

Prevalence of *Legionella* antibodies in immunocompromised patients

Research Article

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Abstract: Introduction: Dialysis patients and patients post-renal transplantation can be predisposed to *Legionella* infections. The aim of this work was to investigate the prevalence of *L. pneumophila* serogroups 1-7 (SG 1-7) antibodies in dialysis patients and in patients following renal transplantation, in order to analyse the potential risk factors for infections. Material and Methods: Commercial ELISA kits were used for detection of serum IgG (SG 1-7, SG 1) and IgM (SG 1-7) present in patients and the control group. Results: In the studied group of patients, positive results (IgM and/or IgG SG 1-7) were obtained in 20 patients (7.12%). One patient only had two classes of antibodies. From the total study group, the antibodies against *L. pneumophila* SG 1 were detected in only one patient on dialysis. Patients with *L. pneumophila* antibodies who are on dialysis or post-renal transplantation did not differ significantly in any of the usually evaluated risk factors of clinical infection. Conclusions: The reported outbreaks of Legionnaires' disease in chronic dialysis patients and those with renal transplants, as well as our results of IgG and IgM antibodies, merit further identification of the sources of this infection but also the ways in which cellular immune system can be managed in the immunocompromised patients with *Legionella* infection.

Keywords: *Legionella pneumophila* antibodies • Risk factors • Dialysis patients • Patients post-renal transplantation

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1. Introduction

Bacteria of the *Legionella* genus are ubiquitous intracellular microorganisms, which may cause a wide spectrum of diseases, including a mild, self-limited influenza-like syndrome (known as Pontiac fever) and

community-acquired or nosocomial pneumonia (Legionnaires' disease) [1-4]. The infection takes place through the inhalation of aerosol or consumption of water contaminated with *Legionella* bacteria [3,5].

From all bacteria of the *Legionellaceae* family, the *L. pneumophila* species account for the majority of

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diagnosed cases of legionellosis (approximately 80-90%), including 60-90% of *L. pneumophila* belonging to serogroup 1 (SG 1) [5]. Serogroups other than *L. pneumophila* serogroup 1 participate in about 20-30% of infections (mainly SG 4 and 6). Only 10-20% of infections are caused by species other than *L. pneumophila* [3].

Legionella is widespread throughout the world. Due to their high adaptability to different environmental conditions, they inhabit natural and artificial reservoirs, and tend to colonise hot and cold water supply systems in the public buildings, households and industrial systems. The presence of the *L. pneumophila* bacilli constitutes a high risk for the water systems found in the health care facilities, e.g. hospitals, dialysis centers, sanatoriums (whirlpools and other water massage equipment used in balneotherapy) as well as dental equipment (dental turbines), where patients are particularly susceptible to infection [3,5,6].

Cigarette smoking, chronic lung disease and immunosuppression (especially that caused by corticosteroid therapy) have been consistently implicated as risk factors. Patients with chronic renal failure and patients post-organ transplantation are deemed high-risk groups. Other factors associated with the development of Legionnaires' disease include old age, cancer and alcohol intake [3,7].

In Poland in 2008, the legal regulation of controlling water systems for the presence of *Legionella* bacteria was introduced. This project was undertaken to evaluate the occurrence of *Legionella spp.* in hot water systems in the Polish health care facilities and large public buildings. Data collected from the State Sanitary Inspection in Poland in the first eight months since the establishment of obligatory water testing in health care facilities show that, the hot water was examined in 411 (51.1%) hospitals. The number of *Legionella* bacteria exceeding the limit value of 100 CFU per 100 ml was found in 241 (58.6%) of the surveyed facilities, which constitutes a real threat for patients' health [8].

So far in Poland, there have been no studies carried out to evaluate as to what extent the patients, especially those with impaired immunity, have come into contact with the *L. pneumophila* SG 1 and the less pathogenic strains *L. pneumophila* SG 2-14.

The aim of this work was, for the first time in our conditions, to investigate the prevalence of *L. pneumophila* antibodies in the two groups of patients – dialysis patients and the patients post-renal transplantation – in order to analyse the generally accepted risk factors for infections.

2. Materials and methods

2.1. Study group

Serum samples were collected from 281 immunocompromised patients between November 2010 and March 2011, i.e. 213 dialysis patients (161 hemodialysis patients and 52 peritoneal dialysis patients) and 68 patients after the renal transplantation. The study was approved by the ethical committee of the Medical University of Lublin.

The mean age of the dialysis patients was 62.5 ± 15.5 years (age range, 19-98 years) and for patients after transplantation it was 49.5 ± 13.4 years (age range, 20-72 years). The mean duration of dialysis prior to the study was 5.4 ± 6.2 years with a range from 1 month to 29.5 years. The mean duration after transplantation in the study group was 4.1 ± 2.9 years with a range from 1-14 years.

