Comparison of Quantitative and Semiquantitative Enzyme-Linked Immunosorbent Assays for Immunoglobulin G against *Chlamydophila pneumoniae* to a Microimmunofluorescence Test for Use with Patients with Respiratory Tract Infections

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We previously reported a high degree of variation in the sensitivities of serodiagnostic kits for the detection of *Chlamydophila pneumoniae* in sera from healthy donors. Since a low predictive value of a test can impair its diagnostic value, we have extended our studies to samples from patients with pneumonia. We focused on the most promising enzyme-linked immunosorbent assays (ELISAs) (SeroCP and SeroCP Quant; Savyon) identified in our previous study and included a new ELISA (sELISA; Medac). The agreement between all ELISAs for immunoglobulin G (IgG) and a reference microimmunofluorescence (MIF) test for IgG (SeroFIA; Savyon) was ≥90% for a collective of 80 patients. The positive predictive values were all ≥93%. The negative predictive values ranged from 68 to 83%. False-negative results were obtained only for samples that had low titers in the MIF test. The correlation of the IgG antibody titers determined by the MIF and SeroCP Quant tests was high ($r_{sp} = 0.9$). Since the semiquantitative SeroCP and quantitative SeroCP Quant ELISAs achieved the highest sensitivities, they were evaluated further by using a second batch of sera from 50 patients with predominantly medium and low antibody titers in the MIF test and a control collection of sera from 80 children with negative MIF results. Again, the tests showed a high concordance with the MIF results (96%), and the antibody titers in the SeroCP Quant and MIF tests correlated well ($r_{sp} = 0.8$). The specificities determined with the negative sera were ≥99% for the SeroCP Quant test and 86% for the SeroCP test. These results show that ELISAs that are fast and objective deliver seroprevalence results, sensitivities, and specificities that are very similar to those of the MIF test.

*Chlamydophila pneumoniae*, a common pathogen found in the respiratory tract, is ubiquitous and has a seroprevalence of ≥70% in the adult population (12, 23, 33). *C. pneumoniae* is considered responsible for about 10% of community-acquired pneumonia cases (1, 11, 15), but most infections remain asymptomatic (12, 14). *C. pneumoniae* lacks the ability to synthesize ATP and thus has an obligatory intracellular infection cycle (19). Only the intracellular forms, i.e., the reticulate bodies, are metabolically active and can be treated with membrane-permeable antibiotics such as macrolides, while the metabolically inactive extracellular elementary bodies (EB) are not sensitive to these agents. The reticulate bodies can differentiate into aberrant forms, which are associated with an intracellular state of persistence, contributing to chronic infections (20). The persistent state is characterized by a metabolically active period without replication (6). Until now, no antibiotics have proven capable of eradicating persistent chlamydial infections (21, 22). An association between persistent *C. pneumoniae* and the development and progression of chronic degenerative diseases such as atherosclerosis, neurological disorders, and asthma has been suggested (14, 27, 34, 37, 38).

The diagnosis of *C. pneumoniae* can be done by immunostaining or isolation and culturing of the organism. PCR is the most sensitive method for the detection of *C. pneumoniae*, but it is not yet in routine use (2, 4, 17, 35). Thus, diagnosis is still based mainly on serology. The microimmunofluorescence (MIF) test represents the “gold standard” (4, 37), but it is a time-consuming method which is only reliable if performed by an experienced operator. Multicenter trials have shown large interlaboratory variances in MIF performance (31).

Recently, some partially automated enzyme-linked immunosorbent assays (ELISAs) have become commercially available. Using sera from 80 healthy donors, members of our laboratory reported that both the sensitivities and specificities of 11 different ELISAs and MIF tests for *C. pneumoniae* serological diagnoses vary considerably (16). Three of four MIF tests (Labsystems, Helsinki, Finland; MRL Diagnostics USA; and Savyon Diagnostic, Ashdod, Israel) provided highly comparable results. Furthermore, some of the ELISAs, such as SeroCP and SeroCP Quant, concurred well with these MIF results, and the SeroCP Quant ELISA could determine antibody titers reliably. We have now extended this study to sera from patients with pneumonia, chronic obstructive pulmonary disease, or upper respiratory tract infections and focused on the evaluation of the ELISAs SeroCP, SeroCP Quant, and the novel sELISA in comparison to a MIF test (SeroFIA).

