Original Article

CD14 Polymorphism Is Not Associated with SARS-CoV-2 Infection in Central European Population

(COVID-19 / CD14 / polymorphism / SARS-CoV-2)

JAROSLAV A. HUBÁČEK[1,2], TOM PHILIPP[3], ONDŘEJ MÁJEK[4,5], DANA DLOUHÁ[2], VĚRA ADÁMKOVÁ[6], LADISLAV DUŠEK[4,5]

[1]Experimental Medicine Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[2]Third Department of Medicine, Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University, Prague, Czech Republic
[3]Clinic of Rheumatology and Physiotherapy, Third Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic
[4]Institute of Health Information and Statistics of the Czech Republic, Prague, Czech Republic
[5]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[6]Preventive Cardiology Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Abstract. A 2021 *in silico* study highlighted an association between the *CD14* polymorphism rs2569190 and increased susceptibility to SARS-CoV-2, which causes coronavirus disease 2019 (COVID-19). The aim of our study was to confirm this finding. We analysed the *CD14* polymorphism (C→T; rs2569190) in 516 individuals who tested positive for SARS-CoV-2, with differing disease severity (164 asymptomatic, 245 symptomatic, and 107 hospitalized). We then compared these patients with a sample from the general population consisting of 3,037 individuals using a case-control study design. In comparison with carriers of the C allele, TT homozygotes accounted for 21.7 % of controls and 20.5 % in SARS-CoV-2-positive individuals (P = 0.48; OR; 95 % CI – 0.92; 0.73–1.16). No significant differences in the distribution of genotypes were found when considering co-dominant and recessive genetic models or various between-group comparisons. The *CD14* polymorphism is unlikely to be an important predictor of COVID-19 in the Caucasian population in Central Europe.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020), has evolved rapidly to become a global pandemic – there have been 772,838,745 confirmed cases and 6,988,678 reported deaths worldwide (covid19.who.int, accessed at 23rd of December 2023), with the Czech Republic being between the most affected (Tuček and Vaněček, 2022).

Common symptoms of COVID-19 (regardless of age) include fever, cough, fatigue, and diarrhoea (Chams et al., 2020; Nayeri et al., 2021). Comorbidities such as obesity, diabetes, and hypertension are recognized as important predictors of increased disease severity. The spread of the disease is also exacerbated by viral shedding, a common biological process observed in symptomatic patients (Long et al., 2021). The COVID-19 course is influenced not only by host immune system variability (Paces et al., 2020), but an important factor is also genetic predisposition, which has been shown to be a predictor of both susceptibility to infection and COVID-19 severity (Kostrouch, 2020; Viza et al., 2020; Hubacek, 2021a; Delanghe and Speeckaert, 2022).

Pati et al. (2021) reported the results of an *in silico* study, which identified a potential association between the functional *CD14* polymorphism rs2569190 (C-159→T, C-260→T relative to the translation start site) and increased susceptibility to COVID-19. Based

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Corresponding author: Jaroslav A. Hubáček, IKEM-EMC, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic. Phone: +420 261 363 379; e-mail: jahb@ikem.cz

Abbreviations: CD14 – cluster of differentiation 14, CI – confidence interval, COVID-19 – coronavirus disease 2019, PCR – polymerase chain reaction, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

on an analysis of epidemiological data from 21 countries, the authors concluded that the T allele may be associated with an increased risk of SARS-CoV-2 infection and a higher COVID-19 mortality rate.

Cluster of differentiation 14 (CD14), also known as monocyte differentiation antigen (OMIM acc. No. 158120), is a protein expressed on the surface of monocytes. CD14 also exists in a soluble form, known as sCD14, which is released from the liver into the serum.

The *CD14* T allele is associated with increased levels of sCD14 (Panda et al., 2020) and CD14 density on monocytes (Hubacek et al., 1999). While the primary role of CD14 is to recognize bacterial lipopolysaccharides, it is also associated with the inflammatory host response in COVID-19 patients – significantly increased numbers of CD14-positive macrophages have been identified in various studies (Al Balushi et al., 2021; Bowman et al., 2021; Zingaropoli et al., 2021). Therefore, the *CD14* polymorphism seems to be a plausible candidate for estimating the course and severity of COVID-19.

Our study examined the *CD14* single-nucleotide variant rs2569190 in individuals with different levels of SARS-CoV-2 infection.

Material and Methods

We conducted a case-control study to validate the findings of an *in silico* analysis (Pati et al., 2021). We compared the frequencies of three *CD14* genotypes (TT, TC, and CC) in a group of adults who all tested positive for SARS-CoV-2 using polymerase chain reaction (PCR) testing (Hubacek et al., 2021b, 2021c, 2023a, 2023b). All of these individuals contracted COVID-19 during the first wave (approximately February 2020 to June 2020) of the disease in the Czech Republic. As a control group representing the general population, we used *CD14* genotype distributions (Hubacek et al., 1999, 2004) from the Czech arm of the post-MONICA study. Information on COVID-19 status and SARS-CoV-2 positivity was not available in control subjects. All examined subjects were of self-reported Caucasian ethnicity.

