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A Multilayer Immune-Inflammatory Genetic Biomarkers in IRF5 Pathway as Contributors in Patient's Outcome with COVID-19

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Abstract

Background: COVID-19 is a disease produced by a viral infection peculiar to the SARS-CoV-2 and which produces an intense inflammatory response after the infectious process with participation of immune pathways, like IRF5, that could enhance post-infection actions. Thus, understanding the aspects of pathophysiological pathways for IRF5 involved in immunoinflammatory responses is essential to search for biomarkers that can help to identify early patients who would have the worst outcome.

Methods and Findings: A comparative study between two groups of patients with cases of COVID-19 divided considering the number of days on which the patient had manifestations that led him to hospitalization (7 days). Blood samples were collected to evaluate IL-6 and

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DNA. Polymorphisms in IRF5 pathways genes (*TLR7*, *IRF5*, *IL6*, *IFNA*, *IFNB1*, *IFNG*) were genotyping by TaqMan® assay using Real-Time PCR (Applied Biosystems). The rs2069849 showed the G allele more frequently in a group that present less than seven days of symptoms, and the same SNP was correlated with highest serum levels of IL-6.

Conclusions: Our results suggest that *IL6* gene polymorphism, involved in the IRF5 pathway, can be associated with the worst patient's outcome in COVID-19. The rs2069849 in the *IL6* gene have been linked and could, in the future, be used as biomarker.

Keywords

COVID-19; IRF5; IL6; Cytokines; Polymorphisms

Abbreviations

COVID: Corona Virus Disease of 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Corona Virus 2; TLR: Toll-Like Receptors; INFR: Interferon-Associated Transcription Factors; IFN- γ : Interferon Gama; IRF5: Interferon Regulatory Factor 5; IRFs: Interferon-Regulatory Factors; INF: Interferon; IFNB: Interferon Beta; DBD: DNA Binding Domain; TNF- α : Tumour Necrosis Factor-Alpha; NF- κ B: Nuclear Factor Kappa Beta; PCR: Polymerase Chain Reaction; HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; DNA: Desoxyribonucleic Acid; SNP: Single Nucleotide Polymorphism; ELISA: Enzyrna-Linked Immunosorbent Assay; IQR: Interquartile Range; ACE2: Angiotensin Converting Enzyme 2; TMPRSS2: Transmembrane Serine Protease 2; MCTD: Mixed Connective Tissue Disease

Introduction

A wide range of knowledge has been produced during the current Coronavirus Disease 2019 (COVID-19) pandemic period. There seems to be a consensus that there is a profile of development of severity in patients and for this reason several approaches are being used in the search for biomarkers that can help in approaches to patients after SARS-CoV-2 infection. In this context the genotypic differences of genes involved in this intricate immune-inflammatory response it could further expand the findings regarding this attempt to individualize the aspects involved with diagnosis, progression and outcome in COVID-19 [1,2].

Several mechanisms have been studied and much is known about the dynamics after infection by SARS-CoV-2, but it is increasingly evident from the multi-layered aspect involved in

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complex list of events that occur in the different tissues. However, the initial presence of the event referred to as cytokine storm and action of the Th1 and Th2 response culminating in the endothelial activation process and thrombogenic events appear to be common in the pathophysiology of COVID-19. Many molecules such as Toll-Like Receptors (TLR), interferon-associated transcription factors (INFR) are involved like secondary players in this context [3,4]. Recently, this group showed that differences between the pattern of severity in COVID-19 was strongly associated with the different levels of IFN- γ presented in the study subjects, evidencing that the differences among host immune responses play a major role in COVID-19 severity [5].

A central role in immune defense can be attributed to interferon. Type I and II works to improve the immunoinflammatory response against virus infection, for example. In this context, Interferon Regulatory Factor 5 (IRF5) belongs to a family of transcription factors, originally implicated in antiviral responses and interferon production. The regulatory factor for interferon 5 is one of nine members that belong to the family of transcription factors initially implicated in antiviral responses and in the production of type I interferon [6-8]. However, several discoveries have imputed that IRF5 acts as a central regulator of the inflammatory response and this becomes evident that when IRF5 can contribute to the pathogenesis of many inflammatory and autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus [9,10]. Moreover, IRF5 may represent a potential therapeutic target due to its relationship with physiological and pathological aspects.

