SARS-CoV-2 Variants and Clinical Outcomes

Subjects: Infectious Diseases

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From the start of the COVID-19 pandemic, new SARS-CoV-2 variants have emerged that potentially affect transmissibility, severity, and immune evasion in infected individuals. Conclusions: SARS-CoV-2 variants can potentially have an impact on clinical outcomes.

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1. Introduction

Variability in organisms leads to important changes which will have an effect on the course of their evolution [1][2]. In viruses, changes can determine their pathogenicity and virulence [3][4]; even single base changes can markedly influence their spread and confer selective advantages [5].

Since the beginning of the COVID-19 pandemic, it has been reported that SARS-CoV-2 has presented multiple changes in its genetic sequence that can potentially increase its infectivity, pathogenicity and antigenic capacity. This could affect the individual's immune response and increase the severity of the clinical outcomes in each of the outbreaks [6][7]. One of the first variants to be recognized was D614G in the spike protein [6][8], and as genome sequencing subsequently progressed in different countries, it was reported that different mutations influence the adaptation of the virus to environmental and population contexts, in addition to conferring various phenotypes of clinical interest [9][10].

The clinical course caused by SARS-CoV-2 is associated with country-specific epidemiological and health contexts, age, pre-existing diseases, comorbidities, and host allelic variations [11][12]. However, meta-analyses and observational studies have shown that the so-called Variants of Concern increase the risk of disease severity and death, compared to other non-VOC variants, including the original Wuhan or "wild-type" variant [13][14]. This opens multiple questions about the interrelationship of the factors that condition the body's responses to SARS-CoV-2 infection and emphasizes the need to study those variables that could impact the outcome of the infection; one question of importance is the interrelationship between variants of the virus and their clinical outcomes, an aspect that, due to the social, biological and methodological heterogeneity of the available evidence, has thus far not been explored in depth [13][14][15].

2. SARS-CoV-2 Variants and Clinical Outcomes

2.1. SARS-CoV-2 Variants

One of the first variants reported in the COVID-19 pandemic was D614G in the spike protein, which is associated with an increase in viral load, immune escape, possible drug resistance and increased pathogenicity. This amino acid substitution has been maintained in the different current variants. [16][17][18][19][20].

It has been pointed out that the region coding for the receptor binding domain (RBD) of the spike protein is prone to accumulate changes in SARS-CoV-2; 13 articles analyzed reported substitutions along this region, among them: N501Y, E484K, N439K, S477N, S399P, and K417V. It has been proposed that changes in this region could alter binding affinity of SARS-CoV-2 for ACE2 [11][13][15][21][22][23][24][25][26][27][28][29][30].

Another reported variant in the spike protein was P681H, which is located near the furin cleavage site and is associated with increased transmissibility and infectivity of SARS-CoV-2 [24][27]. The main Variants of Concern present changes in sequences associated to the spike protein, in the RBD and RBM (receptor binding motif) and the furin cleavage site. Some of the most relevant changes in the spike protein are illustrated in **Figure 1**.

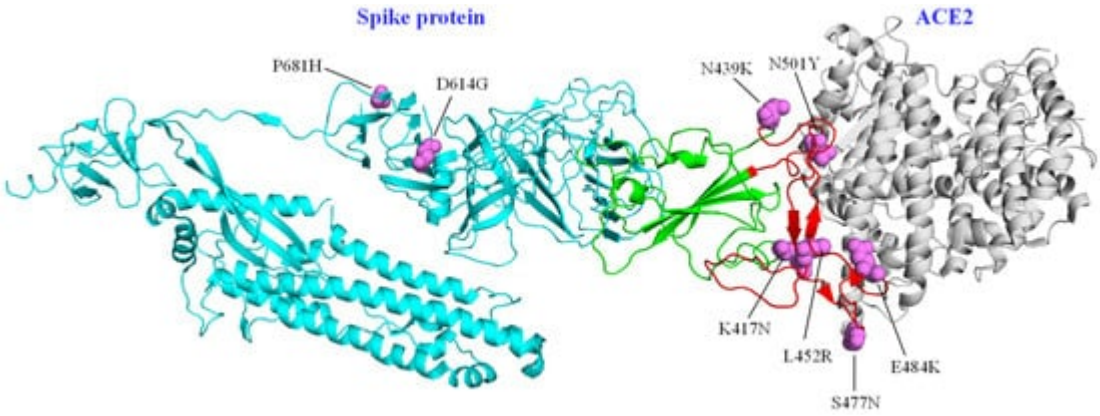

Figure 1. Main changes in spike protein reported in articles analyzed. ● Protomer of the spike protein; ● RBD; ● RBM; ● amino acid substitutions, ● ACE2 protein, (PDB structure [31][32], PyMOL v.4.6).

