The Chemiluminescent Immunoassay (CLIA) for the determination of anti-cyclic citrullinated peptide antibodies (CCP) A comparison with ELISA

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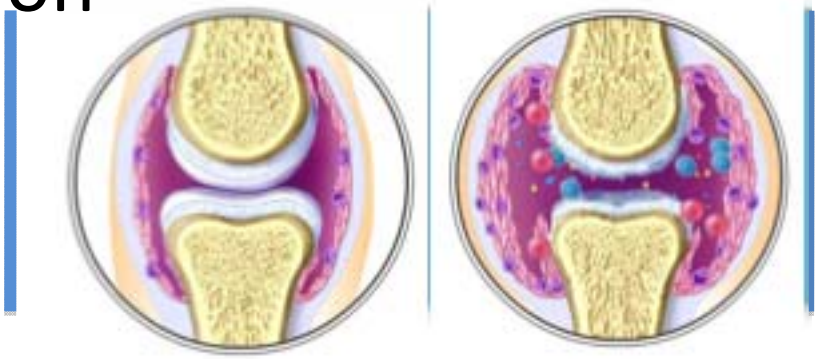
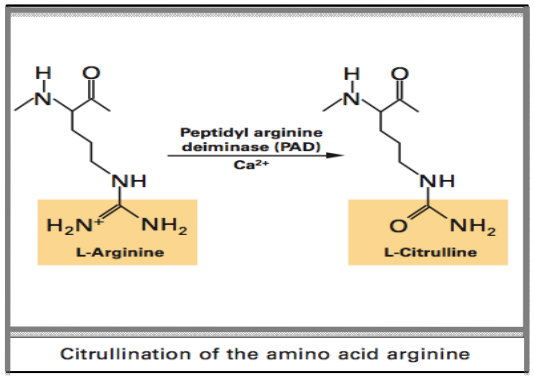
Rheumatoid Arthritis (RA)

Is a common, systemic autoimmune disease characterized by chronic inflammation of the sinovial joints, which leads to progressive joint destruction



Early diagnosis of RA is critical in preventing irreversible joint damage

Autoantibodies against cyclic citrullinated peptide (anti-CCP) are sensitive and highly specific markers for rheumatoid arthritis.



Citrullination of the amino acid arginine

The determination of specific autoantibodies is crucial for the early diagnosis and confirmation of rheumatoid arthritis.

Diagnostic tests are an essential part of clinical medicine, assisting the clinician in formulating diagnosis and judging disease severity, prognosis and likely responses to therapy.

AIM OF STUDY

To compare the clinical performance of the new fully automated **Zenit RA CCP (CLIA)** assay, to the conventional second generation anti-CCP2 **ELISA** (Immunoscan RA) assay, on a clinically well defined group of patients with RA.

Materials and Methods I

A cohort of 186 individuals was enrolled in our study.

56 patients with **ESTABLISHED RA** according to the ACR classification criteria.

