mgr Paweł Kozłowski

Ocena markerów uszkodzenia śródbłonka i rozwoju autoimmunizacji po zakażeniu SARS-CoV-2 u osób nieobciążonych dodatkowymi czynnikami ryzyka

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Promotor: prof. dr hab. n. med. i n. o zdr. Olga Ciepiela

Zakład Medycyny Laboratoryjnej
Warszawskiego Uniwersytetu Medycznego

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autoimmunizacja, autoprzeciwciała, COVID-19, glikokaliks, koronawirus, SARS-CoV-2, selektyna E, uszkodzenie śródbłonka

Keywords

autoimmunity, autoantibodies, COVID-19, glycocalyx, coronavirus, SARS-CoV-2, E-selectin, endothelial damage

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2. Wykaz publikacji stanowiących pracę doktorską

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   IF: 3,9
   punkty MNiSW: 140

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Spis treści

3. Wykaz stosowanych skrótów

AA	ang. arachidonic acid kwas arachidonowy
AC	ang. “anti-Cell” used in ICAP coding ANA indirect immunofluorescence patterns “przeciw komórkowy” – skrót stosowany w ICAP do identyfikowania wzoru fluorescencji przeciwciał ANA
ACE	ang. angiotensin-converting enzyme enzym konwertujący angiotensynę
ACE2	ang. angiotensin-converting enzyme receptor 2 receptor enzymu konwertującego angiotensynę typu drugiego (znaczenie stosowane w kontekście wirusa SARS-CoV-2)
ACE2	ang. angiotensin-converting enzyme 2 enzym konwertujący angiotensynę typu drugiego (znaczenie stosowane w kontekście układu RAA)
ADAMTS13	ang. disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 dezintegryna i metaloproteinaza z motywem trombospondyny typu 1, członek 13
ANA	ang. antinuclear antibodies przeciwciała przeciwjądrowe
Ang II	ang. angiotensin II angiotensyna II
APC	ang. activated protein C aktywne białko C
APC	ang. antigen presenting cell komórka prezentująca antygen

APS	ang. antiphospholipid syndrome zespół antyfosfolipidowy
ARDS	ang. acute respiratory distress syndrome zespół ostrej niewydolności oddechowej
AT	ang. antithrombin antytrombina
AT1R	ang. endothelial angiotensin 2 type 1 receptor śródbłonkowy receptor angiotensyny 2 typu 1
białko E	ang. envelope białko otoczki
białko M	ang. membrane białko błonowe
białko N	ang. nucleocapsid białko nukleokapsydu
białko S	ang. surface białko powierzchniowe
CD62E	ang. E-selectin selektyna E
CD62P	ang. P-selectin selektyna P
CH	ang. central helix centralna helisa
CMV	ang. cytomegalovirus cytomegalowirs
COVID-19	ang. coronavirus disease of 2019 choroba koronawirusowa 2019 roku
CT	ang. C-terminal domain domena C-końcowa

CTD	ang. carboxy-terminal domain domena na końcu karboksylowym
CXCL8	ang. C-X-C motif chemokine ligand 8 ligand 8 chemokiny z motywem C-X-C (interleukina 8)
CXCL10	ang. C-X-C motif chemokine ligand 10 ligand 10 chemokiny z motywem C-X-C
DFS-70	ang. dense fine speckled 70 drobnoziarnisty wzór fluorescencji ANA z dodatnimi obszarami chromosomowymi
DIC	ang. disseminated intravascular coagulation zespół rozsianego krzepnięcia wewnątrznaczyniowego
DNA	ang. deoxyribonucleic acid kwas dezoksyrybonukleinowy
EBV	ang. Epstein-Barr virus wirus Epstein Barr
eNOS	ang. endothelial nitric oxide synthase śródbłonkowa syntaza tlenku azotu
EPCR	ang. endothelial protein C receptor śródbłonkowy receptor białka C
ExoN	ang. exoribonuclease egzorybonukleaza
FP	ang. fusion peptide peptyd fuzyjny
FPPR	ang. fusion peptide proximal region region bliższy peptydu fuzyjnego
HHV-6	ang. human herpes virus 6 ludzki wirus opryszczki typu 6
HHV-7	ang. human herpes virus 7 ludzki wirus opryszczki typu 7

HR1/2	ang. heptad repeat 1/2 1. i 2. sekwencja aminokwasowa białka S wirusa SARS-CoV-2
HSV-1	ang. herpes simplex virus type 1 wirus opryszczki zwykłej typu 1
ICAM-1	ang. intracellular adhesion molecule 1 cząsteczka adhezji międzykomórkowej typu 1
ICAP	ang. International Consensus on Antinuclear Antibody (ANA) Patterns Międzynarodowy Konsensus ds. Wzorów Fluorescencji Przeciwciał Przeciwjądrowych (ANA)
IIa	ang. thrombin trombina
IL-1	ang. interleukin 1 interleukina 1
IL-6	ang. interleukin 6 interleukina 6
JAK	ang. Janus kinase kinaza janusowa
JNK	ang. c-Jun N-terminal kinase N-końcowa kinaza c-Jun
LEDGF	ang. lens epithelium derived growth factor czynnik wzrostu pochodzący z nabłonka soczewki
MAPK	ang. mitogen activated protein kinase kinaza białkowa aktywowana mitogenem
MAS	ang. mitochondrial assembly (G protein-coupled receptor for angiotensin 1-7) receptor dla angiotensyny 1-7 sprzężony z białkiem G
MERS-CoV	ang. Middle East respiratory syndrome coronavirus koronawirus zespołu niewydolności oddechowej Bliskiego Wschodu

MR-proADM	ang. mid-regional proadrenomedullin mid-region proadrenomedulina
NO	ang. nitric oxide tlenek azotu
nsp	ang. non-structural protein białko niestrukturalne
NTD	ang. N-terminal domain domena N-końcowa
OFR	ang. open frame reading otwarta ramka odczytu
p38	ang. p38 mitogen-activated protein kinase kinaza białkowa aktywowana mitogenem p38
PAI-1	ang. plasminogen activator inhibitor-1 inhibitor aktywatora plazminogenu typu 1
PC	ang. protein C białko C
PECAM-1	ang. platelet endothelial cell adhesion molecule pierwsza cząsteczka adhezji płytek krwi do śródbłonka
PGI_2	ang. prostacyclin I2 prostacyklina I2
RAA	ang. renin–angiotensin–aldosterone system układ renina-angiotensyna-aldosteron
RBD	ang. receptor-binding domain domena wiążąca receptor
RBM	ang. receptor-binding motif motyw wiążący receptor
RdRp	ang. RNA-dependent RNA polymerase polimeraza RNA zależna od RNA

RIG	ang. retinoic acid-inducible gene gen indukowany kwasem retinowym
RLR	ang. RIG-like receptors receptory podobne do RIG
RNA	ang. ribonucleic acid kwas rybonukleinowy
RNP	ang. ribonucleoprotein complexes kompleksy rybonukleoproteinowe
ROS	ang. reactive oxygen species reaktywne formy tlenu
RTC	ang. replication and transcription complex kompleks replikacyjny i transkrypcyjny
SARDs	ang. systemic autoimmune rheumatic diseases układowe autoimmunizacyjne choroby reumatyczne
SARS-CoV	ang. severe acute respiratory syndrome coronavirus koronawirus zespołu ostrej ciężkiej niewydolności oddechowej
SARS-CoV-2	ang. severe acute respiratory syndrome coronavirus 2 drugi koronawirus zespołu ostrej ciężkiej niewydolności oddechowej
Src	ang. Src kinase kinaza Src
TF	ang. tissue factor czynnik tkankowy
TFPI	ang. tissue factor pathway inhibitor inhibitor zewnątrzpochodnego szlaku krzepnięcia
TLR	ang. Toll-like receptors receptory Toll-podobne
TM	ang. transmembrane anchor kotwica przezbłonowa (znaczenie używane w opisie białka S wirusa SARS-CoV-2)

