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DOI: 10.7883/yoken.JJID.2015.157

This article has been retracted by the authors under the agreement between the Editor-in-Chief, Masayuki Saijo and authors.

The Editor in Chief of Japanese Journal of Infectious Diseases (June 21, 2016)
Advance Publication by J-STAGE

Japanese Journal of Infectious Diseases

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Received: April 2, 2015. Accepted: October 14, 2015
Published online: November 13, 2015
DOI: 10.7883/yoken.JJID.2015.157

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Pneumonia in Vietnamese Children Aged 1 to 15 years Due to Atypical Pneumonia Causative Bacteria: Hospital-Based Microbiological and Epidemiological Characteristics

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Key words: MCL bacteria, children, pneumonia, epidemiology, Vietnam.

Running title: Atypical pneumonia in Vietnamese children
Summary

Pneumonia caused by *Mycoplasma pneumoniae* (M), *Chlamydia (Chlamydia)* *pneumoniae* (C) and *Legionella pneumophila* (L) (these three bacteria are referred as the MCL, hereafter) is not well-documented in Vietnam, which may limit effectiveness of treatment. We investigated epidemiological & microbiological characteristics of MCL-caused pneumonia in hospitalized children aged 1-15 years.

Multiplex PCR and specific IgM determination were used to detect the target bacteria. Of 722 community-acquired pneumonia (CAP) children, the MCL were the most frequently found pathogens (215 children, 29.78%). *M. pneumoniae* was the most common at 88.4% (190/215), the typing based on *p1* gene for cytoadhesin were both type I and type II, 51% and 28%, respectively. Age is an important factor: more than 77% (167/215) of MCL pneumonia was found in children less than 5 years of age and a high rate (66%; 64/97) of M-caused severe pneumonia cases was in children younger than 2 years. The distribution of MCL-caused pneumonia by age was a statistically significant (*P* < 0.01). In Vietnam, pneumonia due to *M. pneumoniae* in younger children must be interested and appropriately considered in diagnosis and antibiotic utilization. Monitoring of these atypical pneumonia causative bacteria in the system of respiratory infections surveillance is necessary to introduce.

Introduction

Worldwide, pneumonia is a leading cause of child death, killing 6.6 million children under the age of five years in 2012 (1). In Vietnam, community-based studies suggest that every child younger than 5 years suffers from 5 to 8 episodes of acute respiratory infections annually (2), while hospital-based investigations indicate 35-50% of all pediatric patients were hospitalized due to pneumonia. The Vietnamese Ministry of Health has reported that each year 4000 children younger than 5 years die from pneumonia in Vietnam (3).
Bacteria such as *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* can cause mild, moderate or severe community-acquired pneumonia (CAP) in children (these three bacterial species are defined as MCL or atypical bacteria in this paper). These pathogens are increasingly recognized as important causative agents of pneumonia in many countries. However, their role in Vietnam has not been well documented, with neither microbiological nor epidemiological data being available. This could diminish the effectiveness of the general guidelines for treatment of CAP in Vietnamese children (4): antibiotics of beta-lactam group, which do not naturally affect those bacteria, were used as key antibiotics to treat children with CAP, and antibiotics of the macrolide group, which are the first-choice therapeutic agents against MCL infections in children as well as in adults, were not used routinely. Specifically, for younger children, an incorrect treatment inevitably fails to cure lower respiratory tract infections (LRTI).

In general, accurate and prompt etiologic diagnosis of CAP is limited at most Vietnamese hospitals because of inadequate laboratory diagnostic facilities, and this is especially true for the detection of MCL bacteria. While some diagnostic methods have been used in researches (for example serodiagnosis based on specific IgM antibodies for M and C bacteria), these are not available for routine diagnosis and none of the laboratories had diagnostic tools for *L. pneumophila*.