Materials and Methods II

CONTROL GROUP

1. **Patients with RD other than RA**

15 Systemic Sclerosis

18 Primary Sjögren Syndrome

2 Psoriatic arthritis

3 SLE

5 Und connectivite

2 Und spondylartropathy

Materials and Methods III

CONTROL GROUP

2. Samples collected for testing infectious diseases

8 VCA positive

9 CMV positive

6 HCV positive

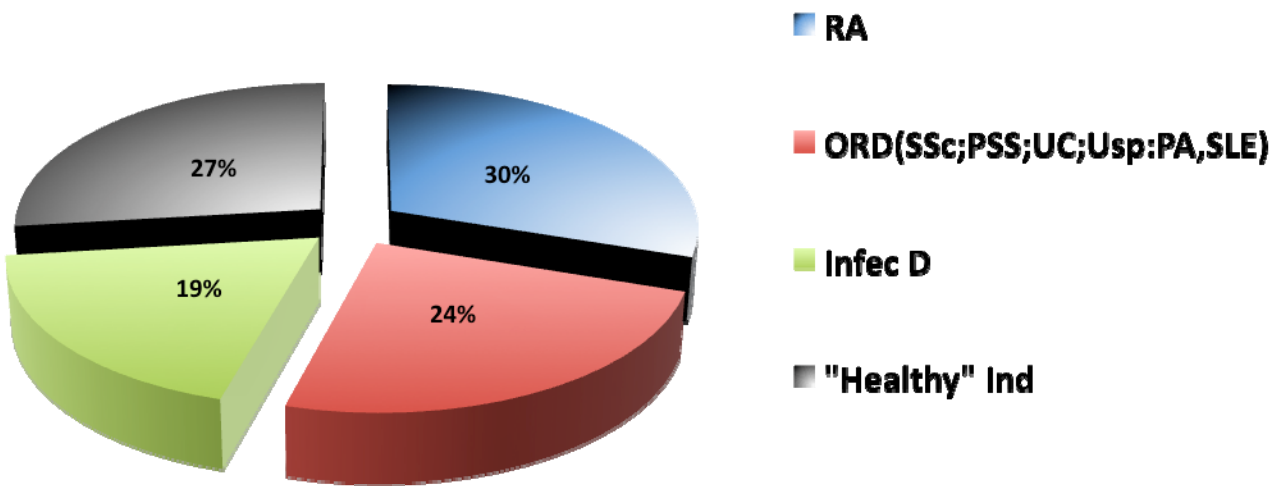
7 HBV Positive

5 negative

3. "Healthy" individuals

50 Blood Donor

STUDIDED SAMPLES
n=186



Materials and Methods IV

Abs to CCP were measured in serum, using the Zenit RA CCP (CLIA) assay from A.Menarini diagnostics and the Immunoscan RA ELISA from Euro – Diagnostica (Arnhem,The Netherlands).

Both assays were performed exactly as described by the manufacturer, including the use of a recommended cut-off value of: 5 AU/ml CLIA, 25Units/ml ELISA(in duplicate measurements).

Materials and Methods V

Statistical Analysis

To study the ability of the two diagnostic tests to predict RA, logistic regression models were fitted to the data and Hosmer-Lemeshow goodness-of-fit test p-values analyzed.

To assess their discriminative performance, the area under the receiver operating characteristic (ROC) curve was used.

Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated as well as the likelihood ratios. A significance level $\alpha = 5\%$ was considered.

Data were analyzed using Intercooled Stata 9.2 for Windows (StataCorp LP, USA).

RESULTS

CLIA

	RA	Non RA	Total
CCP+	55	1	56
CCP-	1	129	130
Total	56	130	186

Sensit. % (95% CI)	Specif. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR^+ (95% CI)	LR^- (95% CI)
98.2 (90.4-100)	**99.2 (95.8-100)**	**98,2 (90.4-100)**	**99.2 (95.8-100)**	**128 (18.1-900)**	**0.018 (0,0026-0.126)**

