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Rheumatoid arthritis

Good to know

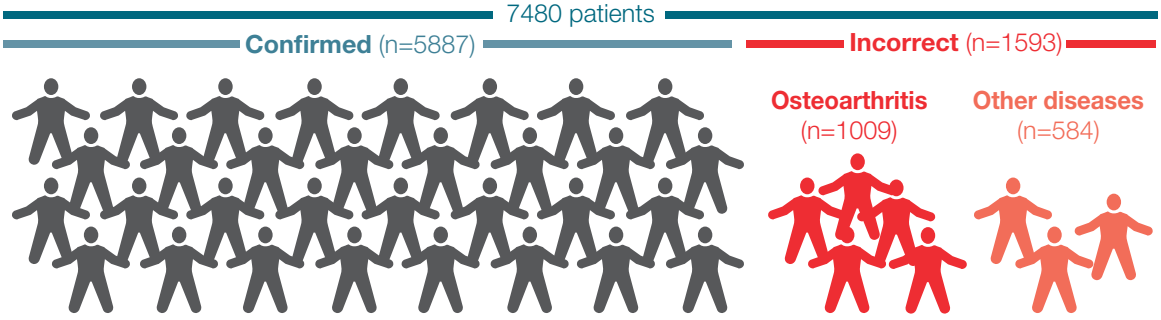
Rheumatoid arthritis (RA)

- Has a prevalence of approx. 1% worldwide[1, 2]
- Is a progressive autoimmune disease that leads to destruction of the joints[2, 3]
- Early treatment can stop its progression and improve quality of life[2-4]
- Differential diagnosis from clinically similar diseases (e.g. osteoarthritis) is needed for proper treatment[2]
- The average time between onset of symptoms and therapy is approximately 12 months[11]
- ACR/EULAR classification criteria for RA are dependent on clinical evaluation and number of positive markers[6]

Often misdiagnosed

Re-evaluation of 7480 patients originally diagnosed with RA revealed:[7]

- Approximately 21% (n=1593) had been misdiagnosed
- Of the misdiagnosed patients, the majority had osteoarthritis (n=1009)



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Testing for ACPA / anti-CCP antibodies

Anti-citrullinated protein antibodies (ACPA)

- The terms “anti-CCP antibodies” and “ACPA” are used synonymously[8]
- Produced against citrullinated proteins mainly in inflamed synovial tissues[5]
- Highly specific markers for RA. ACPA testing supports its differential diagnosis[2, 3, 5, 6]
- Presence in RA patients is associated with earlier and more severe joint destruction as well as a higher risk of extra-articular manifestations[2, 3]
- Can be detected years before the onset of symptoms[2, 5, 6]
- Cyclic citrullinated peptides (CCP) – considered the best antigen for ACPA testing and included in the 2010 ACR/EULAR classification criteria[6, 8]

Second generation cyclic citrullinated peptides (CCP2) – the “gold standard antigen” for ACPA tests[8]

- CCP2 was developed by screening a library of 12 million citrullinated peptides derived from citrullinated synovial proteins[8]
- Most manufacters of ACPA tests use the patented CCP2, one uses CCP3[9]

Clinical performance of anti-CCP antibody tests

	Fully automated	Peptides used	Sensitivity [%]	Specificity [%]	LR (+)*	False positives**
EliA™ CCP test	**Yes**	**CCP2**	**74**	**96**	**18.63**	**198**
Elecsys Anti-CCP test	Yes	CCP2	74	94	11.37	297
AxSYM Anti-CCP test	Yes	CCP2	76	91	7.24	446
Architect Anti-CCP test	Yes	CCP2	83	90	7.98	495
QUANTA Lite™ CCP3 test	No	CCP3	72	94	11.83	297
QUANTA Lite™ CCP3.1 test	No	CCP3	71	92	8.44	396
Anti-CCP-ELISA (IgG) test	No	CCP2	72	96	17.95	198
Axis-Shield Anti-CCP test	No	CCP2	67	95	13.27	248
Immunoscan CCPlus® test	No	CCP2	67	94	11.40	297
EDIA™ anti-CCP test	No	CCP2	72	94	11.96	297

* Positive likelihood ratio ** Assumption: 5000 patients tested/year and a RA prevalence of 1%

Based on the meta-analysis of 83 published studies[9]

What is the impact of a different specificity on ACPA test results?

The lower the specificity, the higher the number of false positive test results[10].

