CHAPTER FIVE

Estimated time of seroconversion in antibody titre in the diagnosis of Legionnaires’ disease

EPF Yzerman¹, RB Geskus², MF Peeters³, JP Bruin¹, JW Den Boer¹

¹Regional Laboratory of Public Health Haarlem, Haarlem, The Netherlands
²Academic Medical Center, Amsterdam, The Netherlands
³Regional Laboratory of Public Health Tilburg, Tilburg, The Netherlands

ABSTRACT

In Legionnaires’ disease (LD), seroconversion is assumed to occur relatively late in the course of disease. The aim of our study was to assess the time to seroconversion of three different antibody tests. Acute phase and convalescent sera of 45 outbreak-related patients with LD caused by L. pneumophila serogroup 1 were tested with three commercially available assays (IFAT, ELISA, and RMAT). Time to seroconversion was estimated using a Bayesian nonparametric estimation procedure. According to this estimation procedure, 90% of patients seroconverted within 3 weeks of onset of disease when using the IFAT. The results of the ELISA showed an estimated seroconversion of 60% after 3 weeks for IgG as well as IgM, with only a slight increase in seroconversion thereafter. Combining IgG and IgM raised the seroconversion rate to 75%. The RMAT showed a slowly increasing seroconversion rate, with an estimated maximum of 60% reached after 7 weeks. We concluded that the IFAT performed better than the ELISA or the RMAT and that seroconversion can be expected within 3 weeks of the onset of disease.

INTRODUCTION

Legionnaires’ disease (LD) is caused by Legionella spp., rod-shaped gram-negative bacteria that were first isolated from patient material in relation to a large outbreak of acute pneumonia of unknown origin among visitors of a convention of veteran soldiers in Philadelphia in 1977 [1]. The microbiological diagnosis of LD is based primarily on the culture of Legionella spp. from sputum samples, detection of antibodies in serum or detection of antigen in urine. Each of these diagnostic methods has its advantages and restrictions with regard to specificity and sensitivity. However, there is general agreement on the required level of specificity for diseases of low incidence: 99% or higher [2]. Studies on the sensitivity of serological methods have yielded varying results [3, 4]. This may be
caused in part by differences in the antigens or antigen preparation used in the assays. This also may be the result of differences in the patient populations studied, leading to a lack of external validity due to non-representative samples. Such a bias can be avoided by active case-finding, as in large outbreaks of LD. In two large outbreaks, the sensitivity of different serologic tests ranged from 61 to 70% [5, 6]. In both outbreaks, the sensitivity of a serum antibody assay was calculated irrespective of the time elapsed since the onset of disease. However, the performance of an antibody test to diagnose LD depends on the timing of acute and convalescent phase sera. The purpose of our study was to assess the time to seroconversion of three different antibody tests for the diagnosis of LD. This study was performed on samples of a sera collection previously used in a study investigating the sensitivity of these antibody assays [7].

PATIENTS AND METHODS

Patients
In this study, 133 hospitalized patients with a confirmed Legionella pneumonia using EWGLI-criteria were included [8]. They were all part of a large outbreak in 1999 in The Netherlands [5]. Of these patients, 104 fulfilled the criteria used in this study. Our gold standard (identical to the EWGLI-criteria, but excluding serological evidence of infection) was defined as a patient who had symptoms compatible with pneumonia, with radiological signs of infiltration, who had visited the site of the outbreak during the incubation period and had laboratory evidence of infection with Legionella pneumophila. Laboratory evidence included isolation of L. pneumophila from a respiratory sample and/or the presence of L. pneumophila antigen in urine specimens. The three tests used in our study were two enzyme immunoassays from Binax (Binax, Portland, Maine, USA), and Biotest (Biotest AG, Dreiech, Germany) and a qualitative immunochromatographic assay, the Binax NOW test. All tests were used according to the manufacturers’ instructions. Urine was concentrated and reread after one hour as recommended [9-11].

Serum samples and serum antibody tests
In total, 62 clinical microbiology laboratories were involved in the diagnosis and treatment of the patients related to this outbreak. All of these laboratories sent available serum samples to the Regional Laboratory of Public Health in Haarlem. The serum samples were stored in portions at -70º C prior to testing. An acute phase serum was defined as a serum taken within the first two weeks after onset of symptoms. We used a serogroup 1 Philadelphia-I subtype rapid microagglutination IgM antibody assay (RMAT), a commercial serogroup 1-6 indirect immunofluorescence antibody assay (IFAT, Meridian Bioscience Europe Srl.) and a commercial IgM and IgG ELISA (Serion, Institut Virion/Serion GmbH,
Würzburg). The sensitivities of these antibody tests had already been investigated on paired serum samples of our patient collection [7]. The criteria for a paired serum sample confirmative for LD were defined as a fourfold rise in antibodies with a final titre of $\geq 1:128$ for the IFAT or a final titre of $\geq 1:32$ for the RMAT [12, 13]. For the ELISA, seroconversion to positive IgM or IgG antibodies was defined confirmative [14]. The presence of standing titres or positive antibodies in the first available serum was defined as indicative of LD.

**Time to seroconversion**

Time from onset of LD to seroconversion was determined for individuals from whom at least two serum samples were available, with the first sample being an acute phase serum. In practice, this means that individuals who died shortly after disease onset were excluded. The moment of seroconversion was known only to be between the time of the last negative and the first positive sample, i.e., it was interval-censored. If the first sample was positive, seroconversion was known to be only before the time of that sample. For individuals who tested negative in all samples, seroconversion likely occurred later than the time at which the last sample was taken (it may be that they never seroconverted). The distribution of time to seroconversion was estimated, taking into account this incomplete observation process. A Bayesian nonparametric estimation procedure for interval-censored data was employed, using DPpackage [15-17].

