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The first, holistic 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 or mild infection, mainly in children, to multi-organ failure, eventually fatal, mainly in the eldest. 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, Mannose Binding Lectin). 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. Low-moderate physical activity can still be recommended. However, extreme physical activity and hyperventilation during the incubation days and early stages of COVID-19, facilitates early direct penetration of high numbers of virus particles in the lower airways and the alveoli, without impacting on the airway's mucosae covered by neutralizing antibodies. This allows the virus bypassing the efficient immune barrier of the upper airways mucosa in already infected, young and otherwise 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 at all ages.

Foreword - *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 experience only mild upper airways symptoms, others develop symptomatic pneumonia, which may progress to 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 pneumonia with or without symptoms, mostly dyspnea.**[1, 17]** 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).**[18]**

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).**[18]** 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.**[19]** 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.**[20]** ARDS, acute cardiac and kidney injury, sepsis, and secondary infection were the most frequent complications.**[20]**

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.**[20]** Among survivors, permanence in intensive care unit (ICU) lasted 7.0 days (2.0-9.0); and discharge from the hospital occurred shortly thereafter.**[20]** 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.**[21] (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. **[22]** SARS-CoV-2 has a similar tropism, and also infects type II pneumocytes, enterocytes and macrophages. **[23,24]** In addition, SARS-CoV-2 can infect cell expressing ACE2 receptor and the serin protease TMPRSS2. **[25]** 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. **[26]** 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, **[27]** SARS-CoV-2 uses its spike glycoprotein to bind to the angiotensin-converting-enzyme-2 (ACE2) receptor. **[28]** 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. **[28]** 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. **[29]** The SARS-CoV spike protein contains 22 N-glycosylation sites. **[30]** The glycosylation pattern of viral particles produced in a human cell line has been already characterized in 13/22 sites. **[30]** 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. **[30]** However, an area which remaining free from glycosylation, is also suitable for ACE2 binding and potentially for other proteins, including neutralizing antibodies. **[30]**

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.**[31]** 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.**[31,32]**

3.2 **Kinetics of the adaptive antibody response**

Typical primary and secondary antibody responses to acute viral infection are efficiently induced. **[32]** 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.**[32,32] (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. **[33,34]** The IgG antibody response starts appearing shortly thereafter (or simultaneously) but persist longer **[31-34]** and may be protective.**[35]**

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.**[32,36]**

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.**[37,38]** Similar effectiveness had been

already demonstrated for plasma from patients having recovered from SARS-CoV and MERS-CoV.**[39,40]** 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.**[41,42]** 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 **[43]** 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. **[44-46]** Among them, hospital doctors frequently exposed to COVID-19 patients are, unfortunately, highly represented. **[47]** Dr. Li Wenliang, the first man alerting China and the World of the new infection, died from COVID-19 at the age of 33.**[48]** Similarly, Dr. Carlo Urbani, i.e. the first man alerting the of SARS-CoV, died of SARS at the age of 46.**[49]** Both doctors cared for weeks patients with severe pneumonia with no personal protection.**[48,49]** In Italy, 114 doctors exposed to SARS-CoV-2, have so far (14th April, 2020) died of COVID.**[50]** 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.**[50,51] (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.**[52]** 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. **[53]** 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.**[54]**

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.**[55]** Moreover, most of the relatively few pediatric cases were classified as mild (81%), only 14% severe and 5% critical.**[55]** Until now (April 14, 2020) the death of only a few humans aged 18 years or less has been attributed to SARS-CoV-2.**[44]** A similar trend has been observed in the United States, where among 149,760 reported cases only 2,572 (1,7%) were children aged <18 years, among which only three died of COVID-19.**[56]**

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.**[55]** In Europe, the virus is still spreading and has already caused over 77,786 deaths (April 14,2020) with a CFR also increasing with age .**[45]** Italy was the first European country facing the pandemic, with 159516 cases of COVID-19 diagnosed up to April 14, 2020 **[46]** 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.**[44] [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. **[57]** In a larger Chinese study, over 90% of all pediatric patients had no severe disease.**[58]**