The respective ethics committees approved the study protocol, and all participants provided their informed consent to take part in the genetic testing.

DNA was isolated from whole EDTA blood samples, and the rs2569190 polymorphism was analysed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as previously described in detail (Hubacek et al., 1999).

Statistical analysis was performed using freely available calculators at www.socscistatistics.com, which are fully compatible with the SPSS statistical program (accessed August 2023). Data were analysed using the χ^2 test with 2 × 2 and 2 × 3 models. The odds ratio (OR) and 95 % confidence interval (CI) for 2 × 2 models were also calculated. The distributions of individual genotypes (TT, TC, and CC; TT as a reference) and alleles (T and C; T as a reference) between the groups were compared using three models: TT vs +C, TT vs TC vs CC, and +T vs CC. The level of P value < 0.05 was considered statistically significant.

Results

We included a total of 516 adults who tested positive for SARS-CoV-2 using PCR-based testing (Hubacek et al., 2021b, 2021c, 2023a, 2023b). Of these, 164 were asymptomatic, 245 were symptomatic without requiring hospital treatment, and 107 were hospitalized due to severe COVID-19. Among the hospitalized group, only samples from survivors were collected. For comparison purposes, we used *CD14* genotype distributions from a larger population of 3,037 adults as a control group (Hubacek et al., 1999, 2004). The general descriptions of the examined groups are given in Table 1.

The detailed *CD14* genotype distribution within each subgroup is summarized in Table 2. The *CD14* genotype distributions within both patients (P = 0.50) and the control group (P = 0.38) were in Hardy-Weinberg equilibrium.

Overall, we found no significant differences in the genotype distributions, regardless of the model or the type of comparison applied. There were no differences in the frequencies of TT homozygotes compared to carriers of at least one C allele in the general population when compared with SARS-CoV-2-positive subjects (P = 0.48, OR = 0.92, 95 % CI [0.73–1.16]). Additionally, we found no differences when comparing the various subgroups of patients categorized by different courses of the disease (Table 2).

Furthermore, when comparing allele frequencies (T vs C) between the control group and patients, we did not observe any statistically significant differences (P = 0.26, OR; 95 % CI –0.93; 0.81–1.06). Similarly, there were no significant differences when comparing the control group with patients exhibiting varying degrees of COVID-19 severity (Table 3).

Finally, we detected no differences in genotype (Table 2) or allele (Table 3) frequencies between the

Table 1. General characteristics of examined subjects

	Control group	SARS-CoV-2 patients	P
Number (N)	3.037	516	
Age (years)	47 ± 16	48 ± 18	0.79
Males (%)	46.5	45.1	0.57
Hypertension (%)	22.1	25.0	0.14
Obesity (%)	28.9	33.1	0.05
Diabetes (%)	8.1	10.2	0.10

Age is presented as the mean ± SD. Hypertension is defined as self-reported treatment or measured SBP/DBP values over 140/90, obesity as BMI over 30 kg/m^2, and type 2 diabetes as self-reported diabetes or diabetes treatment.

Table 2. Distribution of CD14 (rs2569190) genotypes in the control group and SARS-CoV-2-positive individuals

CD14	**Control group (population)**		**SARS-CoV-2 positive (total)**		**COVID-19 asymptomatic**		**COVID-19 symptomatic**		**COVID-19 hospitalized**	
Number	3,037		516		164		245		107	
Genotypes	N	%	N	%	N	%	N	%	N	%
TT	660	21.7	105	20.3	35	21.3	51	20.8	19	17.8
TC	1,485	48.9	247	47.9	72	43.9	123	50.2	52	48.6
CC	892	29.4	164	31.8	57	34.8	71	29.0	36	33.6
P			0.48*		0.91#		0.74§		0.33±	
OR (95 % CI)			0.92 (0.73–1.16)		0.98 (0.67–1.43)		0.95 (0.69–1.30)		0.78 (0.47–1.29)	

Calculated as the 2 × 2 χ^2 test for +C vs T/T individuals; *control group vs all SARS-CoV-2-positive patients; #control group vs COVID-19 asymptomatic individuals; §control group vs COVID-19 symptomatic patients; ±control group vs patients hospitalized with COVID-19.