Testing for Rheumatoid Factor (RF)

RF isotypes – new insights from an established marker

- RF describes antibodies directed against the Fc portion of immunoglobulin G (IgG)[12]
- RF IgM is the most established RF isotype in RA patients, but RF IgA and RF IgG can occur too[13, 14]
- ACPA and RF IgM testing are part of the 2010 ACR/EULAR classification criteria for RA[6]
- RF of all isotypes can occur years before the onset of disease symptoms[15]
- Positivity for only one RF isotype is often not associated with RA[16]
- Positivity for more than one RF isotype is associated with a higher risk for RA[3, 16–19]
- Double positivity for RF IgM and RF IgA was found in 52% of RF positive RA patients, but only in 4% of non-RA patients[3, 20]
- Double positivity for RF IgM and RF IgA provides a high diagnostic confidence for RA[3, 20]

Differentiation and measurement of individual RF isotypes is assay dependent

- Solid-phase based RF assays, e.g. fluorescent enzyme immunoassay (FEIA), can differentiate individual RF isoytpes, depending on the conjugate used[20–22]
- Latex agglutination (Rose-Waaler), nephelometric and turbidimetric RF assays do not differentiate between RF isotypes. None of these assays are solely specific for RF IgM[20–22]
- Only RF IgM specific assays fulfill 2010 ACR/EULAR classification criteria[1]

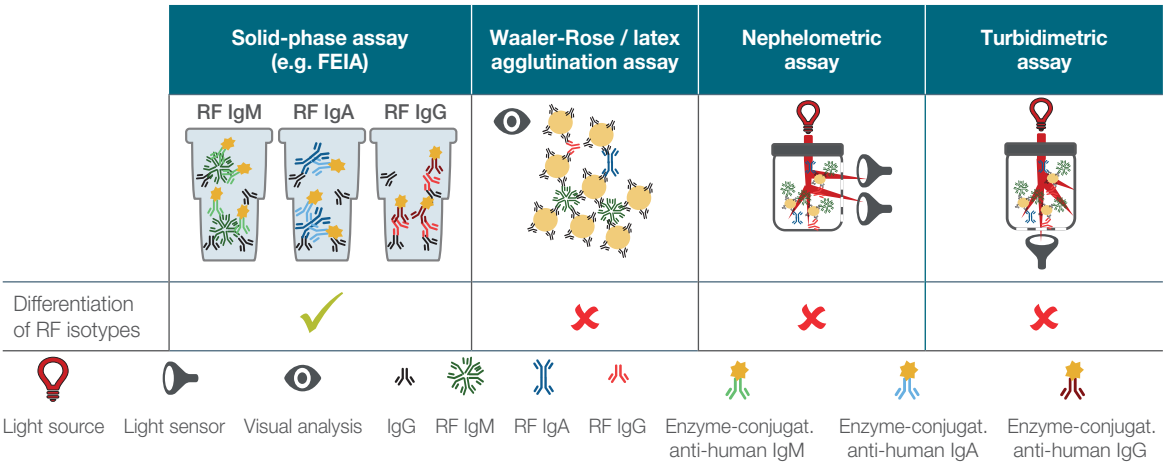

Figure 1: Overview of RF assays and their ability to differentiate and measure individual RF isotypes[20–22]

Additional clinical information can be obtained from the differentiation and measurement of RF isotypes

RF IgM

- High titers of RF IgM correlate with disease activity and extra-articular organ involvement[2,23]

RF IgA

- High titers of RF IgA are prognostic markers for a more severe disease outcome and poor clinical response to TNF-α inhibitors[3,23, 25]

Testing for RF using a solid-phase and a nephelometric assay

Assays included

- EliA™ RF IgM test – FEIA for the measurement of RF IgM [21, 22]
- Nephelometric RF assay – measurement of RF without isotype differentiation [21, 22]

Sample cohort

- 53 RA patients (including RA patients under treatment and in remission)
- 70 autoimmune and non-autoimmune disease control samples

Clinical performance

	Cut-off [IU/ml]	Sensitivity [%]	Specificity [%]	LR (+)*	PPV**
EliA RF IgM test	5.0#	49.1	91.4	5.72	0.81
Nephelometric RF assay	15.9#	45.3	87.1	3.52	0.73
At stratified specificity of 91.4 %##					
EliA RF IgM test	5.0	49.1	91.4	5.72	0.81
Nephelometric RF assay	23.1	39.6	91.4	4.62	0.78

*Postive likelihood ratio **Positive predictive value #manufacturer recommended cut-off ##using Analyze-it, Graphpad Prism 4

