



Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections

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ABSTRACT

In contrast to other respiratory viruses, children have less severe symptoms when infected with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this review, we discuss proposed hypotheses for the age-related difference in severity of coronavirus disease 2019 (COVID-19).

Factors proposed to explain the difference in severity of COVID-19 in children and adults include those that put adults at higher risk and those that protect children. The former include: (1) age-related increase in endothelial damage and changes in clotting function; (2) higher density, increased affinity and different distribution of angiotensin converting enzyme 2 receptors and transmembrane serine protease 2; (3) pre-existing coronavirus antibodies (including antibody-dependent enhancement) and T cells; (4) immunosenescence and inflammaging, including the effects of chronic cytomegalovirus infection; (5) a higher prevalence of comorbidities associated with severe COVID-19 and (6) lower levels of vitamin D. Factors that might protect children include: (1) differences in innate and adaptive immunity; (2) more frequent recurrent and concurrent infections; (3) pre-existing immunity to coronaviruses; (4) differences in microbiota; (5) higher levels of melatonin; (6) protective off-target effects of live vaccines and (7) lower intensity of exposure to SARS-CoV-2.

INTRODUCTION

The novel SARS-CoV-2, which causes the disease called COVID-19, has rapidly spread across the globe. A striking and consistent observation has been the difference in severity of COVID-19 at different ages: severity, the need for hospitalisation and mortality rise steeply with older age while severe disease and death are relatively rare in children and young adults.^{1–3} Most children infected with SARS-CoV-2 are asymptomatic or have mild symptoms, most commonly fever, cough, pharyngitis, gastrointestinal symptoms and changes in sense of smell or taste.^{4,5}

Whether children are also less often infected by SARS-CoV-2 is an ongoing debate. Large epidemiological studies suggest that children comprise only 1 to 2% of all SARS-CoV-2 cases.^{6–8} However, these numbers heavily depend on testing criteria and, in many reports, testing was done only in individuals who were symptomatic or required hospitalisation, which is less often the case for children. Some studies suggest that children are just as likely as adults to become infected with SARS-CoV-2.⁹

What is already known on this topic?

- ▶ Compared with older adults, severe or fatal COVID-19 disease is much less common in infants, children and young adults.
- ▶ This pattern is strikingly different to that for infection with most other respiratory viruses, for which both the prevalence and severity are higher in children.

What this study adds?

- ▶ A number of factors have been proposed to explain the difference between children and adults in the severity of COVID-19, which can be categorised into those that put adults at higher risk and those that protect children.
- ▶ Although, there are several hypotheses for the age-related difference in the severity of COVID-19, the observed age-gradient seems to most closely parallel changes in immune and endothelial/clotting function.

However, more recent studies report that children are less likely to get infected after contact with a SARS-CoV-2-positive individual.^{10–14} It has been suggested that children and adolescents have similar viral loads^{15,16} and may therefore be as likely to transmit SARS-CoV-2 as adults.^{17,18} In addition, the viral load may be similar in asymptomatic and symptomatic individuals.^{19–21} However, reassuringly, transmission in schools from children either to other children or to adults has been rare.^{22–24}

The observation that children are less often infected with SARS-CoV-2 and that they have less severe symptoms is similar to that reported for SARS-CoV-1 and Middle East respiratory syndrome (MERS)-CoV.^{25–27} However, this pattern is strikingly different to that for infection with most other respiratory viruses (eg, respiratory syncytial virus (RSV), metapneumovirus, parainfluenza or influenza viruses), for which the prevalence and severity are both higher in children.²⁸

It remains uncertain whether the age-related difference in clinical features of COVID-19 is due to risk factors for severe COVID-19 that increase with age or due to factors that protect younger age groups. Here, we critically review proposed hypotheses for the age-related difference in severity of COVID-19 (table 1).



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Table 1 Proposed hypotheses for the age-related difference in severity of COVID-19

Hypothesis Key factors	Proposed mechanism	In support of hypothesis	Against hypothesis
(A) FACTORS INCREASING RISK IN ADULTS			
(1) Endothelium and clotting function			
Endothelial damage and hypercoagulable state	<ul style="list-style-type: none"> ▶ Increased endothelial damage with age ▶ Susceptibility to excessive coagulation increases with age 	<ul style="list-style-type: none"> ▶ Importance of endotheliitis and microthrombi in pathogenesis of COVID-19^{29 30} ▶ Association between conditions that affect the endothelium, such as diabetes and hypertension, and severe COVID-19^{35–37} ▶ Age-related changes in coagulation consistent with age gradient of severe COVID-19³⁸ ▶ Thrombotic complications, such as heart attacks and strokes, in COVID-19^{29 31–34} ▶ Vasculitic skin manifestations in COVID-19^{39–44 53 223} 	
(2) ACE2 receptors and TMPRSS2			
Viral entry	<ul style="list-style-type: none"> ▶ Age-related differences in expression, affinity and distribution facilitate SARS-CoV-2 entry into cells 	<ul style="list-style-type: none"> ▶ Expression and affinity of ACE2 increase with age^{55 57–59} ▶ Variants in the ACE2 gene are linked to severity of COVID-19⁶⁸ ▶ Pneumonia caused by CoV NL63 (that also binds to ACE2) is more common in adults compared with young children²²⁴ ▶ TMPRSS2 expression on nasal and lung epithelial cells likely increases with age^{58 59 71} 	<ul style="list-style-type: none"> ▶ ACE2 has anti-inflammatory properties that protect against ARDS, as well as SARS-CoV- and influenza-associated lung injury in animal studies^{64 65} ▶ Less ACE2 leads to higher levels of angiotensin II which is positively correlated with viral load and organ injury in SARS-CoV-2-infected patients⁶⁷
(3) Pre-existing immunity			
Cumulative exposure to commonly circulating HCoVs (229E, HKU1, NL63, OC43)	<ul style="list-style-type: none"> ▶ Non-neutralising HCoV antibodies facilitating cell entry and viral replication (antibody-dependent enhancement) 	<ul style="list-style-type: none"> ▶ Reinfection with commonly circulating HCoV is frequent^{76 77} ▶ Higher levels of neutralising and non-neutralising CoV antibodies have been found in adults, especially elderly compared with children^{78 79 148} ▶ Cellular immunity to SARS-CoV-2 found in some non-exposed individuals^{88–90} ▶ Higher numbers of cross-reactive T cells found in elderly⁸⁰ ▶ Children with COVID-19 have a less robust T cell response to spike protein (lower frequency of CD25⁺ and IFN-γ-producing CD4⁺ T cells), lower neutralising antibody levels and less antibody-dependent enhancement⁷⁸ 	
(4) Immunosenescence and inflammaging			
Age-related changes in the immune system including chronic CMV infection	<ul style="list-style-type: none"> ▶ Decline in innate and adaptive immune function in elderly leads to reduced SARS-CoV-2 clearance ▶ Chronic proinflammatory state associated with age predisposes to cytokine storm ▶ CMV's effect on T cells leading to reduced capacity for immune responses to novel viral infections such as SARS-CoV-2 	<ul style="list-style-type: none"> ▶ Increase in abundance and activity of NLRP3 inflammasome with age might be associated with severe COVID-19^{99 100} ▶ Diseases associated with inflammaging (eg, cardiovascular, diabetes, obesity) are risk factors for severe COVID-19⁹⁷ ▶ CMV causes clonal T cell proliferation and a reduction in naive T cell diversity¹⁰³ ▶ CMV increases inflammatory-mediated cytokines such as TNF-α and IL-6¹⁰⁶ 	
(5) Comorbidities			
Obesity, diabetes, hypertension, chronic lung, heart and kidney disease, and smoking	<ul style="list-style-type: none"> ▶ Likely related to endothelial damage 	<ul style="list-style-type: none"> ▶ Younger age groups less often suffer from the comorbidities that have been associated with severe COVID-19 in adults³⁶ 	<ul style="list-style-type: none"> ▶ Younger age groups with these conditions do not appear to develop severe COVID-19^{7 107–109}
(6) Vitamin D			
Anti-inflammatory and anti-oxidative properties	<ul style="list-style-type: none"> ▶ Lower vitamin D levels 	<ul style="list-style-type: none"> ▶ Vitamin D is reduced in older age groups, as well as in obesity and chronic kidney disease, both of which are associated with more severe COVID-19^{117 118 120} ▶ Vitamin D levels lower in SARS-CoV-2-positive individuals and negatively correlated with severity of radiological findings^{124 125} ▶ Infants less likely to be vitamin D deficient than older age groups, as supplemented in many countries²²⁵ 	

