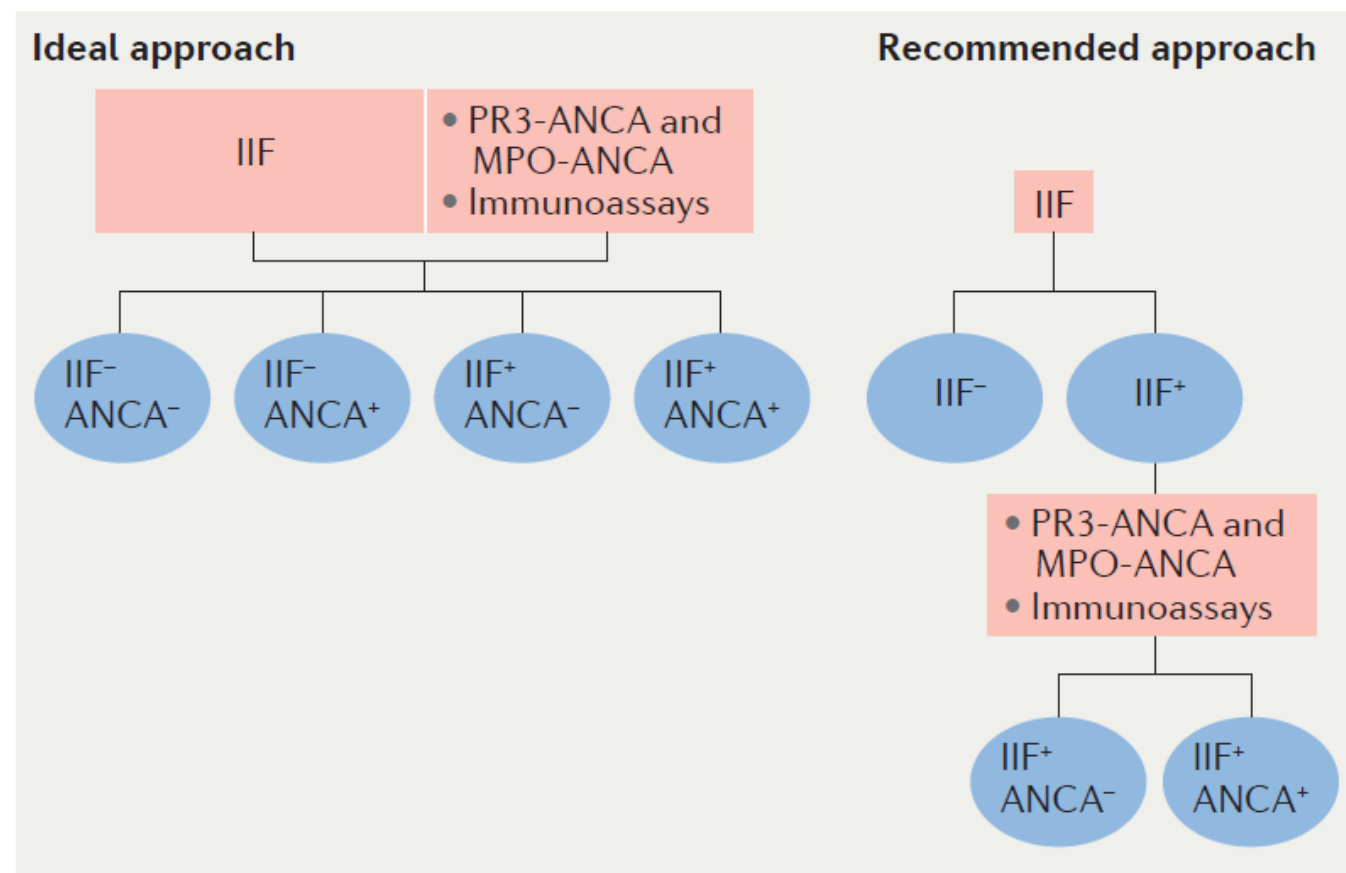
Dr. phil. nat. M. Horn



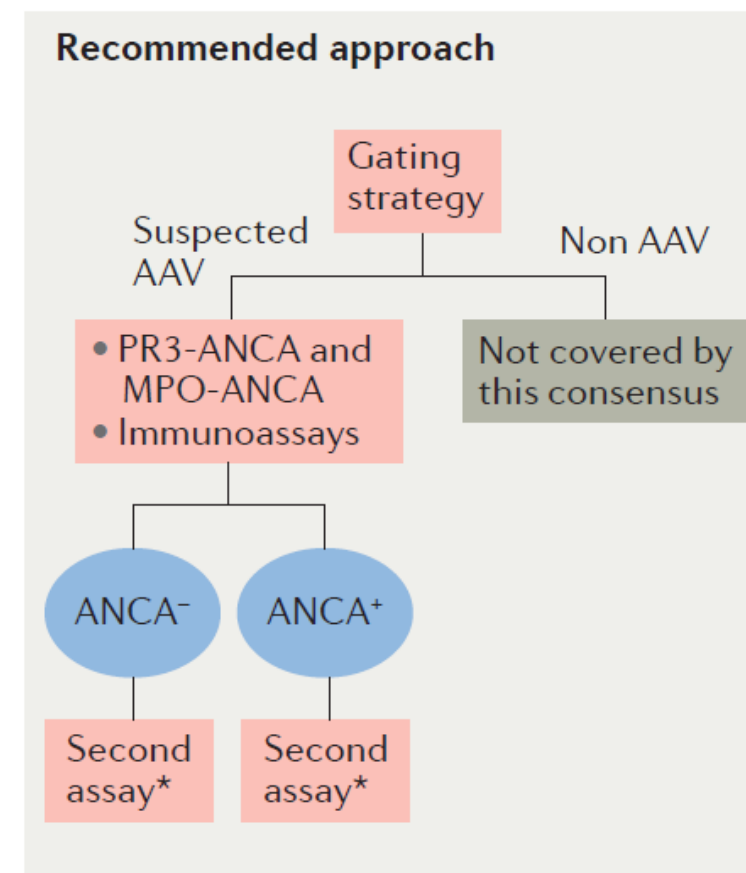
**Universitätsinstitut für
klinische Chemie und
Zentrum für Labormedizin**

Algorithm of ANCA Diagnostics in the context of vasculitis

a 1999 consensus



b 2017 consensus



Bossuyt et al, 2017
 Nat Rev Rheum 13:386-92

... and beyond Vasculitis

b 2

P



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Autoimmunity Reviews 19 (2020) 102618

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Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev



2020 international consensus on ANCA testing beyond systemic vasculitis

Sergey Moiseev^{a,*}, Jan Willem Cohen Tervaert^b, Yoshihiro Arimura^c, Dimitrios P. Bogdanos^d, Elena Csernok^e, Jan Damoiseaux^f, Marc Ferrante^g, Luis Felipe Flores-Suárez^h, Marvin J. Fritzlerⁱ, Pietro Invernizzi^j, David Jayne^k, J. Charles Jennette^l, Mark A. Little^m, Stephen P. McAdooⁿ, Pavel Novikov^a, Charles D. Puseyⁿ, Antonella Radice^o, Alan D. Salama^p, Judith A. Savage^q, Mårten Segelmark^r, Yehuda Shoenfeld^s, Renato A. Sinico^t, Maria-José Sousa^u, Ulrich Specks^v, Benjamin Terrier^w, Athanasios G. Tzioufas^x, Severine Vermeire^g, Ming-Hui Zhao^y, Xavier Bossuyt^z

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Moiseev et al. Autoimm. Rev. 2020;
<https://doi.org/10.1016/j.autrev.2020.102618>

In the context of inflammatory bowel disease

Table 4

Recommendations statements for ANCA testing in non-AAV.

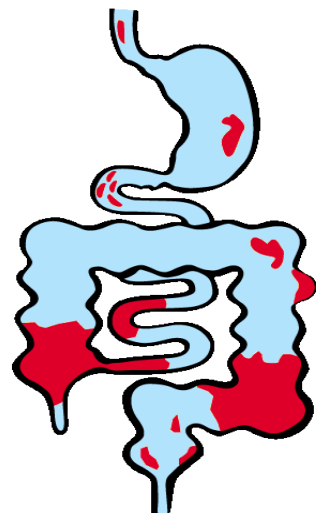
Disease	Statements	LoA, %	Median score
Any disease	ANCA testing* is mandatory for any patient with clinical features suggesting AAV	100	5
Anti-GBM disease	ANCA testing* is mandatory for any patient with anti-GBM disease	100	5
Idiopathic interstitial pneumonia	ANCA testing* is mandatory for any patient with idiopathic interstitial pneumonia	96.6	5
Rheumatoid arthritis	Routine testing is not recommended. Recommended* in patients with kidney disease with a nephritic	96.6	5
AIH-1, PBC, PSC	Routine testing is not recommended. Testing for atypical ANCA by IIF may be useful in patients with suspected AIH-1 in the absence of conventional autoantibodies	89.7	5
IBD (CD, UC)	Routine testing is not recommended. Atypical P-ANCA (IIF) and ASCA may be tested in case of diagnostic uncertainty to discriminate UC from CD	96.6	5
Autoimmune liver disease, AIH-1, PBC, PSC	Routine testing is not recommended. ANCA testing* may be useful in patients with renal impairment, especially associated with infective endocarditis	89.7	5
Inflammatory bowel diseases (CD, UC)	Routine testing is not recommended. Atypical P-ANCA (IIF) and ASCA may be tested in case of diagnostic uncertainty to discriminate UC from CD	96.6	5
Infections	Routine testing is not recommended. ANCA testing* may be useful in patients with renal impairment, especially associated with infective endocarditis	89.7	5
Malignancy	Routine testing is not recommended	96.6	5

Note: *MPO-ANCA and PR3-ANCA according to the 2017 consensus. AIH-1 – autoimmune hepatitis type 1, PBC – primary biliary cholangitis, PSC – primary sclerosing cholangitis, CD – Crohn's diseases, UC – ulcerative colitis, ASCA – anti-*Saccharomyces cerevisiae* antibodies, LoA – level of agreement (%). Scores: 1 – strongly disagree, 2 – disagree, 3 – undecided, 4 – agree, 5 – strongly agree.