Polymerase chain reaction (PCR) assays are now widely available for rapid diagnosis of microbial pathogens, especially for those that are difficult to detect using conventional microbiological techniques. Serodiagnostic methods such as enzyme-linked immunosorbent assay (ELISA) or particle agglutination test (PA test) are simpler and cheaper. But may not be reliable in acute infections because of the time needed for antibody titers to rise. Combining both PCR and serodiagnosis may be a more reliable diagnostic approach. In this study, in order to cover infectious process and acute specific immune responses in
diagnostics we used multiplex PCR and ELISA-based specific IgM antibody determination to detect MCL-caused pneumonia in evaluated patients.

**Materials and Methods**

**Patients and diagnosis:** This study was conducted with the approval of the Research Ethics Committee, National Hospital of Pediatrics (NHP), Hanoi, Vietnam and consent of the patient’s parents. Hospitalized children aged 12 months to 15 years old attending NHP with acute LRTI (CAP) were evaluated prospectively from July 2010 to March 2012. The NHP is a tertiary care facility, with patients referred from different provinces in Vietnam. Baseline data included the age and sex of the patient, the duration of symptoms prior to hospitalization, the duration of antibiotic treatment prior to admission (if any) and the patient outcome (discharge or death).

*Case definition for CAP* was adapted from the guideline of Vietnam Ministry of Health, which are based on principal criteria of the World Health Organization (3) as follows: fever with temperature $\geq 38^\circ$C; cough or sore throat; fast respiratory rate or difficulty breathing, or abnormal breath sounds, infiltrated lung on X-ray. This study did not enroll the children with nosocomial pneumonia (symptom developing after hospital admission), proven immunodeficiency or immunosuppression and without their parental consent. Recruited patients were followed-up until their recovery or death.

*Case definition for a MCL bacteria positive pneumonia case (MCL pneumonia)* was as follows: (1) Patients met the above criteria for case definition, confirmed by radiography and (2) presence of M and/or C and/or L, which was detected in bronchoalveolar lavages by multiplex PCR, or by presence of specific IgM antibodies against M or C or L in one of paired serum samples.

*Chest X-ray examination:* Chest X-ray was taken for all enrolled patients. Two senior
radiologists reviewed all chest radiographs and agreed on the conclusion.

**Sample size and data collection:** Because there was no study of incidence/prevalence of MCL pneumonia in Vietnam, we used the data extracted from the study of Asian multiple centers to calculate the sample size (5). A sample size of 710 was determined to estimate the prevalence of MCL pneumonia of 23.5% within 0.032, with 95% confidence interval, using the following parameters: $\alpha = 0.05$ (value of $\alpha$ is the probability of type I error), $\beta = 0.2$ (value of $\beta$ is the probability of type II error, or (1-power) of the test), and non-responsive rate = 5%. (6). The actual sample size was 722 children hospitalized for pneumonia treatment.

At the time of admission, clinical data (including chest X-ray) were collected uniformly, laboratory specimens (bronchoalveolar lavage and clotted blood) were obtained and medical history and epidemiological information were collected with a standardized questionnaire by interviewing the patient’s parents.

**Laboratory methods**

Sample collection: at admission, bronchoalveolar lavages were taken and transported to the laboratory for detection of MCL bacteria by multiplex PCR.

Two blood samples were taken from enrolled patients in sterilized vacuum tubes without heparin. First blood was taken at their admission. The second blood was taken after 3 weeks. The serum was collected by centrifuging at 2000 rpm for 5 min, and subsequently transported on dry ice to laboratory of the National Institute for Hygiene and Epidemiology (NIHE), Hanoi where they were stored at - 20°C until testing. In addition, blood samples were collected for routine blood counts (WBC and differential) and C-reactive protein (CRP) at their admission.

Detection of three bacterial species causative for atypical pneumonia (MCL bacteria) by multiplex polymerase chain reaction: for testing, stored samples were thawed, mixed well,
and centrifuged at 15,000 rpm for 10 min at room temperature. Most of the supernatant was discarded and 200µl of the pellets were used for DNA extraction with QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer’s instructions. Targeted nuclear detection was performed by an in-house multiplex PCR using the following primers:

For *L. pneumophila*, we used a previously evaluated primer amplifying a region of gene for macrophage infectivity potentiator (7).

