Original Article

Prognostic influence of toll-like receptor 4 gene polymorphism into community-acquired pneumonia course among young patients with cytomegalovirus persistence

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ABSTRACT

Objectives: The aim of this study was to determine the predictive role of TLR4 polymorphism in CAP course among young cytomegalovirus-positive patients. **Subjects and Methods:** One hundred and five patients with pneumonia (age range: 18–44 years) and 61 healthy respondents were observed clinically and specifically (by cytomegalovirus markers and TLR4 + 3725 G/C polymorphism). **Results:** Among CAP patients, there were 51 male (48.6%) and 54 female (51.4%), with average age 34.1 ± 0.8 years, and there were 19 (18.1%) patients with Pneumonia Patient Outcomes Research Team (PORT) I, 46 (43.8%) patients with PORT II, 31 (29.5%) patients with PORT III, and 9 (8.6%) patients with PORT IV. Cytomegalovirus persistence was detected in 80 (48.2%) patients and 34 (20.5%) healthy respondents ($P = 0.003$). G/G genotype of TLR4 signaling was found in 78 (74%) patients with pneumonia, G/C in 24 (23%) patients, and C/C in 3 (3%) patients. Among G/C patients, there were 16.2% cytomegalovirus-positive patients versus 6.7% negative patients ($P < 0.05$), as well as among G/G patients, and there were 59% versus 15,2%, accordingly ($P < 0.01$). The patients of the main group with G/G genotype were characterized by mostly mild (PORT I – 15 [14.3%]) and moderate pneumonia severity (PORT II – 32 [30.5%] and PORT III – 26 [24.8%] patients). The patients with G/C genotype were characterized by mostly PORT II (11 [10.5%] patients). All C/C genotype patients have PORT II ($P < 0.05$). **Conclusions:** Cytomegalovirus persistence worsens the pneumonia course. G/G and G/C TLR4 genotypes are associated with mild pneumonia severity.

KEY WORDS: Community-acquired pneumonia, cytomegalovirus persistence, risk class, TLR4

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INTRODUCTION

Community-acquired pneumonia (CAP) is a common infection associated with significant morbidity and mortality, with an annual incidence of 25 cases/10,000 adults and represents the eighth most common death cause in the USA.[1] The World Health Organization estimates that lower respiratory tract infection is the most common infectious cause of death in the world (the third most common cause overall), with almost 3.5 million deaths yearly.[2] Certainly, CAP rate is not decreased using new innovations in treatment and antibiotic generation. Such statistics among elderly population is explained

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as a result of chronic internal comorbidities influence into the fatal outcome of CAP.[3] The risk CAP factors among young population (18–44 years old according to the World Health Organization Classification) are controversial and are on the hypothesis stage. persistent asymptomatic diseases create an immunological basis for the attachment of the bacterial microflora and due to immunosuppression, worsen the course of CAP.[4] The most frequent cause of such persistent immunodeficiency can be asymptomatic cytomegalovirus infection (CMVI). Every third human of the planet gets persistent CMVI till the 30 years old.[5] Another great problem is late and incomplete investigation measures of CAP, especially its etiology. Several studies have shown that the yield of sputum Gram stain and culture in CAP is very low.[1] The incidence of positive blood culture in adult patients ranges 5%–27%, which leads to underestimation and the use of empirical therapy.[6] On the other hand, nonbacterial (viral and fungal) CAP etiology cannot be statistically recorded due to outpatient treating by different antibiotics.[7]

All these factors have different influence into CAP prognosis which depends on the genetic immunological response to the pathogen. The mutual burden of CMVI and CAP as comorbid states in the conditions of the existing immunodeficiency process due to its own cytopathic effect depends on the functional state of congenital immunity. A special group of innate immune receptors is the so-called "pattern recognition receptors," which play a leading role in the recognition of pathogenic factors. They recognize "pathogen-associated molecular patterns" which are called toll-like receptors (TLRs).[8,9] They contain a variable number of repetitive leucine-rich leukens that provide direct interaction of the receptor with the microorganisms' ligands or their products. The tertiary structure of this domain determines the specificity of TLR binding to certain PAMPs. This domain is involved in the transduction of the signal from the activated TLR inside the cell.[9]