Moiseev et al. Autoimm. Rev. 2020;
<https://doi.org/10.1016/j.autrev.2020.102618>

CD vs. UC

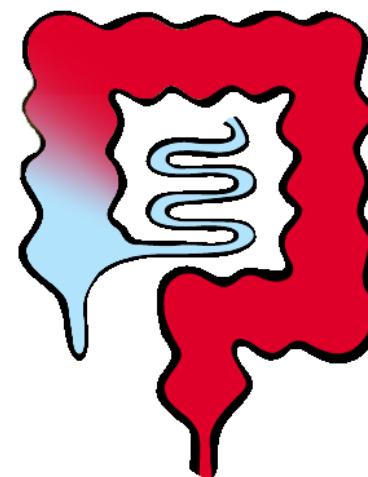
Crohn's Disease



segmental
transmural
entire GI tract



Ulcerative Colitis

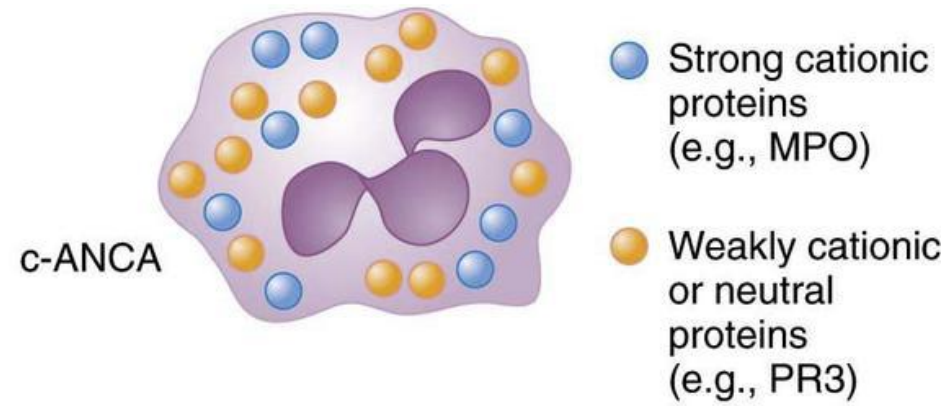


continuously
superficial
Colon only

open points

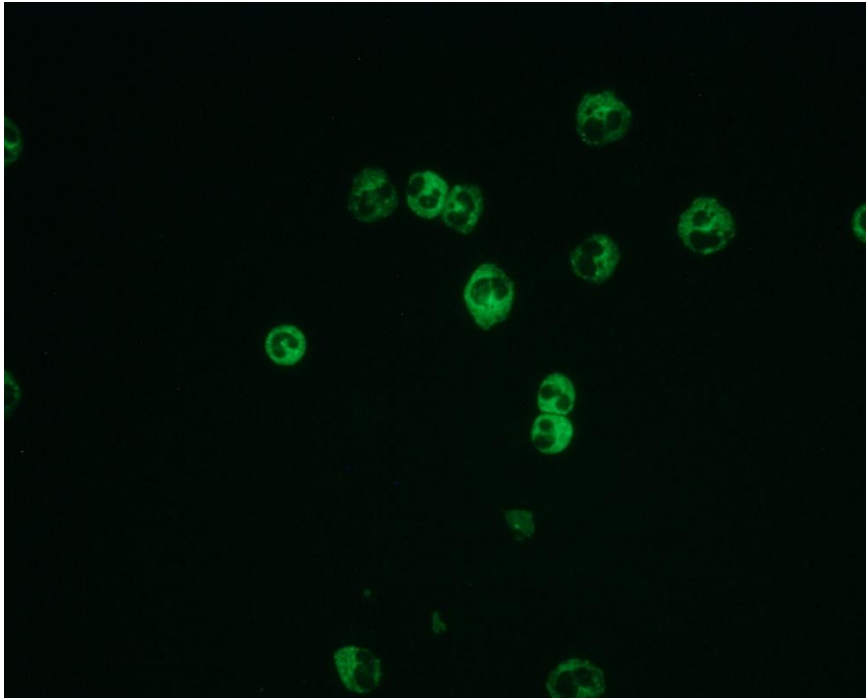
- atypical ANCA by IIF
- the role of PR3 ANCA
- the role of ASCA
- can we combine these markers
- anything more?

ANCA patterns – an *in vitro* Artifact



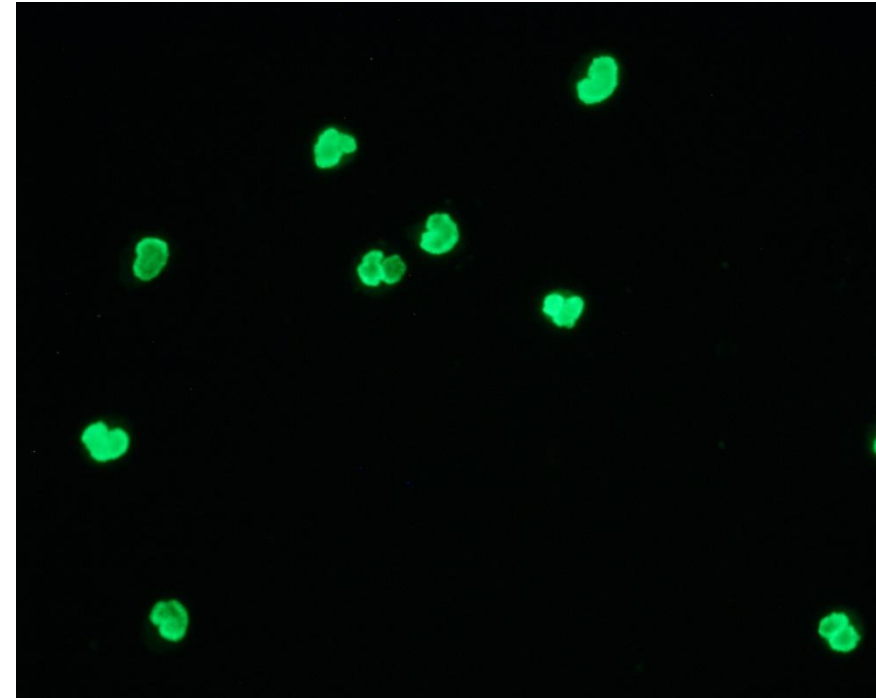
Adapted from Hoffman GS, Specks U.: Arthritis Rheum 1998.

Interpretation of IIF on EtOH fixed Neutrophils



- cANCA: mainly PR3-ANCA

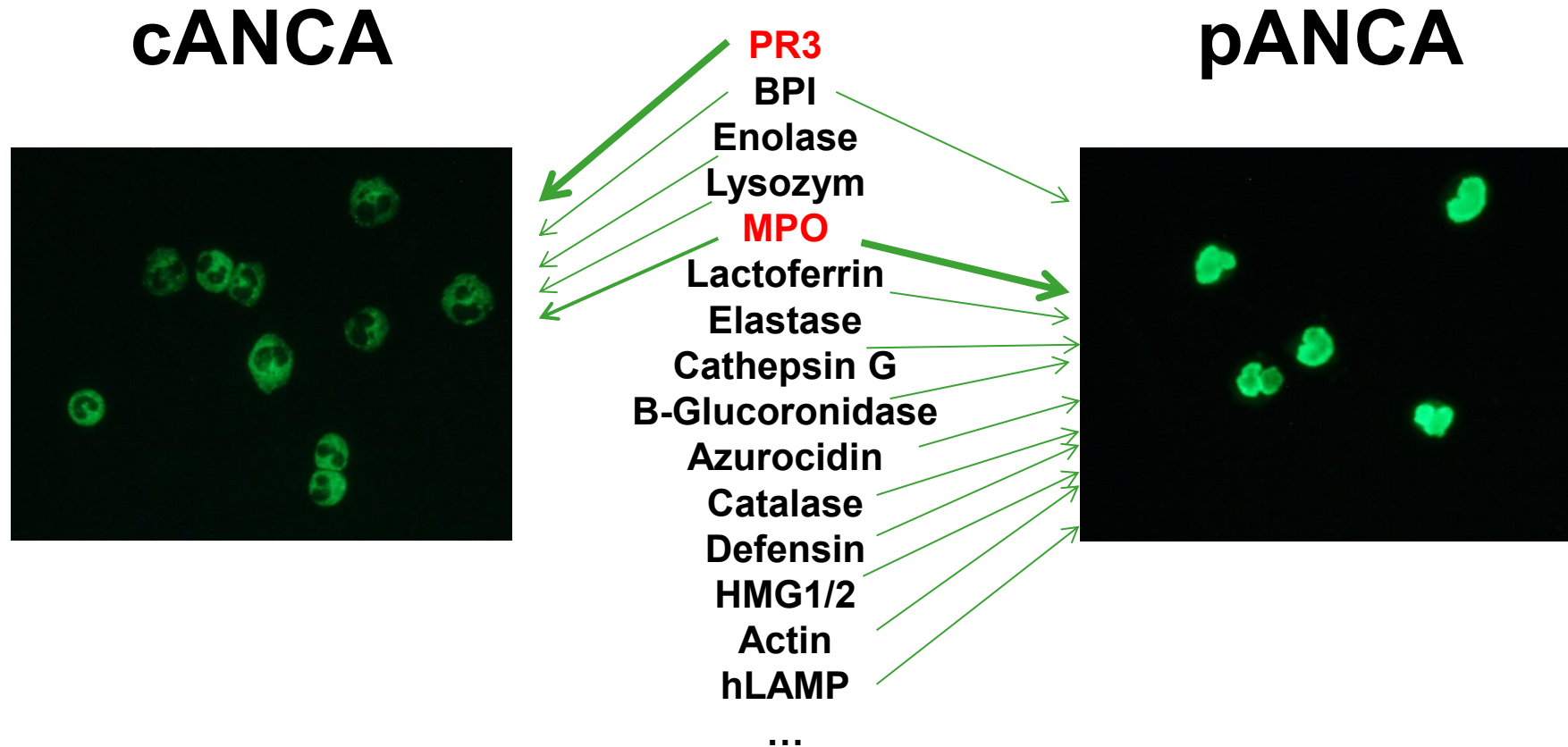
Granulomatosis with Polyangiitis



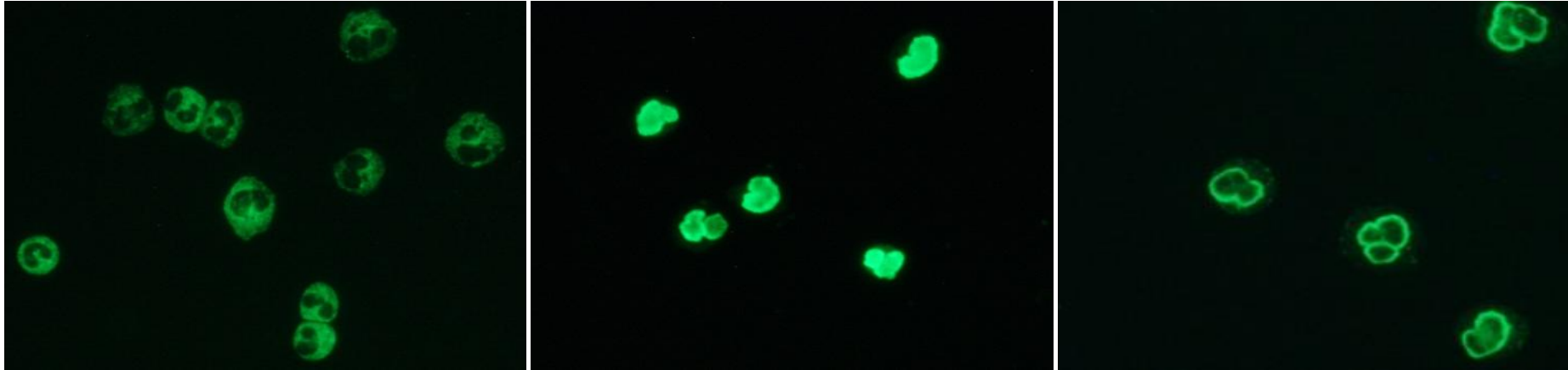
- pANCA: mainly MPO-ANCA

Microscopic Polyangiitis

(there are far more antigens)



