

Genetic and Cell biology Discriminants of Sars-CoV2 Infection and Susceptibility to Covid-19 pulmonary complication

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INTRODUCTION

The agent of Covid19, Sars-CoV-2 has caused thousands of fatalities worldwide and overshadowed the number of deaths of other previous coronavirus outbreaks (Sars-CoV1 2002 and MERS-CoV 2012). Although the new coronavirus pathogenicity is actively under investigation, part of its infectious behavior can be linked to its higher binding affinity to the angiotensin-converting enzyme 2 (ACE2) in the respiratory tracts. However, the expression of ACE2 *per se* may not be sufficient to justify the individual variability observed among affected patients in terms of clinical outcome in apparently non-immune depressed, non-elders subjects. The present update provides an overview of the most recent scientific findings related to genetic factors involved in the Sars-CoV-2 infectious process and their potential role in affecting the virus pathogenicity. The present update can provide valuable hints towards developing a predictive screening/susceptibility profile testing on individuals not yet infected and/or in non-symptomatic positive subjects towards managing the current morbidity and mortality risk and establishing personalized intervention protocols for the early treatment of the Sars-CoV-2-associated life-threatening pulmonary complication.

Relationship between Sars-CoV-2 Infection and Covid19 Pulmonary Complication

The morbidity and the mortality of Coronavirus infectious disease 19 (Covid19) (Zhou et al., 2020) is linked to lung failure observed in those subjects with pulmonary distress requiring intensive care treatment. Radiologically, the observed findings (ground-glass opacities and consolidation at segmental, multilobe bilateral level) are consistent with bilateral interstitial pneumonia (Feng et al., 2020, Liu et al., 2020a). The histopathology of the lungs in these subjects shows diffuse alveolar damage, denudation of alveolar lining cells (typical of acute distress syndromes), reactive pneumocyte hyperplasia (type II), intra-alveolar fibrinous exudates and interstitial loose fibrosis with chronic inflammatory infiltrates with mononucleated cellular elements dominated by lymphocytes (Zhang et al., 2020b, Xu et al., 2020b). Interestingly, in patients with Covid19 showing admitting hospital respiratory problems and which initially resulted negative to the rapid molecular test (RT-

PCR) it has been soon evident that diagnosis could be made by chest X-Ray till the molecular approach would provide diagnostic confirmation (Liu et al., 2020a). On the other hand, it has been also evident that Sars-CoV-2 infection has been associated to a still uncounted and supposedly larger number of non-symptomatic individuals which have caused family foci of symptomatic infections (Lai et al., 2020, Liu et al., 2020b, Bai et al., 2020, Rothe et al., 2020). To date, in regards to the severity of the pulmonary condition linked to Covid19 the literature reports only the positive correlation between viral load and underlying lung injury (Joynt and Wu, 2020, Lescure et al., 2020). Therefore, given the described variability among the individuals infected and presenting pulmonary symptoms, the identification of genetic and cellular discriminants predisposing to lung injury is critical towards early diagnosis and therapeutic intervention, for example, through targeted treatment with IL-6R neutralizing antibody, Tocilizumab (Clinical Trial NCT04317092). Tocilizumab is used in the event of cytokine storm characterized by elevated plasma levels of IL-2R, IL-6, IL-10, and TNF- α which occurs also as a side effect of CAR-T therapy for leukemia (Fitzgerald et al., 2017). The antibody is now in clinical trial for the treatment of Covid-19 as the majority of the clinical SARS-CoV-2 cases confirmed in China, showing lymphopenia and cytokine storm, highly correlate with the progression of the severity of the lung injury (Huang et al., 2020) (Chen et al., 2020a). Another underscored factor potentially affecting the clinical progress of pulmonary disease linked to Covid-19 is the presence of underlying venous thromboembolism (VTE), post venous thrombosis (PVT) or other factors predisposing to thromboembolic pulmonary complications. Indeed, the patients with Covid-19 affected by severe pulmonary complications share the same clinical risk factors to develop pulmonary embolism (PE) associated to DVT and PVT since older age, cancer, systemic hypertension, major trauma are associated to worse clinical outcome in both conditions. Noteworthy, the inflammation associated to VTE shares common markers actually or potentially associated to Covid-19 complications such as IL-6 and ICAM1 (Mukhopadhyay et al., 2019). In this regard, studies focusing on the specific cellular processes by which Sars-CoV-2 infection triggers or induces inflammatory events involving such factors towards co-precipitating VTE in Covid-19 are definitely

