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Original article

Evaluation of three automated enzyme immunoassays for detection of anti-cyclic citrullinated peptide antibodies in qualitative and quantitative aspects

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Abstract

Objective. The anti-cyclic citrullinated peptide (anti-CCP) antibody has been increasingly used in the field of rheumatology, and various manufacturers have developed a variety of anti-CCP assays using mainly ELISA techniques. This study evaluated the performance of recently marketed automated chemiluminescence enzyme immunoassays for anti-CCP.

Methods. We investigated four anti-CCP assays (Diastat anti-CCP ELISA assay, Axsym anti-CCP assay on the Axsym system, the Architect anti-CCP assay on the Architect i2000 system and the Elecsys anti-CCP assay on the Cobas e 411 analyzer). Samples from 64 patients with RA and 152 controls, including patients with various autoimmune diseases, were studied. We assessed the clinical sensitivities and specificities, and compared the qualitative and quantitative results of each anti-CCP assay.

Results. Using the manufacturers' cut-off, diagnostic sensitivities ranged from 90.6 to 93.8% and the specificities ranged from 85.5 to 86.8%. The areas under the curve were comparable among the different assays, and qualitative agreements ranged from 97.2 to 99.1%. In the quantitative results, all anti-CCP assays were significantly correlated ($P < 0.001$), but the correlation coefficient ranged from 0.615 to 0.861. Especially, the correlation coefficients between the automated anti-CCP assays were higher than those between the ELISA assay (Diastat) and the automated assays.

Conclusions. The overall diagnostic performance of the automated anti-CCP assays was comparable, and it provides reliable information on antibody levels, making them useful in monitoring disease activity.

Key words: Anti-cyclic citrullinated peptide antibody, Enzyme immunoassay, Automation.

BASIC SCIENCE

Introduction

In 1998, Schellekens *et al.* [1] discovered that anti-citrullinated peptide antibodies specific for RA bind to antigenic determinants that contain citrulline, a modified form of arginine produced by the action of peptidyl-arginine deaminase. The first generation ELISA was developed for the detection of RA-specific anti-citrullinated peptide antibodies [2]. As peptide cyclization for the generation of a peptide with a higher affinity was used, this RA-specific antibody was called the anti-cyclic citrullinated peptide (anti-CCP) antibody. However, the first-generation anti-CCP assay had low analytical sensitivity (ranging from 48 to 68%) [2–4], and so a second generation anti-CCP assay was developed using highly reactive peptides identified from dedicated libraries of citrullinated peptides screened with RA sera. The second-generation assay showed significant increases in sensitivity compared with the first-generation assay [5]. Although a third-generation assay was developed, previous comparative studies have shown only slightly increased sensitivity [6] or no significant improvement in comparison with that of the second generation [7, 8].

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Table 1 Characteristics of the four anti-CCP assays evaluated in this study

Reagent	Diastat	Architect	Axsym	Elecsys
Manufacturer	Axis-Shield Diagnostics	Abbott Laboratories	Abbott Laboratories	Roche Diagnostics
Technique	ELISA	CMIA	MEIA	ECLIA
No. of calibrators	5	6	6	2
Range of calibrator, U/ml	0–100	0–200	0–200	0–200
Measuring range, U/ml	0–100	<0.5–200	<1.0–200	<7–1000
Reference range, U/ml	0.05–3.8	<0.5–2.5	<1.0–2.9	NA
Cut-off level, U/ml	5	5	5	17

CMIA: chemiluminescent microparticle immunoassay; MEIA: microparticle EIA; ECLIA: electrochemiluminescent immunoassay.

Currently, anti-CCP assays are helpful in various clinical situations, such as early diagnosis of RA, diagnosing RF-negative RA and differentiating RA and other RF-positive arthritis RA, such as hepatitis C virus-related arthritis or undifferentiated polyarthritis [9–13]. Liao *et al*. [14] proposed to include the results of an anti-CCP assay into the existing 1987 ACR criteria. This revised criteria improved RA classification sensitivity, especially in early RA. Recently, the quantitative results of anti-CCP assays have also been investigated. Studies have shown that higher levels of anti-CCP are related to disease progression to RA [15, 16] and the severity of RA symptoms [17]. In spite of the importance of anti-CCP quantitative results, previous studies have evaluated various anti-CCP only using only qualitative or diagnostic performance metrics.