The biological meaning of the activation of mechanisms of innate immunity by endogenous molecules is disturbed under the conditions of CAP with persistent CMVI, which results in an inadequate response of the immune system to signals of immune danger, and accordingly will disrupt antigenic homeostasis.[10] Hyperactivation of TLRs under the action of endogenous ligands can lead to the development of an excessive inflammatory response, accompanied by tissue damage. This can be considered as one of the main mechanisms of CAP-immunological pathogenesis. Pathological hyperreactivation of TLR is usually caused by genetic modifications in their allelic structure, which is genetically determined, manifested only with the active course of controlled pathology and is not subject to correction.[9-11] The effect of polymorphism of alleles such as TLR2, TLR3, and TLR9 on the course of CMVI in conditions of change in immunological reactivity is studied on an example of the characteristics of the course of myocardial infarction, stroke, and thrombosis of the veins of the lower extremities.[11] TLR4 polymorphisms are usually associated with pneumonia caused by *Streptococcus pneumoniae*,[12] septicemia of candidal etiology,[11] and spondyloarthritis. Therefore, our main aim and objectives were to determine the frequency of alleles with mononucleotide substitution of the +3725 G/C of the TLR4 gene in young patients with CMVI to predict the course of the CAP.

SUBJECTS AND METHODS

One hundred and five CAP patients and 61 healthy individuals (age range: 18–44 years) were observed in this study. The CAP diagnosis was established clinically according to the Order No. 128 of the Ministry of Health of Ukraine (2007) and the Clinical Instruction (2013).[13] It took into account the results of the analysis of clinical and functional signs of lung injury, assessing the severity of the clinical symptoms of the disease in the points (cough and its character [productive and unproductive]) and severity in scores of the analog scale from 0 to 3, the nature of sputum, the level of shortness of breath in Medical Research Scale[14] scores (from 0 to 5), and objective symptoms (physical and laboratory). The CAP severity was measured by the Pneumonia Severity Index (PSI) in the scale of Pneumonia Patient Outcomes Research Team (PORT). The PORT calculator added points due to the following criteria: gender, age, nursing home resident, neoplastic disease, liver disease history, congestive heart failure history, cerebrovascular disease history, renal disease history, altered mental status, respiratory rate, systolic blood pressure, temperature, pulse, pH, blood urea nitrogen, sodium level, glucose level, hematocrit, partial pressure of arterial O_2, and pleural effusion. The result was evaluated as follows: 0–50 points – Risk Class I (light CAP course without hospitalization urgency); 51–70 points – Risk Class II (absence of complications and low mortality level [0.6%]; no hospitalization urgency); 71–90 points – Risk Class III (moderate CAP course and mortality level [2.8%]; outpatient and clinical treatment possibility); 91–130 points – Risk Class IV (severe CAP course; hospitalization urgency); and >130 points – Risk Class V (severe CAP course; hospitalization urgency due to high risk of severe complications).[15]

The CMVI-persistence status was evaluated by specific analysis for CMVI-antibodies immunoglobulin G (CMV IgG). For excluding false-positive CMV IgG, the CMVI-persistence duration was measured by CMV IgG-avidity rate: 1%–39% – low avidity, early CMVI; 40%–60% – avidity of middle degree, reconvalescent CMVI; and >60% – high avidity, anamnestic CMV antibodies.[16]

The TLR4 +3725 G/C polymorphism was extracted from DNA samples isolated from peripheral blood lymphocytes of individuals. Electrophoretic fractionation of DNA fragments in agarose gel divided samples of the studied DNA according to the following genetic combinations: G/C heterozygotes, C/C homozygotes, and G/G homozygotes.[17]