The discovery of Interferon-Regulatory Factors (IRFs) dates back to 1988, when a cDNA clone encoding a mouse protein that binds to a virus-inducible enhancer element of the Interferon (IFN) β -coding (IFNB) gene was identified [11]. All IRF family members possess an N-terminal DNA Binding Domain (DBD) that is characterized by a series of five relatively well-conserved tryptophan-rich repeats [12,13]. IRF5 has emerged as another IRF family member that possesses tumor suppressor activity. It has been reported that IRF5 expression is reduced in human leukemia and human ductal carcinoma and that this correlates with disease stage [14,15]. Finally, the evidence indicates that IRF5 is critical for inducing apoptosis in response to DNA damage and therefore IRF5 would end up functioning as a tumor suppressor. Its action may be different from that produced by p53. However, IRF5 will have action on the transcriptional network underlying antiviral immunity [16].

Another important role attributed to IRF5 is its central role in inflammation. IRF5 mediates induction of proinflammatory cytokines such as interleukin-6 (IL-6), IL-12, IL-23 and Tumour Necrosis Factor-Alpha (TNF- α) and its recruitment to promoters of inflammatory genes is assisted by the NF- κ B p65 subunit RelA [17-19]. IRF5 is a key factor in defining the inflammatory macrophage phenotype and it is highly expressed in not only monocytes and macrophages but also in B cells and dendritic cells. Its expression in macrophages can be

upregulated in response to the inflammatory environment and to the stimulation with GM-CSF and IFN- γ [18,20,21].

A striking feature about infectious diseases in humans is their considerable inter-individual phenotypic variability, which can show variations from asymptomatic to lethal infectious processes. Therefore, a very clear hypothesis is that the inter-individual variability in the development of these diseases can be safely attributed in part to the variability observed in the human genes involved in the control of the immune system. Single Nucleotide Polymorphisms (SNP) are described as important causative agents of phenotypic changes resulting from these individual variations [22,23]. Thus, the aim of this study was to investigate polymorphisms in target genes involved with the immunoinflammatory response, having IRF5 as a central point, and their roles as prognostic factors in patients with COVID-19.

Methods

Study Design

This was a comparative study between two groups of patients (n=30) that used a fraction of original studied by Gadotti and colleagues (2020). The study was compounded by hospitalized patients diagnosed with COVID-19 from June to July 2020. The Marcelino Champagnat Hospital is located in Curitiba, Paraná in South region of Brazil. The patient was included after signing the consent form approved by the research ethics committee (3.944.734/2020). Blood samples from patients were collected to evaluate interleukin-6 [5].

Inclusion Criteria

COVID-19 infection was defined by clinical-radiological presentation plus a nasopharyngeal swab Polymerase Chain Reaction (PCR) positive to COVID-19. Inclusion criteria were hospitalized patients with moderate or severe confirmed COVID-19 infection.

Definition of Comparison Groups

The main questioning variable raised in this article was "what the number of days on which the patient had manifestations that led him to hospitalization". The first group included patients who were hospitalized with more than 7 days of symptoms (n=13) and the second group included patients with less than 7 days of symptoms (n=17).

Exclusion Criteria

Patients diagnosed with other viral infections, such as HIV, HCV, HBV, or another common respiratory virus, were excluded, as well as solid organ or hematological transplantation patients. Patients who used tocilizumab were also excluded.

DNA Collection, Purification and Marker Selection and Genotyping

The genetic materials used in the experiments was obtained from whole blood. The samples collected was centrifuged so that it was possible to obtain the buffy coat, which was separated for DNA extraction, according to the protocol for extraction of genomic DNA [24]. Genetic markers in gene locus were selected according to available information in the SNP info website and also for relevance in qualified articles [25]. After this search six SNP to *TLR7* (rs2241044, rs9606615, rs2241049, rs917864, rs879577 and rs2241043), four to *IRF5* (rs11761199, rs1874328, rs752637 and rs3807306), nine to *IL6* gene (rs1524107, rs2069835, rs2069837, rs2069838, rs2069840, rs2069842, rs2069843, rs2069845 and rs2069849), one for *IFNA* (rs10757212), one for *IFNB* (rs1051922) and three to *IFNG* (rs1861493, rs2069716 and rs2069718). The tag SNPs selected were genotyped by polarized fluorescence (Taqman probe-based methodology) using the ABI 7500 platform. After that, each SNP was assessed for genotypic modes of transmission (additive, dominant and recessive models).

