

The potential role of the likelihood ratio approach to support diagnosis and classification of Autoimmune Diseases

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Introduction

What does a weak pos. autoantibody test result mean?

"Medicine is a science of uncertainty and an art of probability."

~ William Osler (1849-1919)

Probability for disease based on laboratory test results

Likelihood ratio (LR)

LR for single cutoff

Anti-CCP	Interval	Fraction of patients	Fraction of controls	LR
	Positive	0.67	0.05	13.4
	Negative	0.33	0.95	0.35

Test result interval-specific LR

PR3-ANCA - MPO-ANCA	Interval (IU/mL)	Fraction of patients (%)	Fraction of controls (%)	LR	95% CI
	0.0, 2.1	9%	95%	0.10	0.07, 0.15
	2.1, 5.0	8.3%	2.5%	3.4	1.9, 6.0
	5.0, 16.0	17.9%	1.5%	11.8	6.6, 21.1
	16.0, 142.0	57.1%	0.97%	58.73	30.4, 113.5
	142.0, 180.0	6.7%	0%	∞	7.5, ∞

Pre-test odds $\times$ LR = post-test odds

$$\frac{p}{1-p} = odds$$

$$Pre\text{-}test\ odds \times LR = post\text{-}test\ odds$$

$$\frac{odds}{1+odds} = p$$

$$\frac{p}{1-p} = \text{odds}$$

$$\frac{0.5}{1-0.5} = 1$$

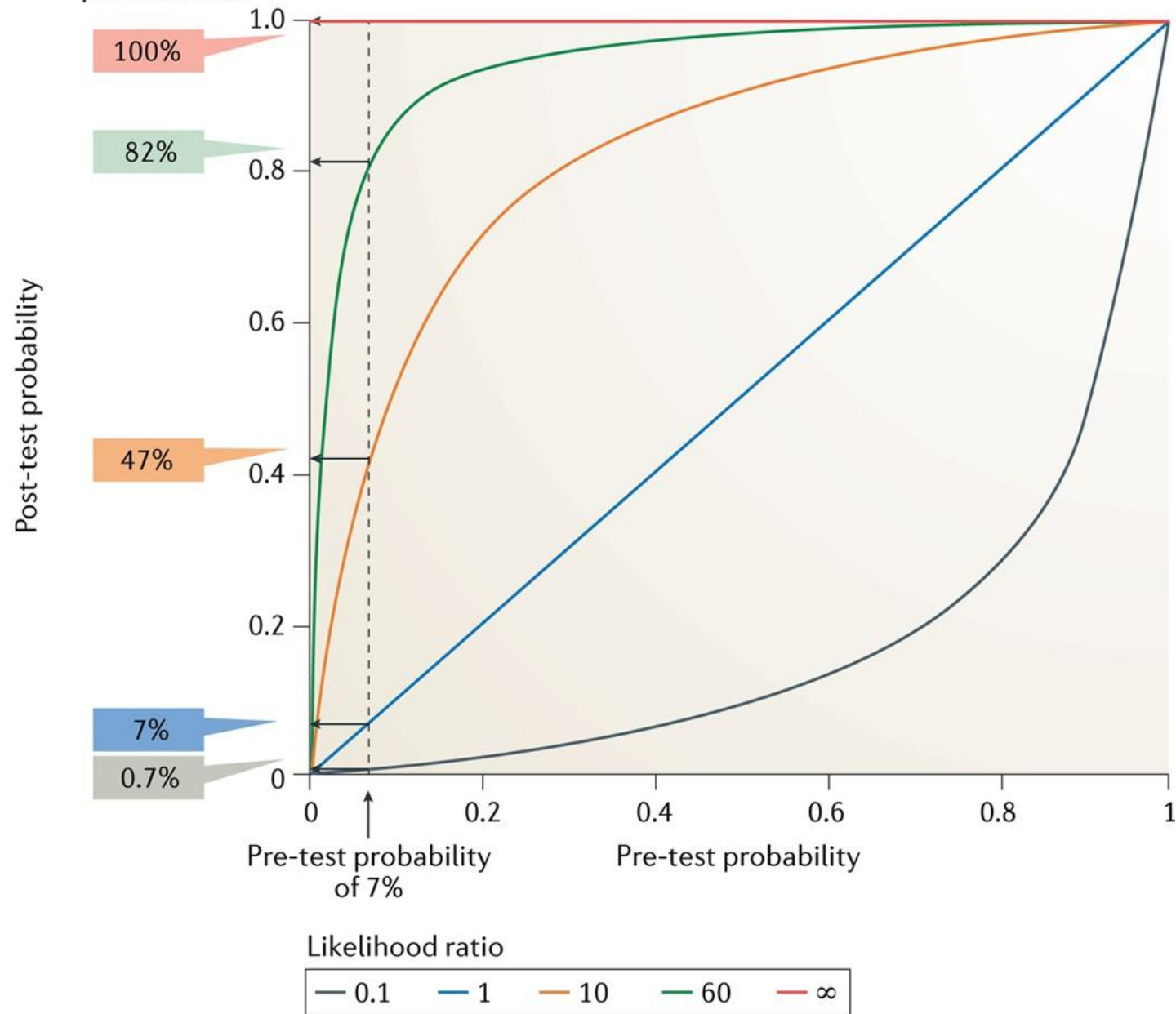
Pre-test odds X LR = post-test odds

$$1 \times 9 = 9$$

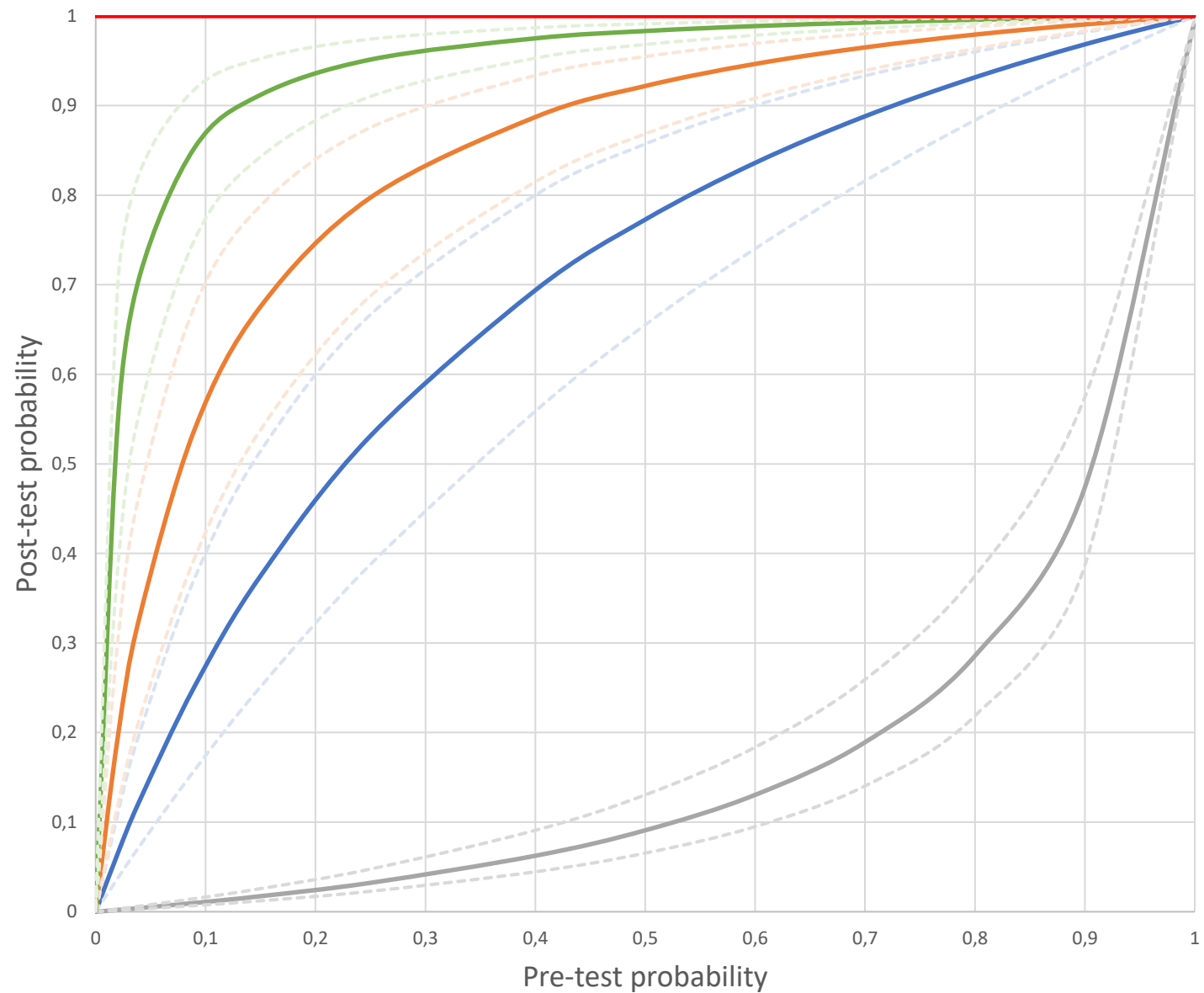
$$\frac{\text{odds}}{1+\text{odds}} = p$$