In a clinical laboratory setting, testing with automated immunoassay analysers is preferred to manual ELISA in terms of technical simplicity, the random-access property, its reduced labour intensity and minimal operator-associated errors. Furthermore, automated immunoassay analysers provide better assay accuracy and precision [18–20]. In the present study, we evaluated the analytical and clinical performances of four anti-CCP assays, including two new, fully automated, random access assays: the Architect anti-CCP assay and the Elecsys anti-CCP assay.

Materials and methods

Patients and sample design

A total of 216 consecutive patients, examined in the rheumatology clinics of a single tertiary-care university hospital from December 2008 to February 2009 and who underwent anti-CCP testing, were enrolled in the study. The study was approved by the ethics committee of the Yonsei University Health System Severance Hospital. Sixty-four patients were diagnosed with RA according to the ACR criteria [21] and constituted the RA group [12 males: median age 56 (range 29–67) years and 52 females: median age 52 (range 20–73) years]. The control group consisted of 152 non-RA patients [33 males: median age 51 (range 4–79) years and 119 females: median age 47 (range 3–83) years]. The non-RA patients were further classified into four groups: OA, undifferentiated arthritis (UA), asymptomatic with positive RF (RFP) and other disease (OD), according to their diagnosis. The diagnosis of OA was based on revised ACR criteria for OA [22], and UA was defined as arthritis that did not fulfil the classification criteria for a definitive diagnosis, according to the ACR criteria for RA [13, 21]. The RFP group of 37 apparently healthy individuals presented with only RF positivity who had negative medical histories for rheumatic or autoimmune diseases. Among the OD group were 48 patients with either juvenile idiopathic arthritis (JIA) ($n = 22$), SS ($n = 8$), Behçet's disease ($n = 8$), SLE ($n = 6$), PMR ($n = 2$), scleroderma ($n = 1$) or gouty arthritis ($n = 1$). All serum samples were stored at -80°C until they were assayed.

Anti-CCP antibody and RF determination

Anti-CCP was determined by one commercially available second-generation ELISA (Diastat anti-CCP; Axis-Shield Diagnostics, Dundee, UK) and the following three commercially available automated EIAs: the Architect anti-CCP assay on the Architect i2000 system (Abbott Laboratories, Abbott Park, IL, USA), the Axsym anti-CCP assay on the Axsym system (Abbott Laboratories) and the Elecsys anti-CCP assay on the Cobas e 411 Analyzer (Roche Diagnostics, Mannheim, Germany). The procedures were conducted according to the manufacturer's recommendations. Key characteristics of each method are shown in Table 1. RF was determined by nephelometry on an IMMAGE 800 (Beckman-Coulter, Fullerton, CA, USA) according to the manufacturer's instruction. A positive result was recorded when RF concentration was >20 IU/ml.

Statistical analysis

The κ statistics, receiver operating characteristic (ROC) analysis, Spearman rank correlations and Passing–Bablok analysis were performed using the statistical software packages Analyze-It for Microsoft Excel (version 2.12) and SPSS for Windows. The sensitivity and specificity of each method were calculated using the manufacturer's cut-off and the optimal cut-off obtained by ROC curve analysis.

Table 2 Number of positive anti-CCP[a] and RF results in RA and non-RA patients according to assay

	Total, *n*	Positive, *n* (%)				
		Diastat	Architect	Axsym	Elecsys	RF
RA	64	60 (93.8)	59 (92.2)	60 (93.8)	58. (90.6)	44 (68.8)
Non-RA	152	22 (14.5)	22 (14.5)	20 (13.2)	20 (13.2)	68 (44.7)
OA	44	2 (4.5)	2 (4.5)	1 (2.3)	2 (4.5)	5 (11.4)
UA	23	9 (39.1)	8 (34.8)	8 (34.8)	7 (30.4)	9 (39.1)
RFP	37	2 (5.4)	2 (5.4)	2 (5.4)	2 (5.4)	37 (100)
JIA	22	6 (27.3)	6 (27.3)	6 (27.3)	6 (27.3)	5 (22.7)
Behçet's disease	8	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)
SS	8	1 (12.5)	2 (25.0)	1 (12.5)	1 (12.5)	5 (62.5)
SLE	6	0 (0)	0 (0)	0 (0)	0 (0)	5 (83.3)
PMR	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gouty arthritis	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Scleroderma	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a]At manufacturer's cut-off.