TM	ang. thrombomodulin trombomodulina (znaczenie używane w kontekście uszkodzenia śródbłonka)
TMPRSS2	ang. transmembrane protease serine 2 przezbłonowa proteaza serynowa 2
TNF-α	ang. tumor necrosis factor α czynnik martwicy nowotworów α
t-PA	ang. tissue-type plasminogen activator tkankowy aktywator plazminogenu
Va	ang. activated factor V aktywny czynnik V
VCAM-1	ang. vascular cell adhesion molecule 1 pierwsza cząsteczka adhezyjna śródbłonka naczyniowego
VIIa	ang. activated factor VII aktywny czynnik VII
VIIIa	ang. activated factor VIII aktywny czynnik VIII
vWF	ang. von Willebrand factor czynnik von Willebranda
vWF:Ag	ang. von Willebrand factor antigen antygen czynnika von Willebranda
WHO	ang. World Health Organization Światowa Organizacja Zdrowia
β2-GPI	ang. β2-glicoprotein I β2-glikoproteina I

4. Streszczenie w języku polskim

W listopadzie 2019 roku w chińskim mieście Wuhan pojawiły się przypadki zakażenia układu oddechowego spowodowane nowym koronawirusem SARS-CoV-2 (ang. severe acute respiratory syndrome coronavirus 2). Chorobę, która rozwija się w wyniku zakażenia nazwano COVID-19 (ang. coronavirus disease of 2019), a w maju 2020 roku Światowa Organizacja Zdrowia ogłosiła stan pandemii ze względu na szybkie rozprzestrzenianie się wirusa i lawinowy wzrost liczby zakażeń. Od początku pandemii do marca 2023 roku odnotowano ponad 700 milionów zakażeń i prawie 7 milionów zgonów z powodu choroby COVID-19 Równocześnie zaczęły pojawiać się doniesienia o pozapłucnych manifestacjach choroby oraz odległych powikłaniach zakażenia wirusem SARS-CoV-2. W patomechanizmie tych powikłań szczególną uwagę zwracają zarówno wywołane przez zakażenie uszkodzenie komórek śródbłonka jak również rozwój procesów autoimmunizacyjnych. Obydwa te zjawiska, które w perspektywie czasu mogą prowadzić do istotnych problemów zdrowotnych w populacji, wymagają pogłębionych badań ze względu na liczbę potencjalnie dotkniętych nimi osób. W pierwszym okresie pandemii uwaga środowiska naukowego skupiona była głównie na chorych, u których przebieg COVID-19 był ciężki. Jednocześnie zakażeniu ulegały także osoby generalnie zdrowe, nieobciążone czynnikami ryzyka ciężkiego przebiegu COVID-19. U osób, które przechorowały COVID-19 rozpoznawano przedłużające się dysfunkcje zdrowotne niezwiązane z żadną inną chorobą niż COVID-19. Takie powikłania nazwano „long COVID". Szacuje się, że na „long COVID" może chorować nawet 45% pierwotnie zakażonych wirusem SARS-CoV-2. W tych szacunkach uwzględnia się przede wszystkim osoby z uprzednio potwierdzonym zakażeniem, z ciężkim lub umiarkowanym przebiegiem choroby. Należy również pamiętać, że zakażenie wirusem SARS-CoV-2 może przebiegać łagodnie lub nawet bezobjawowo. Zasadne stało się więc badanie, czy przebyte zakażenie wirusem SARS-CoV-2 z umiarkowanym, łagodnym lub bezobjawowym przebiegiem u osób bez dodatkowych czynników ryzyka może prowadzić do przetrwałego uszkodzenia komórek śródbłonka i rozwoju procesów autoimmunizacyjnych.

Celem pracy jest pogłębienie wiedzy na temat wpływu zakażenia wirusem SARS-CoV-2 na komórki śródbłonka oraz rozwój autoimmunizacji u osób, które nie są obciążone dodatkowymi czynnikami ryzyka.

Do badań włączono grupę 294 honorowych dawców krwi. Rygorystyczne kryteria kwalifikacji przed donacją krwi pozwoliły na zakwalifikowanie do badania osób nieobciążonych dodatkowymi czynnikami ryzyka. Zakwalifikowanych do badania dawców zbadano na obecność przeciwciał przeciwko białku N wirusa SARS-CoV-2 jako markera przebytego zakażenia. Na podstawie wyników dawców podzielono na grupę badaną (osoby z obecnymi przeciwciałami) n=215 oraz grupę kontrolną (osoby z nieobecnymi przeciwciałami) n=79. W każdej grupie ocenie poddano stężenie wybranych markerów uszkodzenia śródbłonka i glikokaliksu (VCAM-1, ICAM-1, selektyna E, syndekan 1) oraz wykonano badania w kierunku obecności przeciwciał przeciwjądrowych i przeciwko β2-glikoproteinie I.

Wyniki przeprowadzonych badań wykazały, iż u ozdrowieńców nieobciążonych dodatkowymi czynnikami ryzyka, w czasie nie krótszym niż 6 miesięcy od zakażenia, obserwuje się cechy przetrwałego uszkodzenia śródbłonka. Ozdrowieńcy charakteryzowali się wyższym stężeniem E-selektyny (1754 pg/ml vs 1633 pg/ml, p=0.0135) i niższym stężeniem syndekanu-1 (692 pg/ml vs 934 pg/ml, p=0.0082) niż osoby niechorujące na COVID-19. Badania w kierunku obecności przeciwciał przeciwjądrowych oraz przeciwko β2-glikoproteinie I nie wykazały większej częstości ich występowania u ozdrowieńców w porównaniu do grupy kontrolnej.

Powyższe wyniki pozwoliły na sformułowanie wniosku, iż osoby nieobciążone dodatkowymi czynnikami ryzyka po przebyciu infekcji wirusem SARS-CoV-2 mogą być narażone na rozwój chorób związanych z przetrwałym uszkodzeniem komórek śródbłonka. Jednocześnie wyniki pracy nie wykazały zwiększonego ryzyka rozwoju procesów autoimmunizacyjnych, w tym tych prowadzących do zakrzepicy, u badanych ozdrowieńców. Wobec ogromnej liczby osób które przebyły zakażenie wirusem SARS-CoV-2, zmienności samego wirusa, wprowadzenia szczepień ochronnych, a także złożonej natury schorzeń autoimmunizacyjnych, z punktu widzenia zdrowia publicznego konieczne jest pogłębianie badań i poszerzanie wiedzy na temat opisywanych zjawisk.

5. Streszczenie w języku angielskim

Evaluation of markers of endothelial damage and development of autoimmunity after SARS-CoV-2 infection in subjects without additional risk factors.

In November 2019, cases of respiratory infection emerged in the Chinese city of Wuhan. The new disease called coronavirus disease 2019 (COVID-19) was caused be the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In May 2020, the World Health Organization declared a pandemic state due to the rapid spread of the virus and the exponential increase in the number of infections. From the beginning of the pandemic until March 2023, there were more than 700 million infections and almost 7 million deaths from COVID-19. At the same time, reports of extrapulmonary manifestations of the disease and delayed complications of SARS-CoV-2 virus infection began to appear. Infection-induced endothelial cell damage and the development of autoimmune processes contribute to the development of these complications. Both of these phenomena, which in the long term can lead to significant health problems in the population, require in-depth studies due to the number of potentially affected individuals. During the initial period of the pandemic, the attention of the scientific community was focused mainly on patients with a severe course of COVID-19 infection. At the same time, generally healthy people unencumbered by risk factors for a severe course of COVID-19 infection also became infected. Individuals who underwent COVID-19 were diagnosed with prolonged health dysfunctions unrelated to any other disease other than COVID-19. Such complications have been called “long COVID”. It is estimated that up to 45% of those originally infected with SARS-CoV-2 may develop “long COVID”. These estimates include those with previously confirmed infection, with a severe or moderate course of the disease. It is also important to remember that infection with SARS-CoV-2 can be mild or even asymptomatic. It therefore became reasonable to investigate whether a history of asymptomatic to moderate SARS-CoV-2 infection in individuals without additional risk factors could lead to persistent endothelial cell damage and the development of autoimmune processes.