The potential risk factors for *L. pneumophila* infection were analysed in the studied groups of patients. The risk factors were divided into two groups – the risks associated with the patient and risk factors related to the environment. The first group of factors included: gender, cigarette smoking, alcohol abuse, chronic respiratory disease, diabetes, cancer, immunosuppressive therapy, steroid therapy, and other coexisting diseases; and the second group of factors included: place of residence (village or town), visit to the dentist, stay at a sanatorium, travels, use of pools, use of showers, use of a whirlpool, use of water massage, and use of air conditioning for a period of up to 2 months. Complete analysis of the risk factors was performed with the group of dialysis patients only. For the transplant patients, only the information regarding their gender and place of residence was provided.

2.2. Control group

Serum samples were also collected from 100 healthy volunteers. Their ages ranged from 17-74 years with a mean of 36.6 ± 15.2 . In the control group, there were 72 females and 28 males.

2.3. Serological test

Two commercial ELISA kits (EUROIMMUN Medizinische Labordiagnostika AG, Germany) were used for detection of IgG and IgM against *L. pneumophila* SG 1-7

present in the serum of patients and control group. The tests were performed according to the manufacturer's instructions.

IgG antibodies against *L. pneumophila* SG 1-7 were detected in all dialysis patients (n=213) and in all patients post-renal transplantation (n=68). IgM antibodies against *L. pneumophila* SG 1-7 (n=161) were detected in all hemodialysis patients (n=161) and in patients post-renal transplantation who were positive for IgG (SG 1-7).

In case of positive test results (IgG SG 1-7) for IgG *L. pneumophila* in the serum samples, patients and control group were also examined for the most pathogenic serological group (SG 1) *Legionella*, using a commercial ELISA kit (VIRCELL_{MICROBIOLOGISTS}, Spain) according to the manufacturer's protocol.

2.4. Statistical methods

Data was presented as means \pm standard deviation (SD) and analysed using Chi square test. *P* value of < 0.05 was considered to be statistically significant. The statistical analysis was performed using STATISTICA v 9.0 (StatSoft, Poland).

3. Results

In the study group (n=281), the positive results for the antibodies (IgM and/or IgG) against *L. pneumophila* SG 1-7 were obtained in 20 patients (7.12%). In dialysis patients, positive results of IgM and/or IgG antibodies were obtained in 17 cases (7.98%). Among the hemodialysis patients, *L. pneumophila* IgM antibodies were detected in 5 subjects (3.11%). Of the 213 dialysis patients, positive results for *L. pneumophila* IgG antibodies were obtained in 13 subject (6.10%). One patient was found to have both classes of antibodies (IgM and IgG) against the *L. pneumophila* SG 1-7.

Of the 68 patients with renal transplantation, positive results (IgG SG 1-7) were obtained in 3 subjects (4.41%). In these patients the IgM (SG 1-7) and IgG (SG 1) antibodies against *L. pneumophila* were not detected.

Of the whole study group, the antibody against *L. pneumophila* SG 1 were detected in only one patient. This was a 58-year-old diabetic woman with a chronic renal failure (one year dialysis). This patient had no other potential risk factors for *Legionella* infection.

The medical records showed that none of the patients with positive results had *L. pneumophila* infection in the last 2 months prior to the study. During this period no other infections were observed.

Of the 100 control samples (IgM or IgG antibodies against *L. pneumophila* SG 1-7), 9 (9.0%) of the

Table 1. The type and frequency of anti-*L. pneumophila* antibody in the examined groups

	total number of patients	IgG and/or IgM <i>L. pneumophila</i> SG 1-7	
		positive *n (%)	negative n (%)
Dialysis patients	213	17 (7.9)	196 (92.0)
Patients post renal transplantation	68	3 (4.4)	65 (95.6)
Control group	100	9 (9.0)	91 (91.0)

*n – number of positive or negative results

samples were serologically positive: IgM antibodies were detected in 6 (6.0%) and IgG antibodies were detected in 3 of the cases (3.0%). The antibodies against *L. pneumophila* SG 1 were not detected in the control group with positive results of antibodies against *L. pneumophila* SG 1-7.

IgM and/or IgG antibodies anti-*L. pneumophila* SG 1-7 occurred more frequently in the dialysis patients than in the patients post-renal transplantation or control group, but these differences were not statistically significant (p=0.52). Table 1 summarises the type and frequency of anti-*L. pneumophila* antibody in the examined groups.

There was not a significant correlation between patients with positive results of *L. pneumophila* antibodies and the potential risk factors (associated with the patient and risk factors related to the environment). It was observed that positive results of *L. pneumophila* IgM and/or IgG antibodies (SG 1-7) occurred more often in the men (5.65%) than in women (5.11%), but this was not statistically significant (p=0.82).