The changes in SARS-CoV-2 are distributed in various sites in its sequence-like spike protein, N protein, RNA-dependent RNA polymerase (RdRp), NSP3, NSP4 and other open reading frames (ORFs) (**Table 1**).

Table 1. Changes in SARS-CoV-2 sequences reported in the studies. ¤ Changes in nucleotide sequences.

Changes	Location	Sources
▪ D614G, V16F, V367L, K558N, Q675H, A879V, L452R, S939F, V1176F, K1191N, G1219V, S399P, L54F, N501Y, E484K, S477N, L5F, V213A, S689R, A570D, T716I, S982A, D1118H, P681H, N439K, V83L, W258R, Q677H, N811I, S640A, V6FS, H66D, D215G, V483A, H655Y, G669S, Q949R, and N1187D. ▪ m6 A methylation.	Spike protein (S)	[6][16][17][18][19][20] [21][22][24][25][26] [27][28][29][30][33] [34][35][36][37][38]

Changes	Location	Sources
▪ Non-synonymous 21,575; 25,106; 23,403; 24,099, and 24,453. ¤ ▪ Deletion 21,603–21,614. ¤		
▪ R203K, I292T, G204R, S202N, M234I, A376T, S194L, P13L, A119S, Q160R, S193I, R195S, P199S, V30L, G212V, and S197L.	Nucleocapsid phosphoprotein (N protein)	[39][18][22][24][27] [29][30][37][38]
▪ L3606F, and C370R. ▪ Synonymous 19,944, and 20,764. ¤ ▪ Insertion 11,074. ¤	ORF1a	[39][21][26]
▪ A138T.	NSP1	[38]
▪ T85I, A205V, V247A, T256I, Q321K and T814I.	NSP2	[24][27][33][38]
▪ F106F, P822L, P679S, T1022I, A1179V, T1198K, F1354C, P1665L, L916, F924, D1585, N1673, and 8782C.	NSP3	[39][18][27][33][35] [36][38]
▪ F308Y, S76S, A231V, E3073A, and A323S.	NSP4	[18][27][29][33][35] [38]
▪ E3909G.	NSP7	[38]
▪ A21T and T4040I.	NSP8	[27][38]
▪ L42F.	NSP9	[27]
▪ A176S, P314L and V767L.	NSP12	[39][22][35]
▪ P504L, Y541C, T127I, T153I, V169F, M576I, S5398L, and P203L.	NSP13	[27][33][35][38]

Changes	Location	Sources
▪ L7L.	NSP14	[33][35]
▪ H337Y.	NSP15	[27]
▪ Y222C.	NSP16	[27]
▪ G251V, G196V, S253P, Q57H, A54V, A99S, T151I, and D222Y. ▪ Deletion 25,710–25,715. ¤	ORF3a	[39][18][22][24][27][29][30][33][36][37][38][40]
▪ I33T.	ORF6	[18][37]
▪ Deletion 27,508–27,751. ¤	ORF7b	[37]
▪ L84S.	ORF8	[17][18][29][30][33][35][40][41]
▪ A97V, P323L, P232L, P227L, T248I, A656S, H892Y, M906V; G227A; C865T; Y4424; P4715L, 14408C, and C14408T. ▪ Nucleotic substitution nt14408	RdRp	[39][17][18][19][21][24][27][29][30][33][36][37][38][42]
▪ G3728S.	3C-like protease	[38]

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2.2. SARS-CoV-2 Variants and Clinical Outcomes

3. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. N. Engl. J. Med. 2009, 360, 2605–2615.