The aim of this study is to further our understanding of the effects of SARS-CoV-2 virus infection on endothelial cells and the development of autoimmunity in individuals without additional risk factors.

A group of 294 honorary blood donors was included in the study. Strict eligibility criteria prior to blood donation allowed the study to qualify individuals as unencumbered by additional risk factors. Qualified donors were tested for antibodies to the N protein of SARS-CoV-2 virus as a marker of past infection. Based on the results, donors were divided into a test group (those with antibodies) n=215, and a control group (those without antibodies) n=79. In each group, concentrations of selected markers of endothelial damage and glycocalyx were evaluated (VCAM-1, ICAM-1, E-selectin, syndecan-1), and tests for antinuclear antibodies and against β2-glycoprotein I were performed.

Features of persistent endothelial damage were observed in the convalescents without additional risk factors not less than 6 months after infection. Convalescents had higher E-selectin levels (1754 pg/mL vs 1633 pg/mL, p=0.0135) and lower syndecan-1 levels (692 pg/mL vs 934 pg/mL, p=0.0082) than the control group. Tests for the presence of antinuclear antibodies and antibodies against β2-glycoprotein I did not show a higher prevalence in the convalescents compared to the control group.

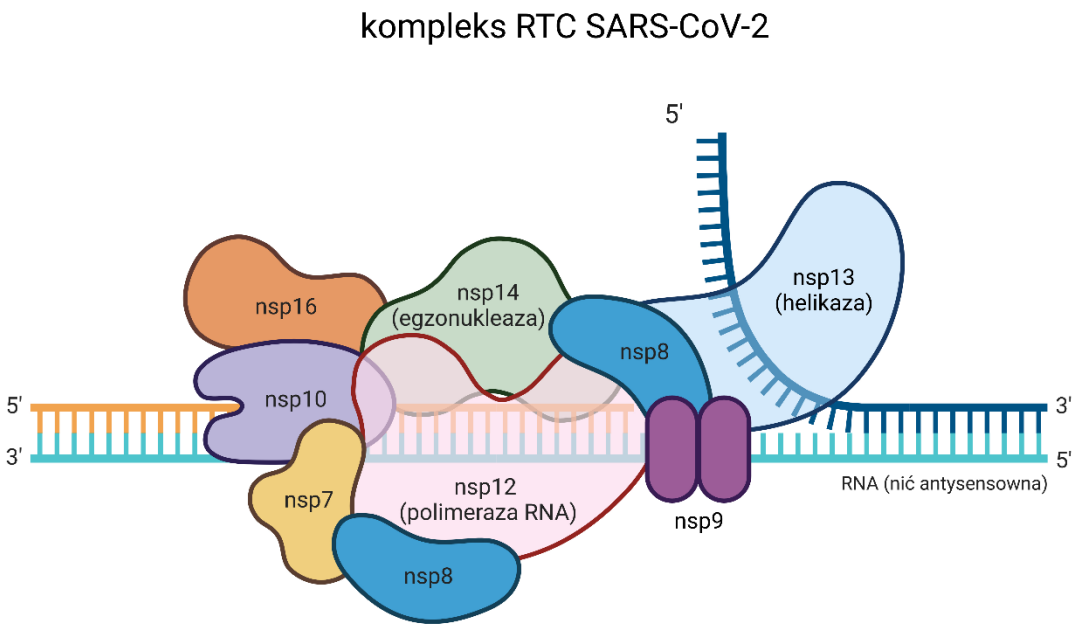
The results allowed us to conclude that people without additional risk factors may be at risk of developing diseases associated with persistent endothelial cell damage after infection with SARS-CoV-2. There was also no increased risk of developing autoimmune processes, including those leading to thrombosis, in the studied convalescents. Due to the huge number of people who have undergone infection with the SARS-CoV-2 virus, the variability of the virus itself, the introduction of immunization, and the complex nature of autoimmune diseases, it is necessary from the public health perspective to deepen research and expand knowledge of the described phenomena.

6. Wstęp

Choroby zakaźne układu oddechowego ludzi powodowane przez koronawirusy opisywane były już w latach 60. XX wieku [1]. Wywoływane przez nie infekcje, powszechnie określane terminem „przeziębienie", mają łagodny charakter [2]. W publicznej świadomości, jako potencjalne zagrożenie, patogeny te zaistniały za sprawą dwóch koronawirusów, które na początku XXI wieku wywołały lokalne epidemie. W listopadzie 2002 roku, w prowincji Guangdong na południu Chińskiej Republiki Ludowej, zidentyfikowano ogniska wywołanej przez wirusa SARS-CoV (ang. severe acute respiratory syndrome coronavirus) choroby zakaźnej układu oddechowego, która u wielu zakażonych osób miała ciężki przebieg, a w około 10% przypadków prowadziła do zgonu [3,4]. Dzięki działaniom podjętym przez służby medyczne udało się zapobiec rozprzestrzenieniu choroby i wywołaniu ogólnoświatowej pandemii. W 2014 roku w Arabii Saudyjskiej zidentyfikowano ogniska zakażeń wirusem MERS-CoV (ang. Middle East respiratory syndrome coronavirus), a sam patogen rozprzestrzenił się na inne kraje Bliskiego Wschodu, Azji, Europy i Ameryki Północnej [5]. Zakażenie MERS-CoV w około 35% przypadków kończyło się zgonem chorego, podjęto więc intensywne wysiłki zmierzające do zatrzymania transmisji wirusa [4]. W listopadzie 2019 roku, w mieście Wuhan – stolicy środkowochińskiej prowincji Hubei, pojawiło się ognisko choroby zakaźnej górnych dróg oddechowych. Za zachorowania odpowiedzialny był koronawirus SARS-CoV-2, a samą chorobę nazwano COVID-19 (ang. coronavirus disease of 2019) [2]. Wirus bardzo szybko rozprzestrzenił się na kolejne prowincje Chin, następnie w szybkim czasie pojawił w Stanach Zjednoczonych i Europie – na skutek masowego przemieszczania się ludzi, którzy opuszczali Chiny, uciekając przed ogłoszoną przez władze kwarantanną całych miast [6]. W marcu 2020 roku obecność wirusa odnotowano w większości krajów Europy, w Stanach Zjednoczonych oraz na Bliskim Wschodzie [7–10]. Z powodu szybkiego rozprzestrzeniania się patogenu oraz lawinowo rosnącej liczby zachorowań, Światowa Organizacja Zdrowia (WHO, ang. World Health Organization) ogłosiła w maju 2020 roku pandemię COVID-19 [11]. Według raportu WHO, od początku pandemii do marca 2023 roku na całym świecie odnotowano około 759 milionów zakażeń SARS-CoV-2, a COVID-19 doprowadził do zgonu 6,9 miliona osób z 337 milionami utraconych lat życia w latach 2020-2021 [12].