Combination of IIF with Antigen-Specific Assays



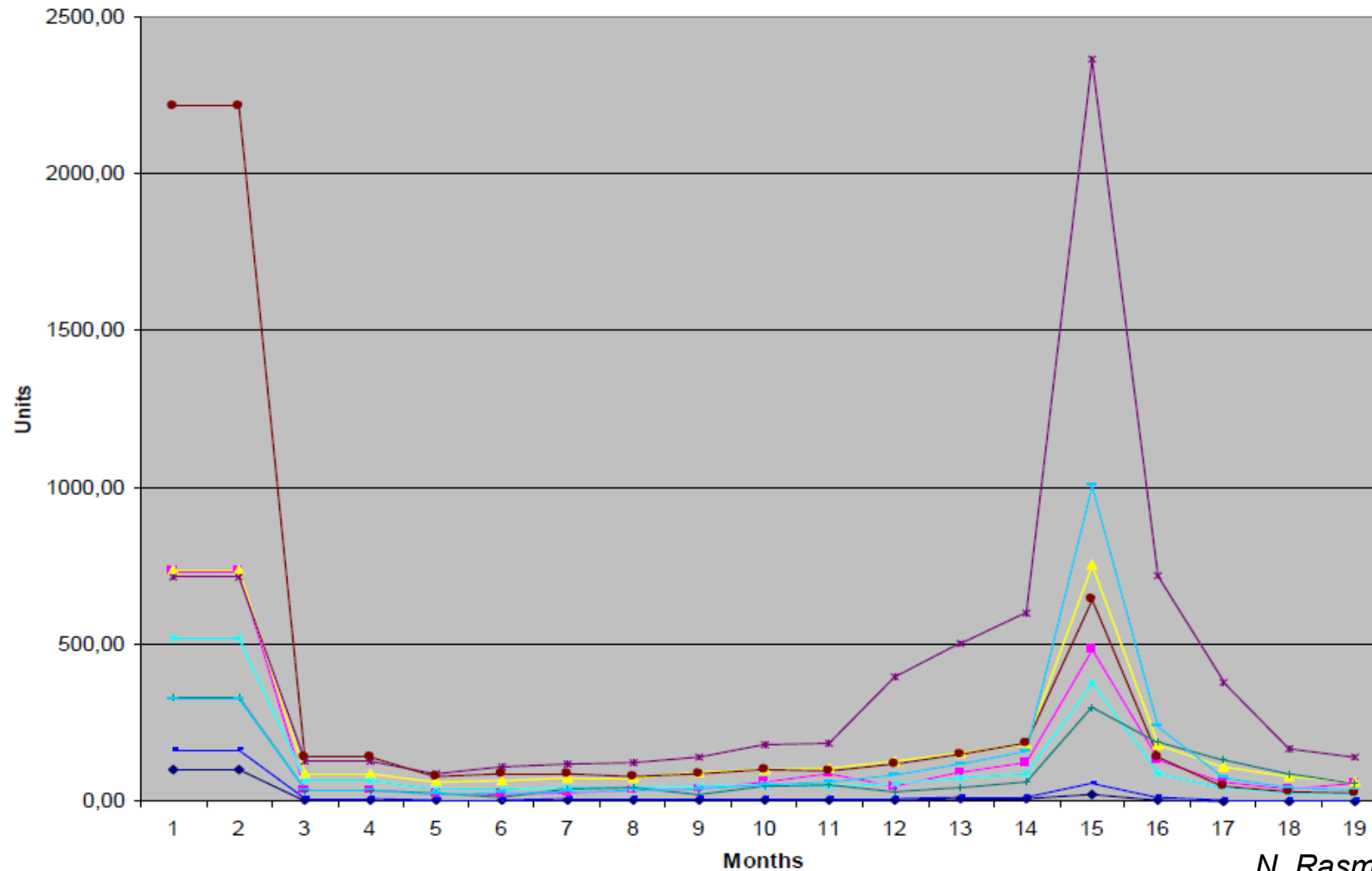
cANCA (cytoplasmic)

pANCA (perinuclear)

xANCA*
(rim-like perinuclear)

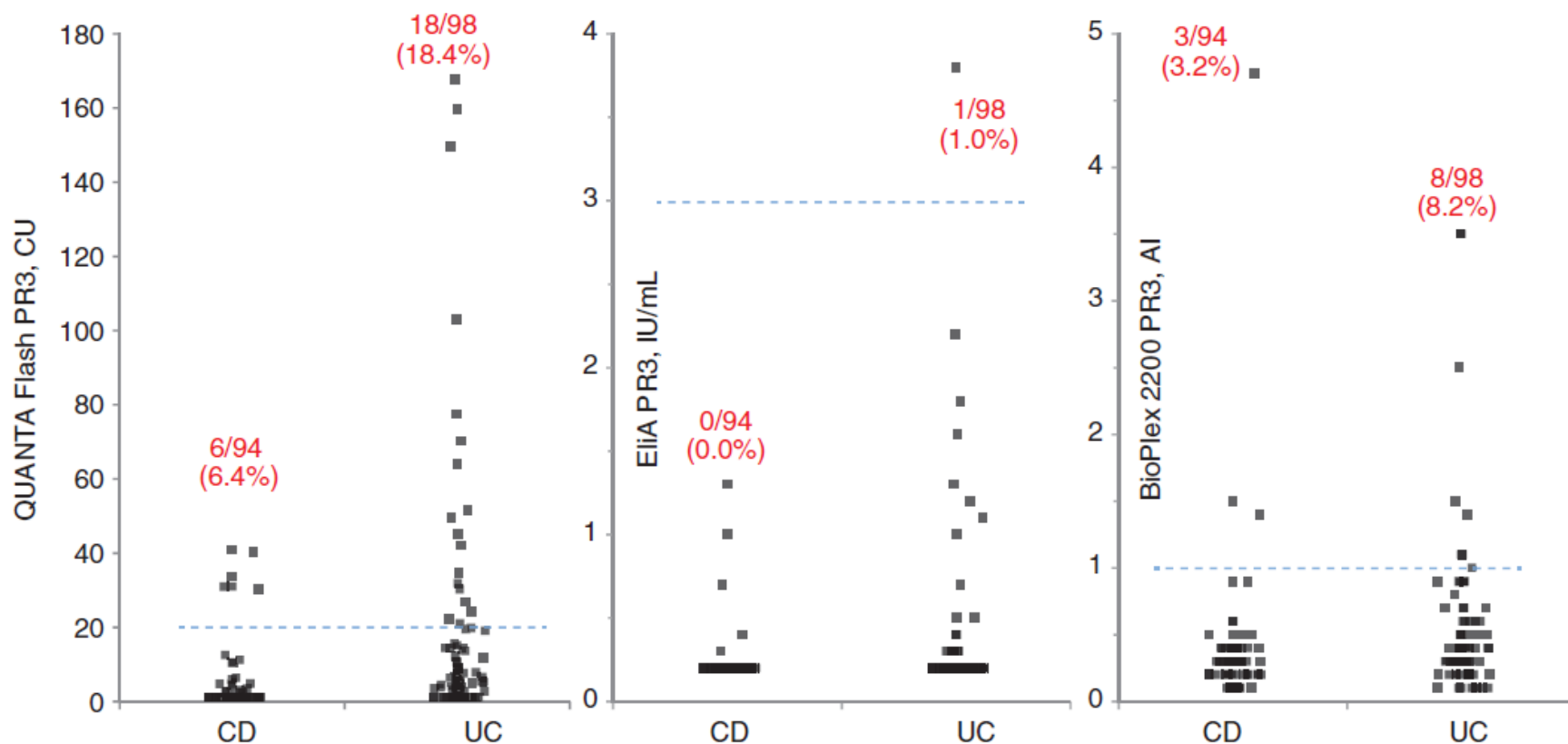
* atypical (p)ANCA, NANA, CU-ANCA,...

Anti-PR3 = anti-PR3??



N. Rasmussen, 2011, personal comm.

is it a CLIA-specific phenomenon?



Mahler et al. CCLM 2018;
<https://doi.org/10.1515/cclm-2017-0346>

The role of PR3-ANCA in the diagnosis of UC – a Meta-Analysis



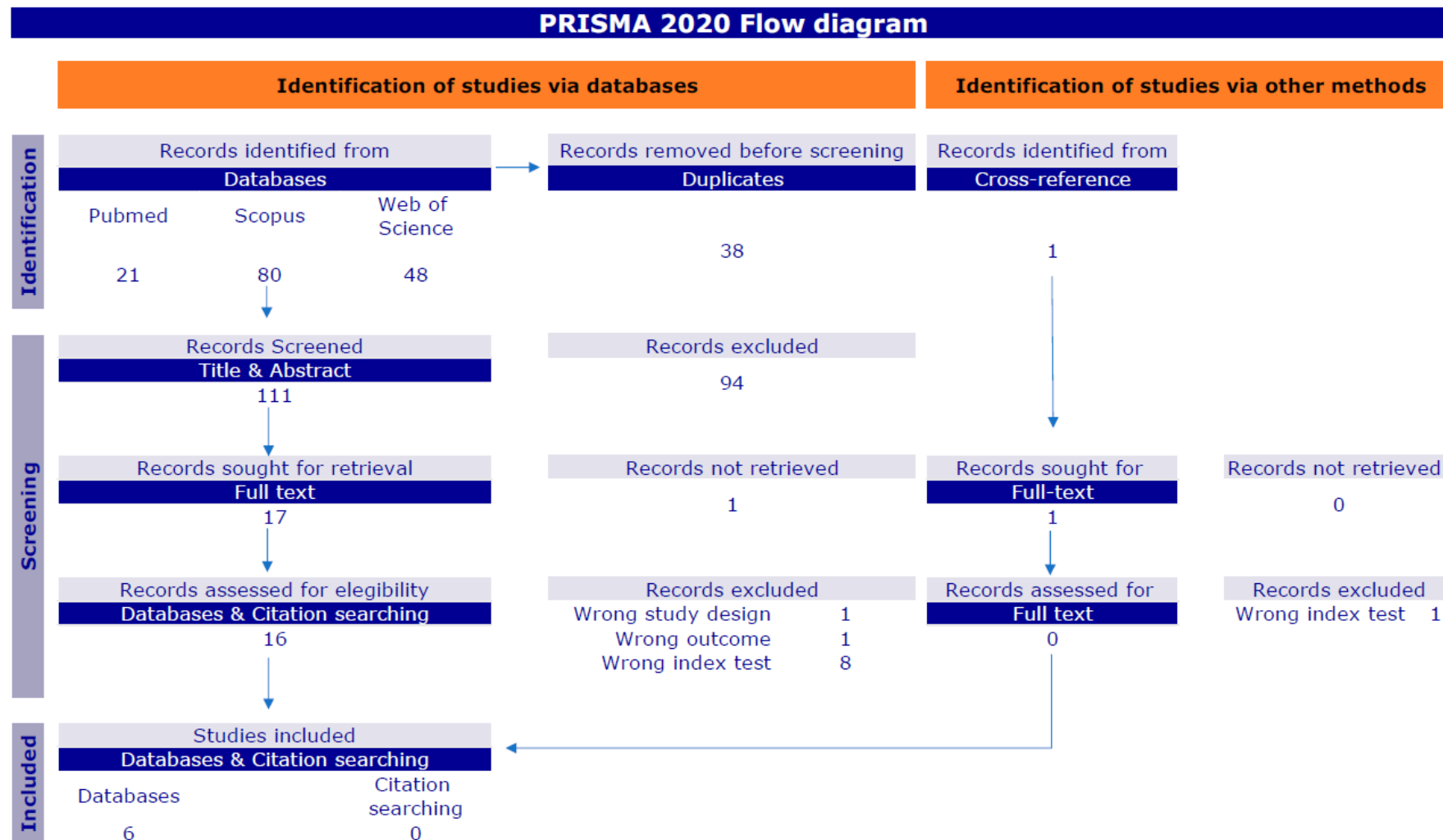
diagn

Systematic Review

Clinical Validity of PR3-ANCA in Inflammatory Bowel Disease

Carmen Andalucía
and Michael Mahl

➤ **Focus**



PR3-ANCA detected by QUANTA Flash have high specificity for UC

Study	UC PR3-ANCA Pos	UC Total	Sensitivity (95% CI)	CD PR3-ANCA Pos	CD Total	Specificity (95% CI)
Laass et al., 2022 [13]	49	121	40.5% (31.7–49.8%)	20	187	89.3% (84.0–93.3%)
Xu et al., 2022 [14]	35	84	41.7% (31.0–52.9%)	1	91	98.9% (94.0–100%)
Horn et al., 2018 [12]	19	33	57.6% (39.2–74.5%)	2	28	92.9% (76.5–99.1%)
Mahler et al., 2017 [7]	18	98	18.4% (11.3–27.5%)	6	94	93.6% (86.6–97.6%)
Mahler et al., 2013 [8]	88	283	31.1% (25.9–36.8%)	4	208	98.1% (95.2–99.7%)
Arias-Loste et al., 2013 [9]	14	48	29.2% (17.0–44.1%)	2	74	97.3% (90.6–99.7%)
Estimates	223	667	34.9% (26.2–44.6%)	35	682	95.9% (92.0–98.0%)

Abbreviations: ANCA = anti-neutrophil cytoplasmic antibodies; CI = confidence interval; CD = Crohn's diseases; Pos = positive; PR3 = proteinase 3; UC = ulcerative colitis; CI = confidence interval.

