The First, Comprehensive Immunological Model of COVID-19: Implications for Prevention, Diagnosis, and Public Health Measures

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List of abbreviations:

ACE2 Angiotensin-converting-enzyme-2
ARDS Acute Respiratory Distress Syndrome
CFR Case-fatality ratio
COVID-19 Coronavirus disease
IgA Immunoglobulin isotope A
ICU Intensive care unit
IgG Immunoglobulin isotope G
IgM Immunoglobulin isotope M
IQR Interquartile range
mAb monoclonal antibody
MBL Mannose binding lectin
MERS Middle East respiratory syndrome
NK natural killer
PCR Polymerase chain reaction
POCT Point of care test
RBD Receptor binding domain
SARS-CoV2 Severe acute respiratory syndrome coronavirus 2
TMPRSS2 Transmembrane protease serine 2
XLA X-linked agammaglobulinemia

Abstract

The natural history of COVID-19 caused by SARS-CoV-2 is extremely variable, ranging from asymptomatic infection, to pneumonia, and to complications eventually fatal. We propose here the first model, explaining how the outcome of first, crucial 10-15 days after infection, hangs on the balance between the cumulative dose of viral exposure and the efficacy of the local innate immune response (natural IgA and IgM antibodies, MBL). If SARS-CoV-2 runs the blockade of this innate immunity and spreads from the upper airways to the alveoli in the early phases of the infections, it can replicate with no local resistance, causing pneumonia and releasing high amounts of antigens. The delayed and strong adaptive immune response (high affinity IgM and IgG antibodies) that follows, causes severe inflammation and triggers mediator cascades (complement, coagulation, and cytokine storm) leading to complications often requiring intensive therapy and being, in some patients, fatal. Strenuous exercise and high flow air in the incubation days and early stages of COVID-19, facilitates direct penetration of the virus to the lower airways and the alveoli, without impacting on the airway's mucosae covered by neutralizing antibodies. This allows the virus to bypass the efficient immune barrier of the upper airways mucosa in young and healthy athletes. In conclusion, whether the virus or the adaptative immune response reach the lungs first, is a crucial factor deciding the fate of the patient. This "quantitative and time-sequence dependent" model has several implications for prevention, diagnosis, and therapy of COVID-19.

***Forward** - This article is dedicated to the memory of Dr. Li Wenliang, who on December 2019 first recognized a new disease and alerted the World of the SARS-CoV-2 epidemic before dying of COVID-19 on 7.February.2020 at the age of 33, of Dr. Carlo Urbani, who on February 2003 first recognized a new disease and alerted the World of the SARS epidemic before succumbing to it on 29.March.2003, and of all the doctors and allied health personnel who have sacrificed their own lives to save those of their patients. We wish to honor their competence, braveness and generosity.*

1. Natural History of COVID-19 & Antibody Response induced by SARS-CoV-2

1.1 Same virus, diverging disease evolution

SARS-CoV-2 is an zoonotic RNA betacoronavirus **[1,2]**, similar to SARS-Cov **[3,4]**, emerging from around November 2019 in humans living in the province of Hubei, China **[5]**, and rapidly spreading with a pandemic trend all over the World.**[6]** The consequences of infection with SARS-CoV-2 broadly varies from benign to fatal.**[1] (Figure 1)** While many infected individuals remain asymptomatic or develop only mild upper airways symptoms, others develop pneumonia and ARDS requiring intubation in ICU, and may undergo complications that can be fatal.**[1]** Viral shedding begins 2-3 days before symptoms onset.**[7]** Infectivity seems to decline significantly already after 10 days from symptom onset **[8]**, but the virus can be detected for a median of 20 days, up to 37 days among survivors.**[9]**

The cumulative amount of virus exposure acquired by the patient at the start of infection cannot be measured. However, it may broadly range from a minimal amount, below the average number of viral particles needed to establish an infection (infectious dose) **[10]**, to higher doses repeatedly acquired from multiple patients in hospitals or overcrowded environments.**[11]** This pattern of exposure has probably been common among health care personnel, especially in the early phases of the pandemic.**[12]**

1.1 First stage: upper respiratory infection - Among infected humans, those developing Coronavirus Disease (COVID-19) show their first symptoms on average 5 to 6 days after infection **[7]** with a 95% confidence interval ranging from 2 to 14 days.**[13]** Initial symptoms are limited to upper airways (cough, sore throat) accompanied by fever, fatigue and muscle ache, while nausea or vomiting and diarrhea are infrequent symptoms at onset.**[14]** At disease onset, the virus RNA is usually detected through swabs from nostrils or pharynx, amplified and detected by PCR with rapid, qualitative POCT or classical, quantitative laboratory methods.**[15]** However, lower viral loads have been detected also in throat swabs from infected but asymptomatic humans.**[15,16]**

1.2 Second stage: pneumonia - While most patients experience only mild fever and symptoms of the upper airways, others develop dyspnea and pneumonia.**[1]** Among 41 patients hospitalized in Wuhan, China, the median time from onset of symptoms to shortness of breath was 8.0 days (interquartile range, IQR, 5.0-13.0).**[17]**

1.3 Third stage: complications - Among the same 41 patients hospitalized in Wuhan, China, the median time from onset of symptoms to acute respiratory distress syndrome (ARDS) was 9.0 days (8.0-14.0), to mechanical ventilation was 10.5 days (7.0–14.0), and to ICU admission was 10.5 days (8.0-17.0).**[17]** Dyspnea associated to decreased oxygenation index were the more frequent signs of respiratory failure or ARDS. Data from China reported that 53% of deaths were related to respiratory failure, 7% to shock (presumably from fulminant myocarditis), 33% to both, and 7% to unclear mechanisms.[18] Among 191 patients admitted in two hospitals in Wuhan, the median time from disease onset and from dyspnea to intubation was 14.5 days (12.0-19.0) and 10 days (IQR 5.0-12.5) respectively.[19] ARDS, acute cardiac and kidney injury, sepsis, and secondary infection were the most frequent complications.[19]

1.4 Fourth stage: exitus or healing - Among patients dying for COVID-19 in the Chinese study, death occurred 18.5 (15.0-22.0) days after disease onset.[19] Among survivors, permanence in intensive care unit (ICU) lasted 7.0 days (2.0-9.0); and discharge from the hospital occurred shortly thereafter.[19] Mortality is associated with older age, comorbidities (including hypertension, diabetes, cardiovascular disease, chronic lung disease, and cancer), higher severity of illness scores, worse respiratory failure, higher d-dimer and C-reactive protein concentrations, lower lymphocyte counts, and secondary infections.[20] (Figure 1)

2 Molecular mechanisms of SARS-CoV-2 cell entry

2.1 Distribution of SARS-CoV-2 - SARS-CoV primarily infects pneumocytes and enterocytes of the small intestine. [21] SARS-CoV-2 has a similar tropism, and also infects type II pneumocytes, enterocytes and macrophages. [22,23] In addition, SARS-CoV-2 can infect cell expressing ACE2 receptor and the serin protease TMPRSS2. [24] Investigations with a holistic data science platform suggested that the virus may infect many other cells types, among which tongue keratinocytes (which may explain dysgeusia reported by some patients), airway club (Clara) cells, and ciliated cells. [25] This outcome also suggests that the exact distribution of SARS-COV-2 in humans may be broader than expected from the solely distribution of membrane receptors directly docked by the virus.

2.2 Molecular mechanisms of SARS-Cov-2 entry - The molecular mechanisms used by SARS-CoV-2 to adhere and penetrate in host cells have been discovered. As SARS-CoV, [26] SARS-CoV-2 uses its spike glycoprotein to bind to the angiotensin-converting-enzyme-2 (ACE2) receptor. [27] The spike molecule, organized as a trimer, is forming a needle whose round tip, formed by the three S1 domains clustered together, is protruding to meet the ACE2. [27] After binding, TGRBSS2, a serine protease, clives the spike glycoprotein between S1 and S2, which allows the virus membrane approaching the cell membrane, fuse with it and enter the cell. [28] The SARS-CoV spike protein contains 22 N-glycosylation sites. [29] The glycosylation pattern of viral particles produced in a human cell line has been already characterized in 13/22 sites. [29] Several N-glycosylation sites, including mannose residues, surround the area of the molecule binding ACE2. These, dense glycosylation is used by the virus to mask surface peptide epitopes which may induce and elicit neutralizing antibody responses, thus preventing docking on ACE2. [29] However, an area which remaining free from glycosylation, is also suitable for ACE2 binding and potentially for other proteins, including neutralizing antibodies. [29]

3 Kinetics of the adaptive antibody response

3.1 **New virus, no memory antibodies -** SARS-CoV-2 is a new virus. The IgG antibodies induced by other common coronaviruses, or by SARS-CoV and MERS, do not recognize and neutralize this new virus.[30] Accordingly, no specific IgG antibodies have been detected against the S glycoprotein in the early stages of the infection, i.e. before an adaptive response was started.[30,31]