Cytokine Evaluation

Blood samples were collected using a standard coagulation tube (SST II Advance, BD Biosciences) to obtain the serum, which was aliquoted and stored at -80°C until analysis. The interleukin-6 were measured using commercially available ELISA kits for IL-6 (ImmunoTools, Friesoythe, Germany), according to the manufacture instructions.

Statistical Analysis

Continuous variables were expressed as median values and Interquartile Range (IQR) and analyzed by Mann Whitney test. Categorical variables were expressed as absolute frequencies with proportions and analyzed by chi-square or Fisher exact test. A p -value < 0.05 was considered significant. All variables in the univariate model meeting a cut-off of $p < 0.1$ were included in the multivariable model. SPSS v23.0 (IBM, Chicago, IL) and GraphPad Prism v7 (GraphPad, San Diego, CA) were used for statistical analysis. The variable days of symptoms was split in > 7 days or ≤ 7 days using the optimal binning procedure on SPSS for all cytokines

included in the analysis. Bonferroni correction was used for multiple testing, and p -values < 0.002 were considered significant for genotype analysis.

Results

Table 1 shows that the group of patients in case group (< 7 days of symptoms before hospitalization) had more IL-6 inflammatory marker, although there is no statistical difference ($p=0.082$). With more than 7 days of symptoms before admission, no patient died, and the group that had less than seven days of symptoms before admission showed that seven patients died.

In Table 2, the *IL6* gene showed that the CC genotype in rs2069849 [C/T] was more frequent (63.0%; $p<0.037$) in the < 7 days group, and no heterozygote was observed in the same group.

Correlation between IL-6 tissue expressions to its gene polymorphisms can be observed in Table 3. In group that presented < 7 days of symptoms the *IL6* gene show six of nine SNP (rs1524107, rs2069837, rs2069838, rs2069842, rs2069843 and rs2069849) had any genotype significantly associated with higher tissue expression values prevailing in this association the presence of the homozygous genotype for the wild allele. Only 3 SNP in this gene no showed association (rs2069835, rs2069840 and rs2069845).

Variables	Days of symptoms >7 (n=13)	Days of symptoms ≤7 (n=17)	<i>p</i> -value
Age*	49.3±11.9	63.5±21.5	0.031 ^a
Gender**- Male	13 (61.9)	8 (38.1)	0.069 ^b
Gender**- Female	17 (89.5)	2 (10.5)	
Died (yes)	0 (0.0)	7 (100.0)	0.008 ^a
Expression of IL-6*	498.5±251.1	1515.7±1068.3	0.082 ^a
* Mean±Standard Deviation; **Absolute number (percentage); a Mann-Whitney U <i>p</i> -value; b Pearson's chi-square test <i>p</i> -value; c Fisher's Exact Test.			

Table 1: Baseline and immunohistochemical characteristics in both groups characterized by the "symptom days before hospitalization" aspect.

Reference SNP † Allele variation [1/2]	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	<i>p</i> -value
rs1524107 [C/T]	CC	CT	TT	
Days of symptoms > 7	12 (42.9)	1 (50.0)	0 (0.0)	0.844
Days of symptoms ≤ 7	16 (57.1)	1 (50.0)	0 (0.0)	
rs2069835 [T/C]	TT	CT	CC	
Days of symptoms > 7	11 (45.8)	2 (50.0)	0 (0.0)	0.435
Days of symptoms ≤ 7	13 (54.2)	2 (50.0)	2 (100.0)	
rs2069837 [A/G]	AA	AG	GG	
Days of symptoms > 7	10 (41.7)	3 (50.0)	0 (0.0)	0.842
Days of symptoms ≤ 7	14 (58.3)	3 (50.0)	0 (0.0)	
rs2069838 [C/T]	CC	CT	TT	
Days of symptoms > 7	11 (47.8)	1 (100.0)	0 (0.0)	0.512
Days of symptoms ≤ 7	12 (52.2)	0 (0.0)	0 (0.0)	
rs2069840 [C/G]	CC	CG	GG	
Days of symptoms > 7	5 (33.3)	2 (33.3)	2 (100.0)	0.182
Days of symptoms ≤ 7	10 (66.7)	4 (66.7)	0 (0.0)	
rs2069842 [G/A]	GG	GA	AA	
Days of symptoms > 7	13 (43.3)	0 (0.0)	0 (0.0)	---
Days of symptoms ≤ 7	17 (56.7)	0 (0.0)	0 (0.0)	
rs2069843 [G/A]	GG	GA	AA	
Days of symptoms > 7	12 (44.4)	1 (100.0)	0 (0.0)	0.240
Days of symptoms ≤ 7	15 (55.6)	0 (0.0)	2 (100.0)	
rs2069845 [A/G]	AA	AG	GG	
Days of symptoms > 7	6 (46.2)	6 (46.2)	1 (25.0)	0.729
Days of symptoms ≤ 7	7 (53.8)	7 (53.8)	3 (75.0)	