... but only limited sensitivity for Ulcerative Colitis

One step back: ANCA in IBD

Table 1. Prevalence of autoantibodies to specific antigens in UC patients.

Study	Healthy controls		Positive antibody test, %	

Duerr et al. (13)
 Nassberger et al. (14)
 Hauschild et al. (15)
 Kaneko et al. (16)
 Schmitt et al. (17)
 Peen et al. (18)
 Mulder et al. (19)
 Broekloelofs et al. (20)
 Kossa et al. (21)
 Yang et al. (22)
 Stoffel et al. (23)
 Sobajima et al. (24)
 Walmsley et al. (26)
 Sobajima et al. (25)
 Roozendaal et al. (27)

Table 2. Prevalence of autoantibodies to specific antigens in patients with CD.

Study	Method	Controls		Patients, n	ANCA ^a	Positive antibody test, %							
		n	SD ^a			BPI	β-Glu ^b	Cathepsin G	Elastase	Lysozyme	Lactoferrin	Catalase	α-Enolase
Mayet et al. (28)	EL	84	3	60				38	3				
Schmitt et al. (17)	EL	120	ND	23						17			
Peen et al. (18)	EL	218	2	52	27								8
Mulder et al. (19)	WB			35	40							17	
Broekloelofs et al. (20)	EL	252	3	35	40			0	0			8	
Kossa et al. (21)	EL	35	3	33	39			12	18	39		3	
Yang et al. (22)	EL	190	2	37	5		8					3	
Stoffel et al. (23)	EL	140	3	44	45	23							
Walmsley et al. (26)	EL	46	3			14		27				3	
Roozendaal et al. (27)	WB			112	19	5					11	26	18

^a Number of standard deviations above the mean of healthy controls that served as a cutoff point for a positive value.

^b β-Glu, β-glucuronidase; EL, ELISA; ND, no data available; WB, Western blot.

^c Colitis controls.

Bossuyt, Clin. Chemistry. 2006;
<https://doi.org/10.1373/clinchem.2005.058560>

pANCA and ASCA in IBD

Table 4. Prevalence of ASCAs and P-ANCAs in CD, UC, diseased controls, and healthy controls.

Antibodies	Study	n ^a	Positive for antibodies, %			
			CD	UC	Diseased controls	Healthy controls
ASCAs	Peeters et al. (40)	147/407/74/157	60	14	11	3.2
	Koutrobakis et al. (42)	97/56/0/150	39	11		0.7
	Quinton et al. (41)	101/100/27/163	61	12	11	0.6
	Ruemmele et al. (43)	36/131/78/0	55	5	5	
	Hoffenberg et al. (44)	25/20/74/0	60		4	
P-ANCAs	Peeters et al. (40)	147/407/74/157	6	50	8	2.5
	Koutrobakis et al. (42)	97/56/0/150	16	67		0
	Quinton et al. (41)	101/100/27/163	15	66	7.5	0.6
	Ruemmele et al. (43)	36/131/78/0	13	57	0	
	Hoffenberg et al. (44)	25/20/74/0		60	11	

^a UC/CD/diseased controls/healthy controls.

Bossuyt, Clin. Chemistry. 2006;
<https://doi.org/10.1373/clinchem.2005.058560>

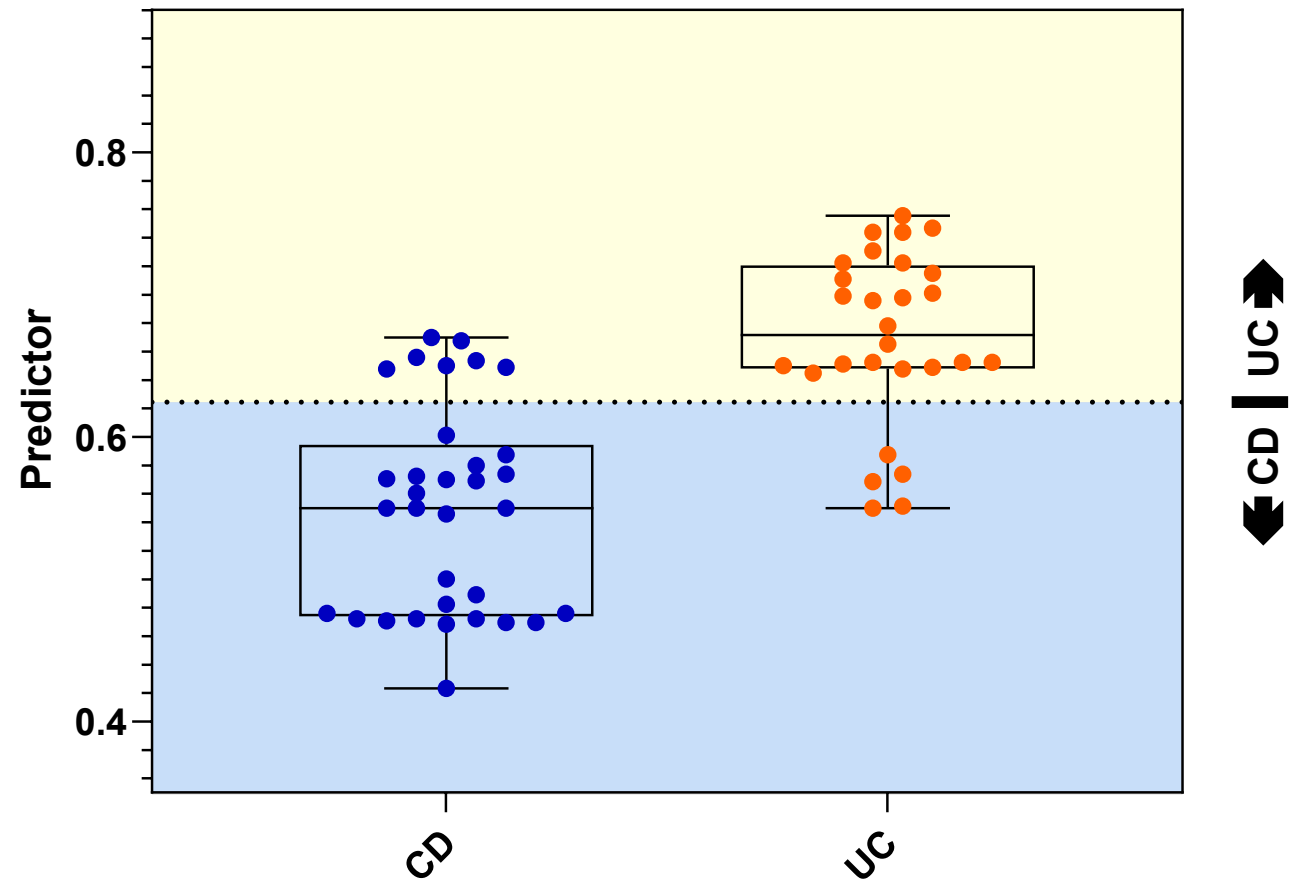
serological distinction of CD and UC



Table 2. Antibody association with ulcerative colitis and Crohn's disease.

Antibody	CD	UC	p-value	c
ANCA				
cANCA, n (%)	2 (7.4)	6 (18.2)	0.276	
PR3-ANCA, n (%)	2 (7.1)	19 (57.6)	<0.001	
PR3-ANCA U/ml, median [IQR]	1 [1, 1.65]	5.8 [1.6,13.8]	<0.001	
pANCA, n (%)	0 (0)	2 (6.1)	0.497	
MPO-ANCA, n (%)	1 (3.6)	3 (9.1)	0.618	
MPO-ANCA U/ml, median [IQR]	1 [1,1]	1 [1,1]	0.585	
xAANCA, n (%)	4 (14.8)	18 (54.5)	0.003	
GAB, n (%)	3 (10.7)	1 (3.6)	0.611	
ASCA				
IgA, n (%)	13 (46.4)	3 (9.1)	0.001	
IgA U/ml, median [IQR]	14.5 [7,72.5]	9 [5,13]	0.038	
IgG, n (%)	15 (53.6)	5 (15.2)	0.001	
IgG U/ml, median [IQR]	37 [6,70]	7 [4,17]	0.001	
PAB, n (%)	10 (35.7)	7 (21.2)	0.208	
gGAB, n (%)	7 (25)	3 (9.1)	0.164	

<https://doi.org/10.1371/journal.pone.0208974.t002>



Horn et al., PLOS One. 2018;
<https://doi.org/10.1371/journal.pone.0208974.t002>

CD vs. UC

Crohn's Disease



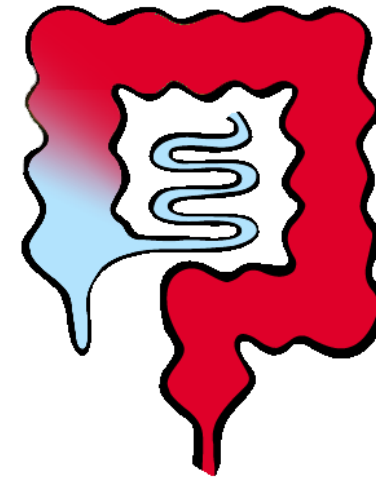
segmental
transmural
entire GI tract

IBD-U

?



Ulcerative Colitis



continuously
superficial
Colon only

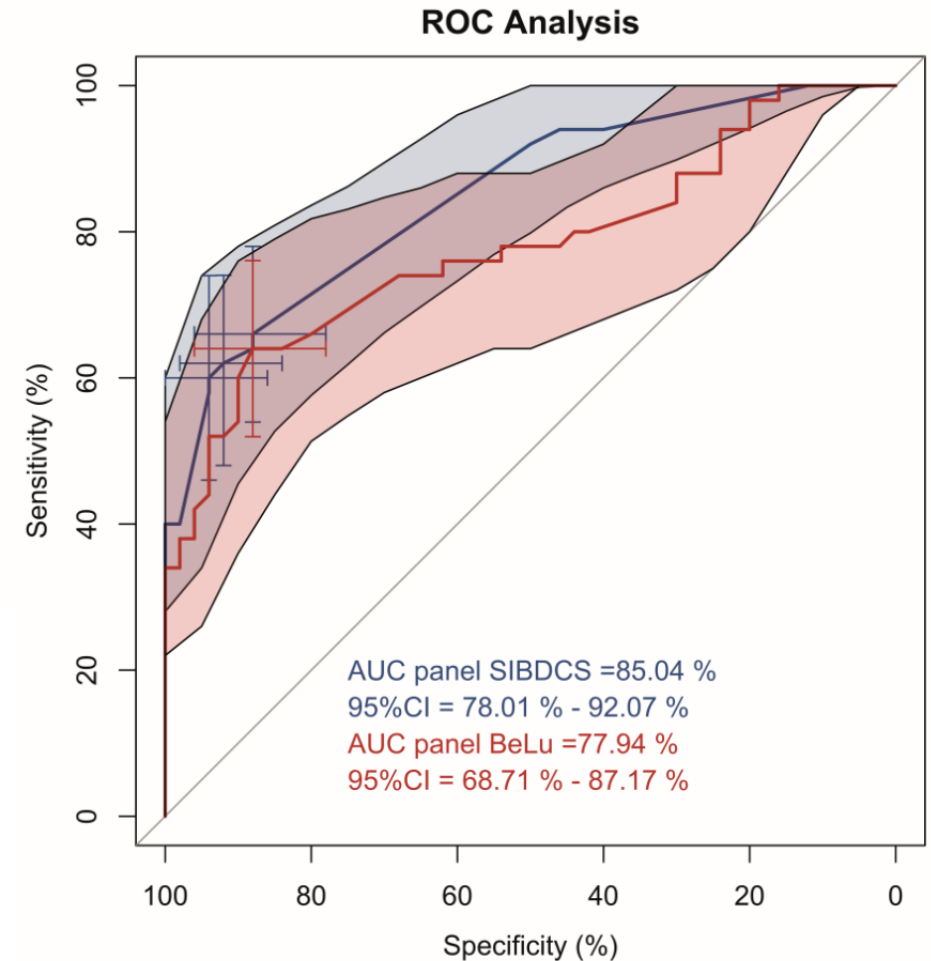
Can re-classification of IBD-U patients be predicted?