Continued

Table 1 Continued

Hypothesis Key factors	Proposed mechanism	In support of hypothesis	Against hypothesis
(B) FACTORS PROTECTING CHILDREN			
(1) Immune system			
Age-related differences in immune response	<ul style="list-style-type: none"> ▶ Stronger innate, trained immune response leading to more effective virus containment/clearance ▶ Weaker adaptive immune response and therefore less hyperinflammation ▶ Lower proinflammatory cytokine responses (cytokine storm) 	<ul style="list-style-type: none"> ▶ Children with COVID-19 have higher levels of IL-17A and IFN-γ⁷⁸ ▶ Some other infections are also less severe in children, for example, dengue, Epstein-Barr virus, hepatitis A, measles, Legionnaires' disease, polio, varicella 	<ul style="list-style-type: none"> ▶ Age-related difference in immune response does not mirror age gradient in COVID-19 in which lower severity extends into early adulthood ▶ Differences in the immune response do not protect against other respiratory viruses to which children are generally more commonly and more severely affected²⁸ ▶ Stronger innate immune response may be both protective but may also worsen cytokine storm^{32 136 138 139} ▶ Children are not less prone to develop a cytokine storm leading to ARDS with RSV and influenza infections^{138 140} ▶ Immunocompromised not at as high risk of severe COVID-19 as would expect if this were principal mechanism²²⁶
(2) Recurrent and concurrent infections			
Viral and mycoplasma infections	<ul style="list-style-type: none"> ▶ More frequent infections with other pathogens may help fight SARS-CoV-2 	<ul style="list-style-type: none"> ▶ Children infected with SARS-CoV-2 often have co-infections with other viruses or mycoplasma^{141 142} ▶ Recurrent viral infections could lead to epigenetic changes in trained immunity making it more effective in clearing SARS-CoV-2¹³⁴ 	
(3) Cross-reactive coronavirus antibodies and T cells			
Exposure to commonly circulating HCoV (229E, HKU1, NL63, OC43)	<ul style="list-style-type: none"> ▶ Pre-existing neutralising antibodies and T cell immunity to commonly circulating HCoV in younger age groups cross protect against SARS-CoV-2 		<ul style="list-style-type: none"> ▶ Antibodies to commonly circulating CoV2 are cross-reactive with SARS-CoV-2, but rarely cross-neutralising¹⁴⁶⁻¹⁴⁸ ▶ No difference in antibody levels against HCoVs between children infected with SARS-CoV-2 and those who are not¹⁵⁰ ▶ Higher levels of neutralising CoV antibodies have been found in adults compared with children⁷⁸ ▶ Higher numbers of cross-reactive T cells found in elderly⁸⁰ ▶ The role of cross-reactive T cells remains unclear⁸⁸⁻⁹⁰ ▶ Unlikely to explain lower severity extending into early adulthood
(4) Microbiota (nasopharyngeal, oropharyngeal, lung and/or gastrointestinal)			
Colonising microbial flora	<ul style="list-style-type: none"> ▶ Differences in the microbiota might influence susceptibility to SARS-CoV-2 	<ul style="list-style-type: none"> ▶ Microbial interactions and competition might limit colonisation and growth of SARS-CoV-2^{154 155} ▶ ACE2 highly expressed in the nasopharynx and gastrointestinal tract^{152 153} ▶ Observed differences in the gastrointestinal microbiota between patients infected with SARS-CoV-2 and healthy controls¹⁶⁰⁻¹⁶² ▶ Administration of probiotics leads to quicker improvement of COVID-19-related symptoms²²⁷ 	
(5) Melatonin			
Anti-inflammatory and anti-oxidative properties	<ul style="list-style-type: none"> ▶ Higher melatonin levels 	<ul style="list-style-type: none"> ▶ Children have higher levels of melatonin^{190 191} ▶ Bats, which suffer from minimal or no symptoms of CoV infection, have higher levels of melatonin compared with humans¹⁸⁹ ▶ Melatonin inhibits calmodulin which increases ACE2 expression and retention on cell surface^{180 181} ▶ In silico studies suggest that melatonin inhibits SARS-CoV-2's main protease¹⁸⁵ 	

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Table 1 Continued

(6) Off-target effects of live vaccines

Trained immunity from BCG, MCV, OPV	▶ More recent vaccination with live vaccines that have off-target effects	▶ Trials show BCG-induced protection against viral infections ^{194 195} ▶ Possible correlation between different BCG countries' vaccination policies and severity of COVID-19 ²⁰⁰⁻²⁰² ▶ More recent BCG, MMR and OPV vaccination in younger age groups might protect against severe COVID-19 ^{199 208 209}	▶ Off-target immunomodulatory effects are unlikely to be long lasting ^{205 206} ▶ Many other explanations for differences between countries' rate and severity of COVID-19 ^{203 205}
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(7) Exposure

Intensity of viral exposure	▶ Severity of COVID-19 associated with initial viral load	▶ Children are predominantly infected by transmission from adults ^{6 208 209} ▶ Children have less workplace, shopping, travel and nosocomial exposure to SARS-CoV-2 ▶ For SARS-CoV and MERS-CoV, subsequent generations of virus with reduced pathogenicity reported ^{217 218}	▶ No evidence for reduced virulence of SARS-CoV-2 in second-generation infections ^{219 220}
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ACE2, angiotensin converting enzyme 2; ARDS, acute respiratory distress syndrome; CMV, cytomegalovirus; HCoV, human coronavirus; IFN, interferon; IL, interleukin; MCV, measles-containing vaccine; MERS-CoV, Middle East respiratory syndrome coronavirus; MMR, measles-mumps-rubella; NK, natural killer; NLRP3, NOD-containing, LRR-containing and pyrin domain-containing protein 3; OPV, oral polio vaccine; RSV, respiratory syncytial virus; TMPRSS2, transmembrane serine protease 2; TNF, tumour necrosis factor.

FACTORS LEADING TO ADULTS BEING AT HIGHER RISK

Differences in the endothelium and clotting function

SARS-CoV-2 can infect endothelial cells and cause vasculitis.^{29 30}

Activation of coagulation pathways and formation of microthrombi, as a result of endothelial damage, as well as angiogenesis play an important part in the pathogenesis of COVID-19 and can lead to thrombotic complications such as heart attacks and strokes.^{29 31-34} This could also explain why patients with conditions that affect the endothelium, such as diabetes and hypertension, are at greater risk for severe COVID-19.³⁵⁻³⁷

The endothelium in children is less 'predamaged' compared with adults and the coagulation system also differs, which makes children less prone to abnormal clotting.³⁸ Of note, the age profile of severe COVID-19 (and the increased risk in men) mirrors that of thrombotic diseases such as deep vein thrombosis.

Reports of children presenting with a more serious Kawasaki disease/toxic shock-like illness ('Paediatric Inflammatory Multisystem Syndrome Temporally associated with SARS-CoV-2 [PIMS-TS]' in Europe; also known as Multisystem Inflammatory Syndrome in Children [MIS-C] in the USA) might seem to also support the concept that vascular function plays an important role in the pathogenesis of COVID-19.³⁹⁻⁴⁷ However, the pathogenesis of PIMS-TS, which usually presents 4-6 weeks after infection, differs from acute COVID-19, as it is likely to be an autoimmune phenomenon.⁴⁸ In addition, the hyperinflammation underlying PIMS-TS is different to that observed in adults with severe COVID-19, which includes higher levels of IL-7 and IL-8 and lower levels of effector CD4⁺ T cells.⁴⁸

Another intriguing observation is that chilblain-like skin lesions (usually on the lower extremities) in association with COVID-19 have been described more often in children and young adults.⁴⁹⁻⁵³ The pathology underlying these lesions has been identified as endothelial viral invasion leading to vasculitis or thrombotic vasculopathy.

Angiotensin converting enzyme 2 receptors and transmembrane serine protease 2

Angiotensin converting enzyme 2 (ACE2) is the main receptor for the entry of SARS-CoV-2 into human cells.⁵⁴ This receptor is present on many cells including epithelial cells of the nasopharynx, lungs, heart, kidney, intestine, liver, testis, placenta,

central nervous system and blood vessels, as well as macrophages.^{55 56} The expression of ACE2 in nasal and lung epithelium increases during childhood and further during adulthood.⁵⁷⁻⁵⁹

Furthermore, it has also been postulated that children have ACE2 receptors with a lower affinity for SARS-CoV-2 and a different distribution across body sites, making the entry of SARS-CoV-2 into cells more difficult.⁵⁵ However, while the number and affinity of ACE2 receptors on epithelial cells increases with age and is influenced by other factors, such as smoking, diet, diabetes mellitus, drugs, gender and genetics,^{37 55-57 60 61} it has not been shown that this leads to differences in the manifestations of COVID-19. Furthermore, ACE2 receptor abundance decreases in the elderly, the age group that is most susceptible to COVID-19. In contrast, in patients who are taking ACE inhibitors or angiotensin receptor blockers for arterial hypertension, ACE2 is overexpressed and it has been postulated that this renders these individuals more susceptible to severe COVID-19.^{62 63} However, this is controversial, partly explicable by the complexity of the regulation of the ACE2-angiotensin system, which is important in regulating the immune response, especially in the lungs, where it plays a role in protecting against acute respiratory distress syndrome (ARDS). In animal studies, ACE2 protects against SARS-CoV-2 and influenza-associated lung injury.^{64 65} After SARS-CoV-2 gains entry into cells through the ACE2 receptor, ACE2 expression is downregulated, which prevents it from exerting its anti-inflammatory properties and from converting angiotensin II to angiotensin (1-7). The consequent excess of angiotensin II might be partly responsible for the organ injury in COVID-19,⁶⁶ as serum levels of angiotensin II are significantly elevated in SARS-CoV-2-infected patients and there is a positive correlation with viral load and lung damage.⁶⁷ This fits with the finding that in patients with diabetes mellitus, in whom ACE2 expression is reduced (likely due to glycosylation), COVID-19 is associated with severe lung injury and ARDS.^{35 36}

While variants in the gene which encodes for ACE2 are linked to the risk of severe COVID-19,⁶⁸ it is still unclear if there is an association between the level of circulating ACE2 and severity of COVID-19. Nevertheless, it is possible that higher circulating levels of ACE2 in blood might neutralise virus and protect against lung damage through inactivation of circulating angiotensin II. In mice, treatment with human ACE2 mitigates lung injury during

influenza infection.⁶⁹ Moreover, serum ACE2 levels are negatively correlated with body mass index and oestrogen levels,⁷⁰ which could contribute to the association between obesity and male sex with severe COVID-19.