4.3 - Gender and Blood group

COVID-19 mortality has been lower among Chinese females than males.**[59]** In Italy, mortality and hospitalization rates have been also more frequent among males than among females.**[60]** Moreover, patients with blood group 0 and A have slightly lower and a slightly higher risk, respectively, of developing COVID-19.**[61]**

4.4 – Physical activity and COVID-19

4.4.1 – The opposite impact of low-moderate versus strenuous exercise

Low-moderate exercise corresponds by general consensus to <60 min exercise for 3-5 days per week and at <80% of maximum capacity **[62]**. This activity is beneficial for the innate immune response against respiratory infections and protects from effort-induced inflammatory and oxidative mediators **[63]**.

By contrast, extreme exercise performed by professional athletes (e.g. bikers, rowers, marathon runners, soccer players) induces a tremendous increase of alveolar ventilation and susceptibility to URI **[62]**. Many professional athletes claim the occurrence of fever, dry cough, malaise and dyspnea a few hours or days after their last performance.**[64]** Nearly 13% of the endurance runners reported viral URTI episodes in the week following their marathon, compared to 2.2% of control runners **[64]**.

4.4.2 – The first COVID-19 case in Italy - 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 the onset of upper airways symptoms and pneumonia was 2 days only. Only 4 days after the onset of COVID-19, the patient was admitted to the intensive care unit of the Policlinico San Matteo in Pavia because of respiratory failure. After weeks of intubation and supportive treatment, the patient luckily recovered and could be discharged in good conditions**.**

The Italian first COVID-19 case is worldwide famous but, surprisingly, no official study on it has been so far published. 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. Indeed, other cases of COVID-19 in (semi-) professional athletes have been described. **[65]** The

problem of SARS-COV-2 infection is part of a more general problem, since athletes and para-athletes have amplified susceptibility to viral respiratory tract infection and chronic respiratory diseases **[66,67]**

4.4.3 - Strenuous exercise and IgA defect - Regular, moderate exercise reduces the risk of acute respiratory infections.**[68]** By contrast, the levels of salivary IgA decline in athletes during and after a training season.**[69]** This observation may explain why elite athlete are at higher risk of upper airway infections.**[70]** A so-called ``open window'' of higher susceptibility to infection ranges between 3 and 72 hours after strenuous exercise.**[71]**

4.4.4 The viral "auto-inhalation" hypothesis - The majority of professional athletes have their lungs that are usually working in near perfect physiological conditions, such as very close to those of the "ideal lung", with an even distribution of their alveolar ventilation.**[72]** These pre-existing ideal conditions together with the frequent and regular practice of extreme and long-lasting workloads can significantly favor the deep inhalation of several irritants, allergens, infectious agents, and also virus particles.**[72]** Aerosols are considered an important mode of transmission for influenza.**[73]** 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.**[73]** 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.**[74]**

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 starts 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 requiring oxygen therapy is a critical event discriminating asymptomatic or mild cases, whose infection remains mostly confined to the upper airways, from those with severe disease, who experience massive viral invasion of their lower airways.**[19]** What makes the difference? What prevents the virus from rapidly reaching the lungs and then causing severe pneumonia? What makes COVID-19 pneumonia a life-threatening disease?

1) No efficient adaptive immune response is available at the time of infection;**[31]**

2) Pneumonia may start before adaptive immune response develops; **[20]**

3) Serious complications begin together with the adaptive immune response.