6.1 Charakterystyka SARS-CoV-2

Koronawirusy są zróżnicowaną grupą wirusów, które mają zdolność do zakażania ludzi, innych ssaków, a także ptaków. Wirus SARS-CoV-2 (rząd: *Nidovirales*, rodzina: *Coronaviridae*, podrodzina: *Orthocoronavirinae*) należy do betakoronawirusów, jednego z czterech rodzajów podrodziny *Orthocoronavirinae* (*alpha-*, *beta-*, *delta-* i *gammacoronavirus*) [13,14]. Zbudowany jest z wirionu otaczającego materiał genetyczny, który stanowi pojedyncza sensowna nić RNA złożona z 29.891 nukleotydów [15]. Genom wirusa zawiera sekwencje kodujące białka strukturalne budujące wirion (białka: S ang. surface, N ang. nucleocapsid, M ang. membrane, E ang. envelope) oraz białka niestrukturalne (nsp 1-16; ang. non-structural proteins) [16]. W genomie wirusa zidentyfikowano 14 regionów otwartej ramki odczytu (OFR; ang. open frame reading), w których znajdują się geny kodujące 16 różnych białek tworzących kompleks odpowiedzialny za replikację i transkrypcję wirusowego RNA (RTC; ang. replication and transcription complex) [17–19] (rycina 1).



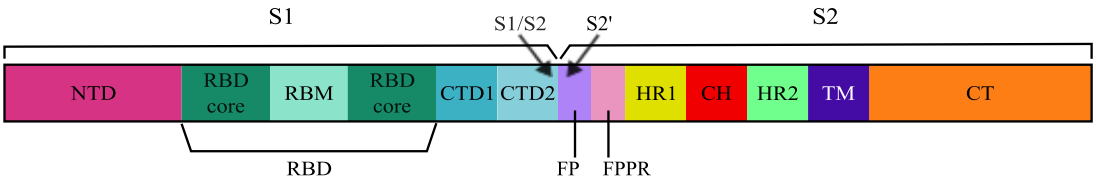
Rycina 1. Kompleks białek wirusa SARS-CoV-2, odpowiedzialny z replikację i transkrypcję RNA (na podstawie Hartenian i wsp. [20], wykonano w programie BioRender.com).

nsp – non-structural protein; RTC – replication and transcription complex.

Białko nsp12 jest wirusową polimerazą RNA zależną od RNA (RdRp; ang. RNA-dependent RNA polymerase), której rolą jest synteza antysensownej nici RNA będącej w następnym etapie matrycą do syntezy sensownej nici RNA,

stanowiącej materiał genetyczny dla nowych wirionów [20]. Białka nsp7 i nsp8 są kofaktorami, które wiążą się z białkiem nsp12 i stabilizują jego miejsce wiążące RNA [21,22], zwiększając tym samym jego aktywność [23]. Białko nsp14 ma aktywność 3'-5' egzorybonukleazy (ExoN, ang. exoribonuclease) o aktywności korektorskiej, która zmniejsza liczbę błędów podczas replikacji genomowego RNA wirusa [20]. Białko nsp13 o aktywności 5'-3' helikazy rozdziela nici RNA, udostępniając białku nsp12 pojedynczą nić RNA jako matrycę [24]. Do fragmentu pojedynczej nici RNA przyłącza się dimer białka nsp9. Białko to jest niezbędne w procesie replikacji RNA wirusa, prawdopodobnie biorąc udział w rekrutacji pozostałych białek do kompleksu RTC [25]. Kompleks białek nsp16 i nsp10 o aktywności 2'-O-metylotransferazy katalizuje na końcu 5' metylację czapeczki RNA [26]. Jest to proces, który pozwala wirusowi upodobnić produkowane przez kompleks RTC transkrypty do RNA komórki gospodarza i przez to uniknąć ich rozpoznania przez obecne w cytoplazmie receptory RLR (ang. RIG-like receptors), a w konsekwencji uniknąć uruchomienia odpowiedzi antywirusowej zależnej od interferonu [27,28].

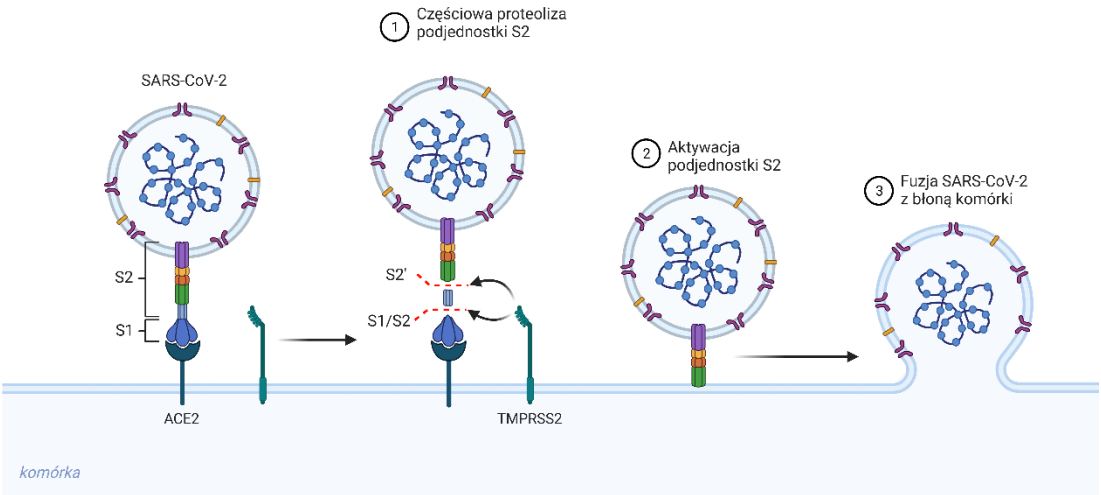
Białko S wirusa jest odpowiedzialne za łączenie wirionu z obecnym na komórkach człowieka receptorem 2 enzymu konwertującego angiotensynę (ACE2; ang. angiotensin-converting enzyme receptor 2) i fuzję wirionu z błoną zakażanej komórki [29]. Białko S składa się z dwóch funkcjonalnych podjednostek. Podjednostka S1 zawiera domenę NTD (ang. N-terminal domain) oraz domenę RBD (ang. receptor-binding domain), wiążącą receptor ACE2 [30]. W obrębie domeny RBD wyróżnia się fragment RBM (ang. receptor-binding motif) oraz tzw. RBM-core. Podjednostka S2 odpowiada za ułatwienie fuzji wirionu z błoną komórkową [31] (rycina 2).



Rycina 2. Schemat białka S (na podstawie Jackson i wsp. [32], wykonano w programie Inkscape). Na schemacie zaznaczono podział białka na podjednostkę S1 i S2 oraz zawarte w nich domeny. Strzałkami zaznaczono miejsca cięcia białka przez proteazę TMPRSS2.

CH – central helix, CT – C-terminal domain, CTD – carboxy-terminal domain, FP – fusion peptide, FPPR – fusion peptide proximal region, HR – heptad repeat, NTD – N-terminal domain, RBD – receptor-binding domain, RBM – receptor-binding motif, TM – transmembrane anchor, TMPRSS2 - transmembrane protease serine 2.