$$\frac{9}{1+9} = 0.9$$

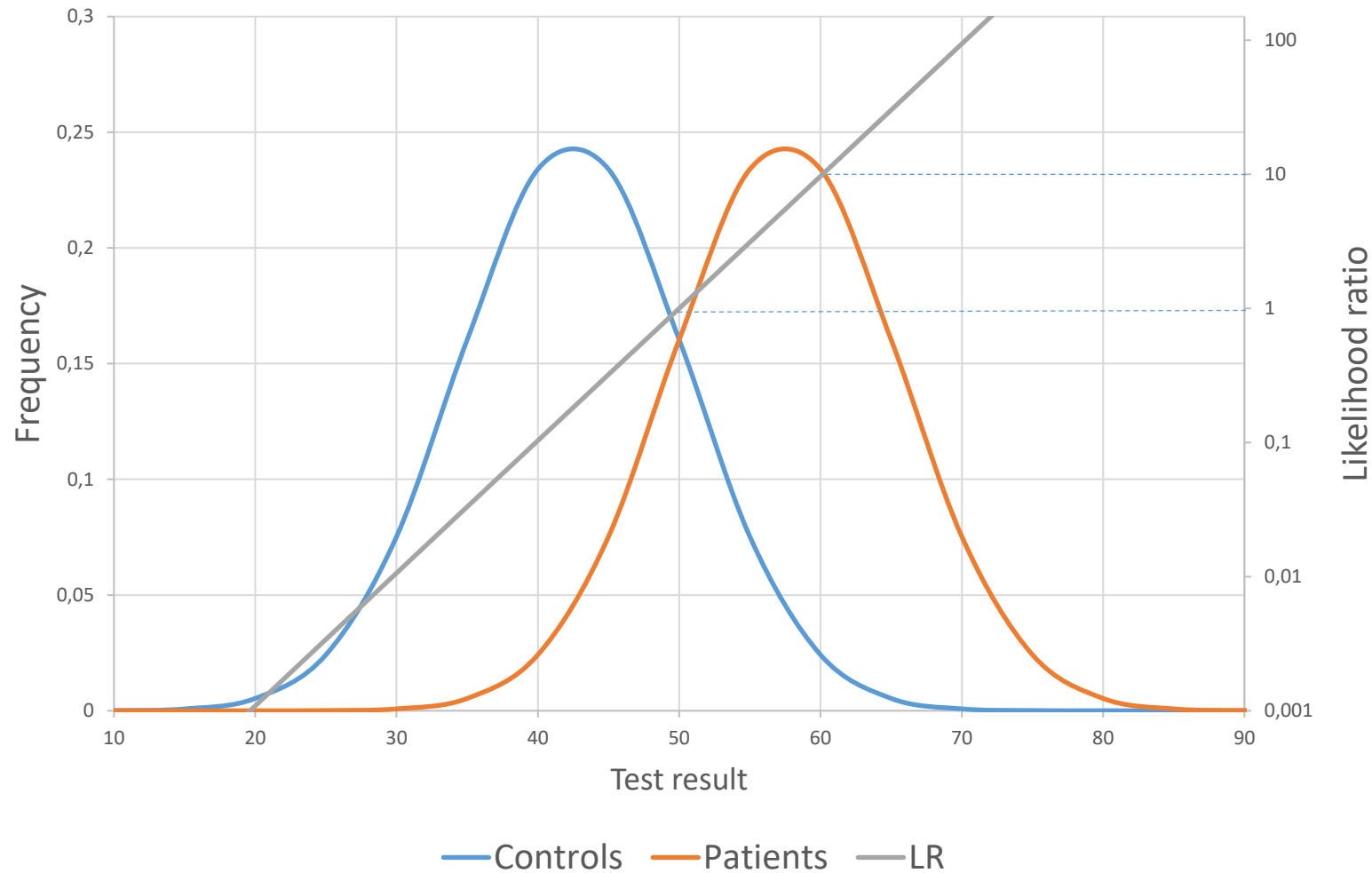
Calculated post-test probabilities:



PR3-ANCA MPO-ANCA	Interval	LR	95% CI
EliA	0.0, 2.1	0.10	0.07, 0.15
	2.1, 5.0	3.4	1.9, 6.0
	5.0, 16.0	11.8	6.6, 21.1
	16.0, 142.0	58.7	30.4, 113.5
	142.0, 180.0	∞	7.5, ∞



Test result-specific LR



Fierz W, Bossuyt X. Likelihood Ratios as Value Proposition for Diagnostic Laboratory Tests. J Appl Lab Med. 2020;5:1061-1069.

Fierz W, Bossuyt X. Likelihood Ratio Approach and Clinical Interpretation of Laboratory Tests. Front Immunol. 2021 Apr 16;12:655262

Reporting LR
in a clinical
setting

Pragmatic approach

Test result interval-specific LR

Intuitive diagnostic information

Includes information on antibody level

Independent of the specific test

Examples of LR values
on lab reports

ANCA: clinical suspicion AAV

ANCA identification

PR3-ANCA

<0.6 U/mL

<2: negative, 2-3: equivocal, >3: positive

MPO-ANCA

12.0 U/mL

<3.5: negative, 3.5-5: equivocal, >5 positive

Likelihood ratio for ANCA-associated vasculitis at diagnosis
(*Rheumatology* 2017; 56:1533-1541)

11.8
(95% CI: 6.6-21.1)

ANCA: clinical suspicion AAV

ANCA identification

PR3-ANCA

<0.6

U/mL

<2: negative, 2-3: equivocal, >3: positive

MPO-ANCA

12.0

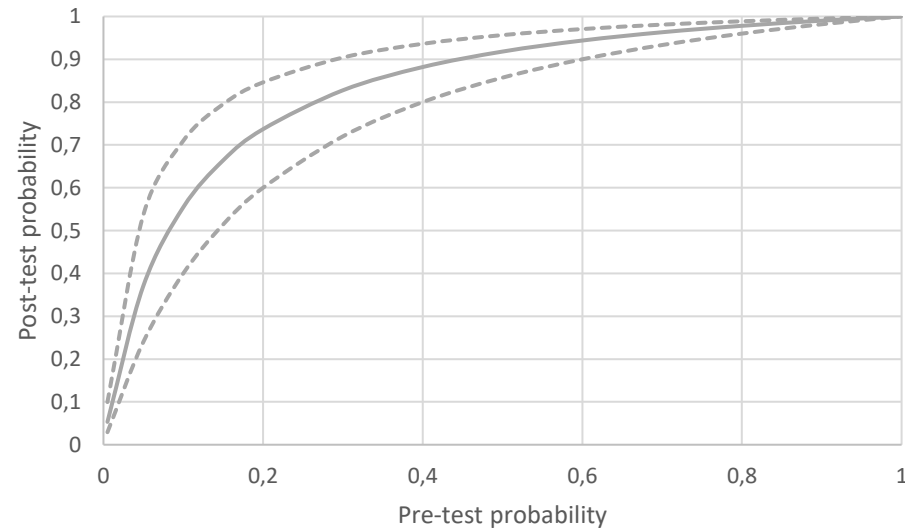
U/mL

<3.5: negative, 3.5-5: equivocal, >5 positive

Likelihood ratio for ANCA-associated vasculitis at diagnosis
(*Rheumatology* 2017; 56:1533-1541)