5.2 The first two weeks after infection are crucial. **[18,20]** 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 the disease. This confrontation also decides whether the infection will be efficiently blocked in upper airways, or how many virus particles reach the lungs, and when. 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.**[75]** Both patients developed pneumonia, and both recovered without any need of receiving oxygen-therapy. **[75]** 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.**[76]** 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.**[77]**

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.**[78]**

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.**[78]**

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 **[79]** but also to the IgA and IgG isotype.**[80]**

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

6.1.1 Natural IgM decline with age. Natural IgM are produced after birth in neonates independently on concurrent infections and rapidly reaches values comparable to adults **[81,82]**. Natural IgM broadly and nonspecifically recognizes diverse microbial determinants and autoantigens **[83]** including A/B blood group antigens. Median values of natural IgM anti-A/B increased during the first years of life to reach adult values in the

children 5 to 10 years old **[84]**. When examined with glycan array, IgG signals remains relatively unchanged with age.**[85]** 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.**[85]** 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.**[44-46]**

A more recent study also found a reduced diversity in natural IgM antibodies in older donors **[86]**, reflecting a similar trend observed in human B-1 B cells, i.e. the cells responsible for natural IgM production, mainly in the spleen **[87]**. This evidence may contribute to explain the increasing prevalence of severe cases of COVID-19 in the eldest.**[44-46]**

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.**[75]** By contrast, anti-glycan IgM were slightly, although not significantly higher in females.**[75]** This outcome is consistent with the observation of higher total IgM levels in females **[88]**. It is well known that blood type has a profound influence on the repertoire of glycan specific IgG and especially IgM antibodies.**[75]** This is also why blood groups are very relevant in regulating host susceptibility to infection.**[89]** In a study among health care workers in Hong-Kong, Group O individuals were remarkably resistant to SARS-CoV infection.**[90]** 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. **[91]** An influence of blood group on susceptibility to severe COVID-19 has been postulated **[61]**, 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 **[61]** or remarkable **[59,60]** 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.**[92]** Thus, MBL: i) activates the lectin complement pathway; ii) promotes opsonophagocytosis **[93]**; and iii) modulates inflammation **[94]**.

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

Serum MBP levels are distinctly higher in children (3-19 years) than in adults (over 20 years) and decline with age (**5**). A considerable amount of serum MBP (about 1 μg/ml) is already present at birth and this level start to increase from day 2 and attained the highest level in a human lifetime (about 2.5 micrograms/ml) within 5 days after birth (**6**). The persistence of increased MBL levels at 3 months and thereafter suggests that a true post-natal maturation takes place in the first 3 months, leading to a sustained MBL increase lasting through childhood (**7**). 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).**[98]**

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.**[99]** Low MBL levels appear to predispose persons to bacterial infectious diseases, particularly in neonatal age and early childhood.**[93]** MBL gene polymorphisms were significantly associated with susceptibility to SARS-CoV infection, possibly explained by the reduced expression of functional MBL. **[100]** 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.**[101]** 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.**[101]** 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 **[101]**. 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) **[102]** 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.**[103]** 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.**[101]** 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.**[102]** 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.**[104]**

8 Immunopathogenesis

8.1. What kills COVID-19 patients with severe 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 **[105]**. 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 **[106]**. SARS-CoV-2 only upregulates IL6, MCP1, CXCL1, CXCL5, and

CXLC10 (IP10). **[106]** Interestingly, ARDS and RF have been associated to increased serum levels of IL-6. **[18]** Elevated serum levels of IL-6 may be an early biomarker of a worsening clinical course.**[107]** Trials with tocilizumab, a monoclonal antibody recognizing IL6-R, started after its efficacy was reported in case reports. **[108]**

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.**[109]** Of relevance, 71.4% of fatalities, but only 0.6% of the surviving patients met ISTH criteria for disseminated intravascular coagulation (DIC) **[110]**, a pro-thrombotic and pulmonary congestion with microvascular thrombosis and occlusion **[111]**. Biomarkers for this process are elevated D-Dimer and plasma thrombomodulin and others, **[112]** and treatments with heparin or Tissue Plasminogen Activator have been suggested. **[111]**

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. **[31,113]** 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. **[75]** 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 mechanisms:

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. **[114]**

8.2.2. IgM and IgG immunocomplexes – It has been suggested that the formation of IgM and IgG immunocomplexes may contribute to inflammation **[115]** and to intravascular coagulation and complement activation. **[116]** Mannose binding lectin can also induce complement activation after binding the viral N-glycans enriched in Mannose. **[101,117]**

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. **[118,119]** 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 a mild disease only;

