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Proficiency testing for the detection of anti-citrullinated protein antibody in China

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ABSTRACT

Background: Anti-citrullinated protein antibodies (ACPAs) are important for the detection of rheumatoid arthritis (RA). There are many laboratories to detect it in their routine work, but their performance is not displayed in China. To examine the performance of ACPA assays from all laboratories, it is necessary to organize a laboratory proficiency test (PT).
Methods: A panel of 5 samples, including 4 positive and 1 negative, was produced by the National Center for Clinical Laboratories, using serum derived from patients, then distributed to 271 clinical laboratories. Quantitative and qualitative results reported by the participating laboratories were compared.
Results: Overall, 80.97% (200/247) of the laboratories had eligible PT scores. Of the kits used, most ELISA and chemiluminescence kits had a high sensitivity and specificity. Regarding intra-assay discrepancy, the Roche and Abbott kit had a better variable coefficient. The ratios of the quantitative results to the kit-specific cut-off values were similar.
Conclusion: Performance varied between laboratories. Reagents and methods are the most important factors. Other factors may affect the intra-assay discrepancy. The similar mean of ratios of the quantitative results to the kit-assigned cut-offs suggests that a national criterion is requisite. It is necessary to organize a PT to identify performances of different laboratories.

1. Introduction

Rheumatoid arthritis (RA) is a relatively common systemic autoimmune disease that affects 0.5–1% of the population worldwide. The pathophysiology of RA involves the production of autoantibodies, which attack tissues and organs, resulting in chronic inflammation with associated cartilage degradation, bone erosion, and physical disability [1]. However, the pathogenesis remains unclear. Inflammation of the synovial joint can lead to progressive structural joint damage and severely reduce quality of life. Early prevention plays a crucial role in preventing irreversible joint damage in RA. It is therefore necessary to diagnose RA earlier. The 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA recommended an algorithm for the classification of definite RA. With this classification system are positive serologic tests for rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) [2]. Of these tests, those for ACPA have a higher sensitivity (88–98%) and a substantially higher specificity (70–80%) than those for RF and other autoantibodies, particularly in terms of early detection of RA [3–6]. In addition, RA can be categorized into 2 subsets based on the ACPA result: ACPA-positive RA which involves a more aggressive and destructive disease course and ACPA-negative RA [7]. Currently, many laboratories are exploring the potential for distinguishing between these 2 subsets [1]. Numerous studies have confirmed the importance of ACPA in RA diagnosis, as reflected by its inclusion in the 2010 RA classification criteria [8,9].

The use of ACPA in the diagnosis of RA is dependent upon accurate detection. Test results may be affected by the reagents used, environmental factors such as temperature and humidity, and operation. Presently, various commercial kits for ACPA detection are available in China; different kits use different antigens and methods. Of these methods, enzyme-linked immunosorbent assay (ELISA), usually manual-operation, is the most commonly used, and the results may be affected by many factors. Chemiluminescence and time-resolved fluorescence immunoassay methods are also used in many clinical laboratories. Furthermore, aside from these internationally recognized kits, many laboratories use kits designed to diagnose RA. Various antigens are used by different manufactures, and the performance levels of different laboratories are

Abbreviations: ACPA, anti-citrullinated protein antibody; CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; PT, proficiency test; NCCL, national center for clinical laboratories; ELISA, enzyme-linked immuno sorbent assay; ACR/EULAR, American college of Rheumatology/European League Against Rheumatism; ECLIA, electrochemiluminescent immunoassay.

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unknown. We conducted a multicenter proficiency test to assess the performance of different laboratories in China, including variability associated with different reagents, instruments, operations, environmental factors, and quality control.

2. Methods

2.1. Preparation of panels

Panels were prepared by the National Center of Clinical Laboratories (NCCL) using human serum-containing ACPA obtained from RA patients. No consent was needed from the patients, and our protocol was approved by the Ethics Committee of the NCCL. Each panel contained 4 samples that were positive for ACPA, and 1 negative sample. The 4 positive samples (1 sample at a low concentration; 2 identical samples at a medium concentration; 1 sample at a high concentration) were quantitated using the Human anti-CCP ELISA KIT (Kexin Biotech, Shanghai, China). Serum from donors negative for hepatitis B surface antigen, anti-hepatitis C virus antibodies, anti-human immunodeficiency virus antibodies, anti-Treponema pallidum antibodies, and ACPA was used as the negative control. The serum samples were dispensed in 0.5-mL aliquots at the appropriate dilutions on ice and were stored at −20 °C until shipment to the participating laboratories.