3.2 Kinetics of the adaptive antibody response

Typical primary and secondary antibody responses to acute viral infection are efficiently induced. [31] An early Chinese study in 173 patients observed a median seroconversion time for Ab, IgM and then IgG at day-12 and day-14, respectively.[30,31] (Figure 1) The SARS-CoV-2 specific IgM antibodies appear around 8-12 days after infection onset and vanish around the end of week 12. [32,33] The IgG antibody response starts appearing shortly thereafter (or simultaneously) but persist longer [30-33] and may be protective.[34]

3.3 Persistence of the antibody levels

Most serological data currently available in the literature refer to patients examined mostly in the acute phase of the disease. Therefore, they are insufficient to exactly establish durability of the antibody titers of each isotype peak when they eventually disappear. The levels of serum IgG antibodies, however seems to be proportional to the intensity of the viral load and to the symptom severity.[31,35]

3.4 Effectiveness of the antibody response

The efficacy of specific Ig and their role in limiting viral spread may be indirectly assumed by observations demonstrating that plasma from subjects recovered from COVID-19 showed a therapeutic efficacy if passively transferred to patients.[36,37] Similar effectiveness had been already demonstrated for plasma from patients having recovered from SARS-CoV and MERS-CoV.[38,39] Consequently, infusion of plasma from convalescent individuals to critically ill COVID-19 patients is a therapeutic option that is being investigated. Although controlled clinical trials are not yet available, several papers report the efficacy of this treatment and the lack of serious adverse events.[40,41] Convalescent plasma was administered in patients with a severe disease, and it is unclear whether earlier administration might have been associated with different clinical outcomes [42] and with the prevention of respiratory distress.

4. Viral and host factors associated with SARS-CoV-2 infiltration of the lower airways

4.1 - Viral exposure dose

Only a small proportion of humans younger than 50, among those who get infected by SARS-CoV-2, suffer from moderate and severe COVID-19. [43-45] Among them, hospital doctors frequently exposed to COVID-19 patients are, unfortunately, highly represented. [46] Dr. Li Wenliang, the first man alerting China and the World of the new infection, died from COVID-

19 at the age of 33.[47] Similarly, Dr. Carlo Urbani, i.e. the first man alerting the of SARS-CoV, died of SARS at the age of 46.[48] Both doctors cared for weeks patients with severe pneumonia with no personal protection.[47,48] In Italy, 114 doctors exposed to SARS-CoV-2, have so far (14th April, 2020) died of COVID.[49] The case fatality ratio among doctors working in hospitals and caring patients developing severe COVID-19 has been therefore much higher than among their age and gender matched peers.[49,50] (Table-1)

Observations in previous viral epidemics, further clarify this aspect. The reliability of high viral loads in nasopharyngeal specimens as a prognostic indicator of respiratory failure or mortality, with or without a high viral load in serum, has been previously characterized in SARS.[51] A link has been established between the initial dose and subsequent severity of the disease to the 1918-19 Spanish Flu pandemic. It was demonstrated by simulation models that the number of simultaneous contacts a susceptible person has with infectious ones are correlated with the infectious dose; that severe cases of influenza result from higher infectious doses of the virus; and that a susceptible person can be easily exposed to very high infectious doses of influenza in over-crowded places. [52] The viral replication is more active and prolonged in patients suffering from severe influenza. Viral clearance is slow when host defenses are weakened, however it is enhanced when antivirals start within the first 4 days of illness.[53]

4.2 - Age

Among over 70 thousand Chinese with COVID-19, most were aged over 30 years (90%), while only 1% were aged 9 years or younger, 1% were aged 10 to 19 years an 8% aged 20 to 29 years.[54] Moreover, most of the relatively few pediatric cases were classified as mild (81%), only 14% severe and 5% critical.[54] Until now (April 14, 2020) the death of only a few humans aged 18 years or less has been attributed to SARS-CoV-2.[43]

The reported case-fatality ratio (CFR) for COVID-19 among Chinese patients increases progressively with age, being 0% below 10y, 8% among patients aged 70 to79 years and 14.8% among those aged 80+ years.[54] In Europe, the virus is still spreading and has already caused over 77786 deaths (April 14,2020) with a CFR also increasing with age .[44] Italy was the first European country facing the pandemic, with 159516 cases of COVID-19 diagnosed up to April 14, 2020 [45] with a CFR steadily increasing with age, with no deaths observed in patients younger than 30 years and 20.1% among those aged 80+ years.[43] [Table 1]

Among 171 Chinese children with proven SARS-CoV-2 infection, only 3 (all with severe comorbidities) required intensive care support and invasive mechanical ventilation. [55] In a larger Chinese study, over 90% of all pediatric patients had no severe disease.[56]

4.3 - Gender and Blood group

COVID-19 mortality has been lower among Chinese females than males.[57] In Italy, mortality and hospitalization rates have been also more frequent among males than among females.[58] Moreover, patients with blood group 0 and A have slightly lower and a slightly higher risk, respectively, of developing COVID-19.[59]

4.4 – Strenuous physical exercise

The first diagnosis of COVID-19 in Europe has been confirmed in a 38 year-old Italian healthy male who regularly participated in running events and soccer games. One day before starting COVID-19 symptoms, he had been training sport. The time-lapse between onset of symptoms and pneumonia was 2 days only. After only 4 days from the onset of COVID-19 he was admitted to the intensive care unit of the Policlinico San Matteo in Pavia for respiratory failure. After weeks of intubation and supportive treatment, the patient luckily recovered and could be discharged in good conditions.

The case of the Italian first COVID-19 case is known worldwide due to media report but, surprisingly, no official study has been published so far. The example of this physically active, young patient offers room for reasoning with regard to the importance of sport for virus transmission and course of disease, especially as other cases of COVID-19 in (semi-) professional athletes have been described and a considerable proportion of the global population is active in sports. [60] The problem of SARS-COV-2 infection is part of a more general problem, since cohorts of athletes and para-athletes are recognized with amplified susceptibility to viral respiratory tract infection and cohorts with known chronic medical conditions, such as airways disease (seen in approximately 20% of all endurance athletes) [61, 62].

4.4.1 Strenuous exercise and IgA defect

Regular, moderate exercise is associated with a reduction of the severity of acute respiratory infections [63], salivary IgA levels decline in athletes during and after a training season.[64] This observation may explain why elite athlete are at higher risk of upper airway infections.[65] A so-called ``open window'' of period is ranging between 3 and 72h hours after the strenuous exercise is finished.[66]

4.4.2 Deep inhalation while performing strenuous exercise (running in particular) and virus spread to lower airways and alveoli

Aerosols are considered an important mode of transmission for influenza.[67] During strenuous exercise, requiring up to 40 l/min of respiratory flow, oronasal (combined nose and mouth) breathing dominates, with the oral component reaching up to 60% of the overall volumes.[67] High flow air and change of breathing from nose to mouth breathing induces progressive cooling and drying of the respiratory tract mucous. Decreasing movement of ciliated cells and increasing mucosal viscosity, finally impairing filtering of microorganisms from the upper respiratory tract system.[68]

The pattern of breathing during strenuous exercise changes dramatically by a tremendous increase of ventilation (i.e.: inspiratory and expiratory volumes of air), and of alveolar ventilation in particular. Obviously, these changes mostly attain to whatever kind of runners belonging to all sport disciplines, being semi-professional and professional athletes particularly exposed (such as much more than individuals of common population) due to their frequent practice of extreme and long-lasting exercise. Furthermore, the majority of these athletes have their lungs that usually work in perfect physiological conditions, such as very close to those of the "ideal lung". In other words, in the absence of any anatomical or physiological factor causing a significant unevenness in distribution of their alveolar ventilation. Paradoxically, these pre-existing ideal conditions significantly favor the deep inhalation of several irritants, allergens, infectious agents. Even the SARS-CoV-2 can then spread more easily to the deepest areas of the lungs (alveolar bronchioles and alveoli) during strenuous exercise, and there start its aggressive action. Not by chance, a great proportion of professional football players claimed the occurrence of fever, dry cough and malaise (and dyspnea in some cases) immediately after, or a few hours following their last official match.

5 The crucial first 10 days from infection: natural immunity is the first-line

5.1 The facts: In COVID-19, the occurrence of pneumonia is a critical event discriminating asymptomatic or mild cases, whose infection remains confined to the upper airways, from those with moderate or severe disease, who experience viral invasion of their lower airways.[19] What makes the difference? What prevents the virus from reaching the lungs and causing pneumonia? What makes COVID-19 pneumonia a life-threatening disease?

1) No efficient adaptive immune response is available at the time of infection;[30]
2) Pneumonia may starts before adaptive immune response develops; [19]
3) Serious complications are associated with adaptive immune response.