In addition to the ACE2 receptor, SARS-CoV-2 entry into cells involves transmembrane serine protease 2 (TMPRSS2), which cleaves the viral spike protein. Like ACE2, TMPRSS2 has been reported to increase with age on nasal and lung epithelial cells.^{58 59 71} However, two recent studies did not confirm these findings.^{72 73}

Pre-existing immunity from coronavirus antibodies and T cells

Commonly circulating human coronaviruses (HCoV)-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43 are responsible for approximately 6%–8% of acute respiratory tract infections.¹ Most individuals develop antibodies to HCoVs during childhood.^{74 75} However, despite seroconversion at an early age, re-infection with HCoVs later in life is common^{76 77} and levels of neutralising and non-neutralising cross-reactive antibodies, as well as cross-reactive T cells increase with age.^{78–80}

Pre-existing non-neutralising antibodies can bind to virions, which can then more easily enter macrophages and granulocytic cells, a mechanism called antibody-dependent enhancement (ADE). Such virions covered in antibodies can also more easily replicate, leading to higher viral loads.⁸¹ Children with COVID-19 have lower neutralising antibody levels and less ADE than adults.⁷⁸ Higher levels of non-neutralising cross-reactive HCoVs antibodies might partly explain increased susceptibility to severe COVID-19 in older adults.⁷⁹ ADE is a phenomenon that also needs to be considered in the development of vaccines, as enhanced disease after viral challenge postvaccination has been observed after vaccination against SARS-CoV and MERS-CoV in animal models,^{82–86} and in the use of convalescent plasma as a treatment option.⁸⁷

Infection with commonly circulating coronaviruses leads to long-lasting T cell immunity to spike (S) protein, nucleocapsid (N) protein and non-structural NSP7 and NSP13 of *ORF1*. However, the role of cross-reactive T cells in relation to SARS-CoV-2 remains unclear.^{88–90} It has been suggested that pre-existing T cells with low avidity, which are often usually present in higher numbers in the elderly, negatively impact T cell responses to SARS-CoV-2.⁸⁰ Children with COVID-19 have been shown to have a less robust T cell response to the spike protein.⁷⁸

Immunosenescence, inflammaging and chronic CMV infection

Ageing is associated with immunosenescence, a gradual decline in innate immunity, exemplified by ineffective pathogen recognition, macrophage activation, reduced neutrophil activity and natural killer [NK] cytotoxic activity and in adaptive immunity, associated with thymic atrophy, lymphopenia, a decrease in naïve T cells and an increase in anergic memory lymphocytes leading to an exhaustion of helper T cells, cytotoxic T cells and B cells.^{91–96} Immunosenescence likely contributes to reduced SARS-CoV-2 clearance.

A second age-related change in the immune system is inflammaging, a chronic pro-inflammatory state that develops with advanced age and has been associated with inflammatory diseases such as atherosclerosis and diabetes, which are associated with severe COVID-19.^{97 98} The predisposition to cytokine storm in the elderly could be explained by an increase in abundance and activity of the NOD-containing, LRR-containing and pyrin

domain-containing protein 3 (NLRP3) inflammasome, which has been associated with severe COVID-19.^{99 100}

Another immunological mechanism that might contribute to the age gradient in COVID-19 severity is the age-related increase in auto-antibodies against type I interferon (IFN) (especially IFN- α and IFN- ω), which are associated with severe COVID-19 pneumonia.¹⁰¹ IFN type plays an important role in the innate antiviral response.¹⁰²

Chronic cytomegalovirus (CMV) infection, which increases with age, may be a significant driver of inflammaging and immunosenescence.¹⁰³ Chronic CMV causes clonal T cell proliferation and a reduction in naïve T cell diversity, and also leads to an advanced differentiation status of CMV-specific CD8 T cells associated with either increased expression or downregulation of surface receptors, cytokine and transcription factors.^{103 104} These effects of CMV on T cells may lead to a reduced capacity for immune responses to novel viral infections such as SARS-CoV-2.¹⁰⁵ CMV also increases inflammatory-mediated cytokines such as tumour necrosis factor (TNF)- α and interleukin (IL)-6,¹⁰⁶ making the elderly with clinically silent CMV infection more susceptible to the cytokine storm associated with severe COVID-19.

Comorbidities

Children have a lower prevalence of the comorbidities that have been associated with severe COVID-19 in adults, such as obesity, diabetes, hypertension and chronic kidney, lung and heart disease.³⁶ Even though, no definite risk factors have been identified in children, those with chronic lung disease (including asthma), cardiovascular disease and immunosuppression more often require hospital compared with previously healthy children.^{7 107–109} However, intriguingly, even children with serious medical conditions, who are on immunosuppressive or cancer treatment, are much less affected by COVID-19 than adults.^{110–113}

Lower levels of vitamin D

Vitamin D has anti-inflammatory and anti-oxidative properties,¹¹⁴ and vitamin D deficiency has been associated with an increased risk for the development of respiratory tract infections.¹¹⁵ Mechanisms by which vitamin D might protect against respiratory viruses include increasing viral killing, reducing synthesis of pro-inflammatory cytokines and protecting the integrity of tight junctions thereby preventing infiltration of immune cells into lungs.¹¹⁶

The overlap between risk factors for severe COVID-19 and vitamin D deficiency, including obesity,¹¹⁷ chronic kidney disease¹¹⁸ and black or Asian origin,¹¹⁹ suggests that vitamin D supplementation may play a role in prophylaxis or treatment of COVID-19.¹¹⁴ In many countries, vitamin D is routinely supplemented in infants younger than 1 year of age and in some countries even up to the age of 3 years. Furthermore, vitamin D levels are lower in older age groups, especially in men, in whom supplementation is less frequent.¹²⁰ Several studies report a negative correlation between estimates of average vitamin D levels in the population and the incidence and mortality from COVID-19.^{121–123} One study found lower vitamin D levels in SARS-CoV-2-positive individuals compared with SARS-CoV-2-negative individuals, even after stratifying for age over 70 years.¹²⁴ Another study found lower vitamin D levels in patients with COVID-19 compared with sex-matched, age-matched and season-matched controls.¹²⁵ Furthermore, the level of vitamin D was negatively correlated with the severity of radiological

findings. Two other studies also found a correlation between low vitamin D levels and COVID-19 severity and mortality.^{126 127}

In vitro studies show that calcitriol, the active form of vitamin D, has antiviral activity against SARS-CoV-2.¹²⁸ A further important finding from a study in rats shows that vitamin D alleviates lipopolysaccharide-induced acute lung injury via the renin-angiotensin system (RAS),¹²⁹ which is important in the pathogenesis of COVID-19, in which the degree of overactivation of the RAS is associated with poorer prognosis. Low vitamin D levels lead to higher RAS activity and higher angiotensin II concentrations.¹³⁰

FACTORS PROTECTING CHILDREN

Differences in innate and adaptive immunity

In addition to direct cytotoxic effects of the virus, immune-mediated mechanisms play a crucial role in the pathogenesis of COVID-19.¹³¹ There are important differences in the immune system between children and adults, which might contribute to the different manifestations of COVID-19.^{78 132}

Children have a stronger innate immune response, which is the first-line defence against SARS-CoV-2, with a higher number of NK cells. Another important factor is 'trained immunity' which involves epigenetic reprogramming of innate immune cells (including NK cells) after exposure to certain stimuli, including infections and vaccinations, leading to 'memory'.^{133 134} These trained cells react faster and more strongly to subsequent pathogen challenge providing enhanced protection. However, this hypothesis would not explain, why this mechanism does not protect children against other respiratory viruses.²⁸ It is likely that immune responses, including IFN production, on mucosal surfaces are also important in the defence against SARS-CoV-2.¹³⁵ To date, no studies have compared IFN production by SARS-CoV-2 challenged epithelial or dendritic cells of children and adults.

In relation to adaptive immunity, children also have a higher proportion of lymphocytes and absolute numbers of T and B cells,¹³⁶ while ageing is associated with a reduction in thymic activity and a reduction in naïve T cells.⁹⁶ Adults infected by SARS-CoV-2 typically have decreased lymphocyte counts¹³⁷ and it could be that higher numbers of lymphocytes, especially the large repertoire of naïve T cells which enables a strong T cell-mediated immune response, play a role in protecting children against SARS-CoV-2.