4. Discussion

The development of infection caused by *Legionella* and its clinical form depends on the interaction between the pathogen and microorganisms. The immune system of a person is exposed to contact with the pathogen as well as the virulence, and number of microorganisms penetrating into the lungs play the main role for development of infection [2,9].

Legionella infection induces innate and specific immune mechanisms, both cellular and humoral immune responses. In the initial phase of infection, the innate immunity is used, which has numerous systems for the early detection of potentially dangerous pathogens. On the other hand, in the late phase of infection, the adaptive immunity is initiated. Although the immunological

mechanisms of *Legionella* infection have not yet been fully understood, it is widely recognised that the cellular immune response is a key host response against this intracellular pathogen. A significant role in inhibiting the growth and elimination of *Legionella* is attributed to macrophages, lymphocytes, dendritic cells and cytokines [2,9].

Therefore, our study, in which the presence of antibodies against *L.pneumophila* was found, are considered to be the indicator of the extent to what an asymptomatic infection occurs in immunocompromised patients. Taking into consideration the severity of infection and high mortality in the patients with Legionnaires' disease (reaching even 20%), those with positive results should be examined further to investigate the sources of infections. These may be due to infection in the water systems in hospitals or at the places of residence [10].

L. pneumophila is an opportunistic pathogen which frequently causes serious infection in immunocompromised patients, particularly those with impaired cell-mediated immunity. Patients with chronic renal failure and patients after organ transplantation are particularly susceptible to *Legionella* infection. This is due to damage to the immune function. Infections in end-stage renal disease patients are caused by immunosuppressive effects of uremia, in turn, in patients after organ transplantation by the immunosuppressive drugs used. In these groups patients have impaired in both cellular and humoral immune response [11-17].

In general, most normal individuals are resistant to this microorganism, which has as its natural habitat warm water containing amoebae or protozoa.

Outbreaks of Legionnaires' disease have been reported in chronic dialysis patients and in patients after renal transplantation [18-22]. In the work of Ongut *et al.*, among 252 chronic hemodialysis patients, antibodies were detected in 13 subjects (5.16%). In the study group infection caused by *L. pneumophila* was not found in the medical history of the previous 6 months. Also analysed were the various risk factors for infection by *L. pneumophila*. There was no statistically significant difference between *L. pneumophila* seropositivity and the potential risk factors [7]. In another study, Morimoto concluded that the titer in hemodialysis patients was higher than in the control group (p less than 0.005) [22]. Boldur *et al.* found in their work that 5 out of 79 chronic hemodialysis patients had titers of 1:512 to 1:2048 of indirect immunofluorescence antibodies indicative of past infections [17].

In our study the results were similar. Among 213 dialysis patients, the positive results of antibodies (IgM and/or IgG) against *L. pneumophila* SG 1-7 were obtained in 17 cases (7.98%). It should be emphasized that IgG antibodies against *L. pneumophila* SG 1 were detected in only one patient from the study group. This serogroup *L. pneumophila* is more virulent than the SG 2-14.

There was not a significant correlation between *L. pneumophila* seropositivity and the analysed potential risk factors (depending on the patient and/or depending on the environment).

Patients post-organ transplantation (especially heart, liver and kidney) have been shown to have a higher incidence of Legionnaires' disease than the general population [11,13]. In the Marshall *et al.* study, 14 cases of Legionnaires' disease were identified in 101 cases with renal transplants [20].

In our study, among 68 patients post-renal transplantation, positive results were obtained only in 3 (4.41%) subjects (only IgG anti-*L. pneumophila* SG 1-7 antibodies). This is probably related to the species of *L. pneumophila* that are not very frequent cause of infection. The other species of *Legionella* are found more commonly amongst transplant patients [11,13].

In our study there were not statistically significant differences in prevalence of *L. pneumophila* antibodies in the studied groups. Studies by others have shown that *L. pneumophila* is a rare cause of infection in our population of patients although, as indicated by the antibodies, the contact with microorganisms was frequent among dialysis patients.

It is worth considering that *Legionella* can colonise in the hospital potable water systems for long periods of time, resulting in an ongoing risk for patients, especially the are immunocompromised ones. Moreover, assessment of the risk of *Legionella* infection in patients with immunosuppression should not only consist in detection of specific antibodies but also the assessment of generally accepted potential risk factors.

The reported outbreaks of Legionnaires' disease in the chronic dialysis patients and those with renal transplants, as well as our results of IgG and IgM antibodies, merit further identification of the sources of infection but also finding the ways in which cellular immune system can cope with the *L. pneumophila* in the immunocompromised patients.

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