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rs2069849 [C/T]	CC	CT	TT	
Days of symptoms > 7	10 (37.0)	3 (100.0)	0 (0.0)	0.037
Days of symptoms ≤ 7	17 (63.0)	0 (0.0)	0 (0.0)	
† SNP identifier based on NCBI dbSNP; Genotype was expressed by number and percentage and a total percentage was show in line; * Logistic regression p-value. After the Bonferroni test, the <i>p</i> -value <0.002 can be considered significant.				

Table 2: Genotypic analysis in *IL6* gene in additive model distribution in symptom days before hospitalization groups.

Reference SNP [†] and allele variation [1/2]	Homozygous 1/1	<i>p</i> -value ^a	Heterozygous 1/2	<i>p</i> -value ^a	Homozygous 2/2	<i>p</i> -value ^a
rs1524107 [C/T]	CC		CT		TT	
>7	498.5±251.1	0.025	NA	NA	NA	NA
≤7	1454.5±1086.1		NA		NA	
rs2069835 [T/C]	TT		TC		CC	
>7	536.1±245.6	0.083	NA	0.102	NA	NA
≤7	1286.9±1038.1		2352.5±280.7		1823.0±1716.8	
rs2069837 [A/G]	AA		AG		GG	
>7	439.5±259.6	0.088	675.5±144.9	0.001	NA	NA
≤7	1259.0±1068.8		2456.6±127.9		NA	
rs2069838 [C/T]	CC		CT		TT	
>7	469.8±256.7	0.013	NA	NA	NA	NA
≤7	1616.0±1061.3		NA		NA	
rs2069840 [C/G]	CC		CG		GG	
>7	593.5±260.9	0.345	317.0±115.9	0.316	636.0±89.0	NA
≤7	1455.3±1156.4		1294.3±1088.3		NA	
rs2069842 [G/A]	GG		GA		AA	

>7	498.5±251.1	0.016	NA	NA	NA	NA
≤7	1515.7±1068.3		NA		NA	
rs2069843 [G/A]	GG		GA		AA	
>7	469.8±256.7	0.007	NA	NA	NA	NA
≤7	1706.5±1033.0		NA		370.5±197.2	
rs2069845 [G/A]	GG		GA		AA	
>7	NA	0.592	557.6±385.9	0.152	479.0±201.3	0.186
≤7	1612.0±1327.9		1711.4±1198.8		1203.2±958.6	
rs2069849 [C/T]	CC		CT		TT	
>7	536.1±245.6	0.029	NA	NA	NA	NA
≤7	1515.7±1068.3		NA		NA	
† SNP identifier based on NCBI dbSNP; * Mean±Standard Deviation for tissue expression of IL-6; NA: not available; a Pearson's chi-square test.						

Table 3: Correlation between tissue expression and genotyping in *IL6* gene in groups characterized by the "symptom days before hospitalization".

Discussion

COVID-19 is a very complex disease which can be considered the outcome of many factors. However, the genetic component seems to have a great weight in the different outcomes among affected patients. Thus, the search for candidate genes has great interest in the knowledge of the pathophysiology of COVID-19. More than 7 days with symptoms before hospitalization, it can be interpreted that the symptoms presented were slight and that is why the patient did not seek the hospital network. Perhaps this profile is of more protected patients.

Due to its strong inflammatory immune component, several pathways are open to research. Thereby, the IRF5 pathway is quite promising, since the signalling end involves in the production of molecules such as IL-6 and others, which are key molecules and already known to be important in the process of beginning, progression and outcome in COVID-19. Thus, understanding the involvement of the multilevel members belonging to this IRF5 pathway could help the search for biomarkers involved with the pathophysiology of COVID-19.