- Swiss IBD-Cohort Study
- 50 Patients with Crohn's disease
- 50 patients with ulcerative colitis
- 76 patients with IBD-U: 26 re-classified during follow-up
 - 18/26 IBD-U → UC
 - 8/26 IBD-U → CD

Table 1. Panel description.

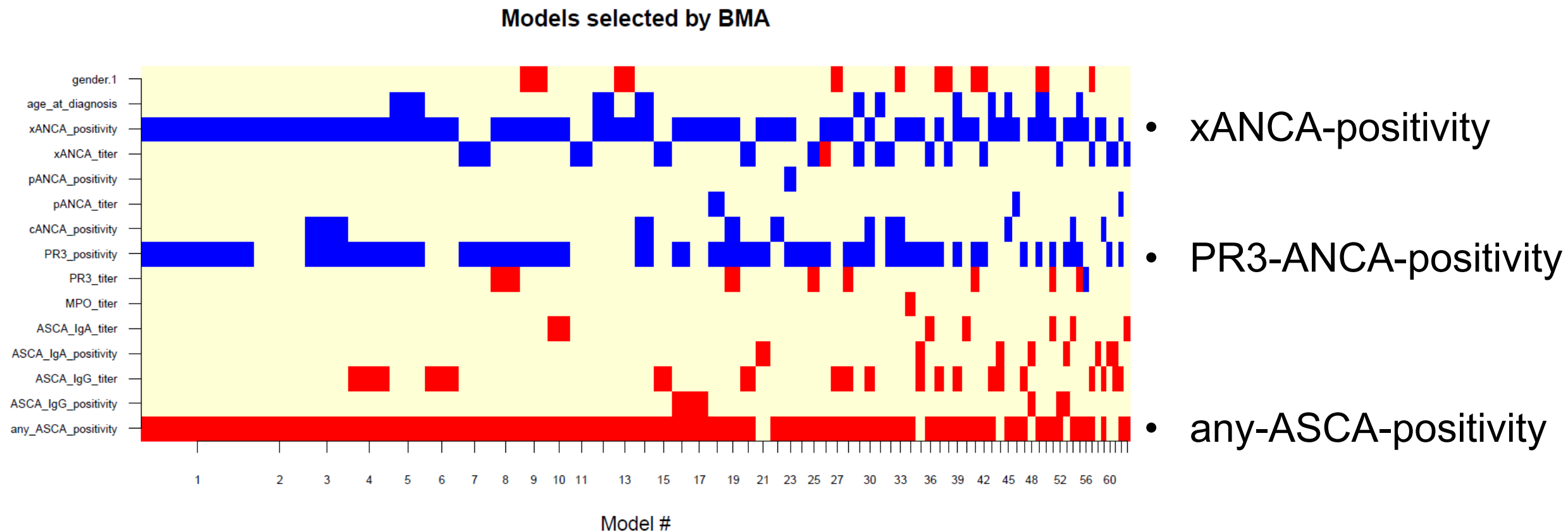
Analyte	BeLu *	SIBDCS
Intercept	0.5937	0.425704
PR-3 ANCA positivity	-0.4085	-0.22856
αANCA positivity	-0.328	-0.25171
pANCA positivity	-0.6299	n.a.
ASCA IgG, Titer	0.0052	n.a.
ASCA IgG positivity	n.a.	0.277831
ASCA IgA positivity	n.a.	0.301052

* BeLu (Bern Lucerne) panel taken from [10]; n.a. not applied; SIBDCS Swiss IBD cohort study.



Sokollik et al., *Diagnostics*. 2023;
<https://doi.org/10.3390/diagnostics13152491>

Machine learning approach to find the best marker combination



Variables with large continuous bars can be read to be included in many models and are therefore very likely to hold high predictive value. *BMA: Bayesian model averaging.*

Sokollik et al., *Diagnostics*. 2023;
<https://doi.org/10.3390/diagnostics13152491>

Again: are all PR3-ANCA the same?

UK-NEQAS

- High discrepancy between methods
- High discrepancy within methods

How comparable are the results?

PR3 IU/mL or U/mL	Returns	Positive	Negative	Mean	Range	
A. Menarini - Zenit EIA	235	234	1	156.72	2.90	500.00
A. Menarini - Zenit RA	1	1	0	500.00	500.00	500.00
AESKU	14	14	0	664.60	500.00	847.00
Alpha Dia	7	7	0	225.49	2.90	363.25
Bio-Rad Bioplex 2200	2	2	0	60.00	57.00	63.00
Biosystems	6	6	0	211.00	8.00	414.00
Diamedix	1	1	0	96.40	96.40	96.40
Euroimmun	1	1	0	0.00	0.00	0.00
HOB-BioCLIA	16	16	0	262.41	8.60	530.40
Inova	4	4	0	493.47	400.00	680.40
Inova QUANTA Flash	11	10	1	174.44	3.20	309.10
ORGenTec Alegria	46	46	0	183.52	76.00	610.00
ORGenTec ELISA	13	13	0	117.86	55.10	200.00
Other ...	6	6	0	102.68	7.50	380.00
Phadia 100 EliA	7	7	0	145.53	100.00	192.10
Phadia 1000/2500 EliA	1	1	0	157.00	157.00	157.00
Phadia 250 EliA	15	15	0	153.52	113.60	177.00
THERADIAG (BMD)	82	82	0	136.11	85.00	183.00
Trinity Biotech	1	1	0	936.00	936.00	936.00
	1	1	0	7.64	7.64	7.64
OTHER UNITS	291	289	2	288.20	0.00	915.00
Aeskulisa PR3 Sensitive	1	1	0	444.00	444.00	444.00
Alpha Dia	6	6	0	93.42	53.00	131.00
Bio-Rad Bioplex 2200	40	40	0	241.25	8.00	468.30
Bio-Rad/Kallestad/Sanofi	1	1	0	8.00	8.00	8.00
D-tek	3	3	0	81.33	67.00	94.00
Euroimmun	7	6	1	74.62	0.00	177.00
Euroimmun PR3-hn-hr	1	1	0	0.00	0.00	0.00
IDS	9	9	0	620.32	500.00	915.00
Inova QUANTA Flash	51	51	0	633.90	534.10	848.00
ORGenTec ELISA	1	1	0	194.15	194.15	194.15
Orgentec PR3 hs	1	1	0	111.00	111.00	111.00
Other ...	3	3	0	426.00	412.00	440.00
Phadia 100 EliA (PR3S)	3	3	0	128.00	121.00	135.00
Phadia 250 EliA (PR3S)	164	163	1	138.39	86.00	182.00

Can we harmonize Specific Test Results?

Likelihood Ratio!