Statistical data description was performed by SPSS 12.0 for Windows (Grand Pack, Serial Number 9593869, Deutschland).[18] Student's parametric test was used to compare two independent samples, and one-dimensional ANOVA dispersion analysis was used to compare >2 independent samples. Descriptive statistics was involved as well. The statistical probability of the investigator criterion was confirmed with an error value of $P < 0.05$.[18,19]

RESULTS

Among examined CAP patients, there were 51 men (48.6%) and 54 women (51.4%) of average age 34.1 ± 0.8 years. The gender classification in the control group found 26 men (42.6%) and 35 women (57.4%) of average age 31.4 ± 0.9 years. The responders of compared groups were representative by gender and age ($P = 0.53$). In the main group, the PORT analysis demonstrated 19 (18.1%) CAP patients with Risk Class I, 46 (43.8%) patients with Risk Class II, 31 (29.5%) patients with Risk Class III, and 9 (8.6%) patients with Risk Class IV.

The specific immunological test for CMV IgG showed the positive result for CMVI persistence among 80 (48.2%) young CAP patients and 34 (20.5%) healthy respondents ($\chi^2 = 8.685$; $r = -0.228$; $P = 0.003$). In the main group, low avidity was established in 7 (6.7%) CAP patients, avidity of middle degree – 23 (21.9%) patients, and high avidity was found in 51 (48.6%) CAP patients. Avidity frequency distribution in control group detected such situation: 1 (1.6%) respondents with low avidity, 5 (8.2%) respondents with moderate avidity, and 28 (45.9%) respondents with high avidity ($\chi^2 = 12.134$; $r = -0.116$; $P = 0.007$). The prevalence of CMVI persistence in CAP patients influenced the CAP severity by increasing of PSI: 80 (76.2 %) CMV-positive CAP-patients (5 [4.8 %] – Risk class I; 38 [36.2 %] – Risk Class II; 28 [26.7 %] – Risk class III; 9 [8,6 %] Risk class IV) versus 25 (23.8 %) CMV-negative ones (χ^2=33.323; r=0.472; P<0.0001).

During comparing the average values of the clinical CAP course, it was found that in the CAP patients with CMVI persistence, the average cough rate was 1.93 ± 0.08 points versus 1.42 ± 0.12 points ($P = 0.001$) in patients without positive markers of viral persistence. Correspondingly, the dynamics of the mean values of dyspnea ($P = 0.004$) and chest pain ($P < 0.0001$) were characterized. The average respiratory rate tended to increase among patients with CMV persistence and was 24.35 ± 0.57/min versus 22.25 ± 0.76/min among the CMV-negative respondents ($P = 0.067$). In addition, the presence of CMV persistence with a high degree of statistical probability increases the hospitalization period for the CAP patient. The average bed day for the CMV-positive respondent was 11.48 ± 0.27 days, whereas for the CMV-negative CAP patient was 9.13 ± 0.64 days ($P < 0.0001$). According to the status objectives among CAP patients with CMVI, the average values of the number of damaged lung segments ($P = 0.002$), the intensity of dull percussion sound ($P < 0.0001$), the severity of wet wheezing ($P = 0.047$) and crepitation ($P = 0.020$), and hyperthermia ($P = 0.013$) were higher compared to the similar indices in CMV-negative CAP patients.

The next step of our scientific work was to classify the respondents according to their TLR4 polymorphism variant and find the connection between CAP severity course genetic predispositions.

Frequency distribution of young CAP patients with TLR4 polymorphism with a monoclonal substitution of +3725 G/C revealed the highest prevalence in the G/G allele population (74.3%) [Figure 1]

Taking into account, the gender composition of the TLR4 polymorphisms surveyed in the group of CAP patients did not reveal any likely differences in the prevalence of a specific type of TLR4 genotype among a specific gender cohort ($P > 0.05$)

We investigated the distribution of respondents from the main group on the variants of the TLR4 of the +3725 G/C gene, taking into account the available CMV IgG to find the probable role of CMVI persistence in predicting the CAP course in patients with different combinations of TLR4 alleles [Figure 2].