The human IRF family contains a conserved DNA binding domain that recognizes a consensus DNA sequence known as the IFN-stimulated response element called ISRE [26]. ISREs are

found in the promoters of various IFN signature genes [12]. The classic production positively regulated by IRF5 of IFN type I (α and β) affects cytokine-producing inflammatory cells, such as macrophages, natural killer cells and cytotoxic CD8 T-cells, which perform the cytotoxic effector function. Activation of these cells by IFN type I results in tissue damage at the place where the cell meets pathogens.

Some studies initially focused on polymorphisms in the elements that help the viral infection process into host cells, such as ACE2 and TMPRSS2 [27]. In the Italy samples, the TMPRSS2 gene have been suggested as novel candidates in the severity of COVID-19 [28]. Using the fundamentals of the pathophysiology of COVID-19, several studies will likely be performed. As an important molecule in the context of the cytokine storm, IL-6 was the target of work involving genetics, even because this gene has been associated with several types of lung disease like chronic obstructive pulmonary disease and pneumonia and certain viral infections such as Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) and influenza virus [29-34].

In group of patients who have had symptoms for less than seven days, that is, patients with more severe symptoms, the wild C allele (*IL6* rs2069849 C/T) was more frequent and can be considered like a risk allele in this analysis (Table 2). Recently, Strafella and colleagues (2020) investigated the distribution of genetic variations in *IL6* and *IL6R* genes. Three synonymous polymorphisms (rs140764737, rs142164099, rs2069849), which may be employed as prognostic and pharmacogenetic biomarkers for COVID-19 and neurodegenerative diseases. Other studies have shown an association of this polymorphism in various pathologies [36-38].

The other important find in our results showed that the serum levels of IL-6 were higher in the group of patients with less than 7 days of symptoms before hospital admission (Table 1). Despite not having statistical significance ($p=0.082$), these results are corroborated by other findings [39-41]. Thus, these results are of biological importance, ratifying the current literature. IL-6 levels were almost 3 times higher in the case group (<7 days). One of the conclusions of this observation is that patients who progressed to more severe conditions initially already have high levels of this important marker. This observation could help to further consolidate these serum markers in COVID-19. Regarding the correlation between serum dosages and polymorphisms in *IL6* (Table 3), it is noteworthy that rs2069849 (C/T) was significant ($p=0.029$) showing higher serum values of IL-6 with this SNP. The same marker showed significance ($p=0.037$) in the univariate analysis (Table 2). The classic functional polymorphism rs1800795 (G174C) was associated with low production of this IL-6 in patients who did not clear HCV infection [42,43]. IL-6 plays a central role regulator for CD4 T cells and the polymorphism studied in the *IL6* gene could be used as an indicator to establish the severity of SARS-CoV-2 infections in COVID-19, including the susceptibility of some individuals to have symptoms or not or to have immunity to infection previously in various diseases (Supplementary Table 1-3) [31-34,44,45].

Our study has some limitations and strengths that merit consideration. As limitations, our study evaluated the first patients admitted during the outbreak in our city. Therefore, changes in clinical management during the evolving epidemics might have a differential impact on our studied outcomes. Our limited sample size might have decreased our power; however, also because of the pandemic, the findings of this study offer new, potentially useful information for this patient population. On the other hand, our grouping by symptom days may have standardized the disease's different stages when patients were admitted. The biological mechanism is a complex system and different phenotypes may be associated with different genetic markers. However, the authors believe that their findings, despite having little statistical significance, are covered with biological plausibility in such an important pandemic context. An important strength of this study was the determination of the correlation between IL-6 dosages and the 9 polymorphisms of the same gene. Even so, it is important to interpret these findings with caution until they are confirmed in larger and ethnically different populations.

Conclusion

Understanding the action of biomarkers in the pathophysiology of diseases can help in several aspects, such as identifying more susceptible individuals in a given group or directing these patients to specific treatments. Thus, the rs2069849 in the *IL6* gene was associated with patients with worse progression characteristics, and thus this polymorphism could be considered an important biomarker associated with worse progression in COVID-19 patients.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary Tables