5.2 The first two weeks after infection are crucial. [17,19] Innate immunity is the only first-line, early defense against the new SARS-CoV-2 virus. Consequently, the early confrontation between host's innate immunity and SARS-CoV-2, at exposure and during the following two weeks, decides the natural history of disease, whether infection will be efficiently blocked in upper airways and how much virus reaches the lungs. To understand which part of the innate immunity involved in early protection from SARS-CoV-2, we have:

1) Examined which Primary Immune Deficiencies are associated with pneumonia.
2) Examined the patterns of risk factors for COVID-19 severity: dose of exposure to SARS-CoV-2; age, gender, ABO group;
3) Identified the innate immunity components fitting the same patterns of risk factors;
4) Examined the biological plausibility that the candidate molecules, emerging from the previous reasoning, are really essential in limiting the consequences of SARS-CoV-2 infection to upper airways or to mitigate the course of pneumonia.

5.3 Lessons from patients with agammaglobulinemia**.**

Two Italian COVID-19 patients with X-linked agammaglobulinemia (XLA), males, aged 26 and 34 years under regular treatment with human gamma-globulin have been recently reported.[69] Both patients developed pneumonia, and both recovered without any need of receiving oxygen-therapy. [69] In another Italian study, the clinical course of COVID-19 in two additional patients with agammaglobulinemia, one XLA and one autosomal recessive, were described. The clinical course resulted milder in patients with agammaglobulinemia when compared to that of other patients.[70] Agammaglobulinemic patients were receiving standard therapy with immunoglobulin preparations, which could not contain SARS-COV-2 specific antibodies, since were prepared from donors before the pandemic and are deprived by natural IgM and IgA. On the other hand, these patients have in general a natural cellular immunity compartment, including NK cells and phagocytes.

These data suggest that the lack of natural IgM and IgA in the upper respiratory airways may have contributed to the rapid viral spread to lungs, causing pneumonia. Unexpectedly in immunodeficient individuals, agammaglobulinemic patients, who are unable to develop specific SARS-CoV-2 Igs, did not develop severe pneumonia, suggesting that the serious complications observed in other patients may be related to the development of acquired immunity.

5.4 Summing-up

Under the circumstances described above, innate immunity become an obvious candidate to act as very first barrier protecting of children, almost all adults and most elders from SARS-CoV-2. Innate immunity is essential to control virus replication early enough, before a very effective adaptive immune response is generated.**[71]**

Anti-viral innate immunity is based on humoral elements, including components of the complement and coagulation systems, soluble proteins not-specifically binding glycans (such as the Mannose Binding Lectin, MBL), natural antibodies (IgM, IgA and IgG), interferons and other cytokines.**[72]**

Cellular elements of the innate immunity that act as anti-viral barrier include Natural Killer cells, MAIT, γ/δ T cells, that contribute to limit pathogen invasion by killing infected cells, secreting inflammatory cytokines or promoting the adaptive immune response.**[72]**

We focused on humoral components and, in particular on natural antibodies and MBL, to ascertain whether these players of the innate immunity fit all the epidemiological and clinical pre-conditions presented in the last three months by SARS-CoV-2.

Finally, we tentatively describe mechanisms beyond the most severe cases of pneumonia as a possible consequence of the development of adaptive immunity in individuals with an early high viral spread in lungs.

6 – Anti-glycan Natural IgM and IgA antibodies

Anti-glycan natural antibodies are detected in serum in the absence of previous immunization, are observed also in gnotobiotic animals, and belong mostly to the IgM isotype **[73]** but also to the IgA and IgG isotype.**[74]**

6.1 Natural IgM concentration mirrors the patterns of host factors associated with COVID-19 severity

6.1.1 Natural IgM decline with age. When examined with glycan array, IgG signals remains relatively unchanged with age.**[75]** By contrast, average anti-glycan IgM signals significantly decrease with age **(Figure 2)** especially after the early 40s, exceeding the expected general reduction in IgM levels with increasing age.**[75]** This evidence may contribute to explain why severe cases of COVID-19 start to be observed in the 4^{th}-5^{th} decade of life and their prevalence increases with age.**[43-45]**

A more recent study also found a reduced diversity in natural IgM antibodies in older donors **[76]**, reflecting a similar trend observed in human B-1 B cells, i.e. the cells responsible for natural IgM production, mainly in the spleen **[77]**. This evidence may contribute to explain the increasing prevalence of severe cases of COVID-19 in the eldest.**[43-45]**

6.1.2 Natural IgM levels are lower in males and blood group "A" individuals.

When examined with glycan array, anti-glycan IgG were not different in females and males.[69] By contrast, anti-glycan IgM were slightly, although not significantly higher in females.[69] This outcome is consistent with the observation of higher total IgM levels in females [78]. It is well known that blood type has a profound influence on the repertoire of glycan specific IgG and especially IgM antibodies.[69] This is also why blood groups are very relevant in regulating host susceptibility to infection.[79] In a study among health care workers in Hong-Kong, Group O individuals were remarkably resistant to SARS-CoV infection.[80] The ability to block SARS-CoV infection in target cells was observed with high-titers of human anti-A (1:256), whilst low-titer anti-A proved to be ineffective. [81] An influence of blood group on susceptibility to severe COVID-19 has been postulated [59], whether this is mediated by differences in the repertoire of glycan specific antibodies remains an interesting hypothesis for investigation. This evidence may contribute to explain why, among humans infected with SARS-CoV-2, those with a blood serogroup group "A" and males respectively have a slight [59] or remarkable [57,58] higher risk of developing severe COVID-19.

7 Mannose Binding Lectin (MBL)

MBL plays a pivotal role in innate immunity interacting with surface sugars of a wide series of microorganisms as a pattern-recognition receptor.[82] Thus, MBL: i) activates the lectin complement pathway; ii) promotes opsonophagocytosis [83]; and iii) modulates inflammation [84].

7.1 Evidence suggesting that MBL may protect in the early stages of SARS-CoV-2 infection

7.1.1 Serum MBL levels decline with age

Values of serum MBL, albumin and the MBL/albumin ratio were significantly lower either in centenarians and in octo-nonagenarians as compared to the general population from the same geographic area (Sardinia and Campania, Italy).[85]

7.1.2 MBL is polymorphic and low levels predispose to SARS-CoV infection

Three polymorphisms in the structural gene MBL2 and two promoter gene polymorphisms are commonly found that result in production of low serum levels of MBL.[86] Low MBL levels appear to predispose persons to bacterial infectious diseases, particularly in neonatal age and early childhood.[83] MBL gene polymorphisms were significantly associated with susceptibility to SARS-CoV infection, possibly explained by the reduced expression of functional MBL. [87] The distribution of MBL gene polymorphisms was significantly different between patients with SARS and control subjects, with a higher frequency of haplotypes associated with low or deficient serum levels of MBL in patients with SARS than in control subjects. Serum levels of MBL were also significantly lower in patients with SARS than in control subjects.[88] MBL could bind SARS-CoV in a dose- and calcium-dependent and mannan-inhibitable fashion in vitro, suggesting that binding is through the carbohydrate recognition domains of MBL. Furthermore, deposition of complement C4 on SARSCoV was enhanced by MBL. Inhibition of the infectivity of SARS-CoV by MBL in fetal rhesus kidney cells (FRhK-4) was also observed.[88] These results suggested that MBL may contribute to the first-line host defense against SARS-CoV and that MBL deficiency is a susceptibility factor for acquisition of SARS [88]. Mutagenesis indicated that a single N-linked glycosylation site, N330, was critical for the specific interactions between MBL and SARS-S.

7.2.3 MBL may interfere with the binding of SARS-CoV to cellular receptor.

The presence of glycans enriched in mannose in the S1 region next to the ACE2 binding site (N234) [89] may led to speculate that MBL could bind and inhibit the S1-ACE2 interaction in SARS-CoV-2, as it did with SARS-CoV. Thus, binding of MBL to SARS-S may interfere with early pre- or postreceptor-binding events necessary for efficient viral entry.[90] Moreover, in a further study, it was observed a potential interaction of polymorphisms in both MBL and CCL2 conferring susceptibility to severe clinical symptoms provoked by SARS-Cov.[88] It is at present not known whether SARS-CoV-2 belong to the category of the "evasion strong" viruses, thanks to an efficient glycan shield.[89] A pre-print paper reinforced the hypothesis of a MBL role in SARS-CoV-2 infection, by showing that extracellular soluble N protein dimers interact with MASP-2 and induce MASP-2 auto-activation and binding to MBL.[91]

8 Immunopathogenesis

8.1. What kills COVID-19 patients with pneumonia?

The most frequent cause of death in COVID-19 is an ARDS with Respiratory Failure (RF). Hypothesis-driven investigations are required to adopt adequate countermeasures and eventually save lives [92]. Two major biological cascades have been observed: the so called "IL-6 cytokine storm" and disseminated intravascular cascade in the lung (DIC) **(Figure 3)**.