Table 3. Distribution of CD14 (rs2569190) alleles in the control group and SARS-CoV-2-positive individuals

CD14	**Control group (population)**		**SARS-CoV-2 positive (total)**		**COVID-19 asymptomatic**		**COVID-19 symptomatic**		**COVID-19 hospitalized**	
Number	6,074		1,032		328		490		214	
Alleles	N	%	N	%	N	%	N	%	N	%
T	2,805	46.2	457	44.3	142	43.3	225	45.9	90	42.1
C	3,269	53.8	575	55.7	186	56.7	265	54.1	124	57.9
P			0.26*		0.31#		0.91§		0.23±	
OR (95 % CI)			0.93 (0.80–1.06)		0.89 (0.71–1.11)		0.99 (0.82–1.19)		0.85 (0.64–1.12)	

Calculated as the 2 × 2 χ^2 test for C vs. T individuals; *control group vs all SARS-CoV-2-positive individuals; #control group vs COVID-19 asymptomatic individuals; §control group vs COVID-19 symptomatic individuals; ±control group vs patients hospitalized with COVID-19.

three groups of patients sorted by the disease severity (asymptomatic, symptomatic, and hospitalized). The frequencies of the genotypes and alleles were similar across different stages of the disease severity.

Discussion

Our study is the first to investigate the *CD14* polymorphism rs2569190 in subjects with SARS-CoV-2 infection. We focused on comparing *CD14* genotypes in individuals who tested positive for SARS-CoV-2 infection (with different COVID-19 severity) and the general control population. Our results did not support the findings of the *in silico* study by Pati et al. (2021), who found that the T allele was associated with increased risk of SARS-CoV-2 infection.

Several other studies that have explored the role of either the *CD14* receptor polymorphism or sCD14 concentrations in the pathogenesis of coronavirus diseases have generated conflicting results.

In 2005 and 2006, the same *CD14* polymorphism came under scrutiny during the outbreak of severe acute respiratory syndrome (SARS) that was caused by a different type of coronavirus (Yuan et al., 2007). However, in the case of SARS, CC homozygotes, not TT homozygotes, were significantly more common among the patients with severe SARS than among the subjects with mild SARS or healthy controls.

The plasma sCD14 concentrations were found to double in COVID-19 subjects upon admission to hospital compared to healthy blood donors (Zingaropoli et al., 2021). Additionally, the frequency of large inflammatory monocytes ($CD14^+CD16^+$) proved to be an early predictor of ICU admission or death in COVID-19 patients (Al Balushi et al., 2021). Finally, Bowman et al. (2021) found that COVID-19 non-survivors had higher levels of sCD14 compared to patients with mild, moderate, or critical illness who recovered.

In contrast, a large multi-ethnic study (Zhu et al., 2021) found that CD14 receptor levels were not among the 18 genetically predicted proteins (out of 1,357 screened) associated with COVID-19 severity. Additionally, a genome-wide association study was unable to confirm the *CD14* region as an important player in COVID-19 aetiology (Severe Covid-19 GWAS Group et al., 2020).

As previously reported, the vulnerability to COVID-19 varies among different ethnic groups (Tai et al., 2021). The frequency of the putatively risky T allele in the *CD14* rs2569190 polymorphism is most common in

Asians at almost 60 % and lowest in Black Africans at about 30 %, while European populations fall between these two values. Interestingly, this distribution does not align with the theory that the *CD14* variant plays a significant role in the development of COVID-19 given that, in the United States, African Americans are at highest risk of COVID-19-associated mortality and White Americans are least susceptible to this disease (Tai et al., 2021).

In summary, these studies suggest that individuals who test positive for SARS-CoV-2 may have stimulated production of sCD14 and an increased number of $CD14^+$ monocytes. Such biological responses could result in these individuals in a poor prognosis of the disease. Increased CD14 concentrations are most likely a consequence of the viral infection. Therefore, as potential biomarkers of COVID-19 severity, they may be of potential use in assessing the progression of the disease. However, whether the T allele is associated with differences in sCD14 production or with an increased number of $CD14^+$ monocytes in COVID-19 patients requires further investigation.

A major limitation of our study is that although we had three different groups of SARS-CoV-2-positive subjects with different disease courses, we were not able (for ethical reasons) to collect a group of COVID-19 non-survivors. Theoretically, if TT homozygotes were at higher risk of COVID-19-associated mortality, we would anticipate a lower frequency of TT homozygotes among the hospitalized subjects. Although we did observe a slight deficiency in TT homozygosity within this group, the difference was marginal. Therefore, it is unlikely that an analysis of the *CD14* polymorphism has any clinical relevance for the risk of mortality associated with COVID-19.

Based on our results and some of the conflicting findings outlined above, a common functional polymorphism within the regulatory sequence of the *CD14* receptor gene (rs2569190) is unlikely to be a major causal determinant of susceptibility to SARS-CoV-2 infection or of the severity of COVID-19 in the Central European Caucasians.

Conflict of interest

There is no conflict of interest.

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