Reference SNP † Allele variation [1/2]	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	p-value *
rs1634323 [A/G]	AA	GG	AG	
Days of symptoms > 7	10 (40.0)	3 (75.0)	0 (0.0)	0.285
Days of symptoms ≤ 7	15 (60.0)	1 (25.0)	0 (0.0)	
rs179008 [A/T]	AA	AT	TT	
Days of symptoms > 7	10 (40.0)	1 (100.0)	2 (50.0)	0.474
Days of symptoms ≤ 7	15 (60.0)	0 (0.0)	2 (50.0)	
rs5741880 [G/T]	GG	GT	TT	
Days of symptoms > 7	10 (52.6)	1 (20.0)	2 (40.0)	0.464
Days of symptoms ≤ 7	9 (47.4)	4 (80.0)	3 (60.0)	
rs179010 [C/T]	CC	CT	TT	
Days of symptoms > 7	3 (37.5)	1 (33.3)	3 (60.0)	0.849
Days of symptoms ≤ 7	5 (62.5)	2 (66.7)	2 (40.0)	
rs179016 [C/G]	CC	CG	GG	
Days of symptoms > 7	6 (66.7)	2 (66.7)	4 (26.7)	0.214
Days of symptoms ≤ 7	3 (33.3)	1 (33.3)	11 (73.3)	
rs179012 [A/G]	AA	AG	GG	
Days of symptoms > 7	4 (66.7)	3 (75.0)	6 (30.0)	0.110
Days of symptoms ≤ 7	2 (33.3)	1 (25.0)	14 (70.0)	
† SNP identifier based on NCBI dbSNP; Genotype was expressed by number and percentage and a total percentage was show in line; * Logistic regression p-value.				

Supplementary Table 1: Genotypic analysis in *TLR7* gene in additive model distribution in symptom days before hospitalization groups.

Reference SNP † Allele variation [1/2]	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	<i>p</i> -value *
rs11761199 [A/G]	AA	AG	GG	
Days of symptoms > 7	4 (40.0)	7 (43.8)	2 (50.0)	0.942
Days of symptoms ≤ 7	6 (60.0)	9 (56.2)	2 (50.0)	
rs1874328 [A/G]	AA	AG	GG	
Days of symptoms > 7	6 (60.0)	4 (28.6)	3 (50.0)	0.289
Days of symptoms ≤ 7	4 (40.0)	10 (71.4)	3 (50.0)	
rs752637 [C/T]	CC	CT	TT	
Days of symptoms > 7	4 (44.4)	7 (50.0)	2 (28.6)	0.644
Days of symptoms ≤ 7	5 (55.6)	7 (50.0)	5 (71.4)	
rs3807306 [G/T]	GG	GT	TT	
Days of symptoms > 7	5 (55.6)	6 (40.0)	2 (33.3)	0.651
Days of symptoms ≤ 7	4 (44.4)	9 (60.0)	4 (66.7)	
† SNP identifier based on NCBI dbSNP; Genotype was expressed by number and percentage and a total percentage was show in line; * Logistic regression <i>p</i> -value.				

Supplementary Table 2: Genotypic analysis in *IRF5* gene in additive model distribution in symptom days before hospitalization groups.

Gene - Reference SNP † Allele variation [1/2]	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	<i>p</i> -value
IFNA - rs10757212 [A/G]	AA	AG	GG	
Days of symptoms > 7	2 (66.7)	7 (87.5)	12 (63.2)	0.448
Days of symptoms ≤ 7	1 (33.3)	1 (12.5)	7 (36.8)	
IFNB - rs1051922 [G/A]	GG	GA	AA	
Days of symptoms > 7	8 (47.1)	4 (40.0)	1 (33.3)	0.877
Days of symptoms ≤ 7	9 (52.9)	6 (60.0)	2 (66.7)	

INFG - rs1861493 [A/G]	AA	AG	GG	
Days of symptoms > 7	8 (53.3)	4 (33.3)	0 (0.0)	0.270
Days of symptoms ≤ 7	7 (46.7)	8 (66.7)	2 (100.0)	
INFG - rs2069716 [C/T]	CC	CT	TT	
Days of symptoms > 7	2 (66.7)	NA	4 (33.3)	0.292
Days of symptoms ≤ 7	1 (33.3)	NA	8 (66.7)	
INFG - rs2069718 [A/G]	AA	AG	GG	
Days of symptoms > 7	4 (50.0)	5 (29.4)	4 (80.0)	0.121
Days of symptoms ≤ 7	4 (50.0)	12 (70.6)	1 (20.0)	
† SNP identifier based on NCBI dbSNP; Genotype was expressed by number and percentage and a total percentage was show in line; Logistic regression <i>p</i> -value. NA: not available.				

Supplementary Table 3: Genotypic analysis in *INFA*, *INFB*, *INFG* genes in additive model distribution in symptom days before hospitalization groups.