A further proposed immunological explanation is that children are less capable of mounting the pro-inflammatory cytokine storm, which plays an important role in the pathogenesis of severe COVID-19 and is responsible for multiorgan failure in critically ill patients.^{32 136 138 139}

Hospitalised children with COVID-19 have higher serum levels of IL-17A and IFN- γ , but not TNF- α or IL-6.⁷⁸ Against this theory, however, is that in RSV or influenza infection, in contrast, children are no less prone than adults to developing a cytokine storm leading to ARDS.^{138 140}

More frequent recurrent and concurrent infections

Children infected with SARS-CoV-2 often have co-infections with other viruses (including commonly circulating HCoVs).¹⁴¹⁻¹⁴³ These viruses could interfere with the replication of the SARS-CoV-2.¹⁴⁴ Frequent recurrent viral infections could also induce an enhanced state of activation of the innate immune system, including epigenetic changes in trained immunity, making it easier to clear SARS-CoV-2.¹³⁴

Cross-reactive coronavirus antibodies and T cells

Although it has been suggested that children might be protected against SARS-CoV-2 as a result of pre-existing cross-reactive antibodies from more frequent and recent HCoVs infections,¹⁴⁵ preliminary data show that, while antibodies to HCoVs cross-react with the spike protein of SARS-CoV-2 and SARS-CoV, these antibodies are rarely neutralising, as they do not bind to SARS-CoV-2 receptor binding domain.¹⁴⁶⁻¹⁴⁹ In accordance with this, no difference in antibody levels against HCoVs was found between children infected with SARS-CoV-2 and those who were not infected.¹⁵⁰ Furthermore, both neutralising and non-neutralising antibody levels are higher in adults, especially elderly adults compared with children.^{78 79} The same is true for cross-reactive T cells to SARS-CoV-2, which are also present in higher numbers in the elderly and might be detrimental.⁸⁰ It is likely that IgA on mucosal surfaces is important for defence against SARS-CoV-2.¹⁵¹ To date, however, no study has compared SARS-CoV-2 IgA levels or avidity between children and adults.

Microbiota

Another potential explanation for the less severe manifestations of COVID-19 in children are the differences in their oropharyngeal, nasopharyngeal, lung and/or gastrointestinal microbiota. The microbiota plays an important role in the regulation of immunity, inflammation, and mucosal homeostasis, as well as in the defence against pathogens. Thus, the microbiota might affect the susceptibility to SARS-CoV-2 infection and severity, or, given that ACE2 is highly expressed in the respiratory and gastrointestinal tract,^{152 153} infection with SARS-CoV-2 might affect the microbiota and therefore inflammation.

Children are more heavily colonised with viruses and bacteria than adults, especially in the nasopharynx, where microbial interactions and competition might limit the growth of SARS-CoV-2.^{154 155} The association between viral load and COVID-19 severity provides some support for this hypothesis.¹⁵⁶⁻¹⁵⁸ While one small study did not find significant differences in the nasopharyngeal microbiota between patients infected with SARS-CoV-2 and healthy controls,¹⁵⁹ other studies have reported significant differences in the oropharyngeal, lung and gastrointestinal microbiota between these groups.¹⁶⁰⁻¹⁶⁴

In relation to the gastrointestinal microbiota, patients infected with SARS-CoV-2 have reduced bacterial diversity with a lower relative abundance of certain bacterial phyla including *Faecalibacterium*, and a higher relative abundance of others including *Bacteroides*.¹⁶⁰⁻¹⁶² While *Faecalibacterium* is known to have anti-inflammatory properties,¹⁶⁵ *Bacteroides* has been associated with decreased gastrointestinal ACE2 expression.¹⁶⁶ The gastrointestinal microbiota differs with age, with children generally having higher numbers of *Bifidobacterium*.^{167 168} Differences in the gastrointestinal microbiota have also been observed between patients who do or do not excrete SARS-CoV-2 in their stool.¹⁶⁹ However, microbiota findings can be influenced by many different factors, including age, hospital admission, antibiotic administration and diet.^{170 171} Therefore, the contribution, if any, of differences in the microbiota to differences in severity of COVID-19 remains unclear and cause versus effect will be difficult to determine.

Higher levels of melatonin

Melatonin has anti-inflammatory and anti-oxidative properties through several different mechanisms.^{172 173} For example, this hormone increases proliferation and maturation of NK cells, T

and B lymphocytes, granulocytes and monocytes in both bone marrow and other tissues,¹⁷⁴ and increases antigen presentation by macrophages.¹⁷⁵ In addition, melatonin decreases serum levels of IL-6, TNF- α and high-sensitivity C reactive protein,¹⁷⁶ and suppresses nuclear factor kappa-B.¹⁷⁷

Melatonin protects against ARDS and haemorrhagic shock during viral infections.^{178 179} Melatonin also inhibits calmodulin, which increases ACE2 expression and retention on the cell surface.^{180 181} Melatonin might also block CD147,¹⁸² which is another cellular receptor for SARS-CoV-2 entry¹⁸³ and involved in regulating chemotaxis and lung inflammation.¹⁸⁴ Furthermore, in silico studies suggest that melatonin inhibits SARS-CoV-2's main protease.¹⁸⁵ It has therefore been suggested that melatonin could be used as prophylaxis or treatment in COVID-19.^{186 187} A randomised trial to evaluate the efficacy of melatonin for prophylaxis against SARS-CoV-2 infection in healthcare workers is ongoing.¹⁸⁸

Bats, which are the main reservoir of coronaviruses and suffer from minimal or no symptoms, have higher levels of melatonin compared with humans.¹⁸⁹ In humans, melatonin secretion is negatively correlated with age, with particularly high levels in infants,^{190 191} which could contribute to the milder symptoms in this age group.

Off-target effects of vaccines

Many live vaccines have off-target (non-specific) immunomodulatory effects beyond protection against their target disease.¹⁹² For BCG and measles-containing vaccines (MCV), this includes reduced all-cause mortality in high-mortality settings and protection against viral infections.^{193–195} The mechanisms underlying the immunomodulatory effects of vaccines are the subject of ongoing investigation. BCG vaccine influences the innate and T cell immunity by epigenetic reprogramming of immune cells and by altering cytokine responses.^{196–198}

As children have generally been received BCG and other live vaccines more recently than adults, it has been postulated that this contributes to age-related difference in COVID-19 severity.¹⁹⁹

Ecological studies identifying associations between countries' BCG vaccination policies and rates and the severity of their COVID-19 outbreak purport to provide evidence in support of this.^{200–202} However, association is not the same as causation and there are exceptions to this observation.^{203 204} In addition, many of the articles claiming an association do not account for likely confounding factors between countries, such as different diagnostic and reporting procedures, different epidemic curves and different capacity of medical systems.^{203 205} It is also unlikely that the beneficial effects of BCG vaccination last for many years as they are likely abrogated by the impact of intervening vaccines and other factors that also modulate the immune system. It is therefore not unexpected that no difference in COVID-19 infection rate was found decades after vaccination in BCG-vaccinated individuals compared with BCG-naïve individuals.²⁰⁶ RCTs of BCG to reduce the severity of COVID-19 are ongoing.²⁰⁷

Like BCG, MCV and oral polio vaccination has also been suggested to contribute to the difference in the severity of COVID-19.^{208 209} Fewer studies have investigated the mechanisms underlying the immunomodulatory effects of MCV, but they have shown an association between MCV and a decrease in circulating leukocytes and lymphocytes, with a decrease in CD4 cells and an increase in CD8 cells.^{210 211} An RCT of measles-mumps-rubella vaccine to reduce the severity of COVID-19 is planned.²¹²

Intensity of exposure to SARS-CoV-2

Viral load influences the severity of COVID-19^{156–158} suggesting that lower intensity of viral exposure might be another factor leading to less severe disease.²¹³ Children might have less intense exposure to SARS-CoV-2 compared with adults who generally have had workplace, shopping, travel and nosocomial exposure.^{6 214–216}

As children are usually infected by an adult, they are infected by a second or third generation SARS-CoV-2. For SARS-CoV and MERS-CoV, subsequent generations of virus were reported with reduced pathogenicity compared with the first-generation virus.^{217 218} However, to date, this has not been reported for SARS-CoV-2. In contrast, an antigen drift through a mutation called D614G in the spike protein has been suggested to lead to higher viral loads and viral transmission without changing pathogenicity.^{219 220} It is unclear whether this was the result of higher fitness or chance. While the D614 variant of SARS-CoV-2 has been the main driver of the pandemic in China, the G614 variant is the main strain spreading through Europe and the USA.²²¹

CONCLUSION

In summary, the observation that, compared with other respiratory viruses, children have less severe symptoms when infected by SARS-CoV-2 is surprising and not yet understood. Furthermore, it is also uncertain why children with the usual risk factors for infections, such as immunosuppression, are not at high risk for severe COVID-19, while previously healthy children can on rare occasions become severely ill.^{110–113 222} Although there are several hypotheses for why children are less affected by COVID-19, with the notable exception of age-related changes in immune and endothelial/clotting function, most do not explain the observed age-gradient in COVID-19 with severity and mortality rising steeply after the age of 60 to 70 years. Unravelling the mechanisms underlying the age-related differences in the severity of COVID-19 will provide important insights and opportunities for the prevention and treatment of this novel infection.

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REFERENCES

- Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. *Pediatr Infect Dis J* 2020;39:355–68.
- Castagnoli R, Votto M, Licari A, *et al.* Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents. *JAMA Pediatr* 2020;174:882.
- Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr* 2020;109:1088–95.
- Zimmermann P, Curtis N. COVID-19 in children, pregnancy and neonates: a review of epidemiologic and clinical features. *Pediatr Infect Dis J* 2020;39:469–77.