11.8

(95% CI: 6.6-21.1)



Reporting LR in RA

Rheumatoid factor

RF – IgM

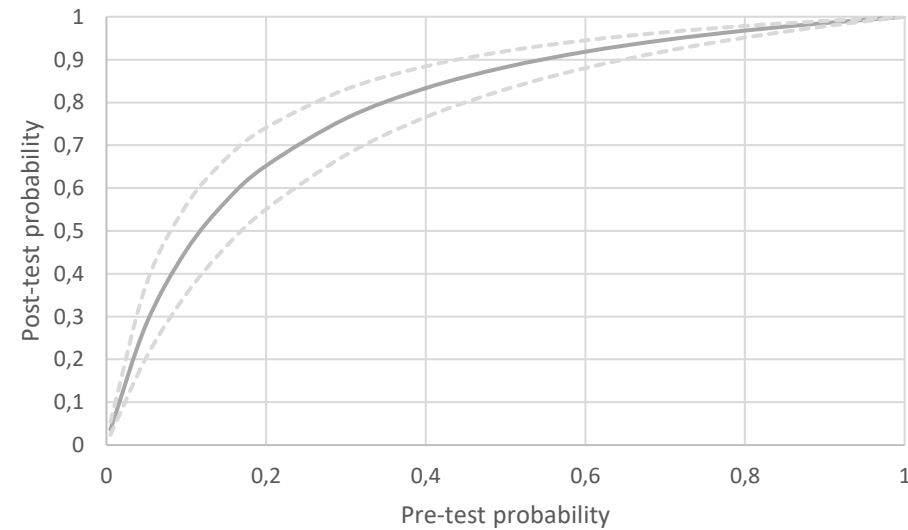
37 U/mL

<3.5: negative, 3.5-5.0: equivocal, >5.0: positive

Likelihood ratio for rheumatoid arthritis at diagnosis

7.5 (95% CI: 4.9-11.45)

(RMD Open 2022 Mar;8:e002099)



Reporting LR in RA

ACPA

Anti-cyclic citrullinated peptide (CCP)

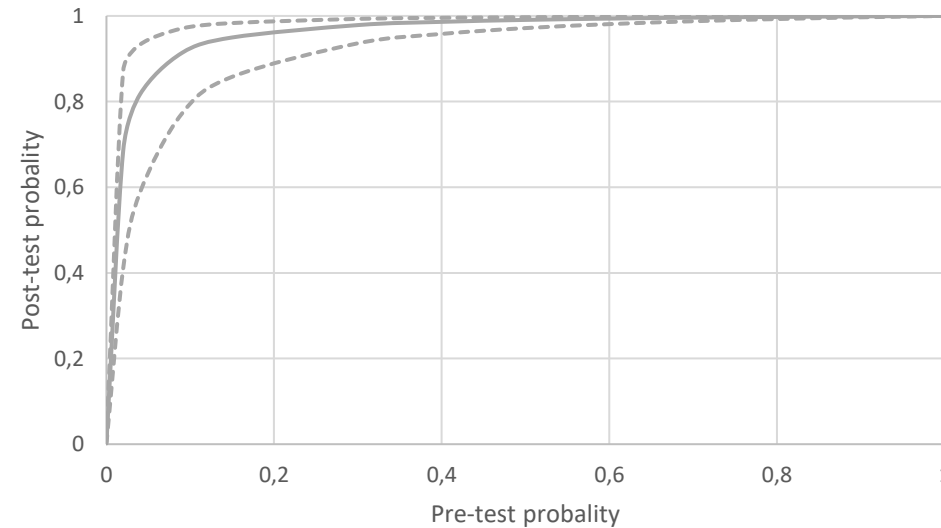
375 U/mL

<7: negative, 7-10: equivocal, >10: positive

Likelihood ratio for rheumatoid arthritis at diagnosis

106.9 (95% CI: 2.58-4425.96)

(RMD Open 2022 Mar;8:e002099)



Reporting LR for ANA / CTD Screen

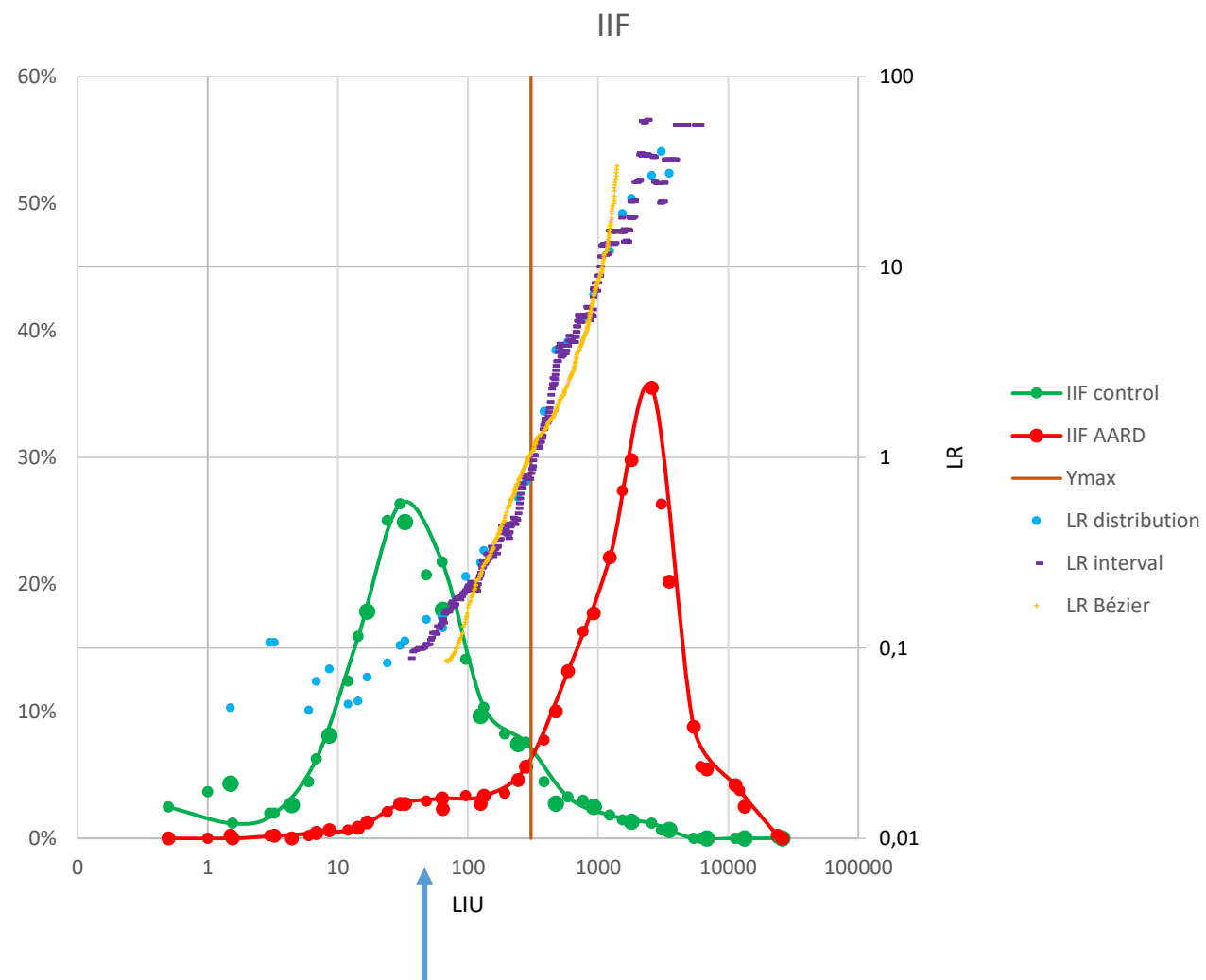
Antinuclear antibodies screening (IIF)