RESULTS

ELISA

	RA	NON RA	Total
CCP+	54	1	55
CCP-	2	129	131
Total	56	130	186

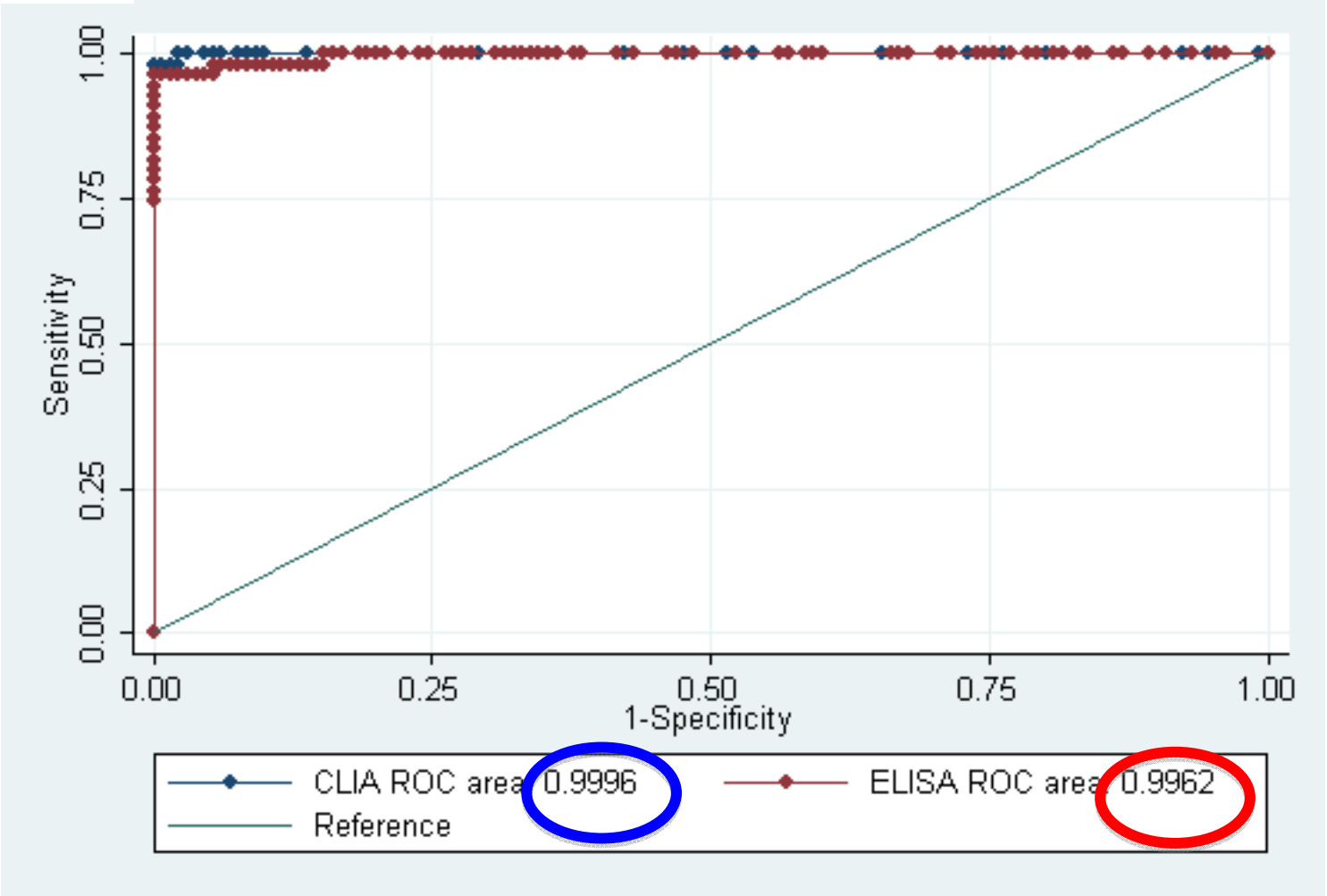
Sensit. % (95% CI)	Specif. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR^+ (95% CI)	LR^- (95% CI)
96.4 (87.7-99.6)	99.2 (95.8-100)	98,2 (90.3-100)	98.5 (94.6-100)	125 (17.8-884)	0.036 (0,0092-0.14)

RESULTS

	Sensit. %	Specif. %	PPV %	NPV %	LR^+	LR^-
CLIA	98.2 (90.4-100)	99.4 (95.8-100)	98,2 (90.4-100)	99.2 (95.8-100)	128 (18.1-900)	0.018 (0,0026-0.126)
ELISA	96.4 (87.7-99.6)	99.2 (95.8-100)	98,2 (90.3-100)	98.5 (94.6-100)	125 (17.8-884)	0.036 (0,0092-0.14)

RECEIVER OPERATOR CHARACTERISTIC (ROC) CURVES

Discriminative performance



RA patients and Control group

RESULTS

RA patients and Control group

	AUC	95%CI	HOSMER AND LEMESHOW TEST P-VALUE
CLIA	**1.000**	**0.999 - 1.000**	**1.000**
ELISA	**0.996**	**0.990 - 1.000**	**0.876**

no statistical difference

P = 0.2584

RECEIVER OPERATOR CHARACTERISTIC (ROC) CURVES
Discriminative performance

RA patients and ORD

RESULTS

RA patients and ORD

	AUC	95%CI	HOSMER AND LEMESHOW TEST P-VALUE
CLIA	**0.999**	**0.997-1.000**	**1.000**
ELISA	**0.998**	**0.995-1.000**	**0.987**

no statistical difference

P = 0,6849

CONCLUSION I

- Zenit RA CCP assay showed excellent diagnostic performance and suggested that the CLIA system has a potential to provide clinically useful data within a short time.

- The clinical performances of the CLIA test as well as the established anti - CCP2 ELISA method were similar. The CLIA test shows a slightly better sensitivity and the same specificity. Both have good discriminative and predictive abilities.

CONCLUSION II

- The CLIA assay seems to be an attractive alternative assay regarding the ELISA method, reduces labor, as well as assay time.

- Further analysis with higher samples sizes are required

Acknowledgements

Dra Zulmira Peerally
Associação Protectora dos Diabéticos de Portugal (APDP)

Prof. Doutora Ana Luísa Papoila
Faculty of Medical Sciences, New University of Lisbon

Dra Susete Pereira
A. Menarini diagnostics – Portugal