(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 leading to a more severe and symptomatic pneumonia;

(C) young but highly exposed patients -the exposure to an excessive cumulative viral dose (i.e. unprotected health care personnel) will overcome their efficient innate immunity. Viral particles will reach the alveoli in early stages and cause symptomatic pneumonia;

9.3 second stage: recover or complications -if a relatively low number of virus particles reaches the alveoli after the establishment and expansion of an efficient adaptive immune response, the patient will probably never require oxygen and will not undergo relevant 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 probability of a better replication in the lung is 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 contribute to tissue damage and organ failure according to the following events:

(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 factors 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 to the S protein of SARS-CoV-2 may cause its conformational changes that make the binding to the ACE-2 receptor more effective for 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. Moreover, the implications for treatment are reported as examples of possible consequences of the model. Evidence-based medicine should also apply for Covid-19 patients, so that "new" or off-label drugs or treatment regimens should only be given in a clinical study context, following approval by relevant national or international agencies. However, we believe that these points are first priorities for intensively and focused clinically oriented research.

10.2 Prevention of severe infection

10.2.1 Identification of high virus spreaders - Symptomatic or asymptomatic high virus spreaders should be identified by quantitative PCR based on viral protein detection in saliva or nasopharyngeal swabs. Quarantine and social distancing to prevent high dose exposure of highly susceptible contacts should be strictly practiced by high virus spreaders. Cumulative high viral dose exposure should be prevented for everybody.

10.2.2 Identification and protection of individuals with low natural antibodies levels – Glycan microarras and other tests aimed at measuring natural antibodies, that might be protective against SARS-CoV-2 and other viruses, should be developed. Individuals with low natural antibody and MBL levels should be identified and specifically protected. Moreover, governments promoting herd immunity must protect individuals, even if young, who may have low levels of natural antibodies. These individuals should be not exposed to the virus, especially if shed at high doses.

10.2.3 Prevention of fast penetration of the virus in the lungs - Intensive fatiguing work, including strenuous sport activities requiring high respiratory volumes and flows, should be avoided during the early stage of infection. when the adaptive immune response is

still not initiated. 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.

10.2.4 **Preventing cross-infection of athletes in team sports or marathons.** Low-moderate physical activity is recommended. However, 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.

10.2.5 **Fostering research on sport and COVID-19 –** Research on the impact of SARS-CoV-2 on athlete populations must be promoted, considering that "robust data needs to be collected to understand the effect of general physical fitness on COVID-19 susceptibility, disease behavior and prognosis."**[120]**

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, might indicate the need of investigating 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.

Chronology and methodology - The idea of elaborating a model of COVID-19 was conceived on March 20th, 2020. A systematic literature search on two open-access platforms (ArchXrv, bioRxiv) and six Journals: NEJM, Lancet, JAMA, Cell, Science, Nature & NGP group, started with the keywords COVID-19 and/or SARS-CoV-2 and/or SARS-CoV and/or MERS. Further articles were retrieved from the references of the selected papers. PMM got also first-hand information from many clinicians (see acknowledgements) treating COVID-19 patients in Northern Italian Hospitals. The first nucleus of the model was generated on April 5th and 6th together with Roberto Nisini. Further publications on the viral load, MBL, natural antibodies (IgM, IgA), B-1 lymphocytes, primary and secondary antibody response, cytokine storm (IL-6), complement, coagulation, pneumonia, nephropathy, myocarditis, plasmapheresis and other treatments was daily updated on PUBMED, ArchXrv bioRxiv. The original Model resisted extremely well to the new information and on April 14th was judged robust enough and almost ready for publication. However, the Italian case nr. 1 still remained unexplained. On April 15th PMM elaborated the "viral auto-inhalation hypothesis" and drafted the divulgation version of the Model. On April 18th Roberto Dal Negro, was contacted and independently formulated exactly the same auto-inhalation hypothesis, thus joined PMM and RN in the final effort of manuscript preparation and submission to Pediatric Allergy and Immunology.