- 5 Waterfield T, Watson C, Moore R, *et al.* Seroprevalence of SARS-CoV-2 antibodies in children - A prospective multicentre cohort study. *medRxiv* 2020:2020.2008.2031.20183095.
- 6 Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020;323:1239-42.
- 7 Bialek S, Gierke R, Hughes M, *et al.* Coronavirus Disease 2019 in Children - United States, February 12-April 2, 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:422-6.
- 8 Parri N, Lenge M, Buonsenso D. Children with Covid-19 in pediatric emergency departments in Italy. *N Engl J Med Overseas Ed* 2020;383:187-90.
- 9 Bi Q, Wu Y, Mei S, *et al.* Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet Infect Dis* 2020;20:911-9.
- 10 Zhang J, Litvinova M, Liang Y, *et al.* Changes in contact patterns shape the dynamics of the COVID-19 outbreak in China. *Science* 2020;368:1481-6.
- 11 Jing Q-L, Liu M-J, Yuan J, *et al.* Household secondary attack rate of COVID-19 and associated determinants. *medRxiv* 2020:2020.2004.2011.20056010.
- 12 Mizumoto K, Omori R, Nishiura H. Age specificity of cases and attack rate of novel coronavirus disease (COVID-19). *medRxiv* 2020:2020.2003.2009.20033142.
- 13 Milani GP, Bottino I, Rocchi A, *et al.* Frequency of children vs adults carrying severe acute respiratory syndrome coronavirus 2 asymptotically. *JAMA Pediatr* 2020.
- 14 Viner RM, Mytton OT, Bonell C, *et al.* Susceptibility to SARS-CoV-2 infection among children and adolescents compared with adults: a systematic review and meta-analysis. *JAMA Pediatr* 2020. doi:10.1001/jamapediatrics.2020.4573. [Epub ahead of print: 25 Sep 2020].
- 15 Jones TC, Mühlemann B, Veith T, *et al.* An analysis of SARS-CoV-2 viral load by patient age. *medRxiv* 2020:2020.2006.2008.20125484.
- 16 Baggio S, L'Huillier AG, Yerly S, *et al.* SARS-CoV-2 viral load in the upper respiratory tract of children and adults with early acute COVID-19. *Clin Infect Dis* 2020. doi:10.1093/cid/ciaa1157. [Epub ahead of print: 06 Aug 2020].
- 17 Park YJ, Choe YJ, Park O, *et al.* Contact tracing during coronavirus disease outbreak, South Korea, 2020. *Emerg Infect Dis* 2020;26:2465-8.
- 18 L'Huillier AG, Torriani G, Pigny F, *et al.* Culture-Competent SARS-CoV-2 in nasopharynx of symptomatic neonates, children, and adolescents. *Emerg Infect Dis* 2020;26:2494-7.
- 19 Lee S, Kim T, Lee E, *et al.* Clinical course and molecular viral shedding among asymptomatic and symptomatic patients with SARS-CoV-2 infection in a community treatment center in the Republic of Korea. *JAMA Intern Med* 2020:e203862.
- 20 Zou L, Ruan F, Huang M, *et al.* SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 2020;382:1177-9.
- 21 Hurst JH, Heston SM, Chambers HN, *et al.* SARS-CoV-2 infections among children in the biospecimens from respiratory virus-exposed kids (brave kids) study. *medRxiv* 2020:2020.2008.2018.20166835.
- 22 COVID-19 in schools - the experience in NSW. National centre for immunisation research and surveillance (NCIRS). Available: <http://www.ncirs.org.au/covid-19-in-schools> [Accessed 04 Mai 2020].
- 23 Children and COVID-19. National Institute for public health and the environment, Netherlands. Available: <https://www.rivm.nl/en/novel-coronavirus-covid-19/children-and-covid-19> [Accessed 04 Mai 2020].
- 24 Ladhani S. Prospective active national surveillance of preschools and primary schools for SARS-CoV-2 infection and transmission in England, June 2020 (SKIDS COVID-19 surveillance in school kids), 2020. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/914700/SKIDS_Phase1Report_01sep2020pdf
- 25 Hon KLE, Leung CW, Cheng WTF, *et al.* Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet* 2003;361:1701-3.
- 26 Leung C-wai, Kwan Y-wah, Ko P-wan, *et al.* Severe acute respiratory syndrome among children. *Pediatrics* 2004;113:e535-43.
- 27 Al-Tawfiq JA, Kattan RF, Memish ZA. Middle East respiratory syndrome coronavirus disease is rare in children: an update from Saudi Arabia. *WJCP* 2016;5:391-6.
- 28 Tregoning JS, Schwarze Jürgen, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev* 2010;23:74-98.
- 29 Varga Z, Flammer AJ, Steiger P, *et al.* Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020;395:1417-8.
- 30 Ackermann M, Verleden SE, Kuehnel M, *et al.* Pulmonary vascular Endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020;383:120-8.
- 31 Tang N, Li D, Wang X, *et al.* Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020;18:844-7.
- 32 Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med* 2020;8:e46-7.
- 33 Oxley TJ, Mocco J, Majidi S, *et al.* Large-Vessel stroke as a presenting feature of Covid-19 in the young. *N Engl J Med* 2020;382:e60.
- 34 Klok FA, Kruij MJHA, van der Meer NJM, *et al.* Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 2020;191:145-7.
- 35 Pal R, Bhansali A. COVID-19, diabetes mellitus and ACE2: the conundrum. *Diabetes Res Clin Pract* 2020;162:108132.
- 36 Zhou F, Yu T, Du R, *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054-62.
- 37 Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med* 2020;8:e21.
- 38 Ignjatovic V, Mertyn E, Monagle P. The coagulation system in children: developmental and pathophysiological considerations. *Semin Thromb Hemost* 2011;37:723-9.
- 39 Verdoni L, Mazza A, Gervasoni A, *et al.* An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 2020;395:1771-8.
- 40 Viner RM, Whittaker E. Kawasaki-like disease: emerging complication during the COVID-19 pandemic. *Lancet* 2020;395:1741-3.
- 41 Jones VG, Mills M, Suarez D, *et al.* COVID-19 and Kawasaki disease: novel virus and novel case. *Hosp Pediatr* 2020;10:537-40.
- 42 Moreira A. Kawasaki disease linked to COVID-19 in children. *Nat Rev Immunol* 2020;20:407.
- 43 Toubiana J, Poirault C, Corsia A, *et al.* Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ* 2020;369:m2094.
- 44 Riphagen S, Gomez X, Gonzalez-Martinez C, *et al.* Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* 2020;395:1607-8.
- 45 Levin M. Childhood multisystem inflammatory syndrome - a new challenge in the pandemic. *N Engl J Med* 2020;383:393-5.
- 46 Moraleda C, Serna-Pascual M, Soriano-Arandes A, *et al.* Multi-Inflammatory syndrome in children related to SARS-CoV-2 in Spain. *Clin Infect Dis* 2020. doi:10.1093/cid/ciaa1042. [Epub ahead of print: 25 Jul 2020].
- 47 Cheung EW, Zachariah P, Gorelik M, *et al.* Multisystem inflammatory syndrome related to COVID-19 in previously healthy children and adolescents in New York City. *JAMA* 2020;324:294-6.
- 48 Consiglio CR, Cotugno N, Sardh F, *et al.* The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell* 2020.
- 49 Rahimi H, Tehranchinia Z. A comprehensive review of cutaneous manifestations associated with COVID-19. *Biomed Res Int* 2020;2020:1-8.
- 50 Colonna C, Monzani NA, Rocchi A, *et al.* Chilblain-like lesions in children following suspected COVID-19 infection. *Pediatr Dermatol* 2020;37:437-40.
- 51 Suchonwanit P, Leerunyakul K, Kositkuljorn C. Cutaneous manifestations in COVID-19: lessons learned from current evidence. *J Am Acad Dermatol* 2020;83:e57-60.
- 52 Criado PR, Abdalla BMZ, de Assis IC, *et al.* Are the cutaneous manifestations during or due to SARS-CoV-2 infection/COVID-19 frequent or not? revision of possible pathophysiologic mechanisms. *Inflamm Res* 2020;69:745-56.
- 53 Colmenero I, Santonja C, Alonso-Riaño M, *et al.* SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases. *Br J Dermatol* 2020;183:729-37.
- 54 Ou X, Liu Y, Lei X, *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020;11:1620.
- 55 Muus C, Lueckel MD, Eraslan G, *et al.* Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *bioRxiv* 2020.
- 56 Li Y, Zhou W, Yang L, *et al.* Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor. *Pharmacol Res* 2020;157:104833.
- 57 Bunyavanich S, Do A, Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *JAMA* 2020;323:2427-9.
- 58 Wang A, Chiou J, Poirion OB, *et al.* Single nucleus Multiomic profiling reveals Age-Dynamic regulation of host genes associated with SARS-CoV-2 infection. *bioRxiv* 2020.
- 59 Saheb Sharif-Askari N, Saheb Sharif-Askari F, Alabd M, *et al.* Airways expression of SARS-CoV-2 receptor, ACE2, and TMPRSS2 is lower in children than adults and increases with smoking and COPD. *Mol Ther Methods Clin Dev* 2020;18:1-6.
- 60 Luo Y, Liu C, Guan T, *et al.* Association of ACE2 genetic polymorphisms with hypertension-related target organ damages in South Xinjiang. *Hypertens Res* 2019;42:681-9.
- 61 Liu D, Chen Y, Zhang P, *et al.* Association between circulating levels of ACE2-Ang-(1-7)-MAS axis and ACE2 gene polymorphisms in hypertensive patients. *Medicine* 2016;95:e3876.
- 62 Mancia G, Rea F, Ludergrani M, *et al.* Renin-Angiotensin-Aldosterone system blockers and the risk of Covid-19. *N Engl J Med* 2020;382:2431-40.
- 63 Reynolds HR, Adhikari S, Pulgarin C, *et al.* Renin-Angiotensin-Aldosterone system inhibitors and risk of Covid-19. *N Engl J Med* 2020;382:2441-8.
- 64 Imai Y, Kuba K, Rao S, *et al.* Angiotensin-Converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;436:112-6.
- 65 Zou Z, Yan Y, Shu Y, *et al.* Angiotensin-Converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Commun* 2014;5:3594.