The next step in the scientific search was the study of the distribution of respondents of CAP patients by variants of the +3725 G/C of the TLR4 gene and CMV IgG avidity. Among the CMV-positive patients, 62 respondents had G/G combination alleles, 2 patients had C/C, and G/C type allelic combination was found in 17 respondents. According to G/G variant of TLR4 polymorphism, 5 (4.8%) CAP patients had low avidity, 18 (1.1%) patients had moderate avidity, and 39 (37.1%) patients had high avidity. According to the G/C variant of TLR4 polymorphism, 1 (1.0%) CAP patient had low avidity, 4 (3.8%) patients

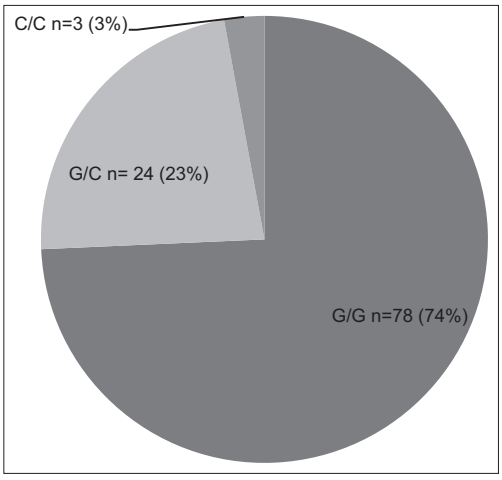

Figure 1: Frequency distribution of young community-acquired pneumonia patients with toll-like receptor 4 polymorphism with a monoclonal substitution of +3725 G/C

had moderate avidity, and 12 (11.4%) CAP patients had high avidity. According to the C/C variant of TLR4 polymorphism, 1 (1.0%) CAP patient had low avidity and 1 (1.0%) more had moderate avidity. No CAP patient with C/C variant of TLR4 polymorphism had high CMV IgG avidity. Certainly, the obtained results were not statistically reliable ($P = 0.40$) and could have been significant in the study of a larger population of patients.

The PORT Scale mortality association study for CAP patients with TLR4 polymorphism showed statistically significant differences in the frequency distribution of respondents [Figure 3].

DISCUSSION

According to our samples, females prevail in both comparison groups, which are suitable to the world gender statistics.[20] The majority (48.3%) of investigated CAP patients has Risk Class I and 8.6% CAP patients are characterized by the most severe CAP course and have Risk Class IV. Such distribution can be explained by young observation category and exclusion severe comorbid pathologies as additional factors for CAP course.

CMVI persistence index is reliably ($P = 0.003$) higher (more than two times) among CAP patients and calculates 48.2% versus 20.5% in control group. In this case, it is explained by widespread CMVI among the general population, and it is reflected in world statistics.[5] On the other hand, we pay attention for percentage differences between the comparison groups. The CMVI persistence predominance can testify for CMVI as a predictor of bacterial adjunctions in such patients. The modern medicine usually treats CMVI as a conclusion disease in immunocompromised individuals. Several studies can show us controversial opinion. CMVI is an independent source for immunological disorders and may have the bad influence into comorbid diseases of any etiology.[4] We also have used the distribution of respondents by CMV IgG avidity for avoiding false-positive results of CMVI persistence. The percentages levels have turned out to be reliably higher among CAP patients ($P = 0.007$). This feature speaks about long-term CMVI persistence in the main group. In addition, CMVI-positive status increases PSI among CAP patients ($P < 0.0001$), so worsens the CAP prognosis. It confirms the hypothesis of multifunctional mechanisms of CMVI influence into the immunological status of patients and the launch of prognostic adverse effects in CAP course.[21,22] The differences of clinical manifestations between CMVI-positive and CMVI-negative CAP patients fully confirm this hypothesis. clinical signs of CAP (cough and sputum) and objective data (percussion and auscultation phenomena) are much more extensive in individuals with CMVI-positive markers, which are statistically proven ($P < 0.05$).