Afterword

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. Berlin (Europe), Verona (Europe) and Rome (Europe), 22.April 2020.

Authors´ Contributions

PMM organized the literature search and conceived the first nucleus of the Model, developed the section of the role of dose of exposure and of natural immunity, conceived the "auto-inhalation" hypothesis as an explanation of pneumonia in the case n1 in Italy. RN contributed to the further definition of the Model and developed the section on the immunopathogenesis of complications. RDN formulated, independently from PMM, the self-inhalation hypothesis developed the corresponding section. PMM and RN developed the section on Implications and RDN contributed to the section deriving from the "auto-inhalation" hypothesis. PMM and RN have prepared the first draft of the manuscript, completed by RDN for the section on respiratory pathophysiology. PMM produced the original hand-made drawings of the figure 1 and 4 and produced the first electronic version of the figure 5; he interacted with the professional infographic designer. RN conceived and produced the figure 3. The three Authors have checked the final version of the manuscript together and prepared together the point-by-point replies to the Editors and Reviewers of Pediatric Allergy and Immunology.

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Disclaimer

The text contains the personal opinion of the Authors, not of their Institutions: Charité Universitaetsmedizin Berlin, Germany (PMM) and Istituto Superiore di Sanità, Rome, Italy (RN). All authors declare no conflict of interest.

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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.[Reprinted unmodified from [85] https://www.nature.com/articles/srep19509, which is available under the Creative Commons License 4.0.**]**

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)**.

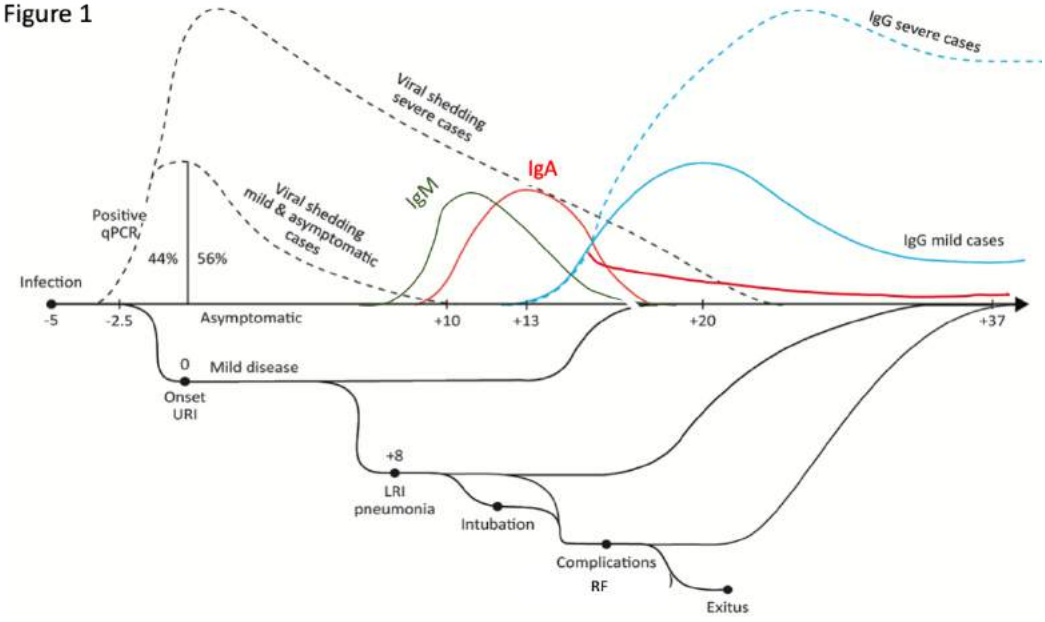
Figure 5 – A "quantitative and time-sequence dependent" model COVID-19. - The natural history of COVID-19 caused by SARS-CoV-2 is extremely variable, ranging from asymptomatic infection, to pneumonia, and to complications eventually fatal. We propose here the first model, explaining how the outcome of 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 airway 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