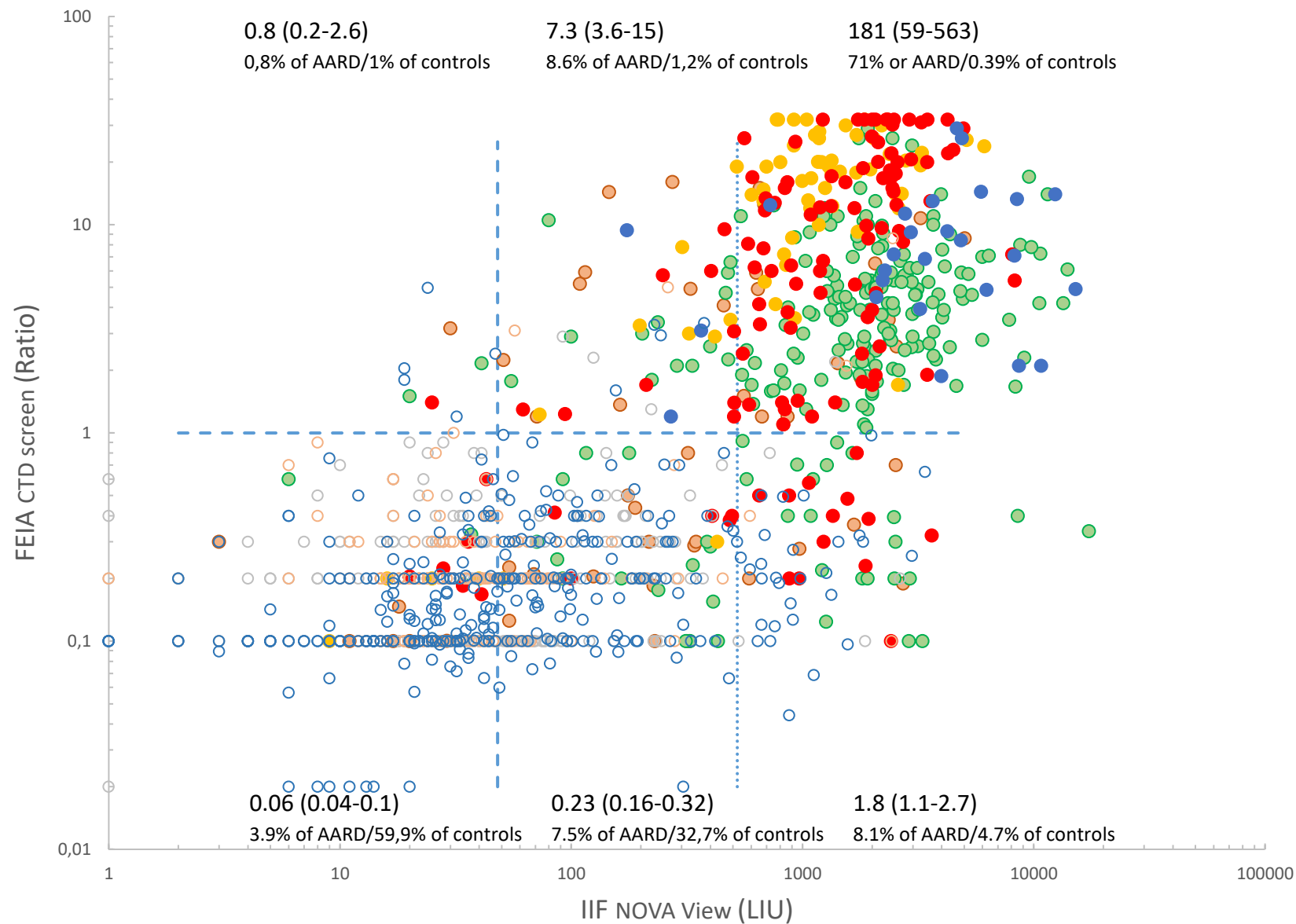
Nucleus	positive
Pattern	fine speckled
Titer	1:320
Cytoplasm	negative
Fluorescence intensity	626 LIU
>48: positive	
LR for ANA-associated rheumatic disease at diagnosis (<i>Autoimmun Rev</i> 2018;17:533-540)	5.2 (95% CI: 3.2-8.6)

Connective tissue disease (CTD) screen

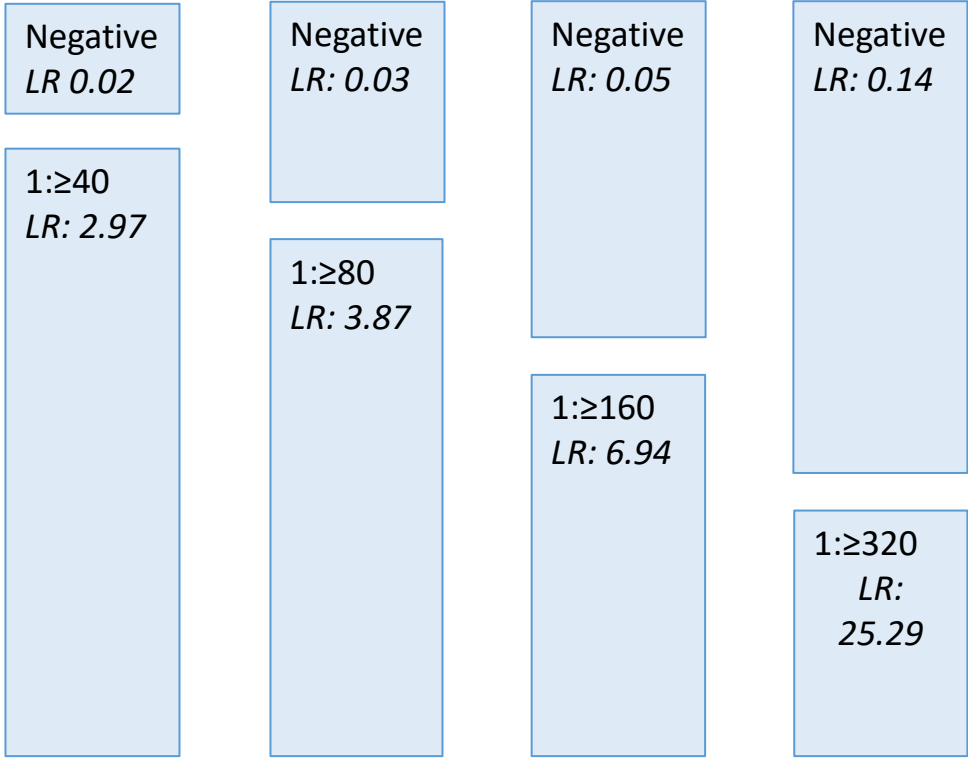
(dsDNA, SSA/Ro 52, SSA/Ro 60, SSB/La, U1-RNP (RNP-70, A, C), Sm, centromere B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA, and Mi-2)