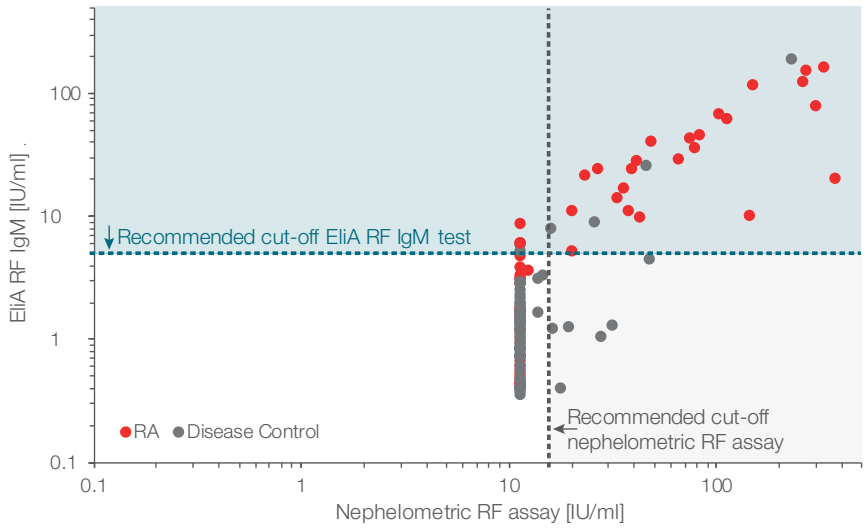

Figure 2: Correlation between EliA RF IgM test and nephelometric RF test. For better visualization, titers are displayed using logarithmic scales.

Internal study; data on file

Summary and conclusion

- In this study, the EliA RF IgM test demonstrated a better clinical performance than the nephelometric RF assay indicated by a higher sensitivity, specificity, LR (+) and PPV
- These results are in line with previously published results that the EliA RF IgM test had a better clinical performance than a nephelometric RF assay [26]

Combining anti-CCP, RF IgM and RF IgA testing

Increasing diagnostic confidence in early RA patients

Assays included

- EliA™ CCP test
- EliA™ RF IgM test
- EliA™ RF IgA test

Sample cohort

- 100 early RA patients (with symptoms < 24 months)
- 149 disease controls (autoimmune and non-autoimmune diseases, cancer, viral and bacterial infections)
- 51 healthy individuals

Clinical performance

	Sensitivity [%]	Specificity disease controls [%]	LR(+)*	PPV**	Specificity healthy individuals [%]
EliA CCP test	62.0	95.3	13.2	0.90	100.0
EliA RF IgM test	62.0	90.6	6.6	0.82	98.0
EliA RF IgA test	50.0	91.9	6.2	0.81	100.0
Double positivity					
EliA CCP test x EliA RF IgM test	56.0	98.7	41.7	0.97	100.0
EliA RF IgM test x EliA RF IgA test	46.0	95.3	9.8	0.87	100.0
Triple positivity					
EliA CCP test x EliA RF IgM test x EliA RF IgA test	45.0	99.3	67.1	0.98	100.0

*Positive likelihood ratio **Positive predictive value

Internal study; data on file

Summary and conclusion

- EliA CCP test had the same sensitivity but a higher specificity than EliA RF IgM test
- EliA RF IgA test had a lower sensitivity but a higher specificity than EliA RF IgM test
- Combining test results increases the positive likelihood ratio and positive predictive value
- The highest positive likelihood ratios and positive predictive values were observed for triple positivity, i.e. when the results for the EliA CCP test, EliA RF IgM test and EliA RF IgA test were combined

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EliA: high quality autoimmunity diagnostics on an intuitive, automated, tailor-made platform

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Increase of operational efficiency and quality of service with the right instrument solution

Technical data

Ordering information	Article No.	Package size	Cut-off negative	Cut-off equivocal	Cut-off positive	Short name
EliA CCP Well	14-5515-01	4 x 12 wells	< 7 U/ml	7-10 U/ml	> 10 U/ml	cp
EliA RF IgM Well	14-5600-01	4 x 12 wells	< 3.5 IU/ml	3.5-5.0 IU/ml	> 5.0 IU/ml	Mrf
EliA RF IgA Well	14-5601-01	4 x 12 wells	< 14 IU/ml	14-20 IU/ml	> 20 IU/ml	Arf