The distribution of CAP young population according to TLR4 +3725 G/C polymorphism shows the statistically reliable domination of homozygote G/G variant, which is observed in 74.3% respondents ($P < 0.05$). As we look for new connections between CMVI and TLR4 signaling, the results of our study show that CMVI-positive patients with G/G polymorphism variant ($P < 0.01$) and G/C polymorphism variant ($P < 0.05$) have higher CAP catching risk than CMVI-negative patients. The same tendency among patients with C/C polymorphism variant is not statistically confirmed. Such connection can be valid only in the condition of exclusion of other comorbid pathologies. Frequency distribution of young CAP patients with TLR4 polymorphism +3725 G/C according to the PORT scale of mortality prediction shows that CAP patients with G/C and G/G genotypes are mostly characterized by mild CAP course (Risk Class II). The role of C/C genotype in CAP severity can be recognized by studying on a larger population. In our opinion, the C-allele appearance in TLR4 +3725G/C polymorphism worsens CAP-course prediction by PSI score elevation at young patients.

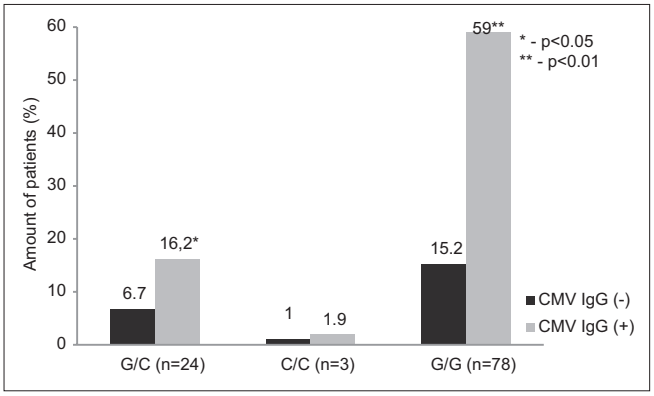

Figure 2: The distribution of respondents from the main group on the variants of the toll-like receptor 4 of the +3725 G/C gene according to the cytomegalovirus immunoglobulin G appearance

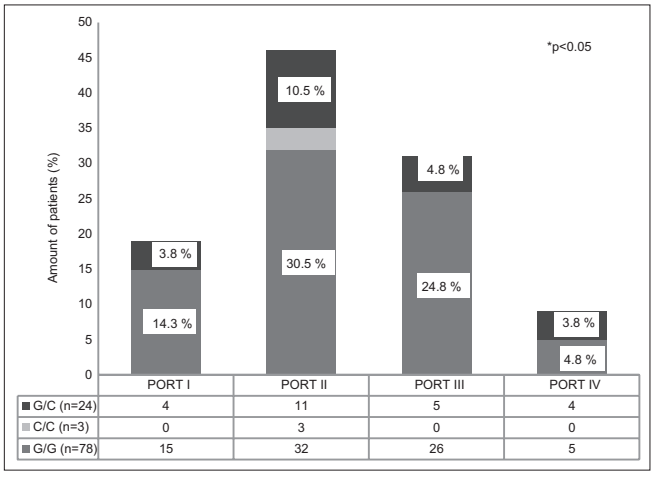

Figure 3: Frequency distribution of young community-acquired pneumonia patients with toll-like receptor 4 polymorphism +3725 G/C according to the Pneumonia Patient Outcomes Research Team scale of mortality prediction

CONCLUSIONS

Despite the large number of studies about CAP, it remains an urgent problem due to the high degree of complications and mortality. Young population is in a high risk group according to specificity of immunological mechanisms and genetic predisposition. The main conclusions of our investigation on this connection are next:

1. Young CAP-patients without comorbid immunological pathology are characterized by significantly high level of CMVI persistence (48.2 %) which may lead to severe complications of CAP-course.
2. The severity of CAP-course directly depends on CMVI-persistence duration.
3. The gomozygote G/G variant of TLR4 +3725 G/C polymorphism predominates among 74.3 % patients.
4. The mild CAP course is observed among G/G and G/C TLR4 signaling types. The influence of C/C genotype into CAP course needs further research.

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Conflicts of interest

There are no conflicts of interest.

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