For *M. pneumoniae*, we used primers that amplify a region of *p1* gene for cytoadhesin protein P1 (MP-F: 5′-aactatgtgtgtgtatgaccagtac-3′; MP-R: 5′-accttgacgagggccgta-3′) (8).

For *C. pneumonia*, we used primers that amplify a region of gene for outer membrane protein (CPTM2r: 5′-cgtgtcgtccagcattta-3′ and CPc1 or HL-1: 5′-gtgttcatgaaggctact-3′) (9). All of the primers were evaluated for specificity due to formation of no specific bands. Based on the referred primers, we optimized the reactive conditions of a conventional multiplex PCR for this study. After optimization, the multiplex PCR assay has a detection limit of ≤ 10 colonies forming units per assay for each target bacterium. Repetition of optimized PCR results was stable in 5 times of testing.

Briefly, the reaction mixture was prepared in a total volume of 25 µl as following: 12.5µl of QIAGEN Multiplex PCR Kit (QIAGEN, Germany), 1.5 µl of 25mM MgCl₂ as supplementation (final concentration was 2.25 mM), 7.5pmol of each MP primer, 30pmol of each CP primer, 10pmol of each LP primer, 1µl of DMSO (99.5%) and 2 µl of extracted nucleic acid from each sample. The multiplex PCR for each sample was performed in PCR machine (Eppendorf AG, Germany) with the following conditions: initial activation of 4 cycles of (96°C for 3 min; 55°C for 35 s; 72°C for 45 s), subsequent to 40 cycles of (94°C for 35 s; 55°C for 40 s; 72°C for 1 min), last extension time of 10 min at 72°C. Amplification products were analyzed by electrophoresis through a 2% combined agarose gel (1g NuSieve GTG agarose mixed with 1g SeaKem GTG agarose (LONZA Group Ltd, Switzerland) in
100ml TAE buffer (Invitrogen, USA). SYBR® Safe DNA gel stain (Invitrogen, USA) was used to visualize the PCR products. The appearance of 229, 287 and 168 base pair (bp) bands corresponding to M, C and L, respectively, were considered positive. In each experiment, negative and positive controls for each bacterium were used. Equivocal PCR results were confirmed by single PCR.

Enzyme-Linked Immunosorbent Assay (ELISA) for specific IgM antibody determination: to supplement the results of M, C and L detection by multiplex PCR, IgM antibody was determined by ELISA using commercial reagents and according to the manufacturer’s instructions (Mycoplasma pneumoniae ELISA IgM Ref No. M1002, Chlamydophila pneumoniae ELISA IgM Ref No. M1007 and Legionella pneumophila ELISA IgM Ref No. M1000 - Vircell S.L, Spain), using acute and convalescent serum samples. According to the manufacturer’s instruction, the result was recorded as positive when the IgM antibody (Ab) index was greater than 11, as equivocal between 9 and 11, and as negative if under 9 (the sensitivity and the specificity of these kits have been described by the manufacturer with reliable results (10).

Typing of M. pneumoniae based on p1 gene for cytoadhesin protein P1 was carried out by PCR (11, 12) with primer set: ADH/4F: 5’-gagegcatcaaccacctttgcgttacg-3 and mixed reverse primers N1: 5’-cgggtgtggaagtatttt-3’ and 2N2C: 5’-tgccttggtcaccggagttg-3’. Essential reagents which were used for typing protein P1 (HotStarTaq Master Mix, Seakem GTG agarose, TAE buffer, DNA ladder 100bp, loading buffer 6x) were provided from the same sources (QIAGEN, Lonza and Invitrogen) as above described.

Detection of point mutations associated with ERY-resistance in domain V of 23S rRNA: for 60 M-positive PCR specimens, identification of point mutation at site 2063, 2064 in the M. pneumoniae 23S rRNA domain V gene region was performed in accordance with the methods reported by Matsuoka et al. (13). We used nested PCR with primer sets
1610LF/2830LR for the first PCR and 1790LF/2185LR for the second PCR, HotStarTaq Master Mix kit (QIAGEN, Germany) and extracted DNA. The second PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Germany), after purification them, they were electrophoresed in a 1.5 Seakem GTG agarose (Lonza), when the single band was confirmed, labeled using a BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems) and applied to a HITACHI 3130 Genetic Analyzer in accordance with the manufacturer’s instructions. The presence or absence of gene mutation at each site was determined by reading using a sequence scanner (Applied Biosystems).