Diagnoses, determinations of outcomes, and the results of
epidemiological studies are all highly dependent on the selected serological test. The choice of test is of utmost importance if treatment decisions must be made.

MATERIALS AND METHODS

Sera. Four different collections of sera were used for this study: (i) 80 sera collected from healthy adult volunteers (healthy collective 1), which were also used in a previous study (16); (ii) 80 sera from adult patients with acute pneumonia (patient collective 2); (iii) 50 sera from 15 adult patients with chronic obstructive pulmonary disease, 29 with pneumonia, and 6 with upper respiratory tract infections (patient collective 3); and (iv) 80 sera obtained from children aged 18 months to 14 years with adenoid vegetations (control collective 4).

Serological studies. Anti-C. pneumoniae antibodies were determined by the SeroCP ELISA for immunoglobulin G (IgG) and IgA, the SeroCP Quant ELISA for IgG and IgA (Savyon and Hain Lifescience, Nehren, Germany), and the Chlamydia sELISA and Chlamydia-IgG-rELISA, both for IgG (Medac, Wedel, Germany). The tests were performed according to the manufacturers’ instructions. All tests are solid-phase assays performed on microtiter plates, but they differ regarding the antigen preparations used, i.e., total antigen (SeroCP and SeroCP Quant), one unnamed antigen (sELISA), or a recombinant lipopolysaccharide (LPS) fragment (rELISA) that is claimed to be genus specific (5).

The SeroFIA MIF tests for IgG and IgA (Savyon) were used as references. Collective 2 was further analyzed with an in-house MIF test for IgG performed at the National Consultative Laboratory for Chlamydia (Institute of Medical Microbiology, University of Jena, Jena, Germany) (13, 28, 29) and with the Chlamydia pneumoniae IgG MIF test (MRL Diagnostics USA and Genzyme Virotech, Rüsselsheim, Germany). The commercial MIF tests were performed and analyzed as recommended by the manufacturers. The tests are solid-phase MIF assays performed on glass slides with purified formalin-treated EBs of C. pneumoniae (TW-183), Chlamydia trachomatis (serotype L2), and Chlamydia psittaci as antigens. The immunological activities of the LPSs from C. pneumoniae and C. trachomatis, but not that from C. psittaci, were reduced in non-specific ways in order to render the tests species specific. All MIF tests were performed and analyzed blindly and in parallel by the same two persons. Samples which did not fulfill the criteria needed to be judged positive according to the manufacturers’ instructions were judged negative for the given test.

Anti-C. trachomatis antibodies were measured by the Savyon and MRL MIF tests (see above) and by an enzyme immunoassay for IgG (Labsystems and Merlin, Bornheim, Germany).

PCR. PCRs were performed on tonsils and adenoid tissues from patients in control collective 4. A seminested PCR with primers for a sequence from the PstI fragment (7) was performed, resulting in one amplicon of 437 bp and a second amplicon of 229 bp. Specificity was checked with an enzyme-labeled gene probe (DEIA; Sorin, Saluggia, Italy). Positive and negative controls were included in each PCR. To exclude the presence of PCR inhibitors, we spiked an aliquot of the samples with a final concentration of 2 × 10^7 inclusion-forming units of C. pneumoniae EB/μl before DNA extraction (24).

Statistics. Statistical analysis was performed with the InStat program (GraphPad Software, San Diego, Calif.). The correlation of the IgG and IgA titers determined by the SeroCP Quant ELISA and the SeroFIA MIF test was determined by a nonparametric correlation (Spearman).