Prior to the reporting of Variants of Interest (VOI) and Variants of Concern (VOC), changes in the SARS-CoV-2 sequence that could have an impact on clinical outcomes had been determined [6]. The D614G variant in the spike protein was initially considered to be related to a higher rate of hospitalizations and moderate to severe clinical outcomes [6][17]; however, analyses in different cohorts showed no relationship with disease severity; this change increases the adaptability of the virus in human populations without necessarily causing more severe disease [19][37]. The same scenario was visualized for the N439K variant in the spike protein, which was also not found to have a direct effect on clinical outcomes, compared to the original virus. However, it was reported that this substitution

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had arisen in different clades independently and that it increased affinity for ACE2 and the resistance against various neutralizing monoclonal antibodies [25].

A study determined that polygenic mutations in SARS-CoV-2 had different outcomes. For mild disease, the following amino acid changes were detected: L84S, G196V in ORF8 and ORF3a, respectively, as well as L37F substitutions in NSP6, F308Y in NSP4 and S197L in the N protein. When analyzing sequences of hospitalized patients, 15 changes distributed in seven genes were found: three in the spike protein, two in RdRp, two in ORF3a, five in NSP3, one in ORF6 and two in NSP3, while in fatal outcomes, L71F changes were found in NSP7 and S253P in ORF3a [18].

In a study where associations between different mutations and clinical outcomes were analyzed, Zekri et al. [38] found in a sample of 50 patients that the V6 deletion in the spike protein was associated with an increased risk and duration of fever and nasal congestion, while the L3606-Nsp6 deletion was associated with an increased presence of diarrhea and conjunctival congestion.

When variants with changes in P504L, as well as Y541C in NSP13 were analyzed, an association was found between these with infection and mortality rates, without correlation with other studies [35]. Likewise, the N501Y variant in the spike protein was found to have an increase, without statistical significance, of 18% in terms of risk of fatal outcome [28].

In silico studies have allowed for a proposal that there are mutation signatures responsible for promoting mild and severe outcomes, in which 20 mutations could be used to separate both groups. These are distributed in the gene encoding the spike protein, as well as in other viral proteins and in untranslated regions (UTRs). [29] This has allowed for development of models to predict the degree of severity by adjusting the age of patients and analyzing their viral sequences (https://covidoutcome.com/, accessible from 27 December 2021).

It has been proposed that mutations in ORF1a, ORF1b and in genes encoding N protein were related to a high prevalence of asymptomatic scenarios. However, when D614G, Q57H (ORF3a) and S194L (N protein) changes were present, they were associated with mild and severe outcomes. Likewise, a single nucleotide change (nt14408) in RdRp was associated with severe cases of the disease [30].

Regarding prolonged viral RNA shedding, which can be up to 100 days in patients with severe disease, one study reported that viral shedding time decreases when A1,430G or C12,473T mutations are present and increases when G227A is present ($p < 0.05$). Likewise, mutations in G227A, C7,392T, C15,324T, and C25,626T were mostly represented in severe disease cases [42].

The analysis of SARS-CoV-2 variants and their impact on clinical outcomes must be seen from an integral perspective; thus, the different levels of structural organization that make up the variants must be evaluated. In this context, it was determined that three structural changes at the RNA and protein levels, specifically A26194T

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(T265I) and C25611A (synonymous mutation) in the ORF3a region and C28854T (S194L) in the N protein were associated with an increase in severe cases and fatal outcomes (p < 0.05) [39].

Methylation at the m6 A loci of the spike protein has been identified in patients debuting with gastrointestinal symptoms, which could provide underlying mechanisms for its change in virulence and transmission capacity during outbreaks and affect the outcome for serious and severe disease [16].

2.3. Rise and Spread of Variants of Concern

Chronologically, the reported VOCs in the studies analyzed were:

Beta (B.1.351): it was first documented in May 2020, in addition to the D614G substitution, this variant presents other changes such as E484K and N501Y that confer the capacity of immune escape by the effect of previous infection or vaccination; the increase in its transmissibility has been estimated at around 50% compared to the Wuhan variant [24].