CTD screen	0.8	ratio
<0.7: negative, 0.7-1.0: equivocal, >1.0: positive		
LR for ANA-associated rheumatic disease at diagnosis (<i>Autoimmun Rev</i> 2018;17:533-540)	0.75 (95% CI: 0.33-1.73)	

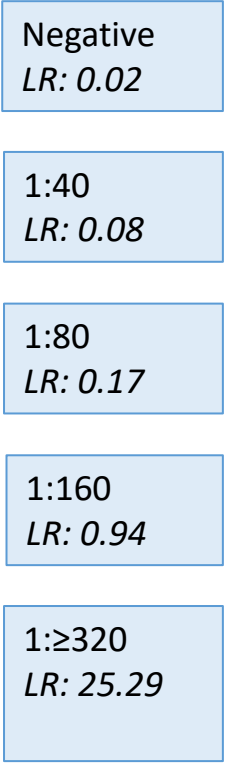




Leuchten et al. 2018
Dichotomous interpretation



Based on Leuchten et al. 2018
Titer-specific LR



cutoff	1:40	1:80	1:160	1:320
Sensitivity	98.4	97.8	95.8	86
specificity	66.9	74.7	86.2	96.6

LR and EQC

first data

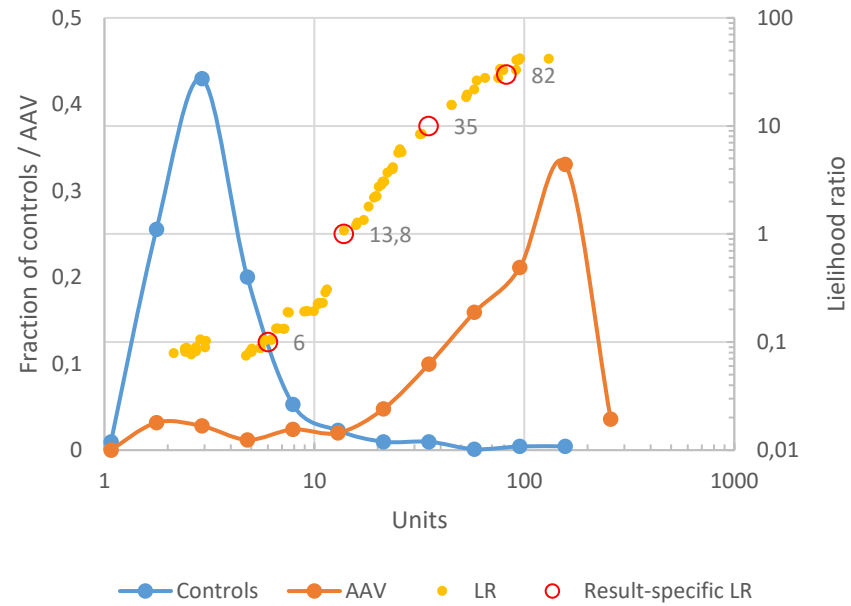
Anti-PR3 and anti-MPO Abs

251 AAV

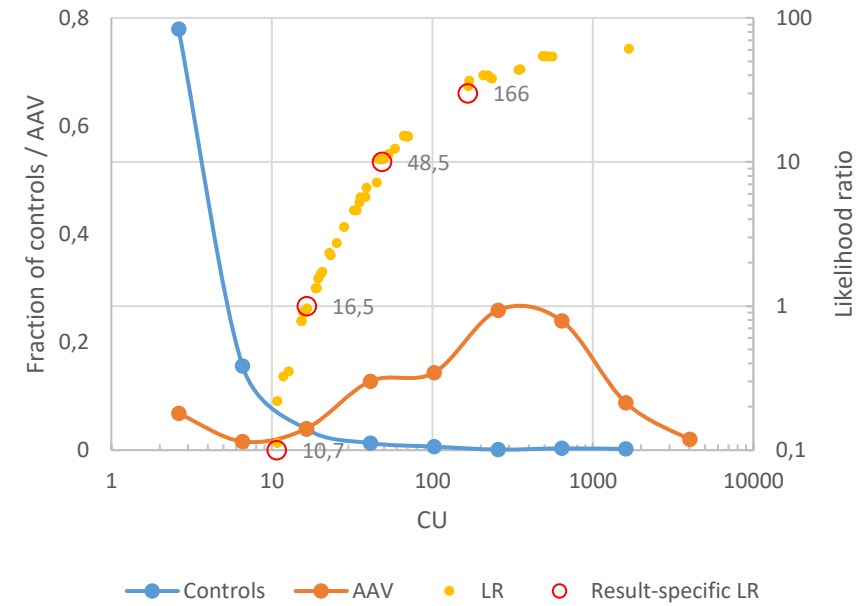
924 controls

Bossuyt X. et al. Rheumatology (Oxford). 2017;56:1533-1541.

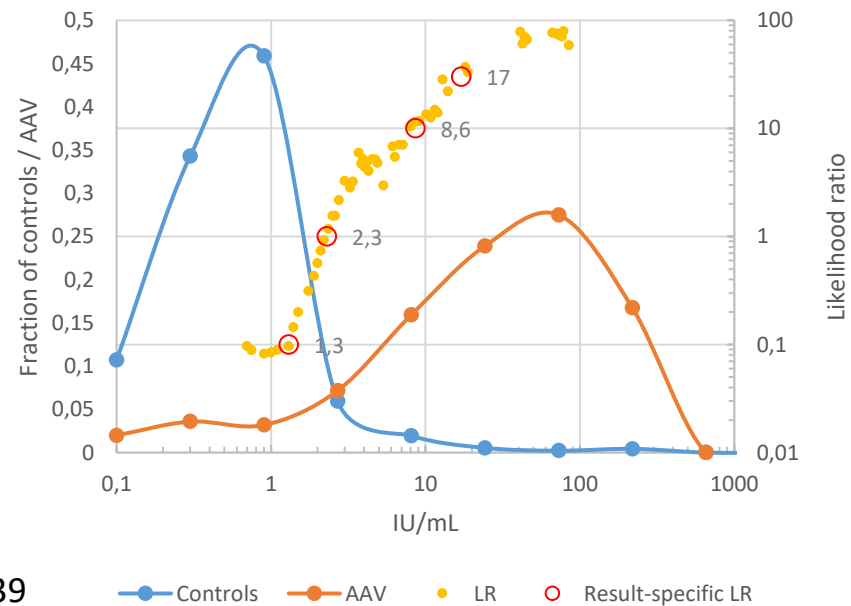
Inova (ELISA - QUANTA Lite)



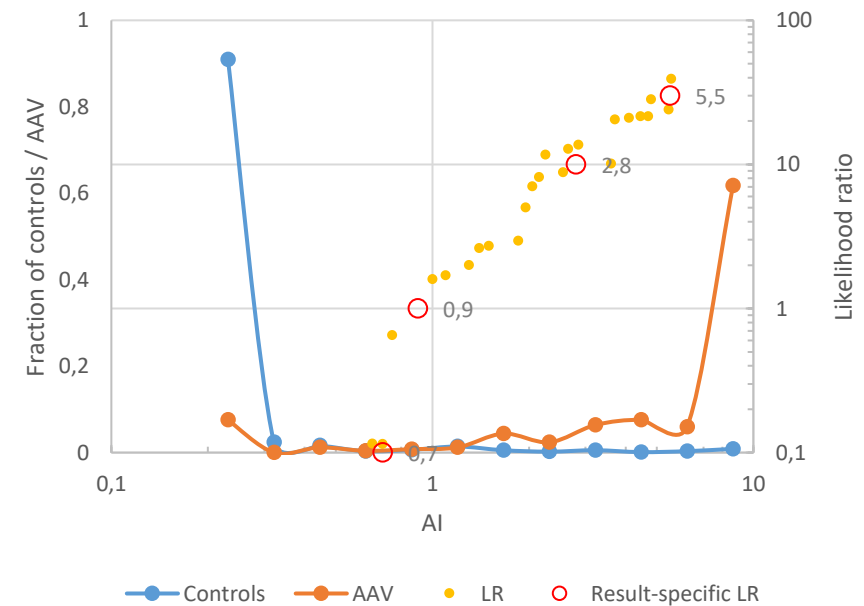
Inova (CLIA - QUANTA Flash)