in need. At the clinical level, the relationship between coagulation dysfunction, cytokine hyperproduction and Covid-19 patients, independently from the potential causative links to VTE (which need confirmatory studies) has provided the rationale for the use of heparin in Covid-19 (Shi et al., 2020). Although the probability of subject affected by Sars-CoV-2 infection with none of the above clinical risk factors (namely younger, oligo-symptomatic) to develop thromboembolism and pulmonary complications is currently unknown, in these cases genetic predisposing factors of thromboembolism (Franco and Reitsma, 2001) should be considered (see Table I). This has provided the rationale for the use of heparin in Covid-19 an Italian clinical trial. Noteworthy, the inflammation associated to VTE shares common markers actually or potentially associated to Covid-19 complications such as IL-6 and ICAM1 (Mukhopadhyay et al., 2019) In this regard, studies focusing on the specific cellular processes by which Sars-CoV-2 infection triggers or induces inflammatory events involving such factors towards co-precipitating VTE in Covid-19 are definitely in need. Although the probability of developing thromboembolism and precipitating conditions by subject affected by Sars-CoV-2 infection with none of the above clinical risk factors (namely younger, oligo-symptomatic) is currently unknown, genetic predisposing factors associated to thromboembolism (and Table I) may play a role towards clarifying

the severity of pulmonary symptoms associated in the group of non-elderly Covid-19 patients predisposed to pulmonary complications and potentially improving the clinical course of the disease in these subjects by early identification of such genetic discriminants.

ACE2 Role in Sars-CoV-2 Infection: from Genetics to Virus-Cell Interactions

An immediate impact on the understanding of the Sars-CoV-2 mode of action has come by the early structural studies showing a very high binding affinity for the ACE2 receptor already used by the 2002 Sars-outbreak associated coronavirus (now referred as Sars-CoV-1) (Zhou et al., 2020, Yan et al., 2020, Chen et al., 2020b, Brielle et al., 2020, Zhang et al., 2020a, Letko et al., 2020). Indeed, based upon the much higher rate of infection observed for Sars-CoV-2 it does not surprise that the measured affinity of the new virus spike (S) protein for ACE2 receptor was five to twenty fold higher than that measured with the Sars-CoV-1 corresponding RBD in the outer spike (S) protein (Wrapp et al., 2020, Walls et al., 2020). In this context it becomes relevant to test also for potential ACE2 genetic evolutionary variants in the population (Li et al., 2020, Li, 2013, Liu et al., 2020c). A few studies have looked at ACE2 variability and/or expression. One study found no variation among different ethnicities at the level of the ACE2 virus binding

Table 1: The genetic discriminants.

Sars-CoV-2 interactor(s)	Sars-CoV-associated role	Reference(s)
Cell Surface/Transmembrane Sars-CoV-2 binders/virus entry receptors		
ACE2	Sars-CoV-2 receptor	(Brielle et al., 2020, Hussain et al., 2020, Ortega et al., 2020, Ou et al., 2020, Yan et al., 2020)
Sialic Acids Receptors	CoVs co-receptors	(Milanetti et al., 2020)
TMPRSS2	CoVs co-receptors	(Iwata-Yoshikawa et al., 2019) (Sungnak et al., 2020)
Cell Surface/Transmembrane Sars-CoV-2 infection modulators		
EGFR	Putative binder/modulators	(Venkataraman and Frieman, 2017)
ICAM1	Potentially Involved in viral pulmonary injury	(Tan et al., 2003)
ITGB6	Potentially Involved in viral pulmonary injury	(Tatler et al., 2016)
ITNs	Putative binder/modulators	(Hanel et al., 2006)
Other CoV receptors		
APN	Putative adaptive binder/modulator	(Wong et al., 2017)
DPP4/CD26	Putative adaptive binder/modulator	(Lu et al., 2013, Vankadari and Wilce, 2020)
Intracellular SarsCoV-2 binders/virus cycle modulators		
ADARB1	Viral process regulation/Genome replication	(Vandelli et al., 2020)
CCNT1	Viral process regulation/Genome replication	(Vandelli et al., 2020)
DDX1	Response defense to virus	(Vandelli et al., 2020)
IFITM3	Sars-CoV-1 IC spread/replication Inhibitor	(Smith et al., 2014)
PROX1	Response defense to virus	(Vandelli et al., 2020)
XRCC5	Putative provirus latency	(Vandelli et al., 2020)
XRCC6	Putative provirus latency	(Vandelli et al., 2020)
ZNF175	Response defense to virus	(Vandelli et al., 2020)
IC Markers of Immune-/Inflammatory/fibrotic-response to viral infection/ pulmonary injury		
EGFR	Potentially Involved in viral pulmonary fibrosis	(Venkataraman et al., 2017, Venkataraman and Frieman, 2017)
IL6R (a-sub)	Ligand binding subunit of IL6	(Garbers et al., 2018)
Hsp70/HSP1B	Potentially Involved in viral pulmonary injury	(Brown et al., 2005)
YAP/TAZ	Potentially Involved in viral pulmonary injury	(Sun et al., 2019)
Other genetic markers linked to Covid19		
RBC A/B/O Ag	Thromboembolic process modulators*	(Tirado et al., 2005) (Zhao et al., 2020)
Factor VIII	Thromboembolic process modulators*	(Tirado et al., 2005)
F2-prothrombin G20210A var/ F5-factor V Leiden var/ F9/ MPL/ PROC/ PROS1/ SERPINC1	Inherited venous Thrombophilia-associated Putative Covid-19 pulmonary injury risk factors	(Goeijenbier et al., 2012) (Franco and Reitsma, 2001)
Natural anticoagulants (AT, PC, PS) genetic deficiency	Putative Covid-19 pulmonary injury risk factor	(Franco and Reitsma, 2001)