**RESULTS**

Two or more serum samples from 55 of the 104 patients who fulfilled the criteria used in this study were available for evaluation. For three patients, the date of onset of LD was not available. For seven patients, no acute phase sera were available. The microbiological diagnosis of the remaining 45 patients was established by culture alone in one case, by culture and urinary antigen test in 12 cases and by urinary antigen test alone in 32 cases. There were no differences in gender, age or mortality between the 59 excluded cases and the 45 included cases. Of the latter, 29 were men with a mean age of 62 years (range 25-78), and 16 were women with a mean age of 59 years (range 21-77 years).

Two serum samples were available from 22 patients, 3 serum samples from 13 patients and 4 to 8 serum samples from 10 patients. The mean interval between the first day of signs and symptoms of pneumonia and the collection of the first serum to be tested for antibodies against *L. pneumophila* was 8 days (range 1-14 days). In the available first serum samples, standing titres were present with the IFAT in 24%, with the ELISA in 11% and with the RMAT in 13%.

The calculated time to seroconversion for the different antibody tests is shown in Figures 1-3. With the IFAT, almost 90% of patients are estimated to seroconvert within 3 weeks of onset of disease. The ELISA shows that an estimated
seroconversion of almost 60% can be expected after 3 weeks for IgM and IgG, with only a minor increase thereafter. Combining the results of IgG and IgM in the ELISA results in a maximum seroconversion rate of 75%, indicating that a number of patients only seroconvert to either IgG or IgM (data presented earlier) [7]. With the RMAT, the estimated seroconversion rate shows a steady but slow increase to about 60% after 7 weeks. If the different tests are combined, the final result is comparable with the IFAT alone: a maximum estimated seroconversion rate of 90%, reached within 3 weeks of the onset of disease (Figure 4).

Figure 1

Estimated time of seroconversion based on sera of 45 outbreak-related LD patients using an IFAT assay

- estimated curve
- 95% confidence interval
LD = Legionnaires disease
IFAT = indirect immunofluorescence antibody test

Figure 2

Estimated time of seroconversion based on sera of 45 outbreak-related LD patients using ELISA IgM and IgG assay

- estimated curve IgG
- 95% confidence interval
- estimated curve IgM
- 95% confidence interval
LD = Legionnaires disease
DISCUSSION

In this study, we defined the acute phase of Legionnaires’ disease as the first two weeks after onset of signs or symptoms. One can argue about the length of this period but, in practice, patients may wait up to a week after the first signs appear before seeking medical attention. Delay in diagnostic serology may take another week. For these reasons, all sera collected within this 14-day period were considered acute phase sera. Using this definition, the IFAT found standing titres
in one-quarter of patients with culture or urinary antigen-confirmed LD. This means that in general practice, serology results for these patients would be considered indicative of LD. With the ELISA and the RMAT, the percentage of standing titres is markedly lower.

Applying a Bayesian nonparametric estimation procedure, with the IFAT, seroconversion is expected to occur in the first two weeks after onset of disease for 50% of the cases. A maximum of 90% seroconversion is reached after 3 weeks. Using the ELISA, 60% of patients are expected to seroconvert within the first 3 weeks for either IgM or IgG, with only a slight increase in the follow-up period; combining IgG and IgM results in a final seroconversion rate of 75%. In contrast, with the RMAT, one can expect a steady increase in seroconversion even after 7 weeks, with an expected maximum of about 60%. In conclusion, the IFAT detects seroconversion early in the course of disease with a high final sensitivity and performed, in our setting, better than the ELISA or the RMAT.

However, using the EWGLI case definition of Legionnaires’ disease, the diagnosis cannot be confirmed with serum antibody assays for patients with a standing titre. Thus, the presence of antibodies early in the course of disease would substantially lower the value of these assays. If standing titres are included in the case definition, sensitivity rises to high levels in Week 3. This finding is in agreement with data from Kallings, who followed 49 outbreak-related LD cases for two years and demonstrated that with the IFAT, a maximum antibody titre was reached within 3 weeks [18]. However, not in line with our results, Kallings reported a relatively sharp decline of the titre after the first month. He concluded that it was impossible to designate a reliable value of a single titre to be used as evidence of LD. In our study, the geometric mean antibody titres declined, but most individual titres remained above the diagnostic cut-off values during the 18-week follow-up period.

In general, antibody detection is thought to be insensitive, and it is recommended that convalescent sera should be collected at 4, 6 and 12 weeks after disease onset [19]. Even then, only about 75% of culture-proven LD patients are expected to seroconvert. These recommendations on serum sampling have been based on studies of selected patient groups, which do not necessarily represent the clinical spectrum of LD. Our study confirms earlier research, which finds that using the IFAT as the most sensitive assay, an acute phase serum is a prerequisite for demonstrating seroconversion and that seroconversion can already be expected within 3 weeks of the onset of disease. It is important to stress that the antigenic composition of the IFAT is in agreement with the antigenic setup of the isolated Legionella strain to reach optimal sensitivity. However, in our study, we estimated time to seroconversion; sensitivity is not likely to influence this estimation. Therefore, it seems plausible that our findings regarding time of seroconversion can be extrapolated to non-outbreak-related LD cases caused by L. pneumophila serogroup 1.
REFERENCES