In addition, routine examinations as quantitative culture method for detection of typical pneumonia causative bacteria and real-time PCR assay for detection of respiratory viruses were performed at the hospital (NHP) (14,15).

**Statistical analysis**

Data were entered and checked using Epidata software. Quantitative variables were checked for normal distribution and compared using One-Way ANOVA or Independent-Sample T test. A Kruskal-Wallis or Mann-Whitney U test was used to compare quantitative variables without normal distribution. Quantitative variables are expressed as mean ± SD or median (interquartile range). Frequencies of category variables were compared by Pearson’s $\chi^2$ test or Fisher’s exact test when appropriate. Associations were considered statistically significant at two-sided $P$ values of less than 0.05 for all the analyses. The above statistical procedures were performed using SPSS version 16.0 (SPSS, Chicago, USA).

**Results**

Seven hundred and twenty-two children, aged 1-15 years met criteria for the definition was included. Most children (502/722, 69%) were aged < 2 years, with 136 children (19%) aged 2 to < 5 years, 66 children (9%) aged 5 to < 10 years and 21 children (3%) aged 10 years or more. The great majority of children (626/722, 87%) had received antibiotics (most
frequently beta-lactam’s, > 86%) for a median of 7 (range 1 to 10) days prior to hospitalization. The median duration of hospitalization was 8 (range 2 -75) days and bronchoalveolar or lobar pneumonia were the most common clinical diagnoses (> 85% children).

Of these 722 patients, MCL bacteria were the most frequently found pathogens (215 children, 29.78%) by PCR and specific IgM detection, pneumonia due to M was the most frequently found one (190/215, 88.37% ) (Table 1).

Of the 215 children with MCL pneumonia, only one cause of infection was found in 144 (67%), in almost 90% of cases being *M. pneumoniae* (129/144) (Table 2).

Almost 56% (120/215) of pneumonia due to M was found in children younger than 2 years old, overall more than 77% (167/215) were found in children under the age of 5 years (Table 3). So that, a relatively high rate (45.12%, 97/215) of MCL pneumonia cases was severe pneumonia, required treatment in intensive care unit. In severe pneumonia, the highest proportion occurred in children younger than two years (66%, 64/97) (data not shown).

Generally, frequency of MCL pneumonia by age was statistically significant (P < 0.0001, Chi-square test) and P value for pneumonia due to M or C or L bacterium alone by age was less than 0.01 (data not shown).

The North of Vietnam has 4 seasons in a year: spring, summer, autumn and winter. Humidity is always high (≥ 85%) in spring and summer. MCL pneumonia was distributed throughout the year. However, remarkable higher rates were found in spring (38.6%; 61/158) and summer (33.5%; 60/179). The differences were statistically significant (P < 0.01) (data not shown).

**Discussion**
*M. pneumoniae* is a global, common bacterial agent causing acute respiratory problems in 10-30% of infected adults and children (16). In *C. pneumoniae* pneumonia, the rate of respiratory problems resulting from infection is 10-20% (17) and in pneumonia due to *L. pneumophila*, it ranges from 2-15% (18, 19).

In this study, an investigation combining serologic and molecular tests was performed to maximize the diagnostic yield.

Seven hundred and twenty-two children hospitalized for treatment of CAP were prospectively evaluated to elucidate microbiological characteristics and epidemiology of CAP due to MCL bacteria.

Overall, within the period examined in this study, a pathogen was found in more than half of enrolled pediatric patients with CAP (data not shown). Notably, MCL bacteria were determined approximately 30% of children with CAP, and *M* was the most common pathogen in 90% MCL-confirmed pneumonia children. These results are identical with our previous study (20) and other studies (21, 22, and 23).