8.1.1. IL-6 cytokine storm - Unexpectedly, ex vivo experiments in human explanted lungs showed that SARS-CoV-2 does not significantly induce types I, II, or III interferons in the infected lung tissues [93]. SARS-CoV-2 only upregulates IL6, MCP1, CXCL1, CXCL5, and CXLC10 (IP10). [93] Interestingly, ARDS and RF have been associated to increased serum levels of IL-6. [17] Elevated serum levels of IL-6 may be an early biomarker of a worsening clinical course.[94] Trials with tocilizumab, a monoclonal antibody recognizing IL6-R, started after its efficacy was reported in case reports. [95]

8.1.2. Intravasal coagulation - Post-mortem analysis of lung diseases showed diffuse alveolar damage, including injury to the alveolar epithelial cells, hyaline membrane formation, fibrin deposition and hyperplasia of type II pneumocytes.[96] Of relevance, 71.4% of fatalities, but only 0.6% of the surviving patients met ISTH criteria for disseminated intravascular coagulation (DIC) [97], a pro-thrombotic and pulmonary congestion with microvascular thrombosis and occlusion [98]. Biomarkers for this process are elevated D-Dimer and plasma thrombomodulin and others, [99] and treatments with heparin or Tissue Plasminogen Activator have been suggested. [98]

8.2 Triggers of the cascades leading to ARDS and Respiratory Failure

The mechanisms triggering an IL-6 cytokine storm or a DIC are still unclear. An intriguing observation is that ARDS symptoms start in coincidence with the onset of the SARS-CoV-2 antibody specific immune response. Interestingly, the serum levels of specific IgA, IgM, and IgG are the highest in patients with the worst clinical course. [30,100] We may hypothesize that in individuals in whom the virus early reaches the lung and actively replicates, a robust adaptive immune response contributes to the tissue damage and severity of the pneumonia. This hypothesis may contribute to explain why patients with agammaglobulinemia had a mild pneumonia and recovered without experiencing complications requiring oxygen therapy. [101] Antibody may be simply a bystander consequence of a powerful viral replication, or rather the direct trigger of a severe inflammation. This may be explained with different mechansims:

8.2.1. The deposition of IgA immune-complexes - COVID-19 patients develop soon high titers of virus specific IgA antibodies. This phenomenon is leading to the deposition IgA immuno-complexes, which may cause inflammation and microthrombosis with mechanisms similar to the IgA nephropathy. [102]

8.2.2. IgM and IgG immunocomplexes – It has been suggested that the formation of IgM and IgG immunocomplexes may contribute to inflammation [103] and to intravascular coagulation and complement activation. [104] Mannose binding lectin can also induce complement activation after binding the viral N-glycans enriched in Mannose. [88,104]

8.2.3. Antibody dependent enhancement - Specific IgG antibodies, if generated against non-neutralizing domains of the SARS-CoV-2 antigens, may have an aggressive, rather than a protective, neutralizing action. These antibodies induce conformational changes of the S protein that facilitate the fusion of viral particles with the cell membrane. [105,106] This “antibody dependent enhancement” may be a dangerous consequence of vaccine or immunotherapeutic approaches and provoke disease exacerbation.

9 A model of the interaction between SARS-CoV-2 and immune system

9.1 Introduction: The confrontation between SARS-CoV-2 and innate immunity, quantitative aspects and the sequence of events is crucial. Natural antibodies and other components of the innate immunity are the first line of defense and must block the virus in the upper airways, in the first 10-12 days from infection (5-7 from the disease onset), i.e. the time required to prepare an efficient adaptive primary antibody response.

9.2 First stage (upper airways): viral clearance or pneumonia. The competition between the virus and natural antibodies may be exemplified with three major scenarios (Figure 4 and Figure 5):

(A) young and healthy people: patients with efficient natural immunity, who have been exposed to relatively low doses; their natural immune response efficiently control the infection for a couple of weeks and the adaptive immune response will complete the clearance mission: the patient remains asymptomatic or develop only an URI.

(B) old patients: viral exposure is probably higher (the source of contagion is also an old person) but the innate immunity is much weaker; a high number of viral particles can reach the alveoli and replicate in type II pneumocytes in coincidence or even much before the expansion of the specific immune response: hence pneumonia.

(C) young but highly exposed patients: an excessive cumulative viral dose of exposure (unprotected health care personnel) will overcome their efficient innate immunity, will reach the alveoli and cause pneumonia.

9.3 Second stage (pneumonia): recovering or complications. If the virus reaches the alveoli well after the establishment and expansion of a very efficient adaptive immune response, the patient will probably never require oxygen and will not undergo complications.

By contrast, if the virus infects the alveoli early enough (i.e. already 7 days from the infection or 2-3 from the first symptoms), the chances of a better replication in the lung are higher. When the specific response is established, massive amount of the virus can interact with massive amounts of antibodies with high affinity. Under these circumstances, immunopathology may concur to tissue damage and organ failure with the following pathways:

(A) The classical pathway of Complement can be activated by immunocomplexes formed by SARS-CoV-2 and specific IgG or IgM. Complement activation causes the release of pro-inflammatory, vasoactive and chemoattractant components that increase local inflammation.

(B) The lectin pathway of Complement may be activated by Virus-IgA immunocomplexes, through MBL binding to both viral N-Glycan and IgA.

(C) Activation of MBL-associated MASP may cause thrombin activation and triggering of coagulation. Both Classic and Lectin pathways of Complement activation on the external membrane of infected cells releasing viruses, may cause deposition of late complement factor and formation of the membrane attack complex (MAC) causing cell damage and release of cellular components.

(D) Non-neutralizing specific IgG and IgA binding the virus may concur to increased infection and inflammation as a consequence of antibody dependent enhancement (ADE) of infectivity. Ig with low affinity or non-neutralizing may cause infection and activation of macrophages via Fc receptors. In addition, Ig binding the S protein of SARS-CoV-2 may cause its conformational changes making more efficacious the binding to the ACE-2 receptor and the viral fusion with the cell membrane.

The local high concentration of cytokines and chemokines that contribute to recruitment of inflammatory cells and vasodilatation, permits to serum natural Igs and MBL to maintain a vicious circle of inflammation with complement activation and immunocomplexes deposition. In this light, it cannot be excluded that MBL or IgM mediated immunocomplexes contribute to activation of platelets or tissue factor leading to coagulation and microthrombosis that have been described in COVID-19 patients with acute respiratory failure. In this phase of the disease, natural IgM and MBL that circulate in serum may have no protective role, but, rather may contribute to tissue damage. Moreover, during this second phase of the disease, the adaptive response is also progressively on the increase. This may be one side protective against further virus spread in the lungs,but may also reinforce the immunological and coagulation cascades provoking complications.

MBL binds to polymeric IgA and initiates complement cascade, a defense against invading pathogens in mucosal immunity. Polymeric IgA also has a role in activating lectin-mediated complement signaling.

The complement cascade links the innate and the adaptive immune system, protecting against invading pathogens during the first phase of the diseases. In this sense, Ab-mediated complement activation flows in parallel between MBL and C1q. Additionally, it can boost proinflammatory effects of IgA deposition with the same mechanism that is supposed to occur in the glomerulus, and results in renal injury.

10 Implications for diagnosis, public health and clinical intervention

10.1 Words of caution

The model of the interaction between the immune system and SARS-CoV-2 in humans is only a first attempt to produce a synthesis of what is known today. The extremely rapid acquisition of knowledge will allow correcting and improving this model very soon. However, the model can be relevant for diagnosis and intervention. The considerations listed below will require further investigation and validation and are open to evidence-based modifications before they can be part of shared guidelines for the prevention, surveillance and control of COVID-19. However, we believe that these points are first priorities for intensively and focused clinically oriented research.

10.2 Prevention of severe infections

10.2.1 Identification of symptomatic or asymptomatic high virus spreaders by quantitative PCR or new rapid tests based on viral protein detection in saliva or nasopharyngeal swabs to promote their quarantine and social distancing to prevent high dose exposure of highly susceptible contacts

10.2.2 Cumulative high dose exposure should be prevented for everybody; however, in this phase in which many governments are pursuing herd immunity, even young individuals who may have low levels of natural antibodies should be not exposed to the virus, especially if shed at moderate/high doses.

10.2.3 To prevent high dose exposure information campaigns should be implemented to promote the attention to fomites in addition to hand washings, medical masks wearing, social distancing and avoid touching eyes, nose and mouth and the promotion of technical devices for contact tracing.

10.2.4 Glycan microarrays should be used in the attempt identifying profiles of natural antibodies associated with milder COVID-19 disease evolution.