$$LR^+ = \frac{\text{Sensitivity}}{1 - \text{Specificity}}$$

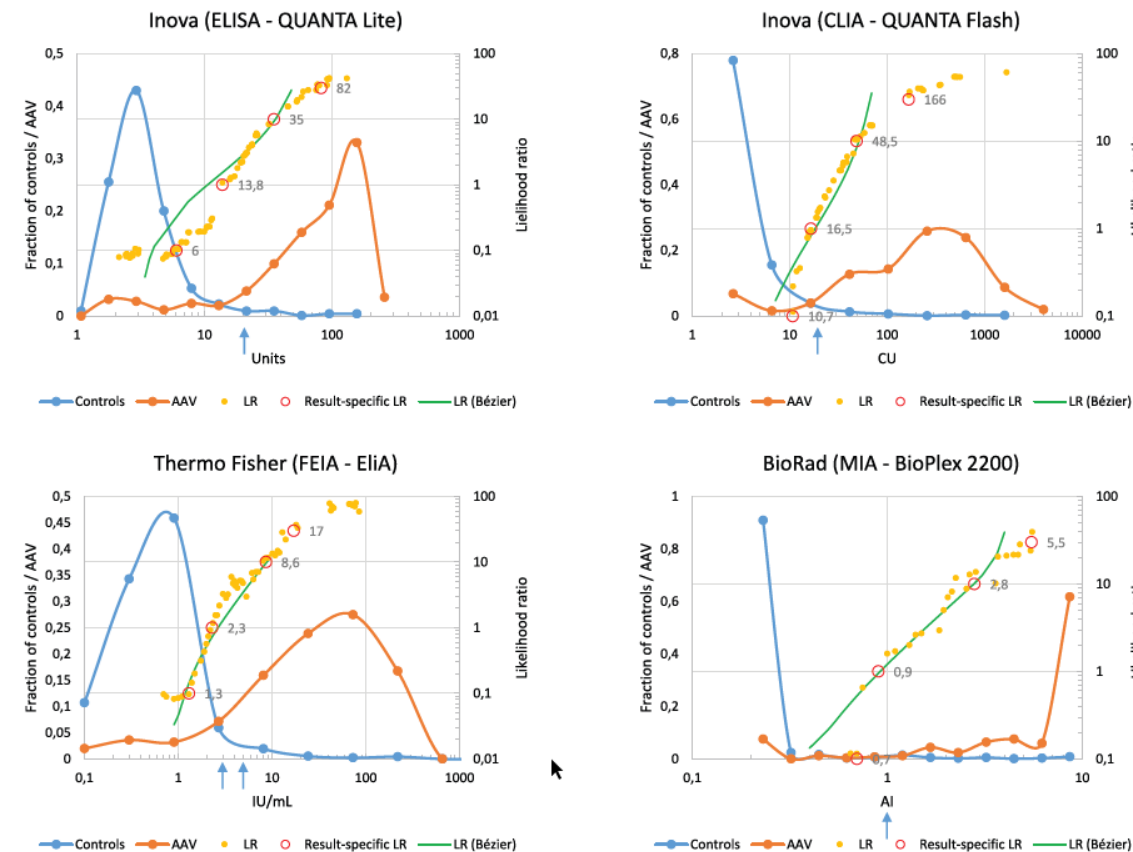


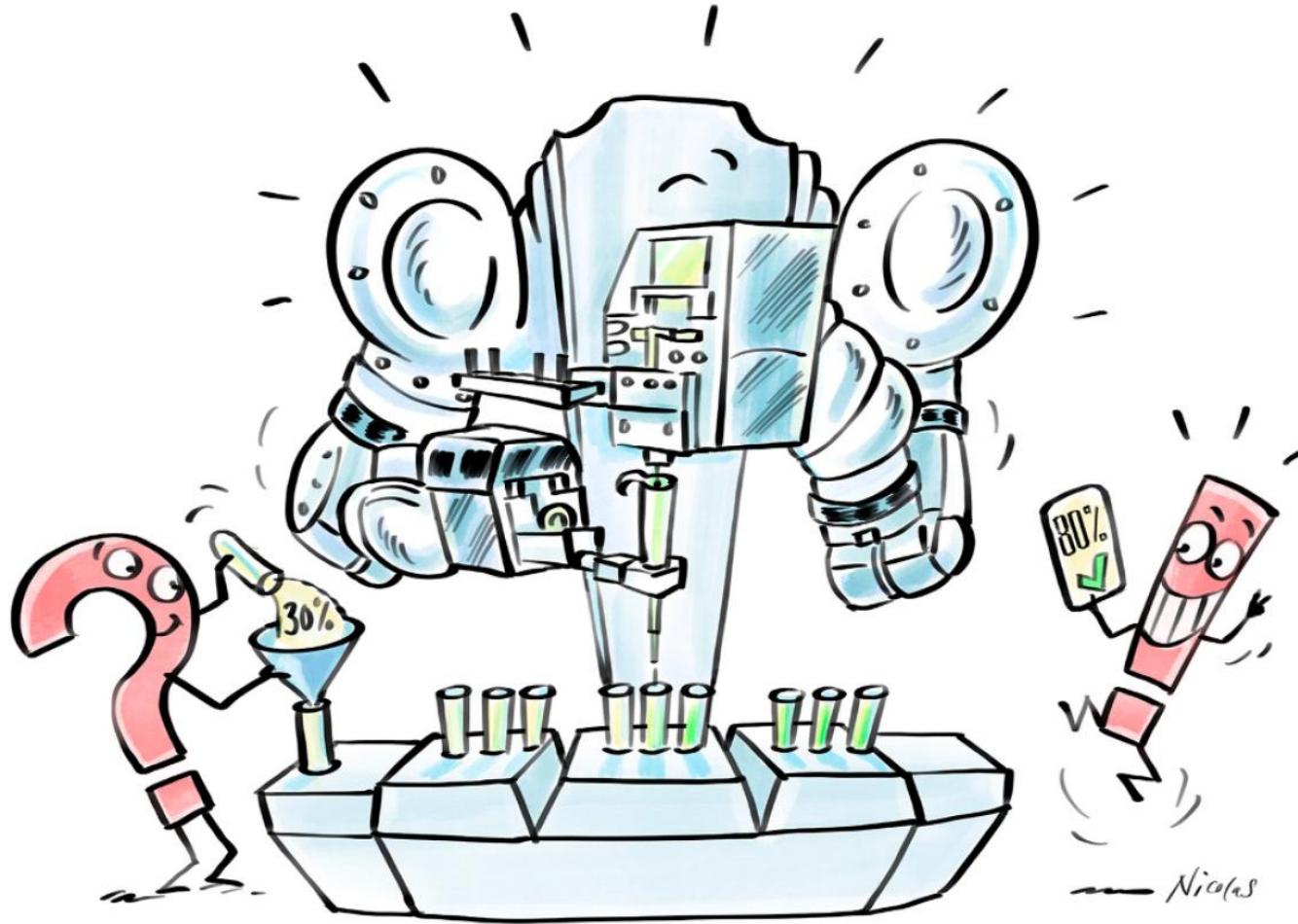
Figure 1: Distribution of test results and test result-specific likelihood ratios (LR). Patients with antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) (n=251) and controls (n=924) were tested for ANCA by eight different assays.



Pre-Test Probability (%) Likelihood Ratio Post-Test Probability (%)

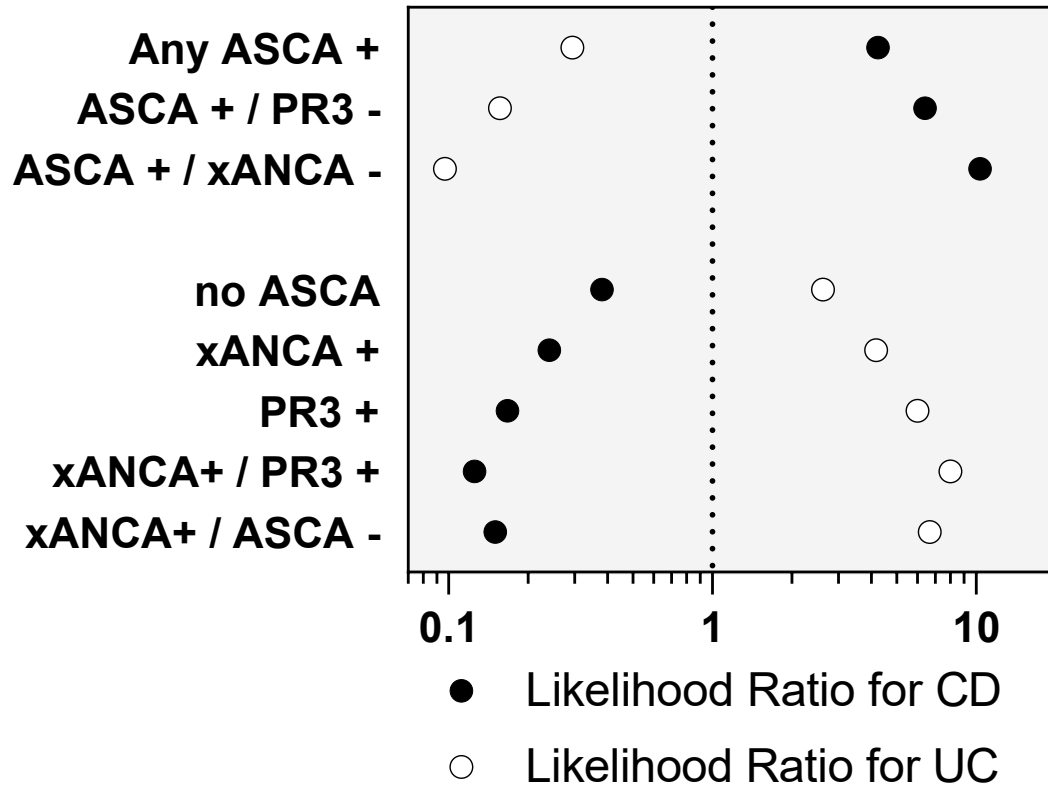
Bossuyt et al, 2021
Clin Chem lab med. 59:e35-39

What the clinician expects from the lab

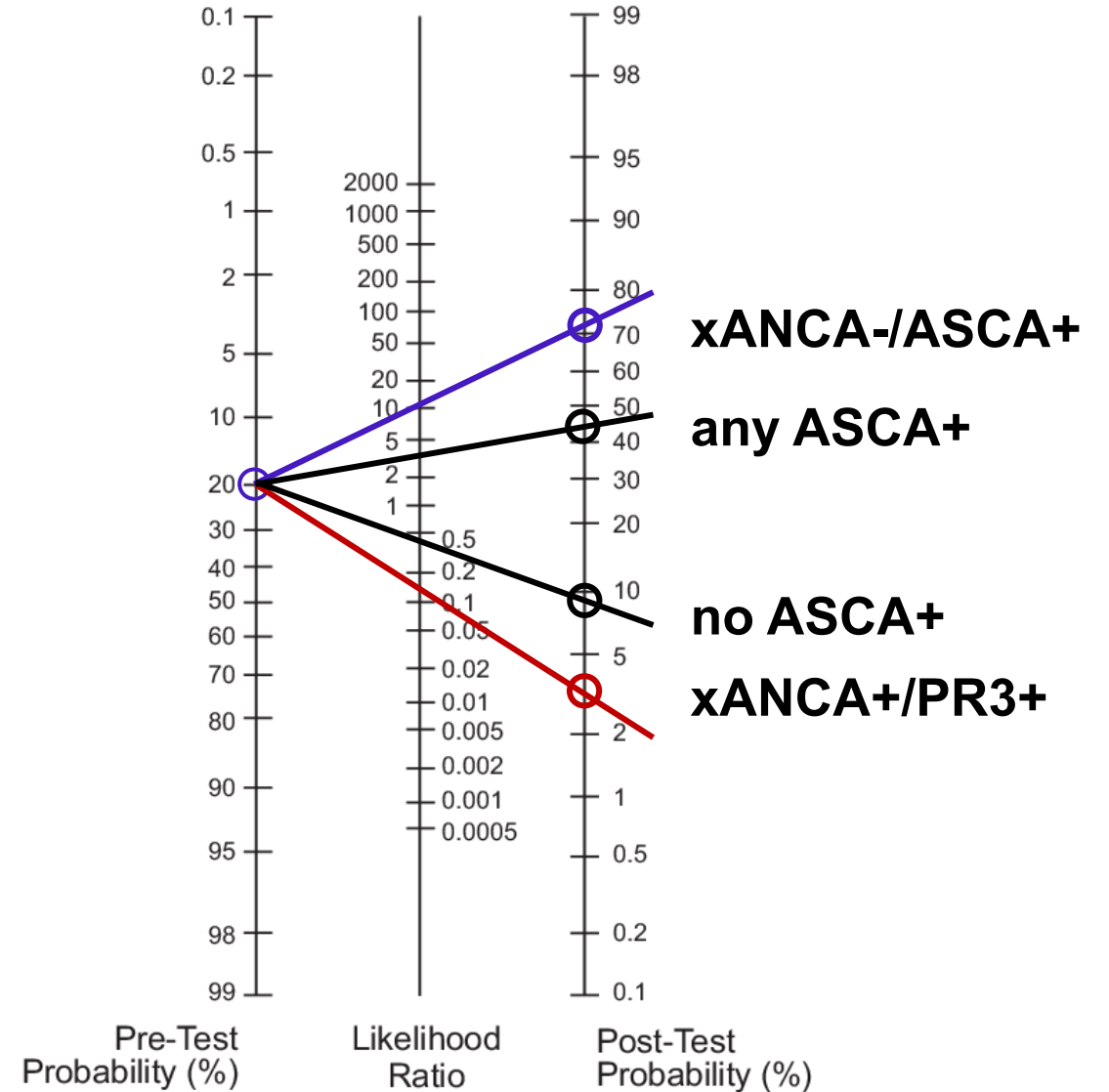


Kolev et al. J. Transl. Autoimmun. 2022;
<https://doi.org/10.1016/j.jtauto.2022.100151>