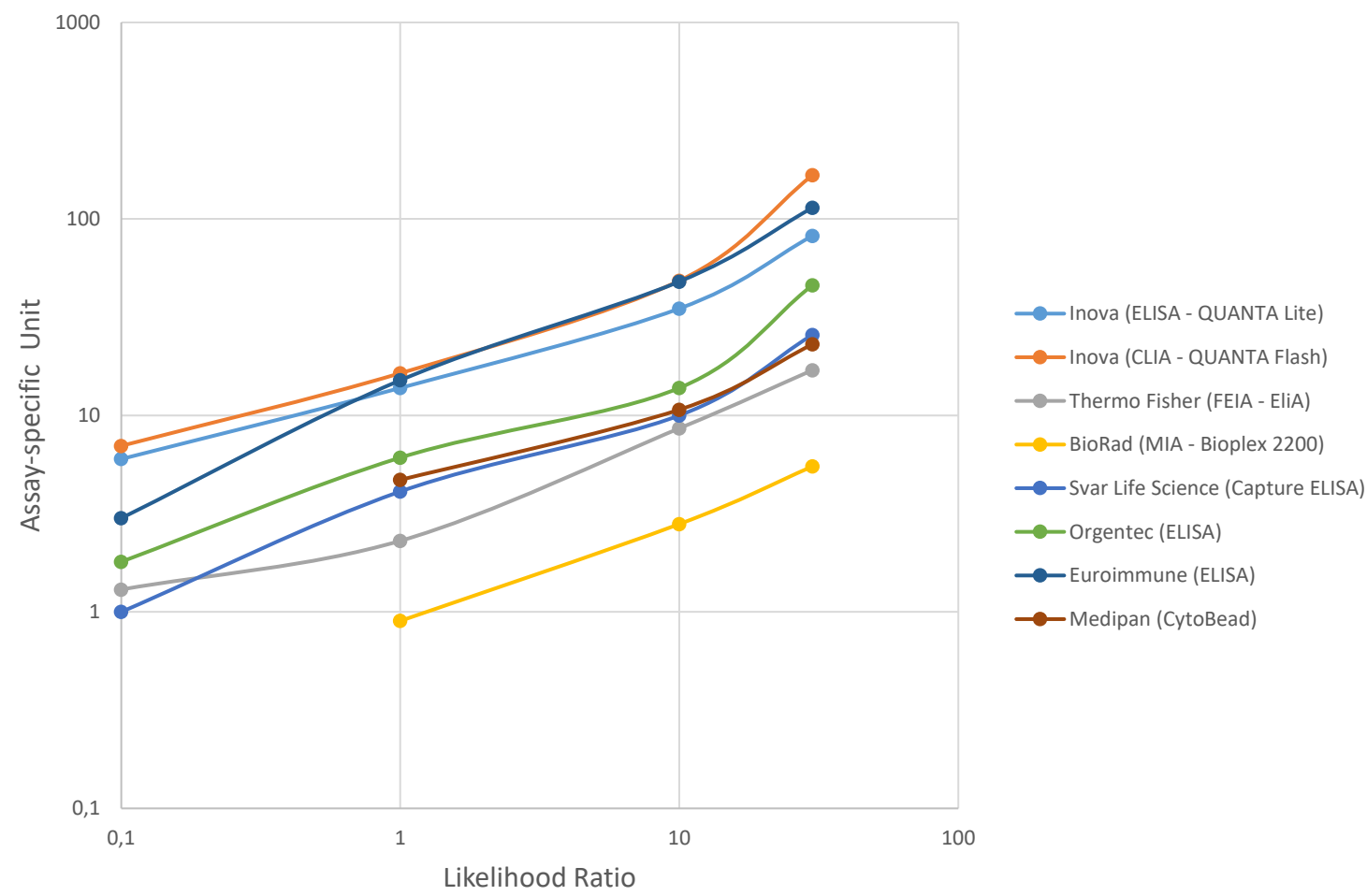
Thermo Fisher (FEIA - EliA)



BioRad (MIA - BioPlex 2200)



LR to align test result interpretation



	Threshold associated with LR				cutoff
	0.1	1	10	30	
Units	6	13.8	35	82	20
CU	7	16.4	48.5	167	20
IU/mL	1.3	2.3	8.6	17	3/5*
AI		0.9	2.8	5.5	1
IU/mL	1	4.1	10	25.7	7
U/mL	1.8	6.1	13.8	46	10/5*
U/mL	3	15.1	48	114	20
IU/mL		4.7	10.7	23	5

* first value for PR3-ANCA, second value for MPO-ANCA

We propose to employ test result-specific LRs to align test result interpretation across assays and manufacturers and to convey clinical information intrinsic to the antibody level.

Pauci-immune vasculitis with acute renal failure

Manufacturer/test	n	PR-ANCA	MPO-ANCA	Comment
Euroimmun	5	LR >30	LR <0.1	-
Werfen - Quanta Flash	7	LR >30	LR <0.1	1 not reported
Werfen - Quanta Lite	3	LR >30	LR <0.1	-
Svar Life Science	1	LR >30	LR <0.1	1 not reported
Thermo Fisher - EliA	43	LR >30	LR <0.1	1 not reported