10.2.5 Prevention of viral faster penetration in the lungs during early stages of infection. Smoking, as well as activities, like intensive fatiguing work, including sport that imply high respiratory volumes should be avoided during the early stage of infection to prevent bypass of the natural immune response in the oral cavity and upper respiratory airways and facilitate penetration of excessive amount of SARS-CoV-2 from the nasal, oral and pharynx mucosa to the lower airways at a stage in which an adaptive immune response is still not initiated. SARS-COV-2 diameter is 100 nm, and aerosols containing it can deposit not only in the tracheobronchial, but also in alveolar regions at rest. The latter probably increases while breathing at very high volumes, like during a marathon or a football soccer game. Especially in the smaller airways, the deposition of particles is inversely proportional to the inspiratory flow.

10.2.6 Particular precautions should be given to athletes performing fatiguing sports, since a portion of sub-micrometer-size, aerosolized particles, are expired by the runner or eliminated by cough or nasal secretions and may contain viruses if the athlete is an asymptomatic but SARS-CoV2 infected individual. These droplets or aerosol might be re-inhaled and facilitate the spread of the virus from the upper to the lower airways. Moreover, in sports were many athletes are in close contact, such as team sports or marathons, the same particles have high chances to be inhaled by other athletes, facilitating viral transmission. To emphasize that strenuous exercise induces a much more frequent spitting of secretions and this can further contribute to the environmental SARS-CoV-2 spreading, particularly if the distancing recommendations are not strictly followed.

10.3 Monitoring and treatment of pneumonia and its complications

10.3.1 Detection of early markers of complement activation, such as C3 and C4 consumption and C4a and C3a plasma increase, would indicate a specific treatment with steroids or new drugs such as Eculizumab, a humanized hybrid IgG2/IgG4 monoclonal antibody directed against human C5, that prevents production of C5a and C5b-9.

10.3.2 Detection of early markers of coagulation such as plasma thrombomodulin and d-dimer would indicate treatment with therapeutic dosages of systemic or nebulized heparin.

10.3.3 Detection of early markers of cytokine storm by routinely measuring levels of inflammatory cytokines in addition to IL-6 would indicate administration of other cytokine inhibitors, such Janus kinases inhibitors.

10.3.4 Detection of non-neutralizing antibodies by specific assays would indicate administration of hyperimmune IgG from convalescent recovered individuals, since high dose of neutralizing Ab are described to reduce ADE, or neutralizing human or humanized monoclonal antibody, upon availability for human use. In fact, plasma administration before development of a humoral response to SARS-CoV-2 would be expected to be most effective in protecting patients from developing severe forms of the disease.

10.4 Population screening for public health measures and immunization

10.4.1 Tests specifically identifying natural (IgM) antibodies directed against the carbohydrate moieties flanking SARS-CoV-2 S1-RBS may be useful, among elders, to identify those at higher risk of severe disease. Glycan microarrays will be instrumental for this target.

10.4.2 Studies on the prevalence of SARS-CoV-2 infections that include asymptomatic and paucisymptomatic individuals could be pursued by measuring SARS-CoV-2 specific serum IgG and IgA that are expected to persist as a memory response to infection. Appropriate and high performing validated tests should be used to retrospectively evaluate the seroconversion status to estimate the herd immunity of a given population.

10.5 Immunization strategy: innate and adaptive immunity

10.5.1 While effective vaccines are being developed, produced, tested, and validated, a strategy to stimulate innate immunity and natural IgM antibody production in particular, would empower the defences of at-risk elderly population. These measures may include influenza, pneumococcal, BCG and other immunizations that have been proved to reinforce natural immunity in general. This can be valid especially considering that pneumococcus is also a frequent cause of co-infection causing severe pneumonia and complications.

10.5.2 Given the relevance of the local immune response to SARS-CoV-2, an immunization strategy based on a mucosal vaccination would assure a higher protection.

Afterwards

SARS-CoV-2 pandemic and COVID-19 are challenging humanity and a quick response is urgent. According to WHO statistics, In the 30 days during which these pages have been elaborated (20 March to 19 April, 2020), may thousand people died for COVID-19 and many countries have been locked down. Scientific and clinical efforts of great scientists and clinicians is producing a storm of knowledge only partially reproduced here. On the basis of their discoveries and observations, the Authors could try to produce a first model of COVID-19. A scientific model is based on observations perceived by humans and on assumptions elaborated by their brains. Hence, a model is only an approximate interpretation of the reality and it is always wrong in some small or relevant elements. The destiny of the model presented here is to be rapidly improved thanks to novel knowledge coming from new observations and better assumptions. The Authors hope that many and more brilliant minds will read the present pages, will identify and highlight putative mistakes, will get inspiration for their research and will produce better, more complete and useful models. of the interactions between our immune system and SARS-CoV-2. If the speculations presented here on implications for surveillance, control and therapy of COVID-19 will contribute, even only minimally, to save some human life and accelerate the end of the pandemic, then the Authors have accomplished their small mission. Rome (Europe) and Berlin (Europe), 22.April 2020.

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Legend to figures

Figure 1 - **Different COVID-19 clinical courses and trajectories of adaptive immune response and viral shedding.** Quantitative polymerase chain reaction (qPCR); Disseminated intravascular coagulation (DIC); Upper respiratory airways infection (URI); Lower respiratory airways infection (LRI); Respiratory failure (RF).

Figure 2 – Variations in anti-glycan IgG and IgM antibody signals with age.[74]

Figure 3 – Evolution of COVID-19 in relation to the cumulative dose of exposure and the natural immune response. Evolution of COVID-19 in dependence of infective viral load, efficacy of natural immunity and protective adaptive immune response. The lines represent the disease evolution of index patients, whose profile is presented in the main text; squares: young patient; circles: old patient; triangles: young doctor exposed to massive doses of virus. Quantitative polymerase chain reaction (qPCR); Disseminated intravascular coagulation (DIC); Upper respiratory airways infection (URI).

Figure 4 – Mediators cascades causing complications during pneumonia in COVID-19 patients. The classical pathway of Complement can be activated by immunocomplexes formed by SARS-CoV-2 and specific IgG or IgM **(A)** Complement activation causes the release of pro-inflammatory, vasoactive and chemoattractant components that increase local inflammation. The lectin pathway of Complement may be activated by by Virus-IgA immunocomplexes, through MBL binding to both viral N-Glycan and IgA **(B)**. Activation of MBL-associated MASP may cause thrombin activation and triggering of coagulation. Both Classic and Lectin pathways of Complement activation on the external membrane of infected cells releasing viruses, may cause deposition of late complement factor and formation of the membrane attack complex (MAC) causing cell damage **(C)** and release of cellular components. Non-neutralizing specific IgG and IgA binding the virus may concur to increased infection and inflammation as a consequence of antibody dependent enhancement (ADE) of infectivity. Ig with low affinity or non-neutralizing may cause infection and activation of macrophages via Fc receptors **(D)**. In addition, Ig binding the S protein of SARS-CoV-2 may cause its conformational changes making more efficacious the binding to the ACE-2 receptor and the viral fusion with the cell membrane **(D)**.

Figure 5 – A "quantitative and time-sequence dependent" model COVID-19. - The natural history of COVID-19 caused by SARS-CoV-2 is extremely variable, ranging from asymptomatic infection, to pneumonia, and to complications eventually fatal. We propose here the first model, explaining how the outcome of first, crucial 10-15 days after infection, hangs on the balance between the cumulative dose of viral exposure and the efficacy of the local innate immune response (natural IgA and IgM antibodies, MBL). If SARS-CoV-2 overcomes this first-line immune barrier and rapidly spreads from the upper airways to the alveoli, then it can replicate with no resistance into the lungs, before a strong adaptive immune defense is established. When high affinity IgM and IgG antibodies are produced, the consequent severe inflammation damages the lungs and triggers mediator cascades (complement, coagulation, and cytokine storm) leading to complications that may be fatal. Strenuous exercise and high flow air in the incubation days and early stages of COVID-19, facilitates direct penetration of the virus to the lower airways and the alveoli, without impacting on the airways mucosae