domain (Cao et al., 2020) while another (Asselta et al., 2020) found no apparent link between ACE2 and severity/sex bias. However, one of the published study has yet evaluated the role of the ACE2 gene promoter region. Indeed, modifications at the genomic and/or epigenomic level on this genomic region might also be taken in consideration towards clarifying the eventual Covid19 predisposing traits in the positive population. Furthermore, the finding that ACE2 is highly expressed in the respiratory tract (Xu et al., 2020a) and that is further increased in response to infections (Jia, 2016) and under preventable conditions such as smoking (Brake et al., 2020) and high blood pressure (Huang et al., 2010, Chamsi-Pasha et al., 2014) further supports the ongoing hypothesis that ACE2 contextual differences in gene expression rather than its mutational status might affect the pathogenicity of Sars-CoV-2 infection and its underlying symptoms penetrance.

Co-receptors, Non-Canonical RBD Binders and Functional Modifying Factors beyond ACE2

Based upon current knowledge, other host factors beyond the canonical RBD mediated Sars-CoV-2 spike (S) protein interaction with ACE2 have already emerged as potentially critical co-factors towards facilitating and potentially diversifying the mechanism of virus infection at the cellular level. In particular, on the host side, among surface proteins acting as co-receptors, TMPRSS2 has been recently found to play a permissive role at the virus recognition and cellular entry phase (Hoffmann et al., 2020, Chao and Zheng, 2020). In agreement with such finding, the results of a study performed on a Italian population sample (Asselta et al., 2020) reports a significant correlation between TMPRSS2 variants and expression levels and Covid-19 progression of the disease. This finding is relevant since TMPRSS2 can be targeted by its inhibitor Camostat mesylate (Kawase et al., 2012) which could be tested for its potential inhibiting effect on the virus entry. Among other cellular binders of Sars-CoV-2, a study (Milanetti et al., 2020) found supporting data for the involvement of Sialic Acid (SA) receptors. SA receptors have been also identified as co-receptors for other Coronavirus strains where they facilitate membrane fusion and virus entry (Qing et al., 2020). Interestingly, the spike (S) protein RBD involved in this physical interaction displays higher structural similarity with the corresponding SA receptor binding region in MERS-CoV while it seems lost in Sars-Cov-1 RBD (Milanetti et al., 2020). Therefore, they may offer another critical mechanism in preventing Sars-CoV-2 infection. Some authors have also suggested a role for Integrins and other ITN-binding adhesion molecules in Sars-CoV binding (Sigrist et al., 2020) (Tatler et al., 2016, Tan et al., 2003). Other authors have speculated potential functional interactions between Sars-CoV-2 and the EGFR on the basis of previous findings of pulmonary tissue fibrosis in Sars-CoV-1 cases which is an hallmark of EGFR signal chronic stimulation coupled to histological presence of EGFR overexpression in the respiratory tracts of the affected patients (Venkataraman and Frieman, 2017). Besides the cellular surface factors involved in the virus recognition and cell attachment, other intracellular permissive factors may likely provide an advantage to the virus intracellular spread and replication. Only one study at this time has addressed the intracellular Sars-CoV-2 interactants (Vandelli et al., 2020). In this study, using a predictive algorithm for (viral) RNA-(cellular) protein interaction the authors have identified a number of possible binders (included in TABLE I). On the virus side, the Sars-CoV-2 genome seems to be quite conserved in its RBD region as shown by its current mutagenic landscape (Wang et al., 2020a). Furthermore, viral genome mutations so far has provided no strong evidences of a role in affecting the pathogenic potential of the infection besides those mutations potentially linked to the species jump (Zhang et al., 2020c). In this context, a study (Bal et al., 2020) has identified a specific deletion in the first Covid19 Cluster in France on the Sars-CoV-2 non-structural protein (nsp) 2 shared by other European clusters in England and the