- 66 Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res* 2020;81:537–40.
- 67 Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;63:364–74.
- 68 Wooster L, Nicholson CJ, Sigurslid HH, et al. Polymorphisms in the ACE2 locus associate with severity of COVID-19 infection. *medRxiv* 2020:2020.2006.2018.20135152.
- 69 Yang P, Gu H, Zhao Z, et al. Angiotensin-Converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci Rep* 2015;4:7027.
- 70 Zhang Q, Cong M, Wang N, et al. Association of angiotensin-converting enzyme 2 gene polymorphism and enzymatic activity with essential hypertension in different gender. *Medicine* 2018;97:e12917.
- 71 Schuler BA, Habermann AC, Plosa EJ, et al. Age-determined expression of priming protease TMPRSS2 and localization of SARS-CoV-2 infection in the lung epithelium. *bioRxiv* 2020.
- 72 Tao Y, Yang R, Wen C, et al. SARS-CoV-2 entry related genes are comparably expressed in children's lung as adults. *medRxiv* 2020:2020.2005.2025.20110890.
- 73 Bunyavanich S, Grant C, Vicencio A. Racial/Ethnic Variation in Nasal Gene Expression of Transmembrane Serine Protease 2 (TMPRSS2). *JAMA* 2020.
- 74 Dijkman R, Jebbink MF, El Idrissi NB, et al. Human coronavirus NL63 and 229E seroconversion in children. *J Clin Microbiol* 2008;46:2368–73.
- 75 Kaye HS, Marsh HB, Dowdle WR. SEROEPIDEMIOLOGIC SURVEY OF CORONAVIRUS (STRAIN OC 43) RELATED INFECTIONS IN A CHILDREN'S POPULATION. *Am J Epidemiol* 1971;94:43–9.
- 76 Isaacs D, Flowers D, Clarke JR, et al. Epidemiology of coronavirus respiratory infections. *Arch Dis Child* 1983;58:500–3.
- 77 Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974;129:271–6.
- 78 Pierce CA, Preston-Hurlburt P, Dai Y, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Sci Transl Med* 2020;12:eabd5487.
- 79 Gorse GJ, Donovan MM, Patel GB. Antibodies to coronaviruses are higher in older compared with younger adults and binding antibodies are more sensitive than neutralizing antibodies in identifying coronavirus-associated illnesses. *J Med Virol* 2020;92:512–7.
- 80 Bacher P, Rosati E, Esser D, et al. Pre-Existing T cell memory as a risk factor for severe COVID-19 in the elderly. *medRxiv* 2020:2020.2009.2015.20188896.
- 81 Tirado SMC, Yoon K-J. Antibody-Dependent enhancement of virus infection and disease. *Viral Immunol* 2003;16:69–86.
- 82 He Y, Zhou Y, Siddiqui P, et al. Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. *Biochem Biophys Res Commun* 2004;325:445–52.
- 83 Hashem AM, Algaissi A, Agrawal AS, et al. A highly immunogenic, protective, and safe adenovirus-based vaccine expressing middle East respiratory syndrome coronavirus S1-CD40L fusion protein in a transgenic human dipeptidyl peptidase 4 mouse model. *J Infect Dis* 2019;220:1558–67.
- 84 Czub M, Weingartl H, Czub S, et al. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. *Vaccine* 2005;23:2273–9.
- 85 Sridhar S, Luedtke A, Langevin E, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. *N Engl J Med Overseas Ed* 2018;379:327–40.
- 86 Liu L, Wei Q, Lin Q, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI Insight* 2019;4:e123158.
- 87 Epstein J, Burnouf T. Points to consider in the preparation and transfusion of COVID-19 convalescent plasma. *Vox Sang* 2020;115:485–7.
- 88 Sette A, Crotty S. Pre-Existing immunity to SARS-CoV-2: the knowns and unknowns. *Nat Rev Immunol* 2020;20:457–8.
- 89 Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020;181:1489–501.
- 90 Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020;584:457–62.
- 91 Shaw AC, Joshi S, Greenwood H, et al. Aging of the innate immune system. *Curr Opin Immunol* 2010;22:507–13.
- 92 Mahbub S, L. Brubaker A, J. Kovacs E. Aging of the innate immune system: an update. *Curr Immunol Rev* 2011;7:104–15.
- 93 Palmer DB. The effect of age on thymic function. *Front Immunol* 2013;4:316.
- 94 Ongrádi J, Kövesdi V. Factors that may impact on immunosenescence: an appraisal. *Immun Ageing* 2010;7:7.
- 95 Caruso C, Buffa S, Candore G, et al. Mechanisms of immunosenescence. *Immun Ageing* 2009;6:10.
- 96 Aspinall R, Andrew D. Thymic involution in aging. *J Clin Immunol* 2000;20:250–6.
- 97 Franceschi C, Garagnani P, Parini P, et al. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* 2018;14:576–90.
- 98 Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging: an evolutionary perspective on Immunosenescence. *Ann N Y Acad Sci* 2000;908:244–54.
- 99 Latz E, Duester P. Nlrp3 inflammasome activation in inflammaging. *Semin Immunol* 2018;40:61–73.
- 100 Freeman TL, Swartz TH. Targeting the NLRP3 inflammasome in severe COVID-19. *Front Immunol* 2020;11:1518.
- 101 Bastard P, Rosen LB, Zhang Q, et al. Auto-Antibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020:eabd4585.
- 102 Takaoka A, Yanai H. Interferon signalling network in innate defence. *Cell Microbiol* 2006;8:907–22.
- 103 Kallemeijn MJ, Boots AMH, van der Klift MY, et al. Ageing and latent CMV infection impact on maturation, differentiation and exhaustion profiles of T-cell receptor gamma/delta T-cells. *Sci Rep* 2017;7:5509.
- 104 van den Berg SPH, Pardieck IN, Lanfermeijer J, et al. The hallmarks of CMV-specific CD8 T-cell differentiation. *Med Microbiol Immunol* 2019;208:365–73.
- 105 Kadambari S, Klenerman P, Pollard AJ. Why the elderly appear to be more severely affected by COVID-19: the potential role of immunosenescence and CMV. *Rev Med Virol* 2020;30:e2144.
- 106 Trzonkowski P, Mysliwska J, Szmit E, et al. Association between cytomegalovirus infection, enhanced proinflammatory response and low level of anti-hemagglutinins during the anti-influenza vaccination--an impact of immunosenescence. *Vaccine* 2003;21:3826–36.
- 107 Mannheim J, Gretsche S, Layden JE, et al. Characteristics of Hospitalized Pediatric COVID-19 Cases - Chicago, Illinois, March - April 2020. *J Pediatric Infect Dis Soc* 2020. doi:10.1093/pids/piaa070. [Epub ahead of print: 01 Jun 2020].
- 108 Shekerdemian LS, Mahmood NR, Wolfe KK, et al. Characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. *JAMA Pediatr* 2020;174:868.
- 109 Zachariah P, Johnson CL, Halabi KC, et al. Epidemiology, Clinical Features, and Disease Severity in Patients With Coronavirus Disease 2019 (COVID-19) in a Children's Hospital in New York City, New York. *JAMA Pediatr* 2020;174:e202430.
- 110 Hrusak O, Kalina T, Wolf J, et al. Flash survey on severe acute respiratory syndrome coronavirus-2 infections in paediatric patients on anticancer treatment. *Eur J Cancer* 2020;132:11–16.
- 111 Marlais M, Wlodkowski T, Vivarelli M, et al. The severity of COVID-19 in children on immunosuppressive medication. *Lancet Child Adolesc Health* 2020;4:e17–18.
- 112 Williams N, Radia T, Harman K, et al. COVID-19 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review of critically unwell children and the association with underlying comorbidities. *Eur J Pediatr* 2020;382:1–9.
- 113 Costi S, Caporali R, Cimaz R. Dealing with COVID-19 in a pediatric rheumatology unit in Italy. *Pediatr Drugs* 2020;22:263–4.
- 114 Martín Giménez VM, Inserra F, Tajer CD, et al. Lungs as target of COVID-19 infection: protective common molecular mechanisms of vitamin D and melatonin as a new potential synergistic treatment. *Life Sci* 2020;254:117808.
- 115 Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;356:i6583.
- 116 Greiller C, Martineau A. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015;7:4240–70.
- 117 Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72:690–3.
- 118 Restrepo Valencia CA, Aguirre Arango JV, Vitamin D. Vitamin D (25(OH)D) in patients with chronic kidney disease stages 2-5. *Colomb Med* 2016;47:160–6.
- 119 Herrick KA, Storaardt RJ, Afful J, et al. Vitamin D status in the United States, 2011–2014. *Am J Clin Nutr* 2019;110:150–7.
- 120 Mosekilde L, Vitamin D. Vitamin D and the elderly. *Clin Endocrinol* 2005;62:265–81.
- 121 Laird E, Rhodes J, Kenny RA, et al. Vitamin D and inflammation: potential implications for severity of Covid-19. *Ir Med J* 2020;113:81.
- 122 Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin Exp Res* 2020;32:1195–8.
- 123 Whittemore PB. COVID-19 fatalities, latitude, sunlight, and vitamin D. *Am J Infect Control* 2020;48:1042–4.
- 124 D'Avolio A, Avataneo V, Manca A, et al. 25-Hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. *Nutrients* 2020;12:1359.
- 125 De Smet D, De Smet K, Herroelen P, et al. Vitamin D deficiency as risk factor for severe COVID-19: a convergence of two pandemics. *medRxiv* 2020:2020.2005.2001.20079376.
- 126 Panagiotou G, Tee SA, Ihsan Y, et al. Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity. *Clin Endocrinol* 2020;93:508–11.
- 127 Maghbooli Z, Sahraian MA, Ebrahimi M, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS One* 2020;15:e0239799.
- 128 Mok CK, YL N, Ahidjo BA, et al. Calcitriol, the active form of vitamin D, is a promising candidate for COVID-19 prophylaxis. *bioRxiv* 2020.
- 129 Xu J, Yang J, Chen J, et al. Vitamin D alleviates lipopolysaccharide-induced acute lung injury via regulation of the renin-angiotensin system. *Mol Med Res* 2017;16:7432–8.