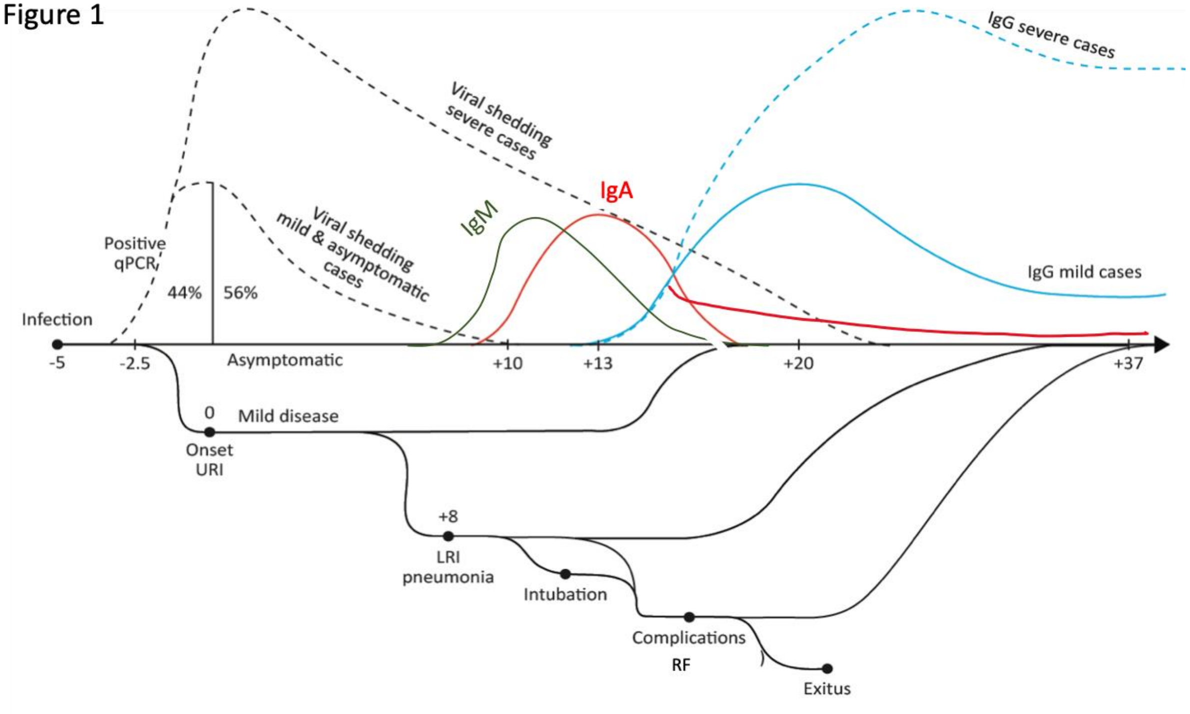
covered by neutralizing antibodies. This allows the virus to bypass the efficient immune berries of young and healthy athletes. In conclusion, whether the virus or the adaptative immune response reach the lungs first, is a crucial factor deciding the destiny of the patient.

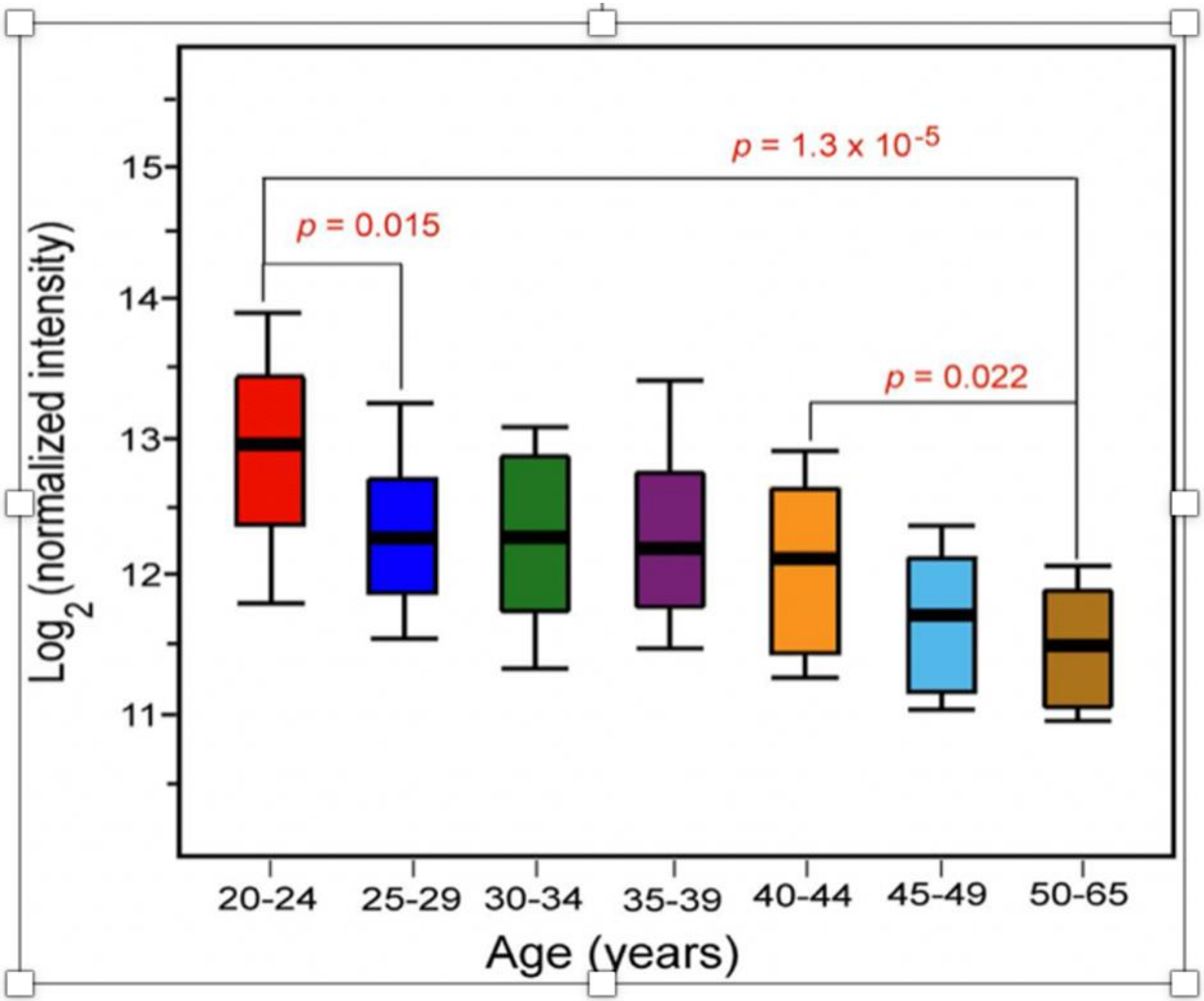
Table 1. COVID-19 Death rates per 1 Million in Italy (April 19, 2020)

Italy			
Age (years)	**Deaths[1]** (n)	**Population** (n)	**COVID-10 Deaths per 1 Million**
≤9	1	4994995	0,2
10 to 19	0	5733448	0,0
20 to 29	7	6103436	1,1
30 to 39	39	6998434	5,6
40 to 49	170	9022004	18,8
50 to 59	712	9567192	74,4
60 to 69	2142	7484862	286,2
70 to 79	5874	6028908	974,3
80 to 89	7534	3699654	2036,4
≥90	2161	828895	2607,1

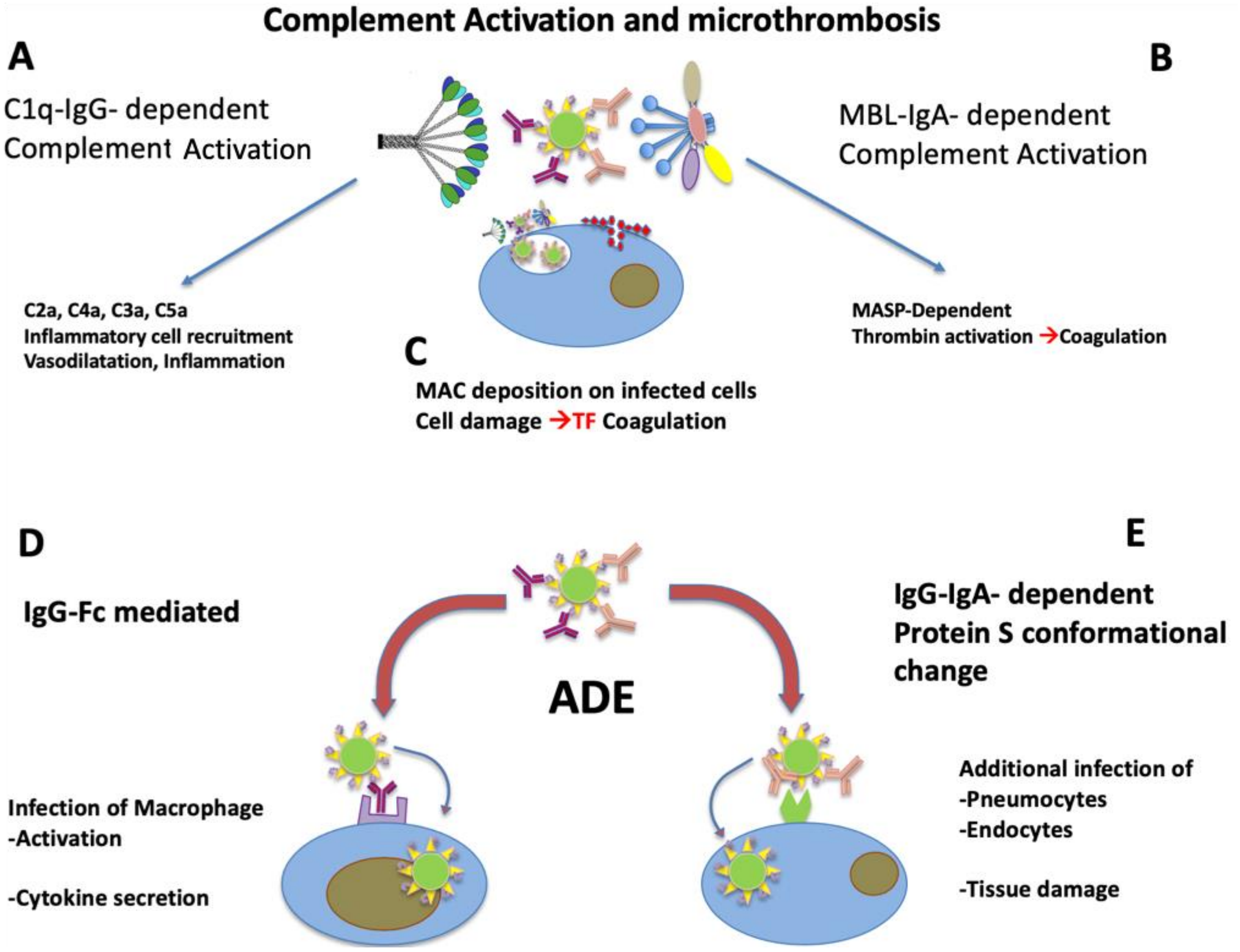
[1]Ref27- https://www.epicentro.iss.it/en/coronavirus/bollettino/Infografica_13aprile%20ENG.pdf