Netherlands while another study (Angeletti et al., 2020) has reported a single residue substitution at positions 723 and 1010 of nsp2, none of which was found to correlate with clinical presentation. Another line of research focusing on structural components outside the RBD focused on the role of the HR1 portion of Sars-CoV2 Spike protein involved in the membrane fusion process in order to validate its molecular targeting potential (Xia et al., 2019, Xia et al., 2020). Overall, the results obtained on Sars-CoV-2 spike protein sequence outside the known Spike (S) RBD-ACE2 mediated interactions towards the observed virus pathogenicity are still to be determined. A single study on more than 3000 positive subjects in China (Zhao et al., 2020) has also evaluated the statistical relationship between red blood antigens of the AB0 group and Sars-CoV-2 infection morbidity and found significant correlations between distinct groups carriers. In particular the study finds subjects displaying the group A blood type carrying the highest risk for Covid19 compared with non-A groups while subjects belonging to group 0 displaying the lowest overall risk independent of age or gender effects on the studied population. A possible causal connection between this finding and the observed worse clinical features in A-type groups could be linked to the relative higher risk for thromboembolic disease observed in subjects co-expressing A type groups along with factor VIII (Tirado et al., 2005). It would be relevant to confirm this causal connection in Covid19 and clarify the possible underlying mechanism(s). The genetic discriminants discussed herein are summarized in Table I.

A Workflow towards Susceptibility Testing for Covid19-Associated Pulmonary Complications

Given that older age and immune-debilitating diseases (such as cancer) have been associated to a worsening of pulmonary symptoms and an overall poorer clinical outcome (Du et al., 2020, Wang et al., 2020b) the cases of relatively younger adults needing intensive care and sometimes losing their life to the above complication by severe hypoxic distress, calls for deeper knowledge in order to early diagnose and treat such patients to reduce the risk of advancing into irreversible states. This population includes the subjects not yet infected (RT-PCR negative and not vaccinated) along with non-symptomatic and early stage Covid-19 patients. Therefore, based upon the current knowledge and the factors reviewed herein, it is pivotal to invoke specific studies on these groups of patients based on omics-based testing to allow the identification of those individuals highly predisposed to the new virus life-threatening interstitial-like lung disease. Such approach would depend on the infection status confirmed by rapid molecular (RT-PCR) test and by immuno-serological test, and include (single cell) nucleic acids extraction of the patient's cellular material isolated by bronco-alveolar brushing, wash or endoscopic biopsy followed by parallel deep RNA-sequencing profiling on both the virus and the host material. For those genetic discriminants expressed in blood cells an appropriate NGS panel could be designed. These, towards screening the targets highlighted in TABLE I. Such type of approach could turn out to be especially valuable in those predisposed subjects identified by validated testing based upon the genetic discriminants summarized herein. Specifically, this strategy would allow to anticipate Tocilizumab treatment to counteract the hazardous cytokine storm and limit the extent of lung injury, therefore, optimizing the clinical outcome of the identified responders. These type of RNA-seq based screening approaches have the potential to importantly reduce the financial burden on the international healthcare system at this unpredictable stage of Covid-19 pandemics.

CONCLUSIONS

A number of genetic and cellular discriminants of Sars-CoV-2 morbidity are emerging in the literature deserving specific and immediate attention under the ongoing pandemics. Along with a few factors

directly related to the virus entry in the target cells of the respiratory tracts, as host receptors, are also intracellular discriminants potentially involved in the intracellular spreading and replication of Sars-CoV-2. Interestingly, a number of signaling components mediating pulmonary fibrotic injury which affects Covid19 complicated cases, besides bearing potential predictive value could turn-out as valuable pharmacological targets towards blocking the mechanisms leading to irreversible pulmonary injury. At last, the finding of a possible role of certain red blood cell antigens of the ABO group in conferring a degree of protection from the infection versus a lack of it calls for additional cause-effect studies. Altogether, the findings summarized herein provide updated rational for a common strategy aimed to speed-up the validation of current genetic and cellular discriminants of the disease associated to Sars-CoV-2 known as Covid19 and support larger studies aimed to reduce its morbidity and mortality.

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