References

1. Aletaha D, Bluml S. Therapeutic implications of autoantibodies in rheumatoid arthritis. *RMD Open.* 2016;2(1):e000009.
2. Littlejohn EA, Monrad SU. Early Diagnosis and Treatment of Rheumatoid Arthritis. *Prim Care.* 2018;45(2):237-55.
3. de Brito Rocha S, Baldo DC, Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. *Adv Rheumatol.* 2019;59(1):2.
4. Smolen JS, Landewe R, Bijlsma J, Burmester G, Chatzidionysiou K, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis.* 2017;76(6):960-77.
5. Bizzaro N. Antibodies to citrullinated peptides: a significant step forward in the early diagnosis of rheumatoid arthritis. *Clin Chem Lab Med.* 2007;45(2):150-7.
6. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569-81.
7. Santos-Moreno P, Villarreal-Peralta L, Gomez-Mora D, Castillo E, Malpica A, Castro C, et al. Osteoarthritis is the Most Frequent Cause of Rheumathoid Arthritis Misdiagnosis in a Rheumatology Center. *Osteoarthritis and Cartilage.* 2017;25:S219.
8. van Venrooij WJ, van Beers JJ, Pruijn GJ. Anti-CCP antibodies: the past, the present and the future. *Nat Rev Rheumatol.* 2011;7(7):391-8.
9. Mathsson Alm L, Fountain DL, Cadwell KK, Madrigal AM, Gallo G, Poorafshar M. The performance of anti-cyclic citrullinated peptide assays in diagnosing rheumatoid arthritis: a systematic review and meta-analysis. *Clin Exp Rheumatol.* 2018;36(1):144-52.
10. Simundic AM. Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC.* 2009;19(4):203-11.
11. Barhamain AS, Magliah RF, Shaheen MH, Munassar SF, Falemban AM, Alshareef MM, et al. The journey of rheumatoid arthritis patients: a review of reported lag times from the onset of symptoms. *Open Access Rheumatol.* 2017;9:139-50.
12. Johnson PM, Faulk WP. Rheumatoid factor: its nature, specificity, and production in rheumatoid arthritis. *Clin Immunol Immunopathol.* 1976;6(3):414-30.
13. Williams DG, Moyes SP, Mageed RA. Rheumatoid factor isotype switch and somatic mutation variants within rheumatoid arthritis synovium. *Immunology.* 1999;98(1):123-36.
14. Falkenburg WJJ, van Schaardenburg D. Evolution of autoantibody responses in individuals at risk of rheumatoid arthritis. *Best Pract Res Clin Rheumatol.* 2017;31(1):42-52.
15. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 2003;48(10):2741-9.
16. Jonsson T, Thorsteinsson J, Valdimarsson H. Elevation of only one rheumatoid factor isotype is not associated with increased prevalence of rheumatoid arthritis–a population based study. *Scand J Rheumatol.* 2000;29(3):190-1.
17. Halldorsdottir HD, Jonsson T, Thorsteinsson J, Valdimarsson H. A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. *Ann Rheum Dis.* 2000;59(2):149-51.
18. Jaskowski TD, Hill HR, Russo KL, Lakos G, Szekanecz Z, Teodorescu M. Relationship between rheumatoid factor isotypes and IgG anti-cyclic citrullinated peptide antibodies. *J Rheumatol.* 2010;37(8):1582-8.
19. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. *Dis Markers.* 2013;35(6):727-34.
20. Jonsson T, Steinsson K, Jonsson H, Geirsson AJ, Thorsteinsson J, Valdimarsson H. Combined elevation of IgM and IgA rheumatoid factor has high diagnostic specificity for rheumatoid arthritis. *Rheumatol Int.* 1998;18(3):119-22.
21. Hamilton RG. Methods (In Vitro and In Vivo): Nephelometry and Turbidimetry. In: Mackay IR, Rose NR, Ledford DK, Lockey RF, editors. Encyclopedia of Medical Immunology: Allergic Diseases. New York, NY: Springer New York; 2014. p. 484-6.
22. Bas S, Perneger TV, Kunzle E, Vischer TL. Comparative study of different enzyme immunoassays for measurement of IgM and IgA rheumatoid factors. *Ann Rheum Dis.* 2002;61(6):505-10.
23. de Angelis V, Meroni PL. Autoantibodies. 2nd ed. Amsterdam ; Boston: Elsevier; 2007. xxxiv, 838 p. p.
24. Mikuls TR, O'Dell JR, Stoner JA, Parrish LA, Arend WP, Norris JM, et al. Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. *Arthritis Rheum.* 2004;50(12):3776-82.
25. Bobbio-Pallavicini F, Caporali R, Alpini C, Avalle S, Epis OM, Klersy C, et al. High IgA rheumatoid factor levels are associated with poor clinical response to tumour necrosis factor alpha inhibitors in rheumatoid arthritis. *Ann Rheum Dis.* 2007;66(3):302-7.
26. Sieghart D, Platzer A, Studenic P, Alasti F, Grundhuber M, Swiniarski S, et al. Determination of Autoantibody Isotypes Increases the Sensitivity of Serodiagnostics in Rheumatoid Arthritis. *Front Immunol.* 2018;9:876.

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