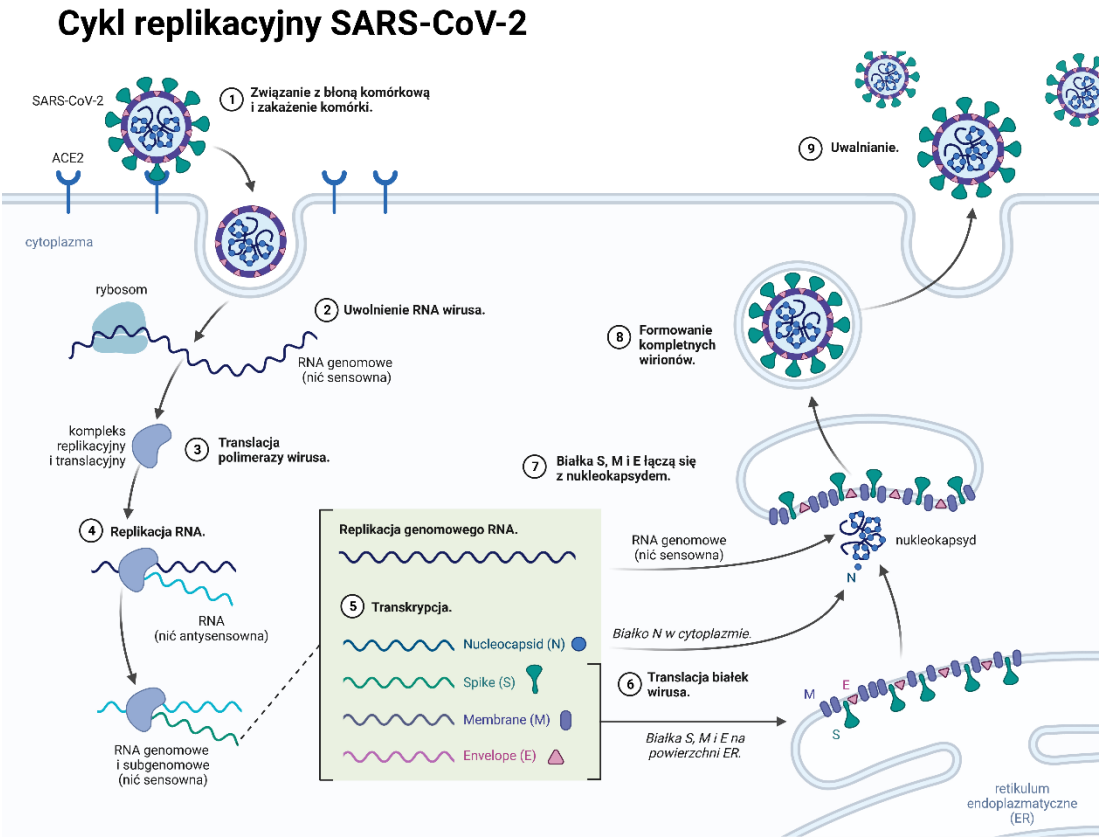
Po związaniu ACE2 przez RBD, obecne w podjednostce S2 regiony HR1 i HR2 (ang. heptad repeat region 1 and 2) tworzą strukturę fuzyjną (ang. fusion core), która pozwala wirusowi wprowadzić jego materiał genetyczny do komórki [33]. Do aktywnego działania podjednostek wirusowego białka S konieczna jest także ludzka transbłonowa proteaza serynowa 2 (TMPRSS2; ang. transmembrane protease serine 2), która po połączeniu jednostki S1 z receptorem ACE2 dokonuje częściowej proteolizy podjednostki S2, umożliwiając tym samym tworzenie struktury fuzyjnej [30,34,35] (rycina 3).



Rycina 3. Fuzja wirusa SARS-CoV-2 z błoną komórkową (na podstawie Hartenian i wsp. [20], wykonano w programie BioRender.com).

ACE2 - angiotensin-converting enzyme receptor 2, TMPRSS2 - transmembrane protease serine 2.

Wirusowe białko N odpowiada za upakowanie materiału genetycznego wirusa w kompleksy rybonukleoproteinowe (RNP ang. ribonucleoprotein complexes), formujące nukleokapsydy [36]. Taka forma organizacji RNA zwiększa jego stabilność oraz ułatwia replikację. Poprzez oddziaływanie z odpowiednimi sekwencjami RNA białko N reguluje procesy jego transkrypcji i translacji [37]. Białko N bierze także, wraz z pozostałymi białkami strukturalnymi, udział w organizacji wirionów i upakowywaniu wirusowego RNA w ich wnętrzu [38,39] (rycina 4).



Rycina 4. Cykl replikacyjny wirusa SARS-CoV-2 (na podstawie V'kovski i wsp. [16] oraz Harrison i wsp. [40], wykonano w programie BioRender.com).

ACE2 - angiotensin-converting enzyme receptor 2, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

6.2 Charakterystyka ostrej choroby COVID-19

Ostra choroba wywołana przez wirus SARS-CoV-2 manifestuje się przede wszystkim nieswoistymi objawami spotykanymi w większości zakażeń wirusowych górnych dróg oddechowych, takich jak: kaszel, gorączka, ból głowy i mięśni, zmęczenie. U niektórych osób w przebiegu COVID-19 występuje także biegunka [41]. U około 15% chorych, w pierwszym etapie pandemii, rozwijała się niewydolność oddechowa. Osoby te wymagały hospitalizacji oraz tlenoterapii w warunkach oddziałów intensywnej terapii [42]. U chorych tych obserwowano kliniczne i laboratoryjne cechy ostrego zespołu wykrzepiania wewnątrznaczyniowego (DIC, ang. disseminated intravascular coagulation) [43], a ich wystąpienie wiązało się z istotnym pogorszeniem rokowania i wyższą śmiertelnością [44]. Pozostali pacjenci, którzy nie wymagali stosowania mechanicznej wentylacji, leczeni byli – w zależności od nasilenia objawów – w warunkach oddziałów szpitalnych bądź izolacji domowej. W miarę gromadzenia coraz większej ilości danych klinicznych pojawiły się doniesienia o pozapłucnych manifestacjach COVID-19, opisujące negatywny wpływ wirusa na układ sercowo-naczyniowy [45–48], nerki [49–52], układ pokarmowy [53–57], centralny i obwodowy układ nerwowy [58–62], układ krwiotwórczy [63], a także na skórę [64–66]. Z upływem czasu, z powodu wprowadzonego programu szczepień ochronnych, zwiększającej się liczby ludzi uodpornionych naturalnie po zachorowaniu, a także na skutek zmian w genomie samego wirusa i pojawianiu się nowych jego wariantów, odsetek chorych na świecie, wymagających intensywnej terapii, zmniejszył się z 15% do około 3% w 2023 roku [42]. Równocześnie zaczęły pojawiać się doniesienia o ozdrowieńcach, którzy skarżyli się na przewlekłe objawy związane bezpośrednio z przebytą infekcją SARS-CoV-2. Zjawisko to określono terminem „long COVID".

6.3 Definicja „long COVID"

Terminem „long COVID" określa się przewlekłe występowanie różnorodnych objawów, które bezpośrednio łączy się z przebytą i zakończoną wyzdrowieniem infekcją wirusem SARS-CoV-2. Do objawów tych zalicza się zarówno te typowe dla ostrej fazy COVID-19, jak i nowe dolegliwości, pojawiające się już po jej ustąpieniu. Zaproponowano kilka definicji „long COVID". Już w 2020 roku Greenhalg i wsp. [67] określili „long COVID" jako obecność objawów choroby powyżej 3 tygodni

od pierwszych objawów ostrej fazy zakażenia SARS-CoV-2. Dodatkowo, autorzy ci wyróżnili w przebiegu „long COVID" dwie fazy:

1. fazę pierwszą, trwającą od 3. do 12. tygodnia od momentu pierwszych objawów ostrej choroby, nazwaną „post-acute COVID-19"
2. oraz fazę drugą, trwającą powyżej 12 tygodnia, określaną jako „chronic COVID-19".

Podobne stanowisko zostało przyjęte przez National Institute for Health and Care Excellence, Scottish Intercollegiate Guidelines Network oraz Royal College of General Practitioners, które za kryterium pozwalające na rozpoznanie „long COVID" przyjęły 5. tydzień po wystąpieniu objawów ostrej fazy choroby, dzieląc jednocześnie „long COVID" na trwający od 5. do 12. tygodnia „ongoing symptomatic COVID" oraz „post COVID", obejmujący czas po 12. tygodniu od pierwszych objawów COVID-19 [68]. W 2022 roku WHO opublikowała definicję „long COVID", opracowaną metodą delficką w ramach konsensusu grupy ekspertów [69]. Definicja ta określa „long COVID" jako występowanie objawów klinicznych u osób z udowodnioną lub prawdopodobną infekcją SARS-CoV-2 w czasie powyżej 3. miesięcy od początku ostrej infekcji. Według definicji, objawy określające „long COVID" mogą pojawić się już w ostrej fazie infekcji lub jako nowe objawy w okresie rekonwalescencji, nie mogą być związane z innymi stanami chorobowymi, a także muszą mieć negatywny wpływ na codzienne funkcjonowanie chorego.