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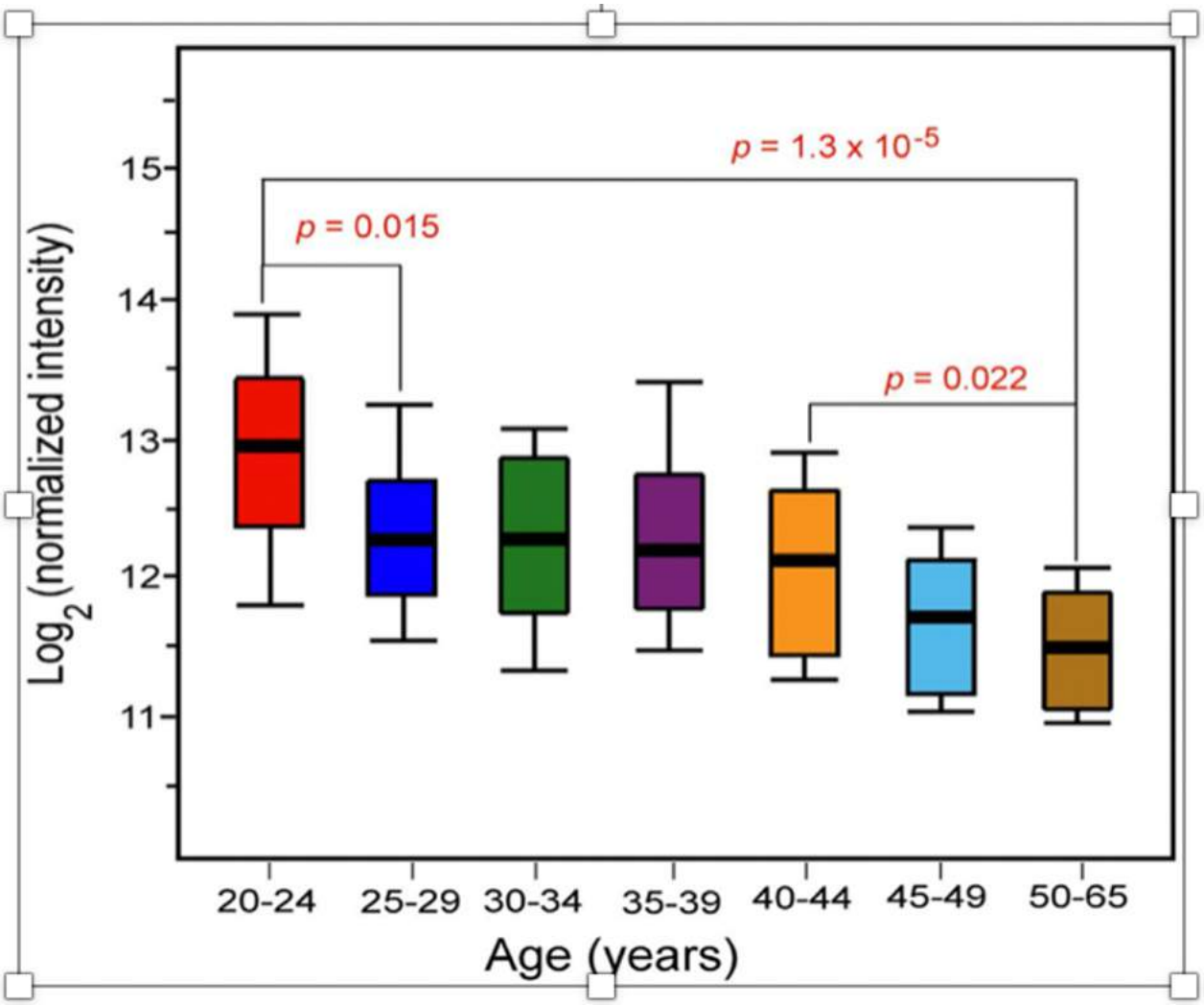