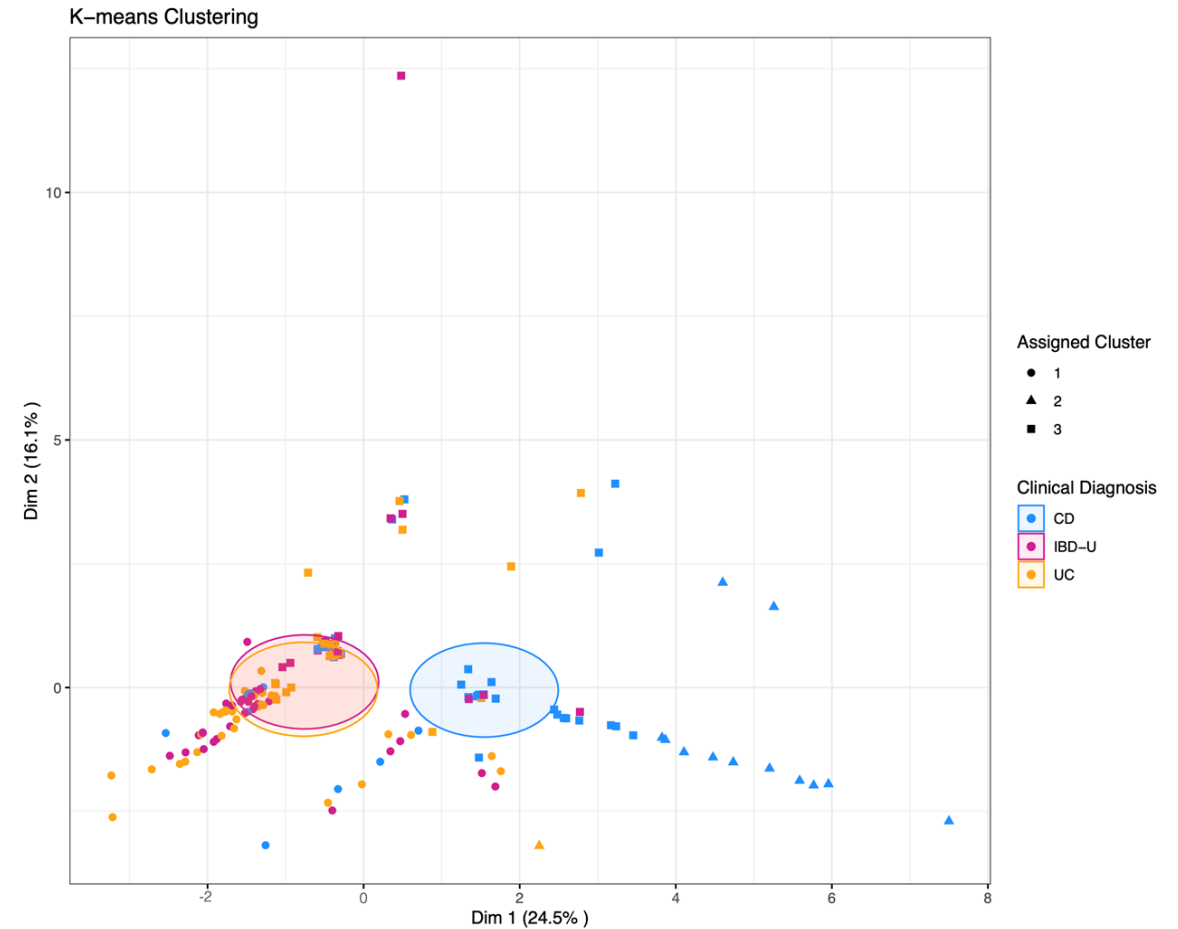
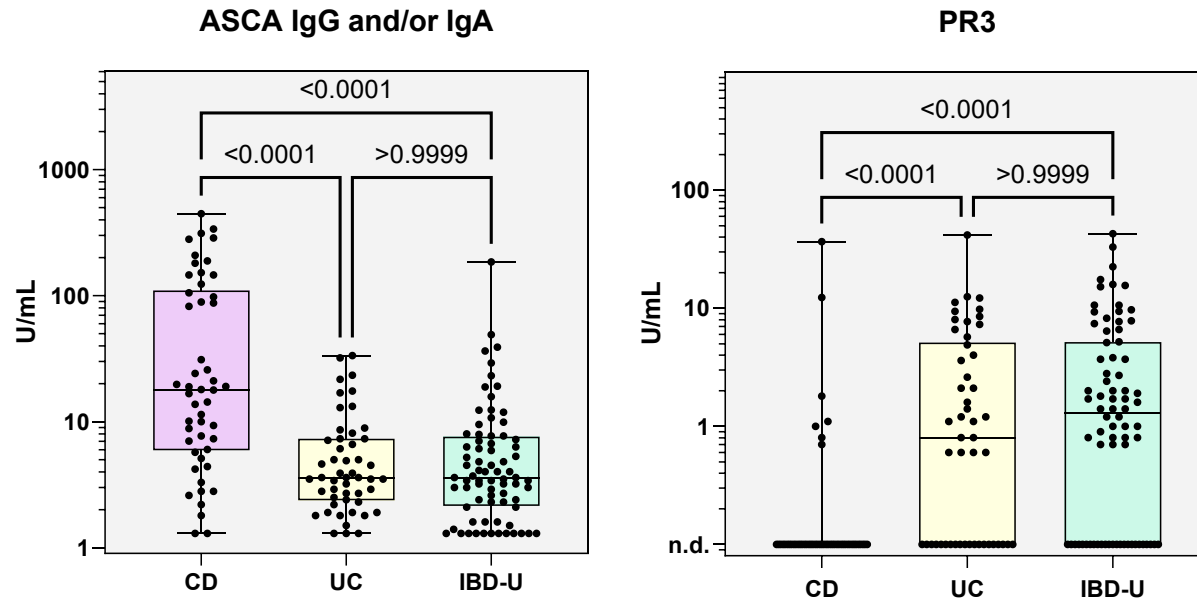
LR of xANCA, PR3-ANCA, ASCA



unpublished data based on the SIBD-Cohort
 (Sokollik et al., Diagnostics. 2023)



Is IBD-U an Independent Disease-Identity?

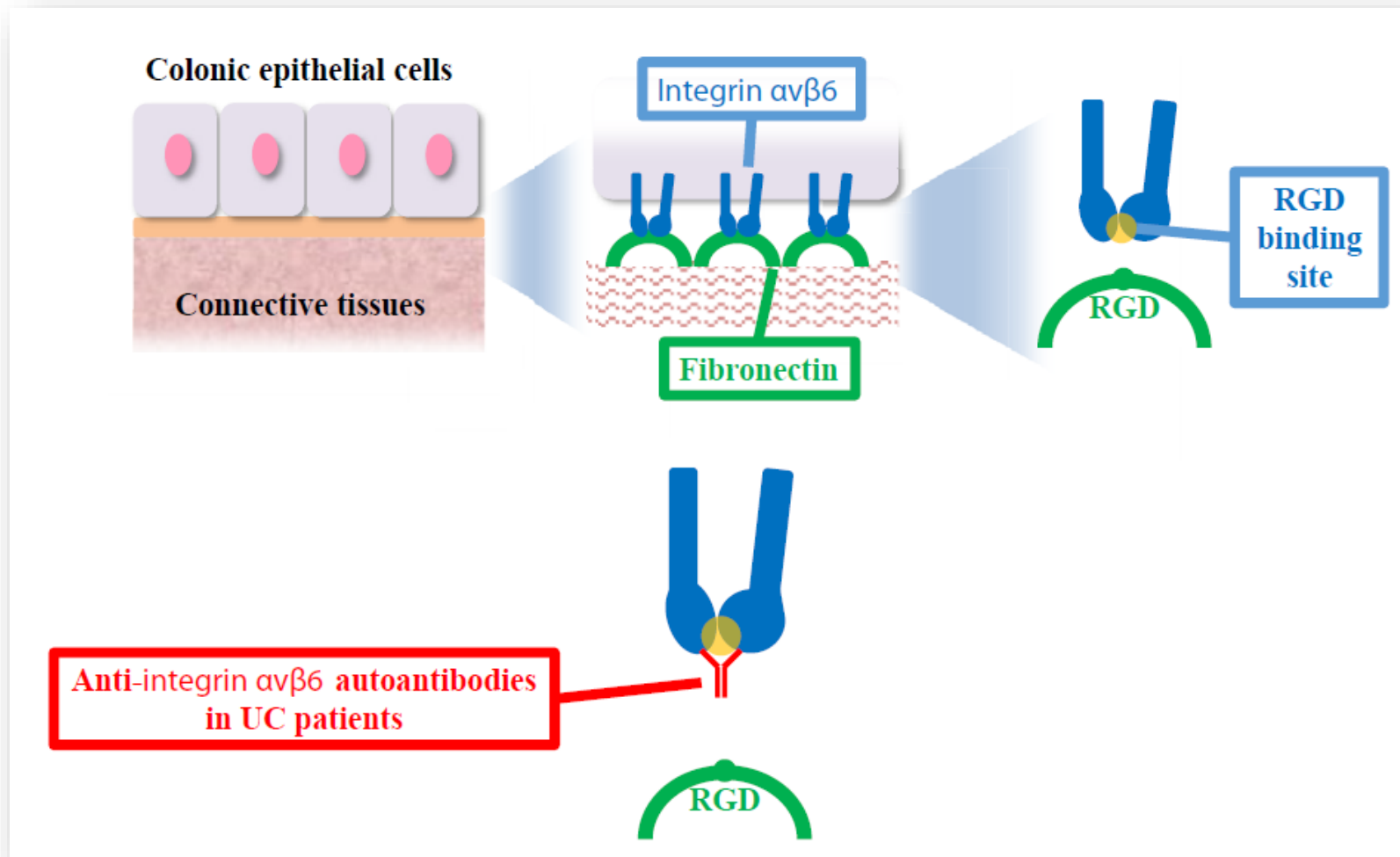


Machine Learning in Antibody Diagnostics for Inflammatory Bowel Disease Subtype Classification

Christiane Sokollik^{1,†}, Aurélie Pahud de Mortanges^{2,†}, Alexander B. Leichtle^{3,4}, Pascal Juillerat^{5,6} and Michael P. Horn^{3,*} on behalf of the Swiss IBD Cohort Study Group

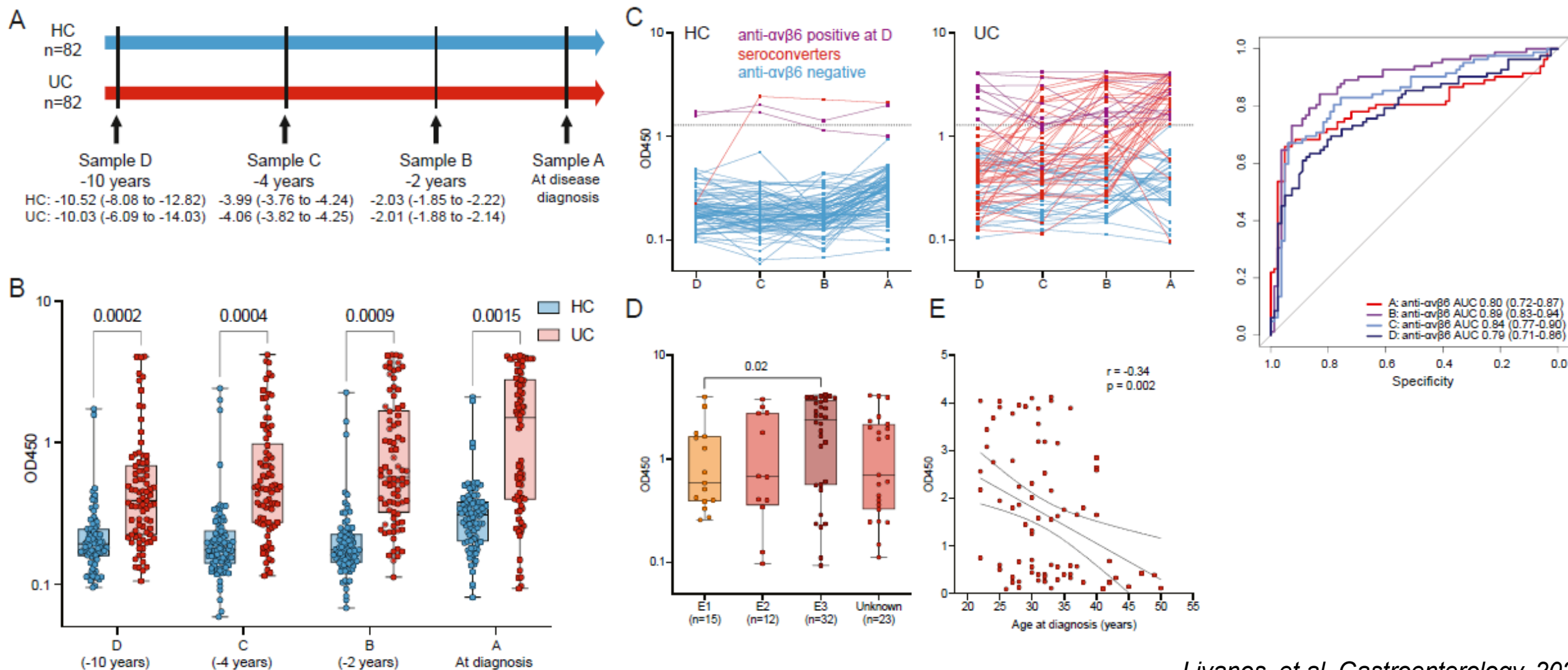
Sokollik, et.al. *Diagnostics*. 2023;
<https://doi.org/10.3390/diagnostics13152491>

Additional Lab Tests?