Results

Comparison of diagnostic performance

Using the cut-off values proposed by the manufacturers, 60 (93.9%), 59 (92.2%), 60 (93.8%) and 58 (90.6%) of the 64 RA patients and 22 (14.5%), 22 (14.5%), 20 (13.2%) and 20 (13.2%) of the 152 non-RA patients tested positive on the Diastat, Architect, Axsym and Elecsys assays, respectively (Table 2). Among the non-RA patients, 2/44 OA, 9/37 UA, 7/22 JIA, 1/8 Behçet's disease, 2/8 SS and 1/1 gouty arthritis patients showed a positive reaction to one or more of the assays. Distributions of anti-CCP concentrations according to disease category (RA, OA, UA, RFP and OD) for each assay are shown in Fig. 1.

The overall correlation of the qualitative results of the four anti-CCP assays is shown in Table 3. The agreement between the qualitative results of the Diastat, Architect, Axsym and Elecsys assays ranged from 97.2 to 99.1%. The κ coefficients of each assay were all over 0.940. Eight discrepant results among the four different anti-CCP assays are shown in Table 4. Irrespective of clinical diagnosis, the Diastat anti-CCP assay showed more positive results (6/8) than did the Architect (5/8), Axsym (4/8) and Elecsys (2/8) assays.

The areas under the curve (AUCs) were 0.903, 0.917, 0.914 and 0.907 for Diastat, Architect, Axsym and Elecsys, respectively (Table 5). We compared the AUCs of all other methods with that of the highest AUC (the Architect assay) and found no significant differences. In the case of RF, the AUC was 0.627, which was significantly different from all other anti-CCP assays ($P < 0.0001$).

The diagnostic sensitivity and specificity of each assay are shown in Table 5. At the manufacturer's cut-offs, sensitivities ranged from 90.6 to 93.8%, and specificities ranged from 85.5 to 86.8%. Optimal cut-off values were estimated based on the highest sum of sensitivity and specificity, and diagnostic performance was calculated for each assay at the optimal cut-off. Especially with the Elecsys anti-CCP assay, the value of the optimal cut-off was lower than the manufacturer's cut-off, and this optimal cut-off improved sensitivity from 90.6 to 92.2% without important losses in specificity.

Comparison of quantitative results

The correlations between the quantitative results are shown in Fig. 2. All anti-CCP assays were well correlated ($P < 0.001$), but the Spearman correlation coefficient (*rs*) between the four assays ranged from 0.615 to 0.861. The best correlation was observed between the Architect and Axsym assays, with a correlation coefficient of 0.886, whereas the correlation between the Diastat and Axsym was the worst ($rs = 0.615$). Interestingly, the correlation between the automated anti-CCP assays (Architect, Axsym and Elecsys) was higher than with the manual ELISA assay (Diastat).

Discussion

Since anti-CCP ELISA was introduced in 2000, it has been increasingly used as a diagnostic test for RA. With the development of the second-generation anti-CCP ELISA, analytical sensitivity significantly increased while maintaining high specificity. A recent meta-analysis reported the sensitivity and specificity of the anti-CCP assay for RA as 67 and 95%, respectively [23]. Besides its use as a diagnostic aid for RA, the presence of anti-CCP is predictive for RA development [24, 25], and the level of anti-CCP is associated with the development of bone erosion in RA [26, 27]. However, the correlation between the reduction of anti-CCP concentration and clinical responses to RA treatment is controversial [28, 29].

Numerous manufacturers produce anti-CCP assays, mainly using the ELISA format. However, an international reference serum of anti-CCP assays has not been prepared. Each manufacturer uses its own calibrators

Fig. 1 Scatter plots of anti-CCP level according to disease category. The lines indicate the manufacturers' cut-off values. OD includes JIA; SS, Behçet's disease, SLE, PMR, scleroderma and gouty arthritis.