- 130 Tomaschitz A, Pilz S, Ritz E, *et al.* Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: the Ludwigshafen risk and cardiovascular health (LURIC) study. *Clin Chim Acta* 2010;411:1354–60.
- 131 Jin Y, Yang H, Ji W, *et al.* Virology, epidemiology, pathogenesis, and control of COVID-19. *Viruses* 2020;12:372.
- 132 Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* 2015;282:20143085.
- 133 Lau CM, Adams NM, Geary CD, *et al.* Epigenetic control of innate and adaptive immune memory. *Nat Immunol* 2018;19:963–72.
- 134 Netea MG, Dominguez-Andrés J, Barreiro LB, *et al.* Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020;20:375–88.
- 135 Fischer A. Resistance of children to Covid-19. how? *Mucosal Immunol* 2020;13:563–5.
- 136 Valiathan R, Ashman M, Asthana D. Effects of ageing on the immune system: infants to elderly. *Scand J Immunol* 2016;83:255–66.
- 137 Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol* 2020;38:1–9.
- 138 Nye S, Whitley RJ, Kong M. Viral infection in the development and progression of pediatric acute respiratory distress syndrome. *Front Pediatr* 2016;4:128.
- 139 Mehta P, McAuley DF, Brown M, *et al.* COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395:1033–4.
- 140 Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza, Bautista E, Chotpitayasonondh T, *et al.* Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 2010;362:1708–19.
- 141 Wu Q, Xing Y, Shi L, *et al.* Coinfection and other clinical characteristics of COVID-19 in children. *Pediatrics* 2020;146:e20200961.
- 142 Zimmermann P, Curtis N. COVID-19 in children, pregnancy and neonates. *Pediatr Infect Dis J* 2020;39:469–77.
- 143 Kim D, Quinn J, Pinsky B, *et al.* Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *JAMA* 2020;323:2085–6.
- 144 Kumar N, Sharma S, Barua S, *et al.* Virological and immunological outcomes of coinfections. *Clin Microbiol Rev* 2018;31:e00111–7.
- 145 Steinman JB, Lum FM, Ho PP-K, *et al.* Reduced development of COVID-19 in children reveals molecular checkpoints gating pathogenesis illuminating potential therapeutics. *Proc Natl Acad Sci U S A* 2020;117:24620–6.
- 146 Lv H, Wu NC, Tsang OT-Y, *et al.* Cross-Reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Rep* 2020;31:107725.
- 147 Miyara M, Sterlin D, Anna F, *et al.* Pre-COVID-19 humoral immunity to common coronaviruses does not confer cross-protection against SARS-CoV-2. *medRxiv* 2020:2020.2008.2014.20173393.
- 148 KK-W T, Cheng VC-C, Cai J-P, *et al.* Seroprevalence of SARS-CoV-2 in Hong Kong and in residents evacuated from Hubei Province, China: a multicohort study. *Lancet Microbe* 2020;1:e111–8.
- 149 Huang AT, Garcia-Carreras B, Hitchings MDT, *et al.* A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* 2020;11:4704.
- 150 Sermet I, Temmam S, Huon C, *et al.* Prior infection by seasonal coronaviruses does not prevent SARS-CoV-2 infection and associated multisystem inflammatory syndrome in children. *medRxiv* 2020:2020.2006.2029.20142596.
- 151 Cervia C, Nilsson J, Zurbuchen Y, *et al.* Systemic and mucosal antibody secretion specific to SARS-CoV-2 during mild versus severe COVID-19. *bioRxiv* 2020.
- 152 Xiao F, Tang M, Zheng X, *et al.* Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 2020;158:1831–3.
- 153 Bunyavanich S, Do A, Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *JAMA* 2020;323:2427.
- 154 Gonzalez AJ, Ijezie EC, Balemba OB, *et al.* Attenuation of influenza A virus disease severity by viral coinfection in a mouse model. *J Virol* 2018;92:e00881–18.
- 155 Nickbakhsh S, Mair C, Matthews L, *et al.* Virus–virus interactions impact the population dynamics of influenza and the common cold. *Proc Natl Acad Sci U S A* 2019;116:27142–50.
- 156 Liu Y, Yan L-M, Wan L, *et al.* Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis* 2020;20:656–7.
- 157 To KK-W, Tsang OT-Y, Leung W-S, *et al.* Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;20:565–74.
- 158 Pujadas E, Chaudhry F, McBride R, *et al.* SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med* 2020;8:e70.
- 159 De Maio F, Posteraro B, Ponziani FR, *et al.* Nasopharyngeal microbiota profiling of SARS-CoV-2 infected patients. *Biol Proced Online* 2020;22:18.
- 160 Gu S, Chen Y, Wu Z, *et al.* Alterations of the gut microbiota in patients with COVID-19 or H1N1 influenza. *Clin Infect Dis* 2020.
- 161 Zuo T, Zhang F, Lui GCY, *et al.* Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology* 2020;159:944–55.
- 162 Xu K, Cai H, Shen Y, *et al.* [Management of corona virus disease-19 (COVID-19): the Zhejiang experience]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2020;49:147–57.
- 163 Budding A, Sieswerda E, Wintermans B, *et al.* An age dependent pharyngeal microbiota signature associated with SARS-CoV-2 infection, 2020. Available: <https://ssrncom/abstract=3582780>
- 164 Shen Z, Xiao Y, Kang L, *et al.* Genomic diversity of severe acute respiratory Syndrome–Coronavirus 2 in patients with coronavirus disease 2019. *Clin Infect Dis* 2020;71:713–20.
- 165 Ferreira-Halder CV, Faria AVdeS, Andrade SS. Action and function of *Faecalibacterium prausnitzii* in health and disease. *Best Pract Res Clin Gastroenterol* 2017;31:643–8.
- 166 Geva-Zatorsky N, Sefik E, Kua L, *et al.* Mining the human gut microbiota for immunomodulatory organisms. *Cell* 2017;168:928–43.
- 167 Derrien M, Alvarez A-S, de Vos WM. The gut microbiota in the first decade of life. *Trends Microbiol* 2019;27:997–1010.
- 168 Ringel-Kulka T, Cheng J, Ringel Y, *et al.* Intestinal microbiota in healthy U.S. young children and adults—a high throughput microarray analysis. *PLoS One* 2013;8:e64315.
- 169 Zuo T, Liu Q, Zhang F, *et al.* Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. *Gut* 2020. doi:10.1136/gutjnl-2020-322294. [Epub ahead of print: 20 Jul 2020].
- 170 Zimmermann P, Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. *J Infect* 2019;79:471–89.
- 171 Zimmermann P, Curtis N. Factors influencing the intestinal microbiome during the first year of life. *Pediatr Infect Dis J* 2018;37:e315–35.
- 172 Hardeband R. Melatonin and inflammation—Story of a double-edged blade. *J Pineal Res* 2018;65:e12525.
- 173 Wu X, Ji H, Wang Y, *et al.* Melatonin alleviates radiation-induced lung injury via regulation of miR-30e/NLRP3 axis. *Oxid Med Cell Longev* 2019;2019:1–14.
- 174 Miller SC, Pandi PSR, Esquifino AI, *et al.* The role of melatonin in immunoenhancement: potential application in cancer. *Int J Exp Pathol* 2006;87:81–7.
- 175 Kaur C, Ling EA. Effects of melatonin on macrophages/microglia in postnatal rat brain. *J Pineal Res* 1999;26:158–68.
- 176 Bazayr H, Gholinezhad H, Moradi L, *et al.* The effects of melatonin supplementation in adjunct with non-surgical periodontal therapy on periodontal status, serum melatonin and inflammatory markers in type 2 diabetes mellitus patients with chronic periodontitis: a double-blind, placebo-controlled trial. *Inflammopharmacology* 2019;27:67–76.
- 177 Sun C-K, Lee F-Y, Kao Y-H, *et al.* Systemic combined melatonin-mitochondria treatment improves acute respiratory distress syndrome in the rat. *J Pineal Res* 2015;58:137–50.
- 178 Huang S-H, Cao X-J, Liu W, *et al.* Inhibitory effect of melatonin on lung oxidative stress induced by respiratory syncytial virus infection in mice. *J Pineal Res* 2010;48:109–16.
- 179 Reiter RJ, Ma Q, Sharma R. Treatment of ebola and other infectious diseases: melatonin "goes viral". *Melatonin Research* 2020;3:43–57.
- 180 Lambert DW, Clarke NE, Hooper NM, *et al.* Calmodulin interacts with angiotensin-converting enzyme-2 (ACE2) and inhibits shedding of its ectodomain. *FEBS Lett* 2008;582:385–90.
- 181 del Río B, Pedrero JMG, Martínez-Campa C, *et al.* Melatonin, an endogenous-specific inhibitor of estrogen receptor α via calmodulin. *J Biol Chem* 2004;279:38294–302.
- 182 Sehrlir AO, Sayiner S, Serakinci N. Role of melatonin in the treatment of COVID-19; as an adjuvant through cluster differentiation 147 (CD147). *Mol Biol Rep* 2020. doi:10.1007/s11033-020-05830-8. [Epub ahead of print: 12 Sep 2020].
- 183 Wang K, Chen W, Zhou Y-S, *et al.* SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxiv* 2020.
- 184 Yurchenko V, Constant S, Eisenmesser E, *et al.* Cyclophilin-CD147 interactions: a new target for anti-inflammatory therapeutics. *Clin Exp Immunol* 2010;160:305–17.
- 185 Feitosa EL, Júnior FTDSS, Nery Neto JADO, *et al.* COVID-19: rational discovery of the therapeutic potential of melatonin as a SARS-CoV-2 main protease inhibitor. *Int J Med Sci* 2020;17:2133–46.
- 186 Zhang R, Wang X, Ni L, *et al.* COVID-19: melatonin as a potential adjuvant treatment. *Life Sci* 2020;250:117583.
- 187 Shneider A, Kudriavtsev A, Vakhrusheva A. Can melatonin reduce the severity of COVID-19 pandemic? *Int Rev Immunol* 2020;39:153–62.
- 188 García IG, Rodríguez-Rubio M, Mariblanca AR, *et al.* A randomized multicenter clinical trial to evaluate the efficacy of melatonin in the prophylaxis of SARS-CoV-2 infection in high-risk contacts (MeCOVID trial): a structured summary of a study protocol for a randomised controlled trial. *Trials* 2020;21:466.
- 189 Haldar C, Yadav R. Annual reproductive synchronization in ovary and pineal gland function of female short-nosed fruit bat, *Cynopterus sphinx*. *Comp Biochem Physiol A Mol Integr Physiol* 2006;144:395–400.
- 190 Iguchi H, Kato KI, Ibayashi H. Age-Dependent reduction in serum melatonin concentrations in healthy human subjects. *J Clin Endocrinol Metab* 1982;55:27–9.
- 191 Waldhauser F, Weissenbacher G, Tatzler E, *et al.* Alterations in nocturnal serum melatonin levels in humans with growth and aging. *J Clin Endocrinol Metab* 1988;66:648–52.
- 192 Pollard AJ, Finn A, Curtis N. Non-Specific effects of vaccines: plausible and potentially important, but implications uncertain. *Arch Dis Child* 2017;102:1077–81.