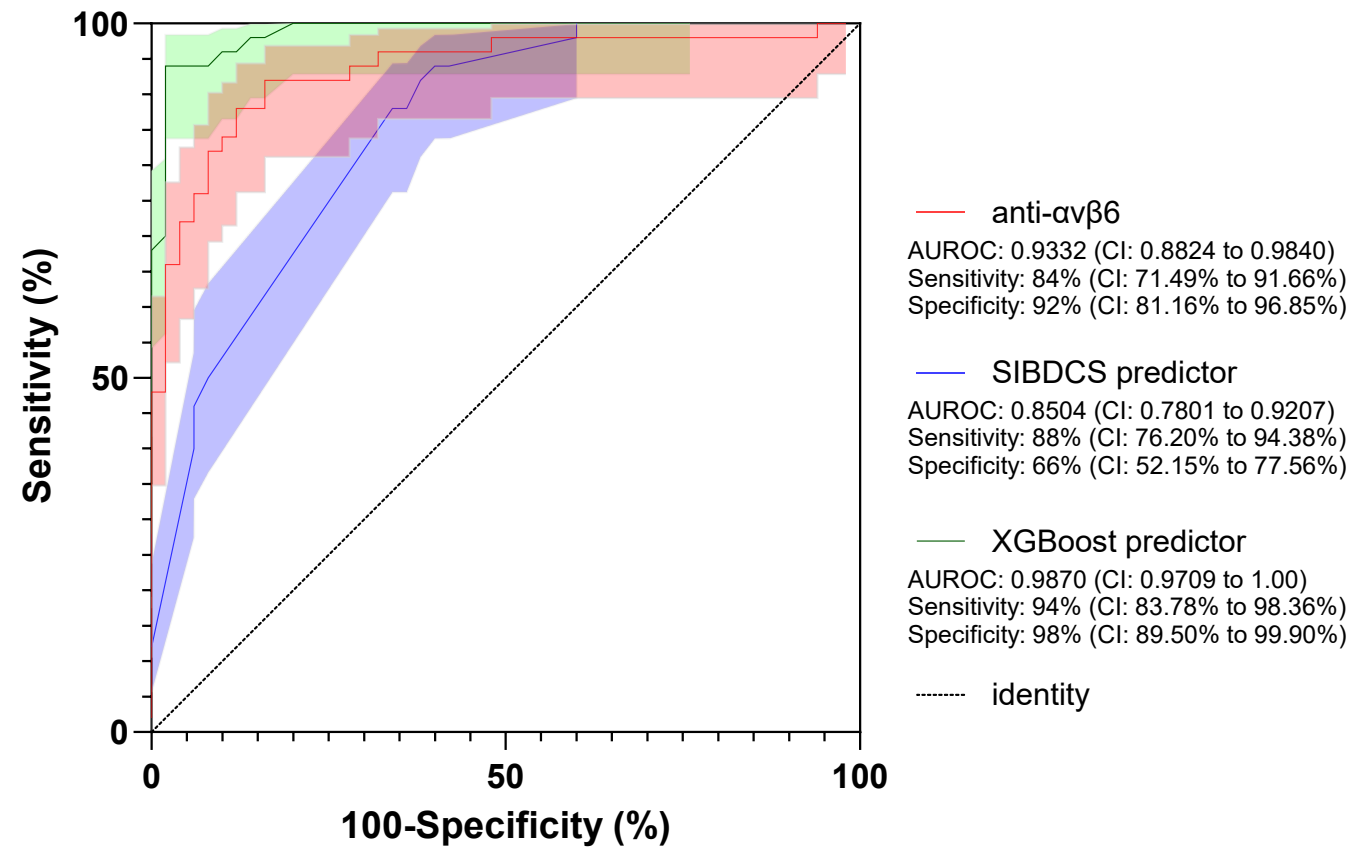
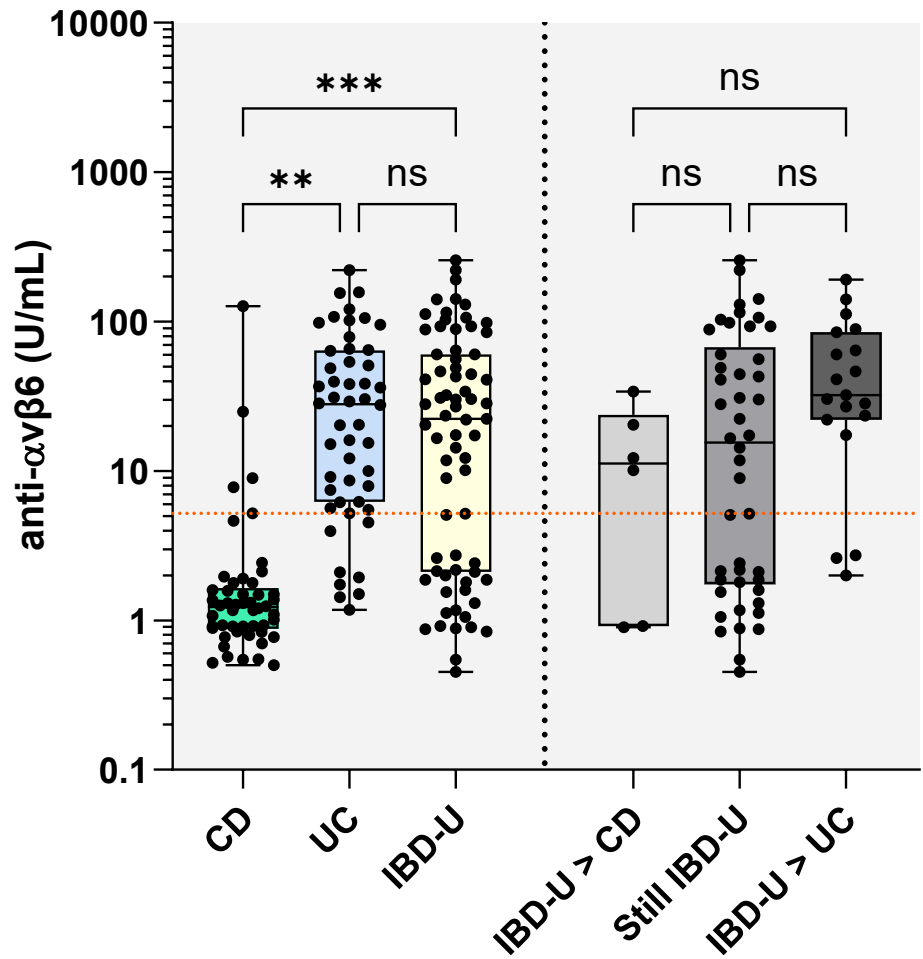
Kuwada, et.al. *Gastroenterology*. 2021;
<https://doi.org/10.1053/j.gastro.2021.02.019>

Anti-Integrin $\alpha v \beta 6$ can predict ulcerative colitis



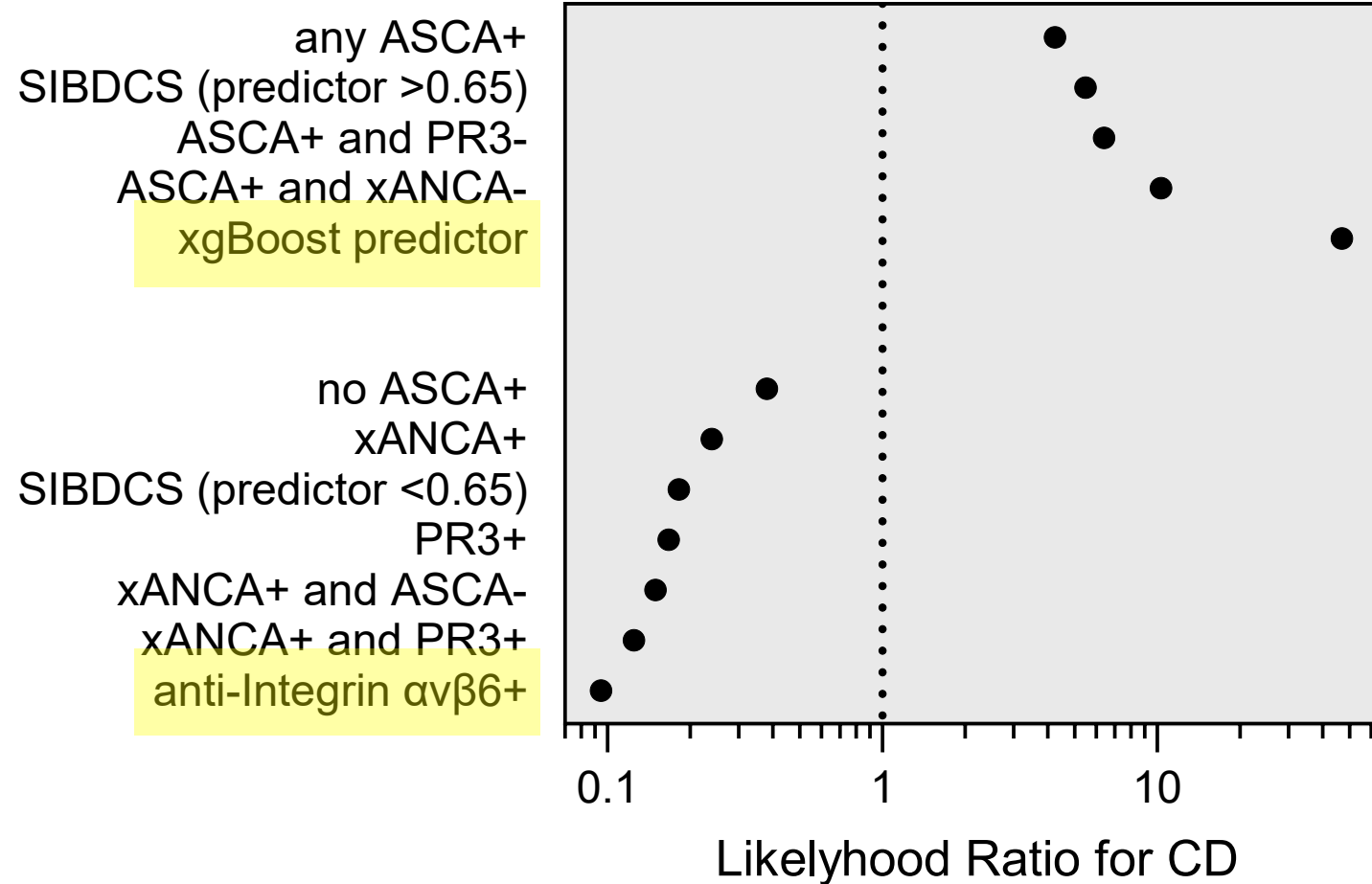
Livanos, et.al. *Gastroenterology*. 2023;
<https://doi.org/10.1053/j.gastro.2022.12.042>

SIBDCS: Anti-Integrin $\alpha v \beta 6$



Horn, et.al. In preparation

Can anti-Integrin $\alpha v \beta 6$ antibodies help to distinguish CD from UC?



Horn, et.al. In preparation

Take Home Messages

- PR3 ANCA determined on QUANTA Flash® can support the serological diagnosis of ulcerative colitis (UC) patients
- Differential diagnostic accuracy can be improved by the additional determination of ASCA IgA and IgG as well as atypical rim-like ANCA (xANCA)
- Unclassified IBD patients (IBD-U) are serologically classified as UC patients
- It is not possible to predict the switch from IBD-U to Crohn's disease (CD)
- Anti-integrin $\alpha v \beta 6$ antibodies might further improve the serological classification of IBD patients in terms of CD vs UC.

Thank you very much!

Werfen

Carmen Andalućía
Laura Martínez-Prat
Chelsea Bentow
Mary Ann Aure
Michael Mahler

Inselspital Bern

Andrea Karolin
Christiane Sokollik
Henning Nilius
Alexander Leichtle
Anna Peter
Franziska Righini Grunder
Johannes Spalinger
Susanne Schibli
Aurélie Pahud de Mortagnes
Pascal Juillerat

Thomas Momot
Daniel Schärer
Olivier Schaerer
Juliette Schlatter
Monika Hurni