2.2. Participants of the PT

In total, 271 clinical laboratories were invited to participate in the study. All laboratories were assigned the same samples with the same code of the panels and were required to detect the samples using their routine procedures. Test samples were shipped on ice to the participating laboratories. Detailed instructions for the panel were also provided to the laboratories, including details regarding the storage conditions, specimen processing methods, and other procedures. We requested that a sample be reported as positive for ACPA if it shows a reaction with the different ACPA screening reagent kits during routine laboratory procedures. The results were returned to the NCCL via e-mail or fax within 4 weeks of receipt of the detection panel. To report the PT results, the laboratories were asked to submit quantitative and qualitative results of the ACPA assay and details of the assay used, including basic information regarding the detection method and manufacturer, as well as the amount of sample used.

2.3. Statistical analysis

The results returned from all participants were compared with the relevant reference results. Results that differed from the reference results were considered as either false-negative or false-positive. Based on the qualitative results, a laboratory could be awarded a maximum of 20 points toward the PT score per sample for a correct detection. We analyzed the percentage of laboratories with PT scores of 100, 80, 60, and <60. The acceptance criteria for ACPA assays were defined according to the PT scores, and were set at >60. This was considered indicative of superior performance for ACPA assays. We analyzed the performance of different laboratories according to the antigens of the different kits, methods, operations, and environments. With regard to the quantitative results, we briefly considered the factors affected by the different kits. The mean, SD, CV, median, and range for different reagent kit groups were calculated for each positive sample, for groups with >10 participants. We also calculated the ratios of the values to their upper limits of normal (ULNs) and compared them according to their assays. We used one-way ANOVA (SPSS 19.0) to compare the results among participants, according to the different kits and methods used. Reagent kit groups with fewer than 10 participants were not evaluated.

3. Results

3.1. Performance based on qualitative results

A total of 247 laboratories returned ACPA detection results, including 206 laboratories that returned qualitative results and 199 laboratories that returned quantitative results. Reported detection methods included ELISA, chemiluminescence immunoassay, the colloidal gold method, and others; a total of 18 reagents were reported to have been used. These reagents applied different antigen in their kits (Table 1). We assigned PT scores only to those laboratories that reported qualitative results. Hence, the majority of participating laboratories had eligible PT scores (97.09%, 200/206); the PT scores of 6 laboratories were unacceptable, in which 5 laboratories used the Kexin Biotech colloidal gold method while 1 laboratories used an unusual kit with a kind of unknown antigen. Only 3/11 laboratories using the colloidal gold method with a kit produced by Kexin Biotech had a PT score of 100 (Table 1).

When we calculated the negative and positive concordance results of the tests among different kits, the majority of kits showed a high positive concordance (>95%) and negative concordance (>90%, Table 1). However, the negative and positive concordance of reagents used by <5 laboratories may have been influenced by the results of individual laboratories. Among these kits, the kit for the colloidal gold method produced by Kexin Biotech had the poorest positive concordance (59.09%). Of the 5 samples provided, 99.03% (204/206) of the laboratories correctly identified the high concentration sample, 97.57% (201/206) and 97.09% (200/206) identified the medium concentration samples, 94.66% (195/206) identified the low concentration sample, and 97.57% (201/206) correctly identified the negative sample.

Regarding the methods, most laboratories utilized ELISA to detect ACPA with manual-operation; the positive concordance was 98.97% (578/584) and the negative concordance was 97.26% (142/146). The positive concordance of chemiluminescence with automated instruments was 100% (196/196) and the negative concordance was 97.96% (48/49).

3.2. Performance based on quantitative results

Only 5 reagent kit groups were used by more than 10 participating laboratories. We calculated the mean, SD, CV, median, maximum, minimum, and mean ± 3SD for the 5 kits for each of the 5 samples (Supplemental Tables 1–5). Laboratories using kits from Roche and Abbott with automated instruments had the lowest CV, particularly in positive samples (Roche, 5.64–9.12%; Abbott, 10.74–12.87%). Others laboratories that used the ELISA method had a similar CV (25.32–35.62%). We compared the quantitative results in the same sample by one-way ANOVA (SPSS 19.0) according to different methodologicals and reagents and obtained a series of p values. The results indicated statistically significant differences in the same sample according to the reagents and methods used (Figs. 1A and 2). We also found a statistically significant difference in the results obtained by participants using Abbott and Roche, which automatically excluded operator variables.

Then, we compared the ratios of the different results for the 5 samples to the response cut-offs recommended by the manufacturers according to reagent by one-way ANOVA (SPSS 19.0). Notably, among the reagent groups, there were no statistical differences in ratios of results detected by Abbott and Roche in highly positive and negative samples and results detected by ELISA methods in either positive samples or negative samples (Fig. 1B). In addition, while the means of the initial results were differed substantially, the means of the ratios were close to each other within the same sample. The SD of the ratios was lower than the initial results (Supplemental Tables 1–5).

4. Discussion

ACPA is considered an efficient diagnostic and prognostic biomarker for RA [10–13]. Here, we report the first nationwide PT scores for ACPA