- 193 Higgins JPT, Soares-Weiser K, López-López JA, *et al.* Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 2016;355:i5170.
- 194 Moorlag SJCFM, Arts RJW, van Crevel R, *et al.* Non-Specific effects of BCG vaccine on viral infections. *Clin Microbiol Infect* 2019;25:1473–8.
- 195 Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, *et al.* Activate: randomized clinical trial of BCG vaccination against infection in the elderly. *Cell* 2020;183:315–23.
- 196 Kleinnijenhuis J, van Crevel R, Netea MG. Trained immunity: consequences for the heterologous effects of BCG vaccination. *Trans R Soc Trop Med Hyg* 2015;109:29–35.
- 197 Freyne B, Donath S, Germano S, *et al.* Neonatal BCG vaccination influences cytokine responses to Toll-like receptor ligands and heterologous antigens. *J Infect Dis* 2018;217:1798–808.
- 198 Messina NL, Zimmermann P, Curtis N. The impact of vaccines on heterologous adaptive immunity. *Clin Microbiol Infect* 2019;25:1484–93.
- 199 Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, *et al.* Trained immunity: a tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. *Cell* 2020;181:969–77.
- 200 Miller A, Reandelar MJ, Fasciglione K, *et al.* Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study. *medRxiv* 2020:2020.2003.2024.20042937.
- 201 Shet A, Ray D, Malavige N, *et al.* Differential COVID-19-attributable mortality and BCG vaccine use in countries. *medRxiv* 2020:2020.2004.2001.20049478.
- 202 Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci U S A* 2020;117:17720–6.
- 203 Riccò M, Gualerzi G, Ranzieri S, *et al.* Stop playing with data: there is no sound evidence that Bacille Calmette-Guérin may avoid SARS-CoV-2 infection (for now). *Acta Biomed* 2020;91:207–13.
- 204 Faust L, Huddart S, MacLean E, *et al.* Universal BCG vaccination and protection against COVID-19: critique of an ecological study Journal Club, coronaviruses: past, present and future web site. Available: <https://naturemicrobiologycommunity.nature.com/users/36050-emily-maclean/posts/64892-universal-bcg-vaccination-and-protection-against-covid-19-critique-of-an-ecological-study> [Accessed 5 Apr 2020].
- 205 Curtis N, Sparrow A, Ghebreyesus TA, *et al.* Considering BCG vaccination to reduce the impact of COVID-19. *Lancet* 2020;395:1545–6.
- 206 Hamiel U, Kozar E, Youngster I. SARS-CoV-2 rates in BCG-vaccinated and unvaccinated young adults. *JAMA* 2020;323:2340–1.
- 207 Curtis N. Bcg vaccination to protect healthcare workers against COVID-19 (brace), 2020. Available: <https://clinicaltrials.gov/ct2/show/NCT04327206>
- 208 Franklin R, Young A, Neumann B, *et al.* Homologous protein domains in SARS-CoV-2 and measles, mumps and rubella viruses: preliminary evidence that MMR vaccine might provide protection against COVID-19. *medRxiv* 2020.
- 209 Polio Global Eradication Initiative. The use of oral polio vaccine (OPV) to prevent SARS-CoV2, 2020. Available: <http://polioeradication.org/wp-content/uploads/2020/03/Use-of-OPV-and-COVID-20200421.pdf> [Accessed 6 Jun 2020].
- 210 Lisse IDAM, Aaby P, Knudsen KIM, *et al.* Long term impact of high titer Edmonston-Zagreb measles vaccine on T lymphocyte subsets. *Pediatr Infect Dis J* 1994;13:109–12.
- 211 Zimmermann P, Perrett KP, van der Klis FR, *et al.* The immunomodulatory effects of measles-mumps-rubella vaccination on persistence of heterologous vaccine responses. *Immunol Cell Biol* 2019;97:577–85.
- 212 Avidan MS. ROWN CORONATION: COVID-19 research outcomes worldwide network for coronavirus prevenTION (crown corona), 2020. Available: <https://clinicaltrials.gov/ct2/show/NCT04333732>
- 213 Dalton CB, Corbett SJ, Katelaris AL. COVID-19: implementing sustainable low cost physical distancing and enhanced hygiene. *Med J Aust* 2020;212:443–6.
- 214 Dong Y, Mo X, Hu Y, *et al.* Epidemiology of COVID-19 among children in China. *Pediatrics* 2020;145:e20200702.
- 215 Posfay-Barbe KM, Wagner N, Gauthey M, *et al.* COVID-19 in children and the dynamics of infection in families. *Pediatrics* 2020;146:e20201576.
- 216 Lu X, Zhang L, Du H, *et al.* SARS-CoV-2 infection in children. *N Engl J Med* 2020;382:1663–5.
- 217 Chowell G, Abdirizak F, Lee S, *et al.* Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Med* 2015;13:210.
- 218 Breban R, Riou J, Fontanet A. Interhuman transmissibility of middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 2013;382:694–9.
- 219 Zhang L, Jackson CB, Mou H, *et al.* The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. *bioRxiv* 2020
- 220 Korber B, Fischer WM, Gnanakaran S, *et al.* Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell* 2020;182:812–27.
- 221 Easwarkhanth M, Al Madhoun A, Al-Mulla F. Could the D614G substitution in the SARS-CoV-2 spike (S) protein be associated with higher COVID-19 mortality? *Int J Infect Dis* 2020;96:459–60.
- 222 Zimmermann P, Goetzinger F, Ritz N. Additional concerns regarding children with coronavirus disease 2019. *JAMA Pediatr* 2020. doi:10.1001/jamapediatrics.2020.2916. [Epub ahead of print: 26 Oct 2020].
- 223 Basatneh R, Vlahovic TC. Addressing the question of dermatologic manifestations of SARS-CoV-2 infection in the lower extremities: a closer look at the available data and its implications. *J Am Podiatr Med Assoc* 2020. doi:10.7547/20-074. [Epub ahead of print: 20 Apr 2020].
- 224 Huang S-H, Su M-C, Tien N, *et al.* Epidemiology of human coronavirus NL63 infection among hospitalized patients with pneumonia in Taiwan. *J Microbiol Immunol Infect* 2017;50:763–70.
- 225 Centers for Disease Control and Prevention CDC. Vitamin D, 2019. Available: <https://www.cdc.gov/breastfeeding/breastfeeding-special-circumstances/diet-and-micronutrients/vitamin-d.html> [Accessed 16 Jun 2020].
- 226 Russell B, Moss C, George G, *et al.* Associations between immune-suppressive and stimulating drugs and novel COVID-19—a systematic review of current evidence. *Eancermedalscience* 2020;14:1022.
- 227 d’Ettorre G, Ceccarelli G, Marazzato M, *et al.* Challenges in the management of SARS-CoV2 infection: the role of oral bacteriotherapy as complementary therapeutic strategy to avoid the progression of COVID-19. *Front Med* 2020;7:389.