Although, it has been reported that *M* causes mild respiratory infections (24) and is a cofactor in severe pneumonia (25), in our study, *M*-caused pneumonia were present in almost 90% cases and using the classification for severe pneumonia of PIDS and IDS of America (26), more than 45% (97/215) children with MCL pneumonia were severe pneumonia and rate of severe pneumonia due to *M. pneumoniae* alone was 56% when compared with the rate of severe pneumonia with co-infection (44%) (data not shown).

Therefore, apart from its co-infecting role in CAP requiring hospitalization, *M. pneumoniae* alone is also an important agent causing severe pneumonia requiring hospitalization in Vietnamese children.

Pneumonia due to *M, C* and *L* can occur in all age. It has been reported that: the high rate of atypical pneumonia was in school-age (27, 28, 29); *M. pneumoniae* and *C. pneumoniae*
were often etiologic agents in children older than five years and in adolescents (30); and children older than 2 years were particularly susceptible to *M. pneumoniae, C. pneumoniae* caused pneumonia (31, 32).

In Vietnam, there was not any basically epidemiological study on M-caused pneumonia. Based on analyzed reports of the published papers (31, 33, 34, 35), Vietnamese pediatricians often omit MCL bacteria in their diagnosis and treatment of pneumonia in children younger than two and also under the age of five years.

Our findings showed that out of 215 children with MCL pneumonia, about 56% children were aged younger than 2 years, and more than 77% children under the age of 5 years. The distributions of MCL-caused pneumonia by age were statistically significant (*P* < 0.0001 and *P* < 0.01) (data not shown). Our findings corroborate those of a previous report (36).

For this reason, although it has been stated that the child’s age is important in making the diagnosis of causative agent (30), but it is not exclusive factor in diagnosis of MCL pneumonia, especially, pneumonia due to M in younger children (under 5 years of age).

Generally, accepted national consensus statements and individuals’ recommendations for treatment of CAP in children are based on available evidence that ranges from informal expert opinion to well-designed prospective studies. Before this study, diagnosis and treatment of M-caused pneumonia in younger children were not much considered. As a result, the analyzed data which were associated with pathogenic role of MCL bacteria, especially *M. pneumoniae* causing LRTIs in children must be considered to guide appropriate diagnosis and antibiotic utilization.

In fact, most of the patients with result positive for MCL bacteria showed good outcome with appropriate antibiotic therapy (98.6% - data not shown).

Limitations of the study: many previous studies showed that *M. pneumoniae* infections have peak in 4 to 5 year cycles. We should have performed this study for a long time to see
full features of the disease in Vietnam. But, in our condition, the funding was unable to satisfy maximal requirement. Therefore, we could not extend the study in both time and adequate diagnostic tools (e.g. conventional PCR used instead of real-time PCR for detection of MCL bacteria, not any antigen detection test used for Legionella).

Because of limited funding for detection of MCL bacteria by real-time PCR, we optimized a multiplex PCR in both composition of the reaction and temperature condition with evaluated primer sets. Reliability of the multiplex PCR was also confirmed during performing the assay and evaluation of patient’s response to treatment. In our study process, no specimen displayed discordant results, the results of PCR positive for M, C and L bacteria were almost concordant with positive results in IgM antibody detection and most of the patients with positive results showed good outcome with appropriate antibiotic therapy except for three severe cases.

In conclusion, atypical pneumonia causative bacteria are important agents of CAP in children with a considerable rate, and were significantly associated with severity of pneumonia in Vietnam. Among those, *M. pneumoniae* was the most common pathogen that could be detected in numerous CAP children younger than two and/or five years old. Therefore, in Vietnam, pneumonia due to *M. pneumoniae* in younger children must be interested and appropriately considered in diagnosis and antibiotic utilization. It is necessary to monitor these atypical pneumonia causative bacteria in the system of respiratory infections surveillance.

**Acknowledgements**
This study was supported by The National Foundation for Science and Technology Development (NAFOSTED), grant no. 106.03-2010.36 from The Ministry of Science and Technology, Vietnam.