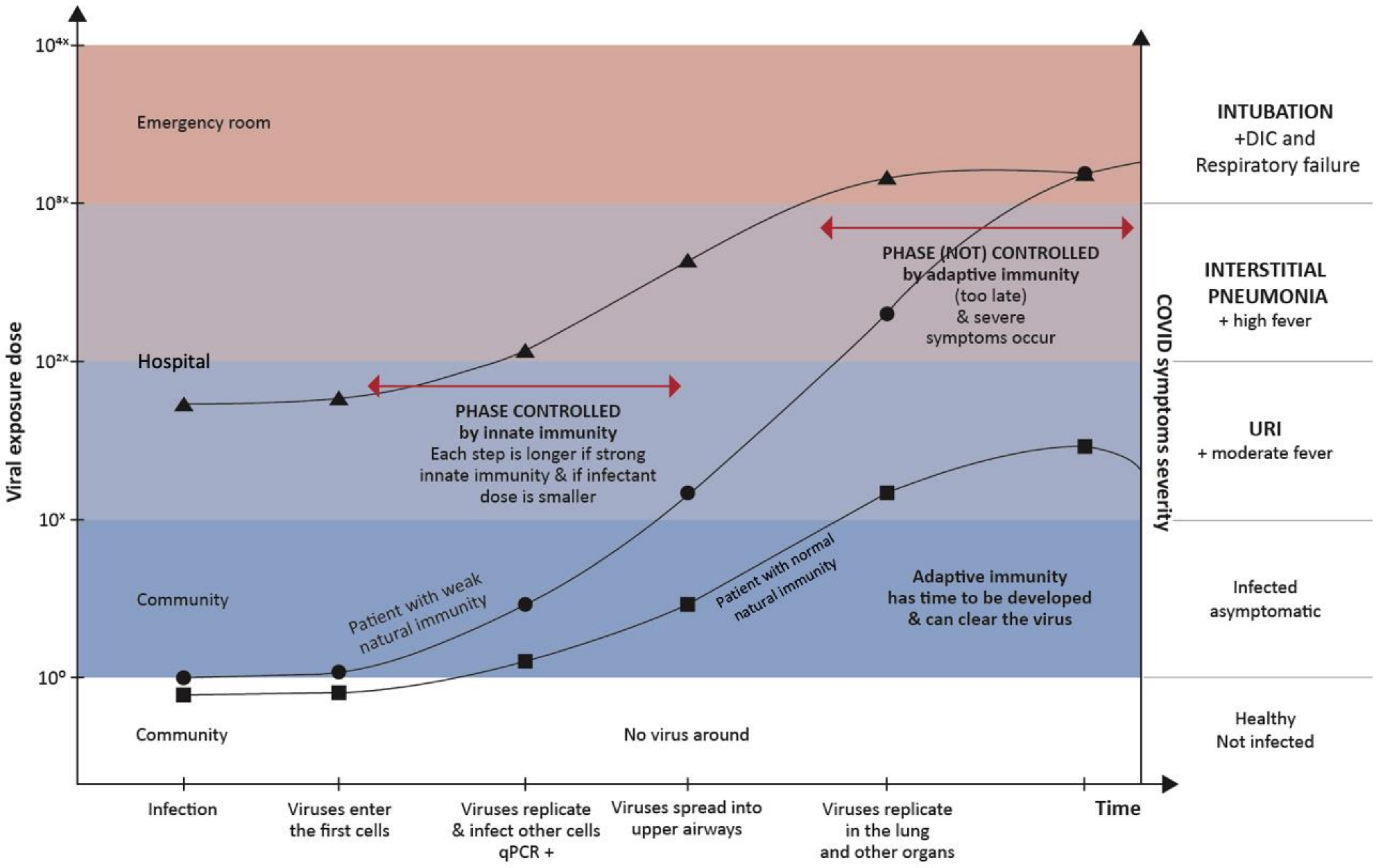
Figure 1


IgG severe cases
Viral shedding
severe cases
IgA
IgM
Viral shedding
mild & asymptomatic
cases
IgG mild cases
Positive
qPCR
44%
56%
Infection
-5
-2.5
Asymptomatic
+10
+13
+20
+37
0
Mild disease
Onset
URI
+8
LRI
pneumonia
Intubation
Complications
RF
Exitus



p = 1.3 x 10-5
p = 0.015
p = 0.022
Log2 (normalized intensity)
15
14
13
12
11
20-24
25-29
30-34
35-39
40-44
45-49
50-65
Age (years)


Complement Activation and microthrombosis
A
C1q-IgG- dependent
Complement Activation
B
MBL-IgA- dependent
Complement Activation
C2a, C4a, C3a, C5a
Inflammatory cell recruitment
Vasodilatation, Inflammation
C
MAC deposition on infected cells
Cell damage →TF Coagulation
MASP-Dependent
Thrombin activation →Coagulation
D
IgG-Fc mediated
ADE
E
IgG-IgA- dependent
Protein S conformational
change
Infection of Macrophage
-Activation
-Cytokine secretion
Additional infection of
-Pneumocytes
-Endocytes
-Tissue damage


Viral exposure dose
10⁴ˣ
10³ˣ
10²ˣ
10ˣ
10⁰
Emergency room
Hospital
Community
Community
No virus around
PHASE CONTROLLED
by innate immunity
Each step is longer if strong
innate immunity & if infectant
dose is smaller
PHASE (NOT) CONTROLLED
by adaptive immunity
(too late)
& severe
symptoms occur
Patient with weak
natural immunity
Patient with normal
natural immunity
Adaptive immunity
has time to be developed
& can clear the virus
Infection
Viruses enter
the first cells
Viruses replicate
& infect other cells
qPCR +
Viruses spread into
upper airways
Viruses replicate
in the lung
and other organs
Time
COVID symptoms severity
INTUBATION
+DIC and
Respiratory failure
INTERSTITIAL
PNEUMONIA
+ high fever
URI
+ moderate fever
Infected
asymptomatic
Healthy
Not infected