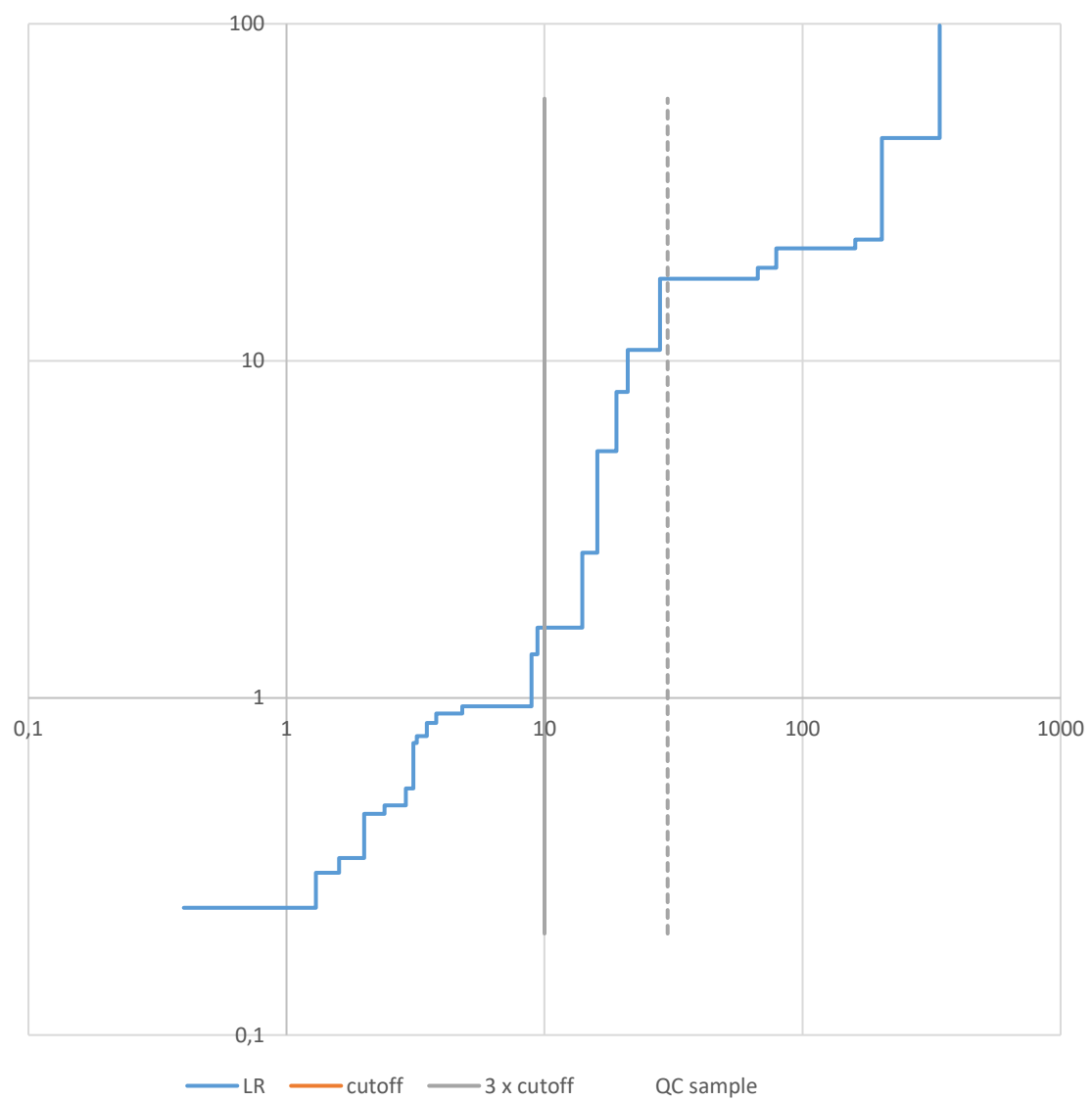
*Final
considerations*

ACPA - RF

398 RA at diagnosis

1073 controls

Van Hoovels et al. RMD Open. 2022 Mar;8(1):e002099

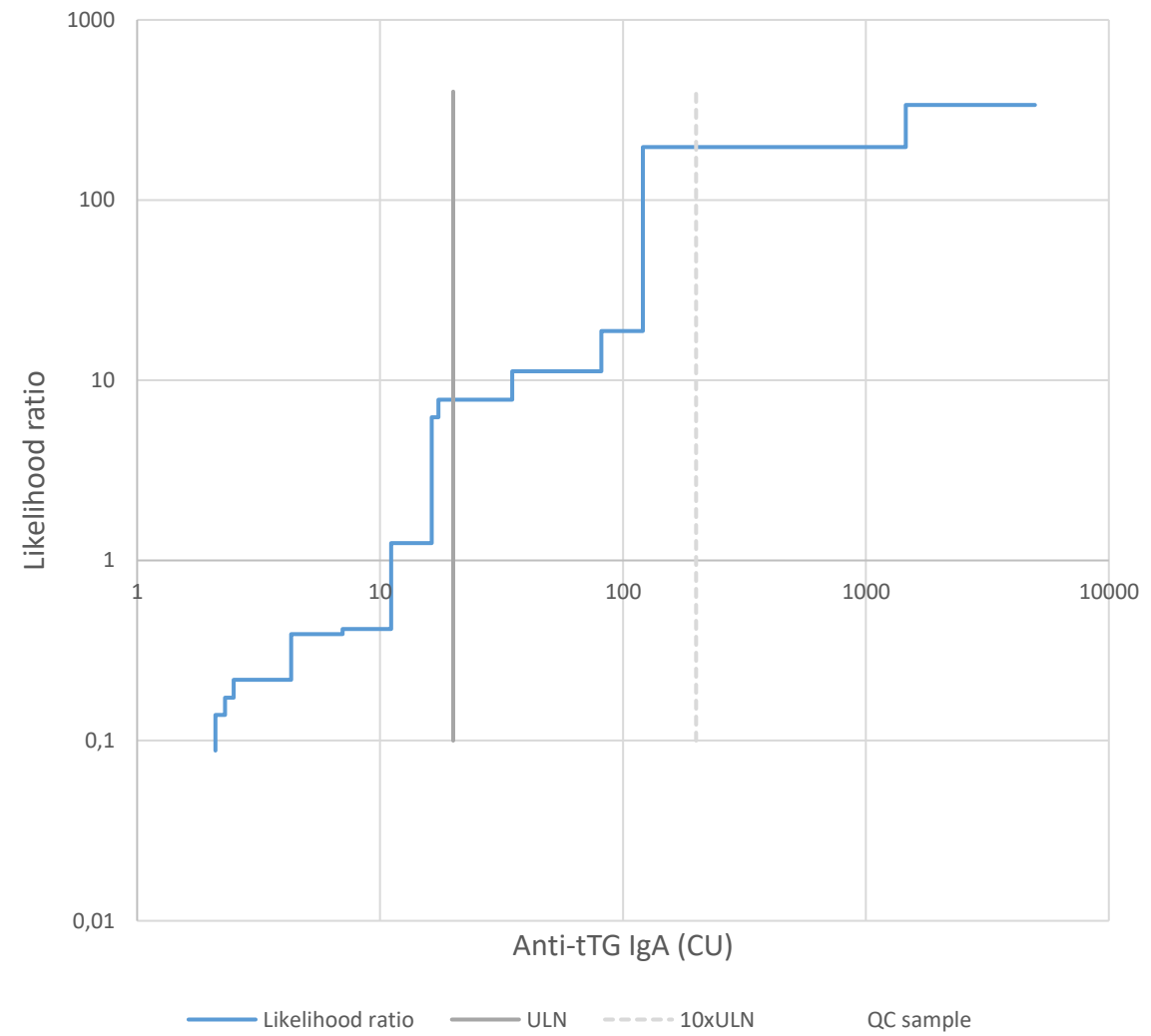


Anti-tissue transglutaminase

156 CD at diagnosis (biopsy confirmed)

974 diseased controls

Quanta Flash tTG IgA

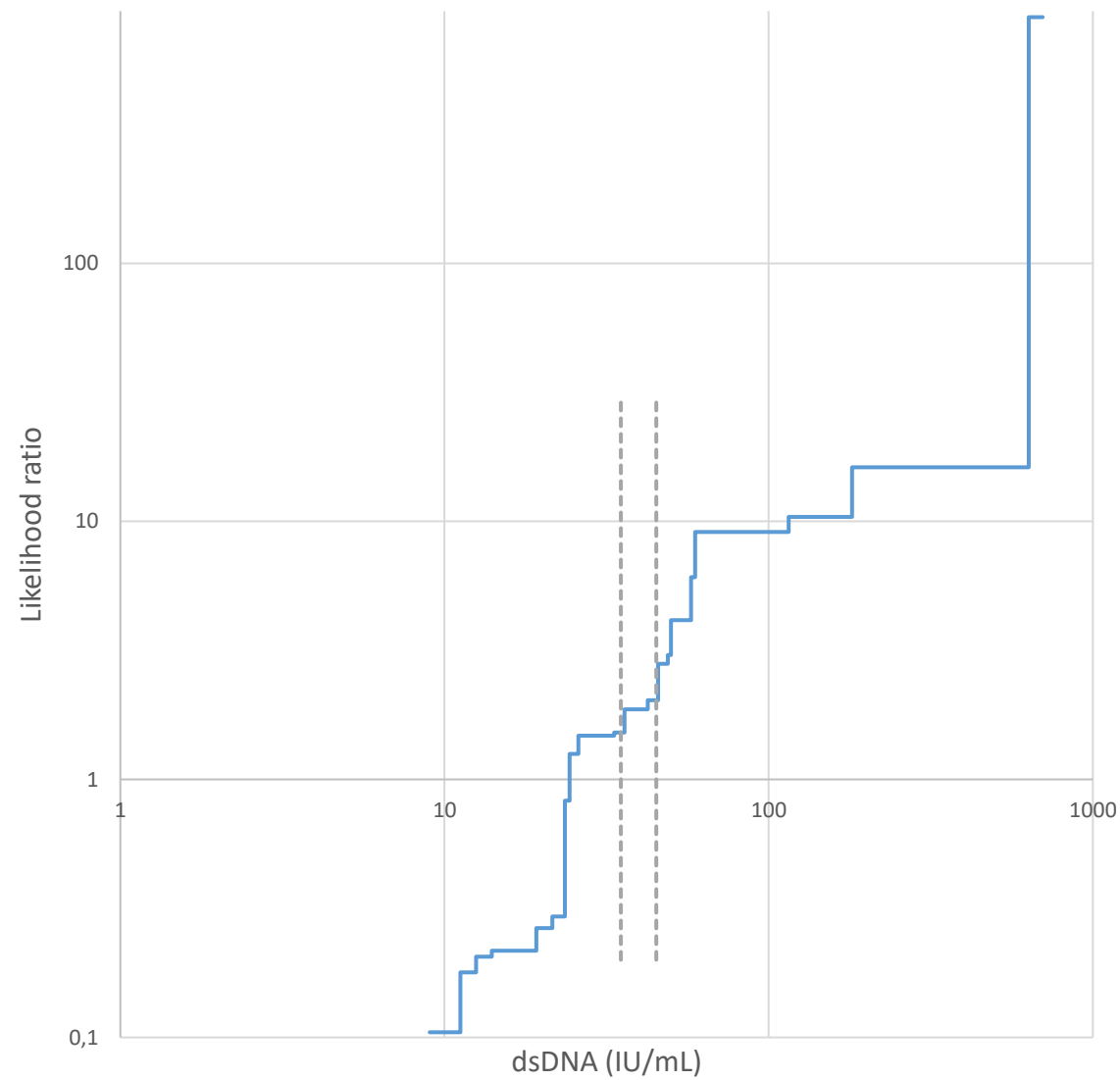
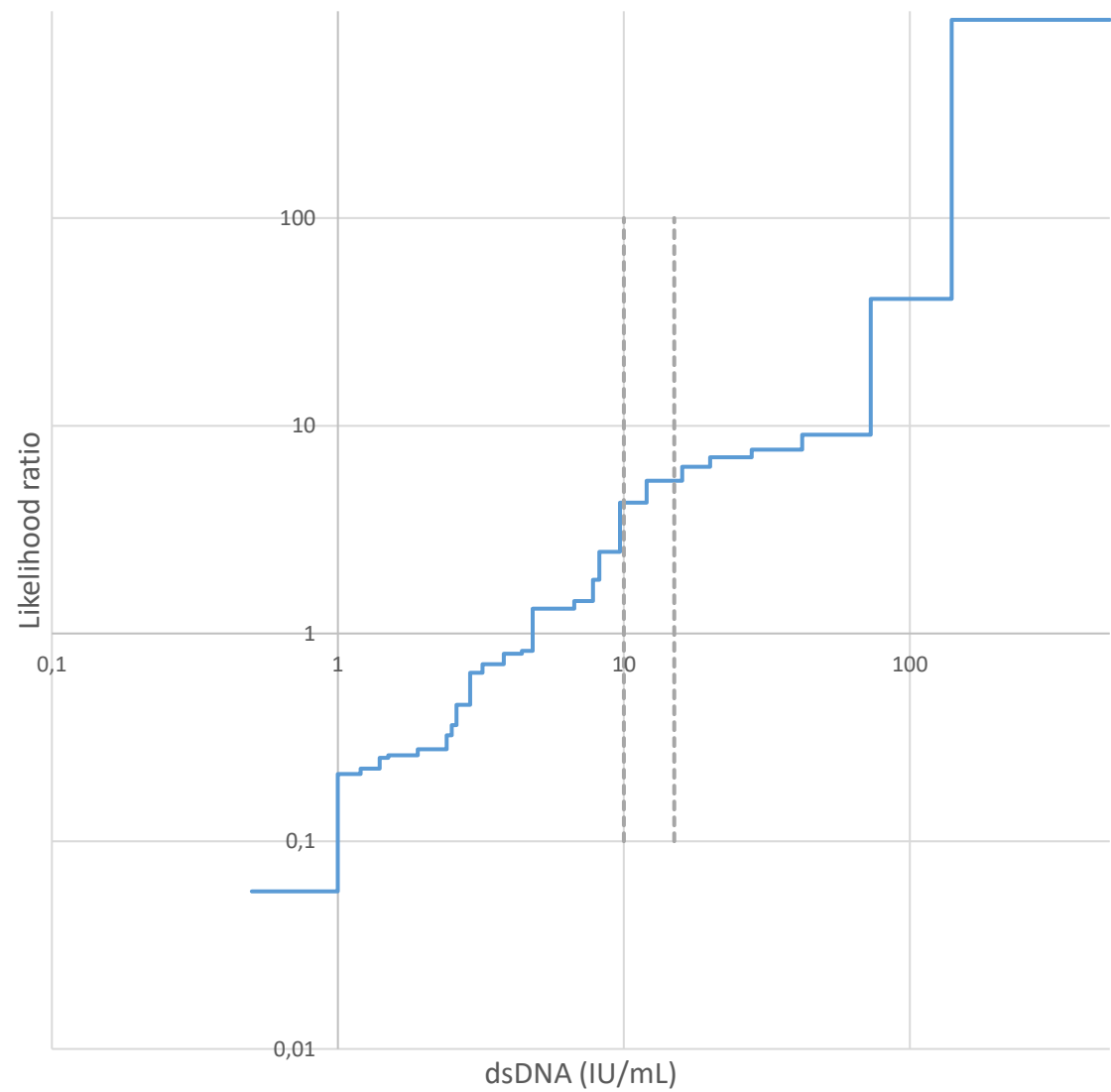


Anti-dsDNA

133 SLE at diagnosis

961 diseased controls

200 blood donors



Conclusions

Likelihood ratio

- provides intuitive diagnostic information
- gives an idea how much more or less likely a particular test result occurs in patients compared to controls
- is unit-independent
- link with pre-test - post-test probability

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L. Nevejan, P. Dobbels, G. Norman, A. Voreck

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Cučnik, C. Dahle, I. Heijnen, L. Bernasconi, F. Benkhadra, L.
Bogaert, A. Van Liedekerke, G. Vanheule, J. Robbrecht, L.
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In vitro diagnostic companies: Orgentec, Euroimmun,
BioRad, Thermo Fisher, Werfen, Svar Diagnostics, Generic