Sincere thanks to Prof. Nguyen Thanh Liem – Former Director of National Hospital for Pediatrics, Hanoi, Vietnam for supporting the study, Ms. Do Thi Bich Ngoc and to our colleagues at National Institute of Hygiene & Epidemiology and National Hospital of Pediatrics, Hanoi, Vietnam. The authors wish to thank Dr. Elaine Pamela Wright, Dr. Victor Fitzmaurice and Dr. Vu Tan Trao for critically reading the manuscript and English correction.

**Conflict of interest**
The authors declare that they have no competing interests.

**References**


10. Vircell Microbiologists: Mycoplasma pneumoniae ELISA IgM. Page 3; Legionella pneumophila Serogroup 1 ELISA IgM: 2-3; Chlamydophila pneumoniae ELISA IgM. Page 3. Vircell S. L., Granada, Spain


14. Keizo Matsumoto and Tsuyoshi Nagatake. Clinical Microbiology of Respiratory Infections. Text Book, Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Japan. 1994.


**Table 1.** Proportion of Patients with MCL Bacteria Positive Community-Acquired Pneumonia on the Basis of PCR and ELISA Findings

Total number of enrolled patients with community acquired pneumonia: **722**

Total number of atypical pathogen positive patients: **215 (29.78%)**

<table>
<thead>
<tr>
<th>Detected Pathogen</th>
<th>By only ELISA</th>
<th>By only PCR</th>
<th>By both PCR and ELISA</th>
<th>Total of atypical bacteria positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pneumoniae</em></td>
<td>9</td>
<td>42</td>
<td>139</td>
<td>190</td>
</tr>
<tr>
<td><em>C. pneumoniae</em></td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td><em>L. pneumophila</em></td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>
### Table 2. Distribution of MCL Bacteria-Positive Pneumonia by Infected Patterns

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Pneumonia due to MCL bacteria only*</td>
<td>154</td>
<td>71.63</td>
</tr>
<tr>
<td><strong>M, C, L bacterium alone</strong></td>
<td>144</td>
<td>67</td>
</tr>
<tr>
<td>(or <em>M. pneumoniae</em>, or <em>C. pneumoniae</em> or <em>L. pneumophila</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. pneumoniae</em></td>
<td>129</td>
<td>(89.58%)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em></td>
<td>08</td>
<td>(5.55%)</td>
</tr>
<tr>
<td><em>L. pneumophila</em></td>
<td>07</td>
<td>(4.86%)</td>
</tr>
<tr>
<td>Coinfection among M, C, L bacteria</td>
<td>10</td>
<td>4.65</td>
</tr>
<tr>
<td>+ <em>M. pneumoniae</em> + <em>C. pneumonia</em></td>
<td>09</td>
<td></td>
</tr>
<tr>
<td>+ <em>M. pneumoniae</em> + <em>L. pneumophila</em></td>
<td>01</td>
<td></td>
</tr>
<tr>
<td>(2) Pneumonia due to co-infection with other pathogens</td>
<td>61</td>
<td>28.37</td>
</tr>
<tr>
<td>(with typical pneumonia causative bacteria; respiratory viruses)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>100</td>
</tr>
</tbody>
</table>

* MCL bacteria only: pneumonia is caused by only one kind of MCL bacteria or ≥ 2 kinds of those MCL bacteria (typical pneumonia causing bacteria or viral pathogens were absent)

** M, C, L bacterium alone: pneumonia is caused by *M. pneumoniae* or *C. pneumoniae* or *L. pneumophila*
Table 3. Distribution of MCL Bacteria Positive Community-Acquired Pneumonia by Age

<table>
<thead>
<tr>
<th>Age</th>
<th>MCL bacteria positive cases</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- &lt; 2 y</td>
<td>120</td>
<td>55.81</td>
</tr>
<tr>
<td>2 - &lt; 5 y</td>
<td>47</td>
<td>21.86</td>
</tr>
<tr>
<td>5 - &lt;10 y</td>
<td>39</td>
<td>18.14</td>
</tr>
<tr>
<td>≥ 10 y</td>
<td>9</td>
<td>4.19</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>100</td>
</tr>
</tbody>
</table>