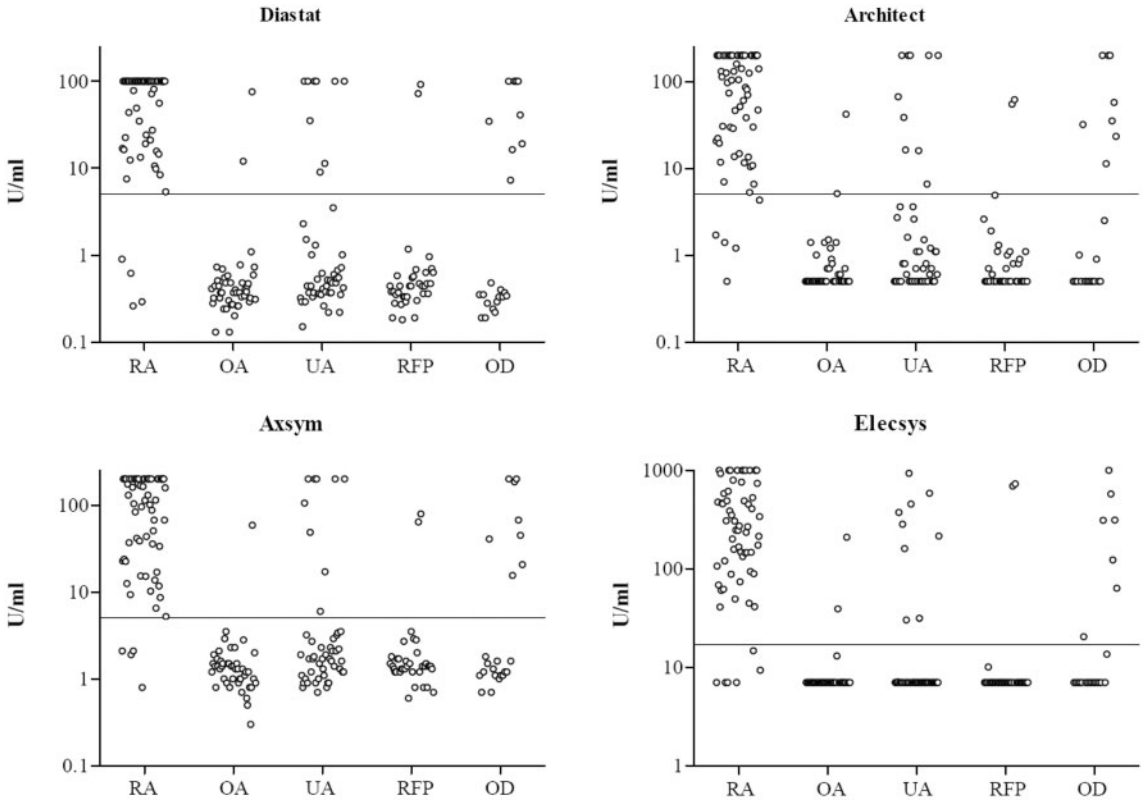

Table 3 Agreement and κ statistics between the anti-CCP assays

Assay	κ statistics and percent agreement between two anti-CCPs (no. of identical results/total)			
	Diastat	Architect	Axsym	Elecsys
Diastat	–	0.951	0.980	0.940
Architect	97.7 (211/216)	–	0.950	0.970
Axsym	99.1 (214/216)	97.7 (211/216)	–	0.940
Elecsys	97.2 (210/216)	98.6 (213/216)	97.2 (210/216)	–

Table 4 Discrepant results between the four anti-CCP assays[a]

No.	Sex/age	Results of anti-CCP (measured value, U/ml)				Clinical diagnosis
		Diastat	Architect	Axsym	Elecsys	
1	F/57	Pos (14.5)	Pos (10.8)	Pos (11.7)	Neg (14.7)	RA
2	F/47	Pos (5.4)	Neg (4.3)	Pos (5.2)	Neg (9.4)	RA
3	F/49	Pos (7.3)	Neg (2.5)	Neg (1.6)	Neg (<7.0)	UA
4	F/35	Pos (16.3)	Pos (11.3)	Pos (15.5)	Neg (13.6)	UA
5	F/14	Pos (9.0)	Neg (2.6)	Pos (6.0)	Neg (<7.0)	JIA
6	M/15	Neg (1.0)	Pos (16.3)	Neg (2.7)	Pos (30.4)	JIA
7	F/53	Pos (12.0)	Pos (5.1)	Neg (2.8)	Pos (39.3)	OA
8	F/39	Neg (3.5)	Pos (6.6)	Neg (2.9)	Neg (<7.0)	SS

[a]At manufacturer's cut-off.