6.4 Epidemiologia „long COVID", czynniki ryzyka jego wystąpienia i objawy kliniczne

Kilka czynników wpływa na fakt, iż ustalenie dokładnej częstości występowania „long COVID" jest bardzo trudne. Pierwszym z nich jest sama definicja, która opisuje to zjawisko dość niejednoznacznie, pozostawiając lekarzom i chorym dużą swobodę w przypisywaniu związku pomiędzy różnorodnymi objawami a przebytą infekcją SARS-CoV-2. Drugim powodem jest zmienność wirusa i pojawianie się wielu jego nowych wariantów, które wywołują odmienne zespoły objawów o różnym nasileniu. Obraz kliniczny COVID-19 zmienia się znacząco także ze względu na masowe uodpornienie populacji w wyniku przechorowania i szczepień, co z kolei przekłada się na zmianę częstości występowania możliwych powikłań. Istotnym ograniczeniem jest też

heterogenność grup badanych, na podstawie których określano prewalencję „long COVID". Analizy danych zebranych z badania dużych grup dorosłych chorych określają średnią prewalencję „long COVID" na poziomie około 42-45% [70–72], wskazując przy tym wyższe wartości wśród chorych hospitalizowanych – w porównaniu do chorych niehospitalizowanych: odpowiednio 54% *vs* 34% [70] oraz 47,5% *vs* 26,4% [71].

Do czynników ryzyka rozwoju „long COVID" zalicza się: płeć żeńską, wiek, nadwagę, cukrzycę, nadciśnienie tętnicze oraz ciężki przebieg ostrej fazy infekcji z występowaniem co najmniej pięciu objawów klinicznych [73]. Dodatkowym czynnikiem ryzyka rozwoju „long COVID" jest wysoka wiremia w trakcie ostrej fazy zakażenia, która powoduje silną stymulację układu odpornościowego, nasilając uszkadzanie tkanek [74]. Potencjalnym czynnikiem ryzyka może być też reaktywacja zakażeń latentnych wirusami: EBV, CMV, HSV-1, HHV-6 i HHV-7 w czasie ostrej fazy infekcji SARS-CoV-2 [74–77]. Wachlarz objawów „long COVID" jest szeroki. W tabeli I (str. 27) zebrano dane o prewalencji najczęściej zgłaszanych objawów ze strony układu oddechowego, układu nerwowego oraz układu ruchu [78–84]. Inne opisywane objawy to m.in.: biegunka, wymioty, mdłości, jadłowstręt, ból mięśni, zmiany skórne, wypadanie włosów, pogorszenie funkcji poznawczych oraz pamięci, zaburzenia afektu, zaburzenia lękowe [85–89].

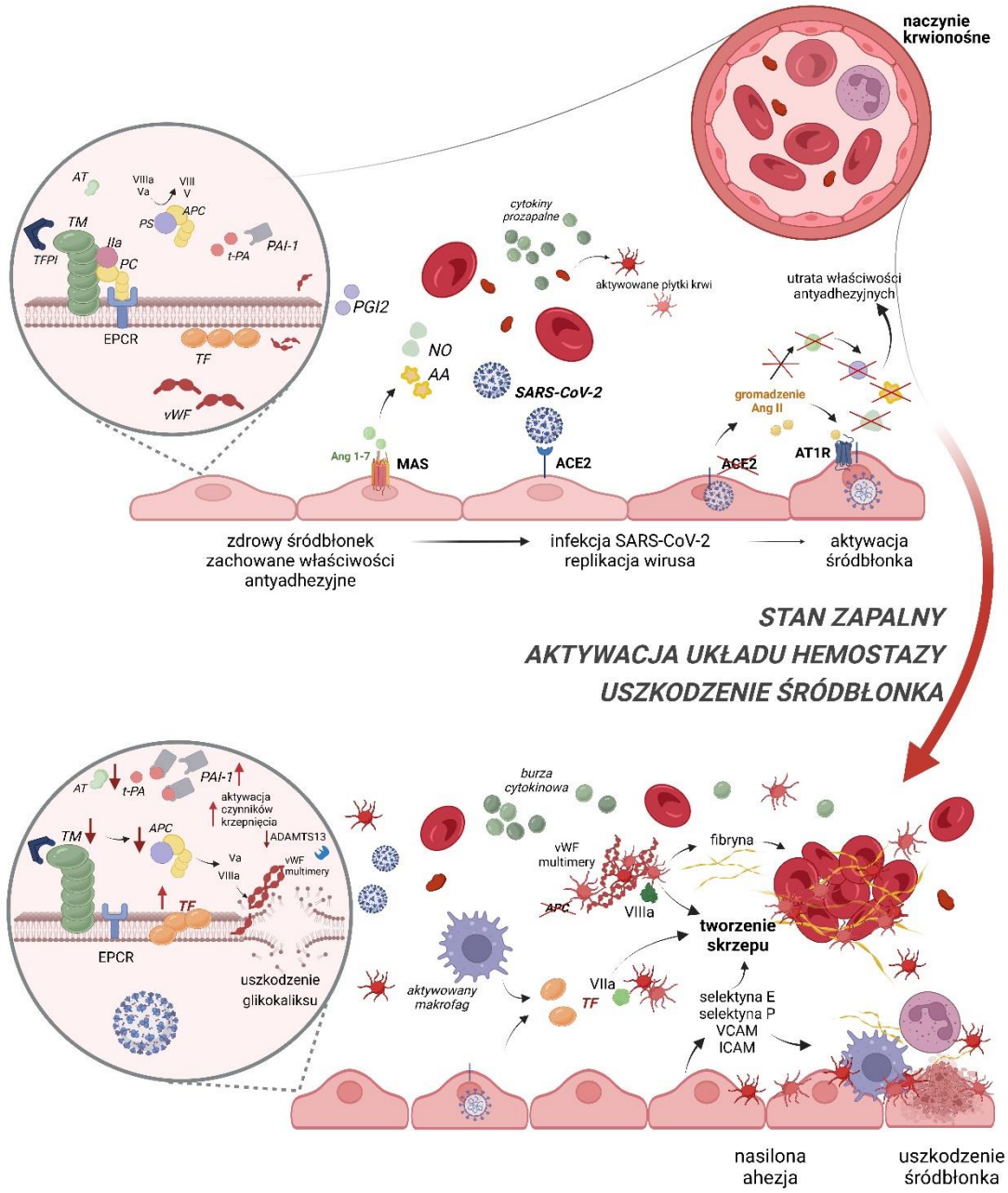
Tabela I. Częstość występowania zgłaszanych objawów związanych z „long COVID”.

				Układ oddechowy [%]			Układ nerwowy [%]		inne [%]		
	Liczba pacjentów	Mediana wieku [lata]	% kobiet	Duszność	kaszel	Zmniejszona wydolność płuc	Zaburzenia uwagi	Utrata węchu i smaku	Ból stawów	Przewlekłe zmęczenie	Ból w klatce piersiowej
Groff D. [78]	250 351	54	44	30	13	30	24	13	10	37	13
Sanchez-Ramirez D. [79]	5 323	55	44	32	13	39	-	-	38	16	-
Nalbandian A. [80]	3 398	57	47	34	15	-	-	13	16	53	13
Michelen M. [81]	10 951	56	48	25	-	26	26	-	-	31	-
Lopez-Leon S [82]	48 009	52	55	24	19	10	27	23	19	58	16
Garg M. [83]	6 924	52	77	43	20	-	-	24	27	66	17
Kessel S. [84]	3 000	46	-	29	36	-	-	16	-	47	22

6.5 Uszkodzenie śródbłonka jako następstwo infekcji wirusem SARS-CoV-2

Komórki śródbłonka pełnią ważną rolę w regulacji procesów związanych z napięciem ścian naczyń, z ich przepuszczalnością, a także w procesach hemostazy oraz odpowiedzi immunologicznej. Obserwowane w przebiegu ostrej choroby COVID-19 zjawiska rzuciły nowe światło na rolę uszkodzenia komórek śródbłonka i zniesienia ich naturalnych funkcji regulacyjnych w rozwoju objawów choroby i jej powikłań (m.in. zespół ostrej niewydolności oddechowej (ARDS, ang. acute respiratory distress syndrome) oraz związane z COVID-19 powikłania zakrzepowe) [90–94].