RESULTS

To determine whether the high concordance of the MIF results reported previously (16) also translates to patient samples, we measured C. pneumoniae IgG antibody titers in samples from collective 2 by using three different MIF tests. The concordance between the SeroFIA MIF test and the in-house MIF test or the MIF test from MRL Diagnostics was 94 and 96%, respectively (data not shown). The seroprevalence of IgG antibodies was 81% according to the SeroFIA MIF test (Table 1). These results were used as a reference for the ELISAs. In contrast to the MIF test, all ELISAs showed some false-positive and false-negative results. All false-negative results were obtained with samples that had the lowest titers (1:64) in the MIF test (71, 86, and 91% of these 21 samples were judged correctly by the sELISA, SeroCP Quant, and SeroCP tests, respectively), while all samples with medium (1:256 and 1:512) or high (1:1,024) titers in the MIF test were judged correctly. Five samples were positive for C. trachomatis (not shown). All of these were also positive for C. pneumoniae by all three ELISAs and the MIF test. The concordance of the different ELISA results with the MIF results is shown in Table 2. The best concordance was achieved by the SeroCP Quant ELISA (94%).

The sensitivities, specificities, and positive and negative predictive values (PPV and NPV) of the different ELISAs, calculated on the basis of the MIF test, are given in Table 2. The PPVs of all species-specific ELISAs, i.e., all but the rELISA, were ≥93%, while their NPVs were 81% for SeroCP Quant, 83% for SeroCP, and 68% for sELISA.

In contrast to SeroCP and sELISA, the SeroCP Quant ELISA also determines antibody titers. The SeroCP Quant and MIF antibody titers correlated well (r = 0.9, P < 0.001).

The genus-specific rELISA only detects anti-LPS antibodies, which occur early during infections (32, 36). For 80 sera, only 41 of 65 positive samples were positive by rELISA, resulting in an NPV of 31%. Of the samples with high antibody titers in the MIF test (1:1,024), which probably indicates active infections, only 77% were considered positive by the rELISA. The weak
concordance with the MIF test is in line with a previous study using sera from healthy donors (16). Since the sELISA was not available for the first study, it was now additionally evaluated with the sera from collective 1. The results of the sELISA showed a concordance of 83% with those of the MIF test, and the NPV and PPV were 61 and 100%, respectively. Due to these poor results, the sELISA and rELISA were not included in further studies.

Currently, the role of IgA antibodies is not clearly defined (9). IgA has been suggested to represent mucosal immunity (8), and due to its shorter half-life, seems to represent a better marker than IgG for chronic infections (26). As determined by the MIF test, the seroprevalence of IgA in patient collective 2 was 76% (Table 1) and all samples positive for IgA were also positive for IgG. The concordance of the ELISA results with the MIF results, as well as sensitivities, specificities, NPVs, and PPVs, are given in Table 2. Again, false-negative results were only obtained with samples that had low IgA titers (1:32) in the MIF test. The correlation between the MIF test and SeroCP Quant results for IgA quantification had an $r_{sp}$ value of 0.8 ($P < 0.001$).

Because correct diagnoses of IgG in sera from patients with very low and medium antibody titers are most critical, a second batch of sera from patients with respiratory diseases (patient collective 3) was collected and the IgG titers were determined by the SeroFIA MIF test (6 of 50 were negative, 12 of 50 had a titer of 1:64, 17 of 50 had a titer of 1:256, and 15 of 50 had a titer of 1:512). The concordance of the SeroCP and SeroCP Quant ELISA results with those of the MIF test and the NPVs and PPVs were $\geq 96\%$ (Table 2). The correlation between the SeroCP Quant and MIF results had an $r_{sp}$ value of 0.8 ($P < 0.001$).

In order to determine the specificities of the SeroCP and SeroCP Quant ELISAs in more detail, we used a collection of sera from children with adenoid vegetation (control collective 4). The sera were all negative in the MIF test. Since the MIF test often produces false-negative results for children’s sera (10), a PCR test for chlamydial DNA was performed on adenoid tissue and tonsil samples to confirm the MIF results. In SeroCP Quant assays for IgG and IgA, negative results were obtained for all 80 sera, except for one sample that had a low IgG titer, while the SeroCP assay had some false-positive results (11 of 80 positive for IgG and 4 of 80 positive for IgA). The specificities obtained for this control collective were 99% for the SeroCP Quant IgG test, 100% for the SeroCP Quant IgA test, and 86 and 95% for the SeroCP IgG and IgA tests, respectively.