Table 5 Clinical diagnostic performances of the four anti-CCP assays and RF

	Diastat	Architect	Axsym	Elecsys	RF
ROC AUC (95% CI)	0.903 (0.855, 0.951)	0.917 (0.878, 0.956)	0.914 (0.872, 0.956)	0.907 (0.864, 0.951)	0.627 (0.546, 0.707)
Manufacturer's cut-off	5.0	5.0	5.0	17.0	20.0
Sensitivity (95% CI)	93.8 (84.8, 98.3)	92.2 (82.7, 97.4)	93.8 (84.8, 98.3)	90.6 (80.7, 96.5)	68.8 (55.9, 79.8)
Specificity (95% CI)	85.5 (78.9, 90.7)	85.5 (78.9, 90.7)	86.8 (80.4, 91.8)	86.8 (80.4, 91.8)	57.2 (49.0, 65.2)
Positive predictive value, %	73.2	72.8	75.0	74.4	40.4
Negative predictive value, %	97.0	96.3	97.1	95.7	81.3
Optimal cut-off	3.6	5.1	3.5	13.6	22.5
Sensitivity (95% CI)	93.8 (84.8, 98.3)	92.2 (82.7, 97.4)	93.8 (84.8, 98.3)	92.2 (82.7, 97.4)	67.2 (54.3, 78.4)
Specificity (95% CI)	85.5 (78.9, 90.7)	86.2 (79.7, 91.2)	86.8 (80.4, 91.8)	86.2 (79.7, 91.2)	59.9 (51.6, 67.7)
Positive predictive value, %	73.2	73.8	75.0	74.1	41.3
Negative predictive value, %	97.0	96.3	97.1	96.3	81.3

and control materials without standardization between assays. Several previous studies have compared the technical and diagnostic performance of anti-CCP assays produced by diverse manufacturers. Although the overall diagnostic performance of anti-CCP assays was comparable across the different assays, they recommended careful selection of methods in a clinical laboratory setting [7, 8, 18, 30]. Recently, novel anti-CCP assays using automated platforms have been developed. Automated immunoassay analysers offer great advantages over traditional ELISA (especially for high-volume hospital laboratories), such as improved precision, reliability, technical simplicity, short turnaround time and high-speed throughput. Because previous evaluations were limited to aspects of qualitative and diagnostic performance for each anti-CCP assay, we decided to assess the diagnostic performance and quantitative results of four anti-CCP assays. One anti-CCP assay used the ELISA methodology (Diastat), whereas the other three (Architect, Axsym and Elecsys) were available in automated immunoassay analysers using CMIA, MEIA and ECLIA, respectively.

The overall agreement between the qualitative results of the four anti-CCP assays was good. The best agreement (99.1%) was observed between Diastat and Axsym, and the worst agreements (97.2%) were observed between Diastat and Elecsys and Axsym and Elecsys. The difference between the best and worst agreement was 1.9%, which is minimal.

Regarding diagnostic performance, the Architect assays showed the highest AUC (0.917). However, the other three anti-CCP assays showed similar performances when compared with the Architect assay. The AUC was not statistically different among the assays evaluated, suggesting that the diagnostic performances of the four anti-CCP assays were comparable. At the manufacturer's cut-off, diagnostic sensitivities and specificities ranged from 90.6 to 93.8% and from 85.5 to 86.8%, respectively. Previous meta-analysis has presented the sensitivities and the specificities of second-generation anti-CCP assays as 68% (range 39–93%) and 95% (range 81–100%), respectively [31]. In this study, the clinicians knew the anti-CCP results of enrolled patients and thereafter confirmed the final diagnosis, a study design that would probably generate higher sensitivities than those of previously published reports. However, anti-CCP assays are already widely used in clinics and so this study design reflects real everyday rheumatology practice.

The correlation between the quantitative results of the four anti-CCP assays was good ($P < 0.001$). Especially, the correlation coefficients (range 0.861–0.886) between three automated anti-CCP assays were higher than that of the manual ELISA and automated assays (range 0.615–0.788). The measurement ranges of the four anti-CCP assays were different from each other. The Diastat anti-CCP assay showed the narrowest measuring range (0–100 U/ml), with the results of 47/82 (57.3%) anti-CCP-positive patients (>5 U/ml) exceeding the measuring range (>100 U/ml). Contrarily, the Elecsys anti-CCP assay showed the widest measuring range (<7–1000 U/ml), with only 12/78 (15.4%) anti-CCP-positive patients (>17 U/ml) exceeding the measuring range (>1000 U/ml). This may be important in quantitative measurement of anti-CCP levels and in the clinical application of the quantitative results related to disease progression or treatment outcome.

The lack of interchangeability of the various anti-CCP assays may prevent objective judgement and unbiased comparison of the results of previous studies. Clinicians and researchers should be alerted to the fact that results from the various anti-CCP assays now available could differ, especially quantitatively. Although a limited number of batches of reagent and calibrators were used, our study showed that the quantitative results of one automated assay could be interchangeably converted to the results of the other two automated assays using the correlation equation. International standardization or harmonization of anti-CCP assays should be achieved as soon as possible.