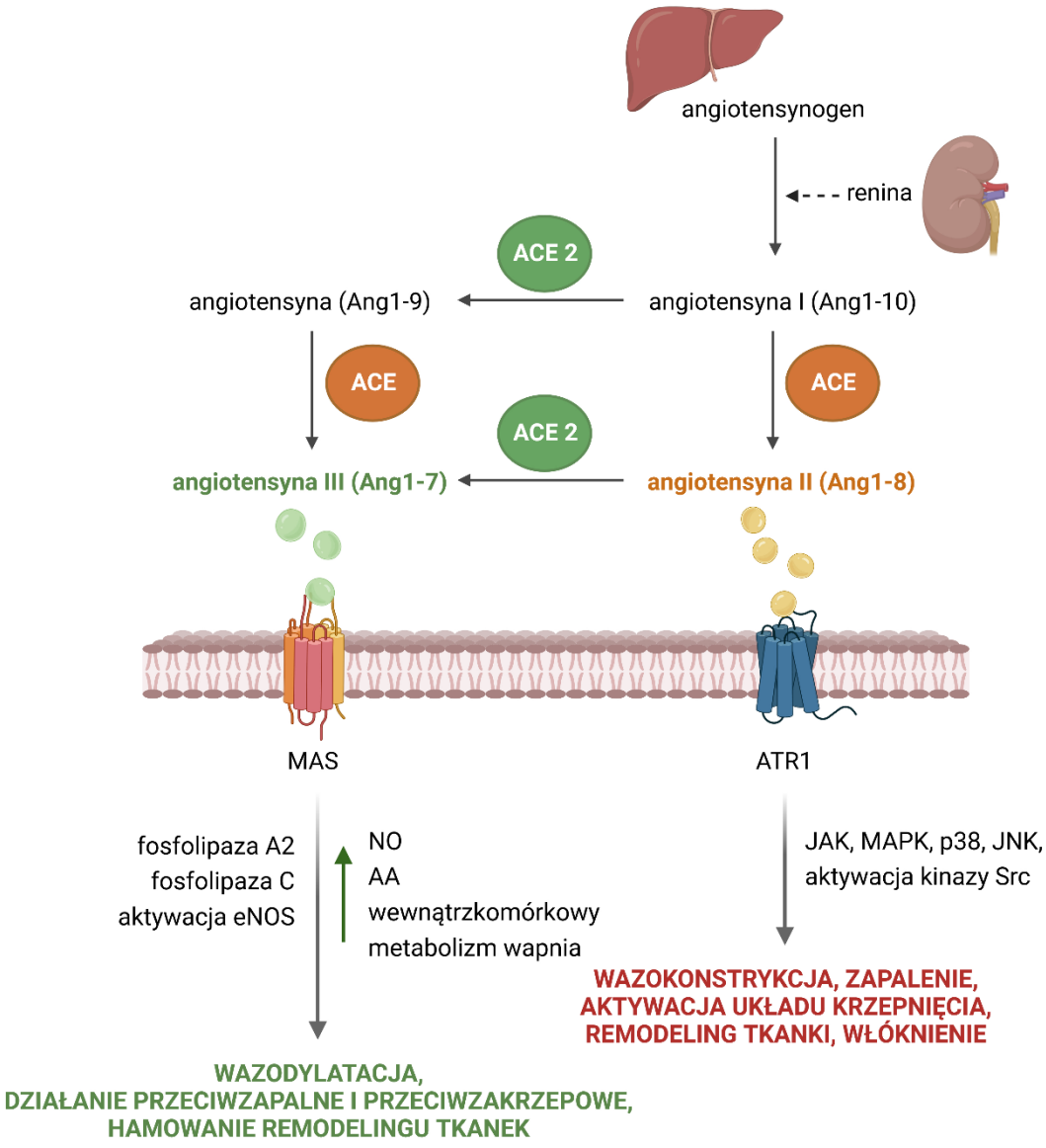
Komórki śródbłonka wykazują ekspresję receptora ACE2 [95,96], dlatego ulegają zakażeniu wirusem SARS-CoV-2. Zakażenie to powoduje uszkodzenie komórek endotelium i utratę jego naturalnych funkcji regulujących układ hemostazy [97], napięcie ściany naczyń [98] i warunki reologiczne w naczyniach [99,100], a także udział w odpowiedzi immunologicznej [100] (rycina 5).



Rycina 5. Wpływ zakażenia wirusem SARS-CoV-2 na komórki śródbłonka (wykonano w programie BioRender.com).

AA – arachidonic acid, ACE2 - angiotensin-converting enzyme receptor 2, ADAMTS13 - disintegrin and metalloproteinase with a thrombospondin type 1 motif, Ang II – angiotensin II, APC – activated protein C, AT - antithrombin, AT1R - endothelial angiotensin 2 type 1 receptor, EPCR - endothelial protein C receptor, ICAM-1 - intracellular adhesion molecule 1, IIa - thrombin, MAS - G protein-coupled receptor for angiotensin 1-7, NO – nitric oxide, PAI-1 - plasminogen activator inhibitor-1, PGI_2 - prostacyclin I2, PS – protein S, TF – tissue factor, TFPI - tissue factor pathway inhibitor, TM - thrombomodulin, t-PA - tissue-type plasminogen activator, Va - activated factor V, VCAM-1 - vascular cell adhesion molecule 1, vWF – von Willebrand factor, VIIa - activated factor VII, VIIIa - activated factor VIII.

Połączenie wirusa ze śródbłonkowym receptorem ACE2 powoduje internalizację powstałego kompleksu. Prowadzi to w konsekwencji do zmniejszenia ekspresji ACE2 na powierzchni endotelium, zaburzenia równowagi w układzie RAA (renina-angiotensyna-aldosteron) [101,102] i nasilenia procesu zapalnego oraz zaburzenia w regulacji napięcia ściany naczynia (rycina 6).



Rycina 6. Schemat układu renina-angiotensyna-aldosteron (wykonano w programie BioRender.com).

AA – arachidonic acid, ACE – angiotensin-converting enzyme, ACE2 – angiotensin-converting enzyme 2, AT1R – endothelial angiotensin 2 type 1 receptor, eNOS – endothelial nitric oxide synthase, JAK – Janus kinase, JNK – c-Jun N-terminal kinase, MAPK – mitogen activated protein kinase, MAS – mitochondrial assembly (G protein-coupled receptor for angiotensin 1-7), NO – nitric oxide, p38 – p38 mitogen-activated protein kinase.