**DISCUSSION**

The sensitivity and specificity of a serological test have a large impact on its predictive value for diagnosis and on the resulting seroprevalence data gained in epidemiological studies. Thus, this study aimed at evaluating different serological assays. We first evaluated the quality of four ELISAs for the diagnosis of *C. pneumoniae* antibodies in sera from 80 patients with pneumonia compared to that of MIF tests. The SeroCP and SeroCP Quant ELISAs were chosen for their excellent correlation with the MIF test in a previous study (16). The sELISA, which was not available for the previous study, was employed to test sera from healthy collective 1 and patient collective 2. Since no convalescent-phase sera had been used, this part was not a diagnostic study, but a mere assay evaluation. The concordance of the ELISA results with the MIF test results regarding IgG detection was high, although all ELISAs gave a few false-positive results. None of those samples was positive for *C. trachomatis* and only one of them was positive for anti-LPS antibodies by rELISA, so cross-reactivity with *C. trachomatis* antibodies can nearly be excluded. However, cross reactions of antibodies with *Bartonella* antibodies in the *C. pneumoniae* MIF test and in Western blots have been reported (25), and it cannot be excluded that other infections may raise antibodies that are cross-reactive (18, 30).

The NPVs for IgG detection in sera from pneumonia patients ranged from 68 to 83% for the species-specific ELISAs. The evaluation of samples with low titers of IgG and IgA, such as 1:64 or 1:32, showed the most variance between tests, since all false-negative samples by each test were samples that had very low titers in the MIF test. This lack of sensitivity may not be of major significance for the diagnosis of acute infections, in which at least a fourfold rise in IgG titer is expected and the use of paired samples is recommended as a diagnostic criterion, but it would strongly affect the patient composition of collects analyzed retrospectively or for prospective seroepidemiological studies, in which no acute-phase sera or paired samples are assessed. This poor accuracy of diagnosis for samples with low titers is not restricted to the ELISAs evaluated here but is a general problem that has also been reported for the analysis of samples with low titers by different MIF tests (16, 31). However, it has been discussed that these low titers may be an indication of chronic infections (4). The results of the rELISA differed most from the MIF results. This might be explained by the fact that only a subset of anti-*C. pneumoniae* antibodies, i.e., the anti-LPS antibodies, which are synthesized with different kinetics, are detected. Whether the rELISA is convenient for the detection of acute infections has been controversial. The fact that in our study only 77% of subjects with very high IgG titers (1:1,024) had anti-LPS antibodies raises further doubts about whether the rELISA is appropriate for acute diagnoses.

In conclusion, the evaluation of the SeroCP ELISA and the

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* Eighty samples from collective 2 and 50 samples from collective 3 were analyzed for *C. pneumoniae* antibodies by the indicated ELISA, and assay performance was evaluated on the basis of the gold standard MIF test.
SeroCP Quant ELISA by using two collections of sera from patients with respiratory tract diseases as well as one control collection of sera from children revealed the high specificities and sensitivities of these ELISAs as well as a very good agreement with the MIF results. Furthermore, the antibody titer values determined by the SeroCP Quant assay showed an acceptable correlation with those determined by the MIF test. Most commercially available MIF tests, even though they are based on different antigen preparations, have been shown to produce comparable results (3, 16). However, the major advantage of ELISAs is that they are less time-consuming and more objective, since they are not dependent on the quality of the fluorescence microscope used or the experience of the reader.

The putative association of persistent C. pneumoniae infections with various disorders calls for a method for reliable risk evaluation and treatment monitoring. Only strict quality control of diagnostic measures will lead to a clarification of these controversies.

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REFERENCES