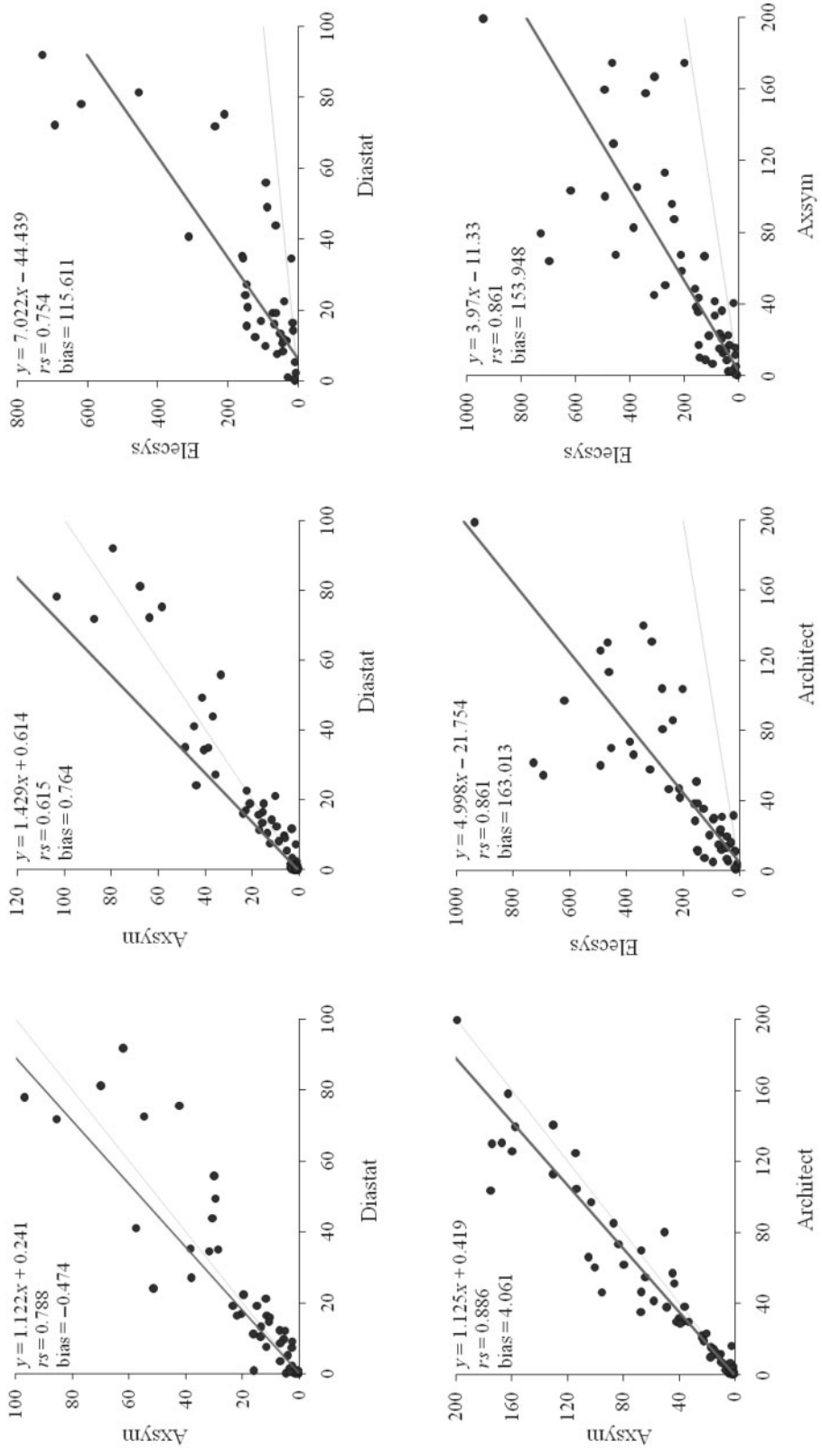

Fig. 2 Passing–Bablok regression analysis of the four anti-CCP assays. Results exceeding the measuring range were excluded from the statistical analysis.

Rheumatology key messages

- Quantitative value of anti-CCP could differ according to assay format.
- Automated anti-CCP assays provide more reliable quantitative results.

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