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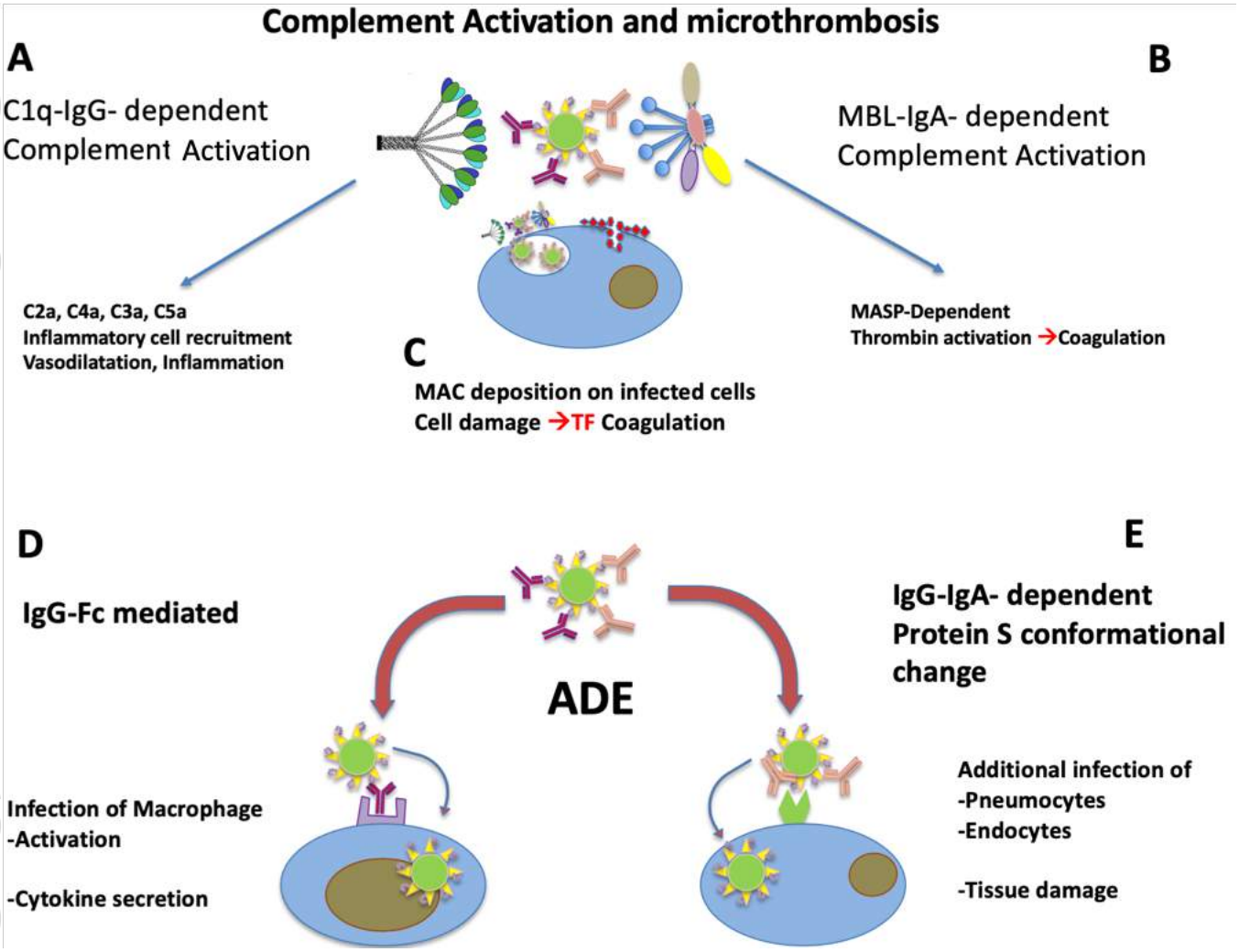