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Science, Abbott, Ortho-Clinical diagnostics, Beckman Coulter,
Siemens, Diazym, Werfen

ANCA

Bossuyt X, Rasmussen N, van Paassen P, Hellmich B, Baslund B, Vermeersch P, Blockmans D, Cohen Tervaert JW, Csernok E, Damoiseaux J. A multicentre study to improve clinical interpretation of proteinase-3 and myeloperoxidase anti-neutrophil cytoplasmic antibodies. *Rheumatology (Oxford)*. 2017;56:1533-1541.

ACPA - RF

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ANA

Claessens J, Belmondo T, De Langhe E, Westhovens R, Poesen K, Hüb S, Blockmans D, Mahler M, Fritzler MJ, Bossuyt X. Solid phase assays versus automated indirect immunofluorescence for detection of antinuclear antibodies. *Autoimmun Rev*. 2018 Jun;17(6):533-540.

Bossuyt X, Claessens J, De Langhe E, Belmondo T, Westhovens R, Hue S, Poesen K, Blockmans D, Mahler M, Fritzler MJ. Antinuclear antibodies by indirect immunofluorescence and solid phase assays. *Ann Rheum Dis*. 2020;79:e65.

IgA tissue transglutaminase – DGP

Oyaert M, Vermeersch P, De Hertogh G, Hiele M, Vandeputte N, Hoffman I, Bossuyt X. Combining antibody tests and taking into account antibody levels improves serologic diagnosis of celiac disease. *Clin Chem Lab Med*. 2015;53:1537-46.