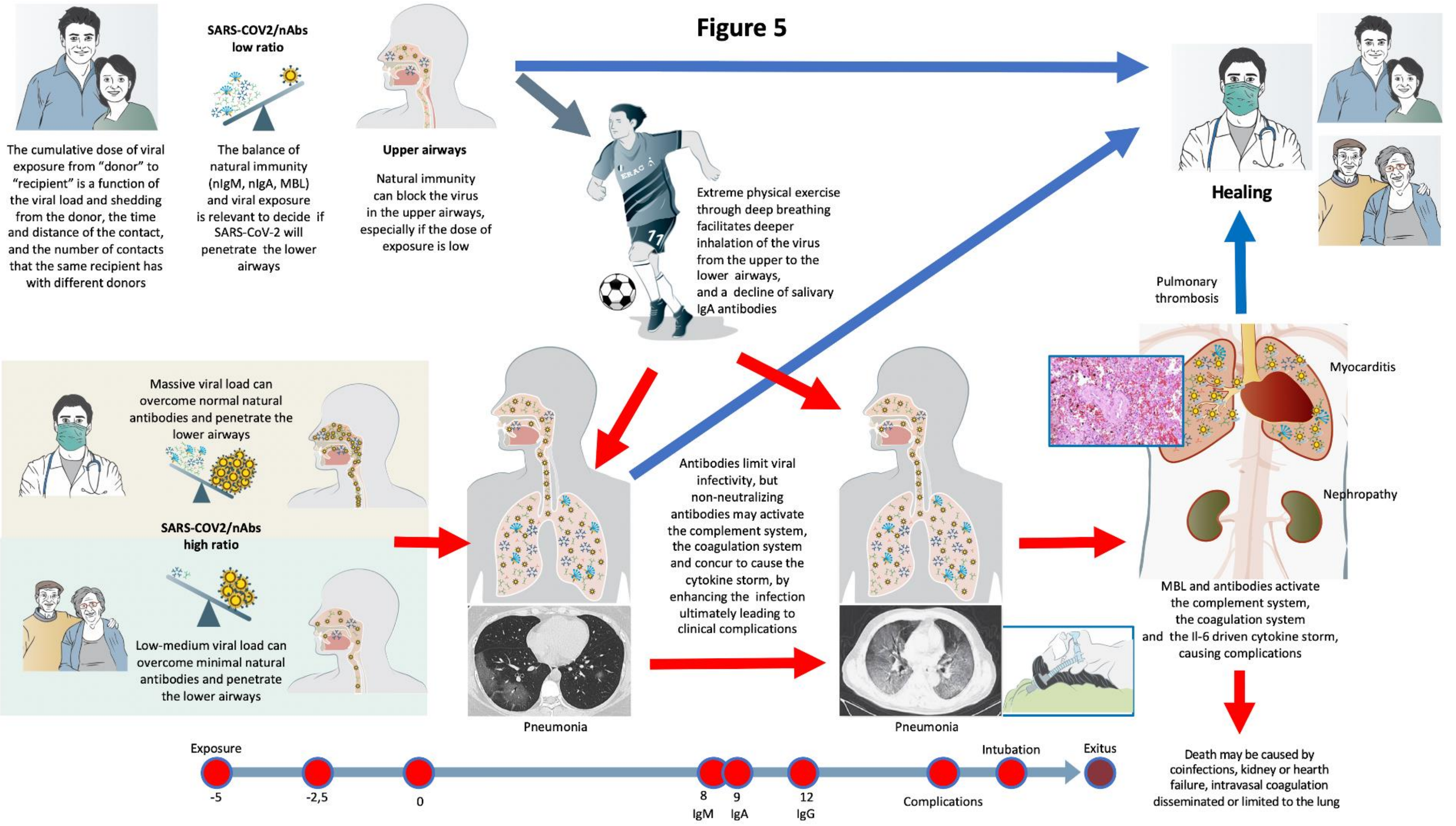


Figure 5
SARS-COV2/nAbs low ratio
The cumulative dose of viral exposure from "donor" to "recipient" is a function of the viral load and shedding from the donor, the time and distance of the contact, and the number of contacts that the same recipient has with different donors
The balance of natural immunity (nIgM, nIgA, MBL) and viral exposure is relevant to decide if SARS-CoV-2 will penetrate the lower airways
Upper airways
Natural immunity can block the virus in the upper airways, especially if the dose of exposure is low
Extreme physical exercise through deep breathing facilitates deeper inhalation of the virus from the upper to the lower airways, and a decline of salivary IgA antibodies
Healing
Pulmonary thrombosis
Myocarditis
Nephropathy
Massive viral load can overcome normal natural antibodies and penetrate the lower airways
SARS-COV2/nAbs high ratio
Low-medium viral load can overcome minimal natural antibodies and penetrate the lower airways
Antibodies limit viral infectivity, but non-neutralizing antibodies may activate the complement system, the coagulation system and concur to cause the cytokine storm, by enhancing the infection ultimately leading to clinical complications
MBL and antibodies activate the complement system, the coagulation system and the Il-6 driven cytokine storm, causing complications
Pneumonia
Pneumonia
Exposure
-5
-2,5
0
8 IgM
9 IgA
12 IgG
Complications
Intubation
Exitus
Death may be caused by coinfections, kidney or hearth failure, intravasal coagulation disseminated or limited to the lung

ELECTRONIC REPOSITORY

The first, comprehensive immunological model of COVID-19:

implications for prevention, diagnosis, and public health measures

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Anti-glycan Natural IgM and IgA antibodies

Anti-glycan natural antibodies are detected in serum in the absence of previous immunization, are observed also in gnotobiotic animals, and belong mostly to the IgM isotype [1] but also to the IgA and IgG istopype.[2]

Their low affinity is compensated by a higher avidity and efficient complement activation, so that anti-glycan natural IgM play a crucial role in immune-surveillance against tumors, autoimmunity, and first-line-of-defense against invading pathogens, including viruses.[3] Studies based on a highly sensitive microarray-based assay found that high purity, anti-glycan IgM antibodies are prevalent in cord-blood and recognize a broad range of non-human and human glycans, with a pattern of recognition independent from that of the mother.[4] Humans harboring rare disorders resulting in selective IgM (SIgMID) [5] or IgA [6] immunodeficiency defects are predisposed to recurrent infections, especially of the upper respiratory tract. Natural IgM antibodies recognize glycans, and outcompete IgG and IgA in this property.[2,3] These natural antibodies are produced mainly in the spleen by B1-cells.[7] By studying with glycan array technology, free serum anti-glycan and anti-glycopeptide IgM (and IgG) have been deeply examined in 135 healthy subjects of varied age, race, gender, and blood type.[3]

Starting with the ‘80s, natural IgM have been regarded as almost irrelevant because of their low affinity, cross-reactivity and pentameric structure which are now interpreted as great advantage to economically recognize and bind surface structures in previously never seen pathogens.[8] Sera from non-immune mice can neutralize influenza virus. This property is lost in sera deficient in natural IgM.[9] Natural IgM binds and activate complement, facilitating virion aggregation and coating of the viral hemagglutinin receptor.[9] RAG1-deficient mice, when reconstituted with natural IgM, had a prolonged incubation time and symptoms provoked by the influenza virus.[9] This evidence suggests that natural IgM and the early components of the classical pathway of complement may work in concert to neutralize also SARS-CoV-2, have a significant and beneficial impact on the course of COVID-2 and prevent viral pneumonia. Given this premise, we have matched the pattern of distribution of natural IgM antibodies in the population and checked whether it fits the patterns of risk factors associated with severe COVID-19.

Mannose Binding Lectin (MBL)

MBL plays a pivotal role in innate immunity interacting with surface sugars of a wide series of microorganisms as a pattern-recognition receptor.[10] Thus, MBL: i) activates the lectin complement pathway; ii) promotes opsonophagocytosis 11]; and iii) modulates inflammation [12].

In detail: the binding of MBL and MBL-associated serine proteases (MASPs) to carbohydrates on the surface of microorganisms led to the activation of C2 and C4 that are cleaved to the C4bC2a complex that has a C3 convertase activity. [13] An antiviral role of MBL has been less investigated. Native and recombinant human MBL exhibited neutralization activity against Influenza virus (H3N2) with a plaque focus reduction assay at the viral attachment phase.[14] Interestingly, recombinant MBL seems to be effective against Ebola in mice [15] because MBL binds to Ebola and Marburg envelope glycoproteins contributing to neutralization of the virus.[16] It has been demonstrated that Carbohydrate-binding agents (CBAs) play an active role in defenses against viruses such as HIV, human hepatitis C virus, coronavirus and influenza virus [17] MBL and defensins directly bind the O- and N-linked glycans of several viruses, while the mannose receptor and DC-SIGN are the most widely known cell associated CBA [18] MBL is secreted in the blood by the liver and can be produced by macrophages.[19] Although it is present in sera, it may be detected in gut [20] as well as in upper and lower airways secretions, following inflammation-promoted exudation [21]

Diagnostic tests

Detection of specific antibody responses induced by SARS-CoV-2 can be useful for research purposes and for epidemiologic studies, aimed at acquiring reliable information for public health measures.[22] The best molecular targets for these tests are the spike (S) and the nucleocapsid protein (N), considered the most specific immunogenic components of SARS-CoV-2.[23]

Many tests are being marketed [24] but only a few of these tests have been already (14.April.2020) validated in properly designed studies.[22,25] At disease onset, a general problem is given by a weak test positivity that may be generated by IgM antibodies already induced in the general population not by SARS-CoV-2, but by other, endemic coronaviruses.[22,26]

The antibody levels of the primary response induced by SARSCoV-2 are lower after mild infection than after severe infection, so that the use of two different antigens is recommended to increase test sensitivity, when mildly infected patients are examined.[22] Given their longer persistence in circulation, tests measuring IgG antibodies are preferable in sero-surveillance studies.[27]

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