Alpha (B.1.1.7): identified in September 2020, presents a 70% increase in transmissibility, consequence of key changes, specifically in the RBM (N501Y) and near the furin cleavage site (P681H), which could increase the affinity for ACE2 and have an impact on infection and transmission, respectively [24]. This could have contributed to the rapid dispersion and dominance of this variant in the world before the arrival of the Delta variant (B.1.617.2). [11][43][44]

Delta: identified in October 2020, it has become the most common variant globally, its main changes are D614G, E484Q and L452R, it has been reported that this variant has biological and clinical implications such as increased risk of hospitalization, longer duration of virus release by infected persons, low Ct values in PCR, greater affinity to the ACE2 receptor, mechanisms of escape to the effect of antibodies and transmissibility increased by 50% [43][44].

Gamma (P.1): first documented in November 2020, highlighting the presence of three changes that confer affinity for the ACE2 receptor, these are K417T, E484K and N501Y, which contribute to its increased transmissibility estimated at 40% in relation to the first variants [24].

When independently analyzing the clinical outcomes associated with VOC, it was identified that the Alpha and Delta variants affect individuals with similar demographic and comorbidity characteristics, while patients infected with the Gamma variant are older people, mainly between 45 and 64 years old, with a higher probability of presenting cough and anosmia, compared to the other variants [23][24].

2.4. Other Variants Related with Clinical Outcomes

The dynamics of the SARS-CoV-2 variants analyzed throughout the pandemic has been complex. In France, after the first outbreak there were new variants that had an epidemiological impact, in the comparative study by Fournier et al. [22] it was determined that the Marseille-4 variant had 13 changes, one of which (S477N) was associated with

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hypothesis (p < 0.05). This mutation could be associated with changes in the affinity for ACE2 and decrease the sensitivity of the virus to neutralizing antibodies. In this same context, a cohort study conducted in France determined that lineages B.1.177 and B.1.160, Marseille-2 and Marseille-4, respectively, during the second phase of the pandemic, were associated with more severe clinical outcomes and consequently higher mortality and hospitalization rates [23], however in this study the association between variants and disease severity was not clear.

Conversely, the B.1.243 lineage was found to be significantly associated with higher disease severity and fatal outcomes. This lineage shows several substitutions in NSP12:P323L, N:S194L as well as D614G and P681H changes in the spike protein [24].

The B.1.616 lineage whose differences from the original SARS-CoV-2 are centered on nine changes and one deletion in the spike protein (H66D, G142V, Y144del, D215G, V483A, D614G, H655Y, G669S, Q949R, N1187D), as well as changes in other regions, was associated with a higher risk of hospitalization when compared to VOC and other known lineages (p < 0.05) [26].

Conversely, when analyzing the degree of disease severity with SARS-CoV-2 variants, Al Khatib et al. [21] identified changes in specific regions of the B.1 and B.2 lineages associated with severe symptoms; patients who developed worse clinical scenarios had greater variability in the SARS-CoV-2 analyzed sequences (*p* value 0.001).

When different clades were analyzed with respect to their clinical outcomes, it was determined that the LV clades (variant of the ORF3a coding protein NS3:G251V) were associated with more severe outcomes as they had more pronounced systemic inflammation with higher concentrations of proinflammatory cytokines, chemokines and growth factors compared to the G, S and O clades [41]. Conversely, when outcomes were analyzed with respect to infection by the G and S/L clades, it was observed that, regardless of clade, the results were similar in terms of rate of hospitalizations and death [33]. One study reported that clade V was statistically related to increased mortality in comparison with other clades [36].

It has been reported that the M1V variant has lower rates of dyspnea, rhinitis and hospitalizations, which has been related to its infection in younger age groups, while the M4V variant infects mainly older adults and has a higher probability of producing fever, lower frequency of cough, rhinitis and olfactory and gustatory disorders, as well as a higher rate of hospitalization associated with hypoxemia. It has also been noted that the M4V variant confers some immunological escape and has been the responsible for cases of reinfection [22][45].

3. Conclusions

The most identified SARS-CoV-2 variants presented changes in the spike protein, N protein, RdRp, NSP3, as well as in different ORFs sequences. In most of the articles, possible associations between SARS-CoV-2 variants and clinical outcomes were found. However, only eight articles reported significant associations adjusting for age, sex, comorbidities, and other variables. There are multiple factors, such as age and pre-existing diseases, involved in the course of COVID-19 disease, that have been determinant in the degree of severity. Nevertheless, the

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