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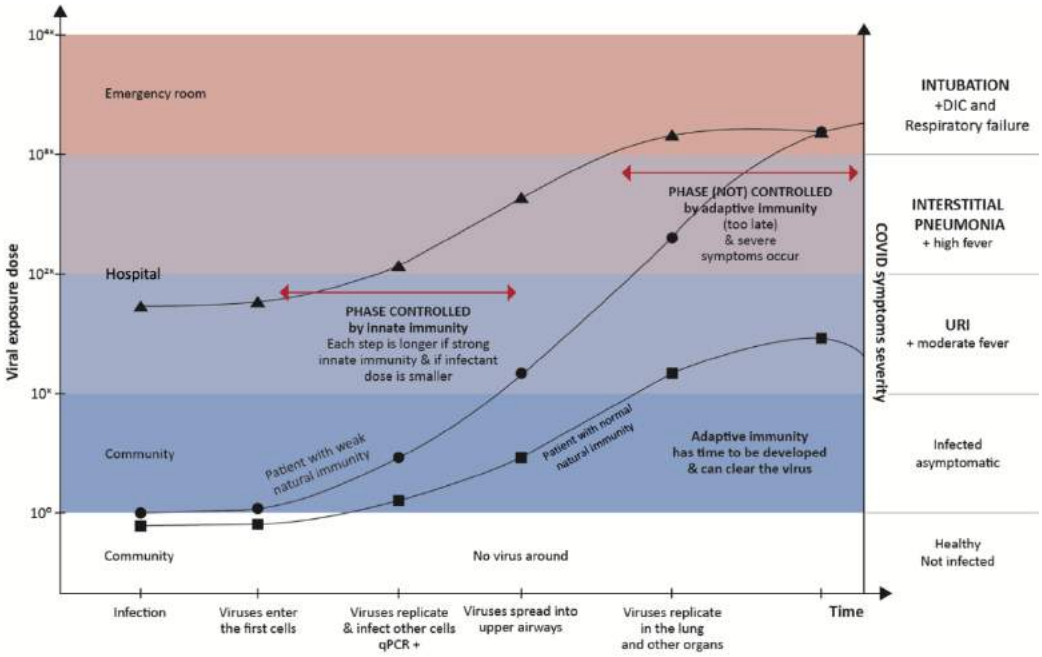

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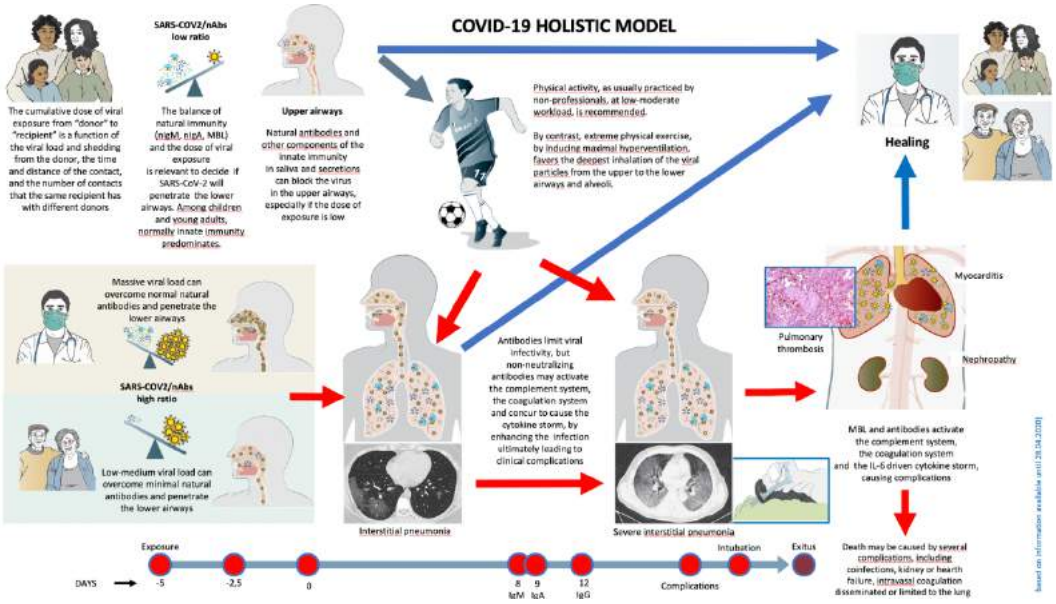

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