Angiotensyna I (Ang 1-10) jest przekształcana przez enzym konwertujący angiotensynę (ACE, ang. angiotensin converting enzyme) do angiotensyny II (Ang 1-8), oddziałującej ze śródbłonkowym receptorem typu 1 dla angiotensyny 2 (AT1R, ang. endothelial angiotensin 2 type 1 receptor), co wzmaga napięcie ściany naczynia, efekt prozapalny oraz przebudowę tkanek z włóknieniem [103]. Szlak alternatywny z udziałem enzymu konwertującego angiotensynę typu 2 (ACE2, ang. angiotensin-converting enzyme 2) przekształca angiotensynę I (Ang 1-10) i angiotensynę II (Ang 1-8) do angiotensyny III (Ang 1-7), prowadzi do relaksacji ściany naczynia, nasilenia procesów przeciwzapalnych oraz zahamowania przebudowy tkanek i włóknienia [104]. Przesunięcie równowagi w układzie RAA powoduje także zmniejszenie syntezy tlenku azotu (NO) oraz prostacykliny I_2 (PGI_2), co z kolei ułatwia aktywację i agregację płytek krwi [105,106]. Po wniknięciu wirusa SARS-CoV-2 do komórki śródbłonka jej wewnątrzkomórkowe receptory TLR (ang. Toll-like receptors) i RLR rozpoznają wirusowe RNA i uruchamiają mechanizmy nieswoistej odpowiedzi immunologicznej, prowadząc do syntezy interferonów typu I oraz cytokin prozapalnych (.in.. IL-1, IL-6, TNF-α) (tzw. burza cytokinowa), chemokin (m.in. CXCL8, CXCL10), selektyny E (CD62E), selektyny P (CD62P) oraz cząsteczek ICAM-1(CD54, ang. intracellular adhesion molecule 1) i VCAM-1 (CD106, ang. vascular cell adhesion molecule 1) [107–110]. Cytokiny prozapalne stymulują na powierzchni śródbłonka ekspresję czynnika tkankowego (TF, ang. tissue factor) [111]. Infekcja wywołuje w komórkach endotelium stres oksydacyjny, w którym zwiększona produkcja reaktywnych form tlenu (ROS, ang. reactive oxygen species) jest dodatkowym czynnikiem zwiększającym syntezę cytokin prozapalnych oraz ekspresję cząsteczek adhezyjnych [112]. Uszkodzeniu ulega także pokrywający powierzchnię komórek glikokaliks, który bierze udział w regulowaniu procesów hemostazy, przepuszczalności drobnych naczyń oraz oddziaływania elementów morfotycznych krwi z komórkami śródbłonka [113,114]. Uszkodzenie glikokaliksu prowadzi do znacznego obniżenia aktywności endogennych inhibitorów krzepnięcia krwi (antytrombiny i kofaktora II heparyny), zwiększenia przepuszczalności drobnych naczyń oraz ułatwionego przylegania do śródbłonka leukocytów i płytek krwi [115]. U chorych na COVID-19 zaobserwowano zwiększone stężenie czynnika von Willebranda (vWF:Ag, ang. von Willebrand antigen) [116–118]. Zjawiska te powodują utratę naturalnych antyadhezyjnych właściwości komórek śródbłonka, które pod wpływem zakażenia stają się istotnym czynnikiem promującym adhezję płytek krwi i ich agregację, co w konsekwencji prowadzi do niekontrolowanej

nadmiernej aktywacji układu hemostazy, tworzenia zakrzepów i ostatecznie zaburzenia ukrwienia tkanek i narządów [119]. Opisywane zmiany w komórkach śródbłonka, wywołane zakażeniem wirusem SARS-CoV-2, wiążą się ze zwiększonym ryzykiem wystąpienia w ostrej fazie zakażenia incydentów sercowo-naczyniowych [120,121], ostrego uszkodzenia nerek [120,122], niedokrwienia ośrodkowego układu nerwowego [123–125] oraz niewydolności wielonarządowej, związanej z rozsianym tworzeniem zakrzepów w mikrokrążeniu [126–128]. Opisywane zmiany w endotelium nie ograniczają się jednak tylko do ostrej fazy choroby. Uważa się, iż u części pacjentów, na skutek m.in. przetrwałej replikacji wirusa, utrzymuje się aktywacja komórek śródbłonka oraz synteza cytokin prozapalnych wraz ze wszystkimi opisanymi wyżej konsekwencjami [112,129]. Zjawisko to może być odpowiedzialne za rozwój części objawów składających się na „long COVID", zwłaszcza tych związanych z makro- i mikroangiopatiami – m.in. chorób sercowo-naczyniowych, chorób nerek czy zaburzeń neurologicznych, a także objawów ogólnych, takich jak przewlekłe zmęczenie i mialgia [130]. Z tego powodu poszukuje się markerów uszkodzenia śródbłonka, które mogłyby być przydatne do oceny jego uszkodzenia przez wirus SARS-CoV-2 (tabela II, str. 34). Uwagę w tych poszukiwaniach skupiono dotychczas na grupach chorych obciążonych czynnikami ryzyka rozwoju „long COVID", przede wszystkim na osobach z wielochorobowością oraz tych, u których przebieg ostrej choroby COVID-19 był ciężki.

Tabela II. Wybrane markery uszkodzenia śródbłonka badane w chorobie COVID-19 (ostrej i/lub umiarkowanej postaci) oraz u ozdrowieńców.

Angiopoetyna 2 [131–139]
Selektyna E, selektyna P [139–152]
Cząsteczka adhezji międzykomórkowej typu 1 (ICAM-1) [138–141,147,150,151,153–157]
Mid-region proadrenomedulina (MR-proADM) [158–164]
Inhibitor aktywatora plazminogenu typu 1 (PAI-1) [137,140,149,165–169]
Pierwsza cząsteczka adhezji płytek krwi do śródbłonka (PECAM-1) [141,170]
Syndekan-1 [155,171–175]
Tkankowy aktywator plazminogenu (t-PA), inhibitor zewnątrzpochodnego szlaku krzepnięcia (TFPI) [171,176]
Trombomodulina [134,150,171,177]
Pierwsza cząsteczka adhezyjna śródbłonka naczyniowego (VCAM-1) [139–141,147,150,153–155,157]
Czynnik von Willebranda [134,150,152,156,171,174,177–182]

6.6 Infekcja wirusem SARS-CoV-2 a rozwój autoimmunizacji

Rozwój autoimmunizacji jest procesem złożonym i zależy od wielu czynników (m.in. predyspozycje genetyczne, płeć, ostre i przewlekłe choroby w wywiadzie, przyjmowane leki, narażenie na szkodliwe czynniki fizyczne i chemiczne), które w określonych warunkach mogą przełamać fizjologiczne mechanizmy zapobiegające zniesieniu tolerancji na własne antygeny. Pewną rolę w indukowaniu autoimmunizacji przypisuje się infekcjom wirusowym, w tym zakażeniu wirusem SARS-CoV-2 [183]. Rozpoznanie wirusowego RNA przez receptory TLR uruchamia silną odpowiedź nieswoistą z tworzeniem inflammasomów i syntezą dużych ilości cytokin prozapalnych. Powoduje to uszkodzenie komórek. Uszkodzenie to – w przypadku zakażenia wirusem SARS-CoV-2 – dotyczy w dużym stopniu także komórek endotelium. Śmierć komórek śródbłonka w mechanizmie pyroptozy pociąga za sobą zniesienie sekwestracji autoantygenów (m.in. DNA, białek histonów, białek cytoplazmatycznych) oraz ich modyfikację. Tak zmienione autoantygeny mogą być przetwarzane przez komórki prezentujące antygeny (APC, ang. antigen presenting cell) i indukować odpowiedź autoreaktywnych limfocytów T (ang. bystander activation) [184,185]. Odpowiedź ta może rozszerzać się na inne epitopy danego autoantygenu oraz na inne autoantygeny o podobnej strukturze – tzw. zjawisko rozprzestrzeniania się epitopów (ang. epitope spreading) [186]. Czynnikiem indukującym rozwój odpowiedzi autoimmunizacyjnej jest też

podobieństwo antygenów patogenu do antygenów organizmu zakażonego – zjawisko mimikry molekularnej (ang. molecular mimicry) [187]. Odpowiedź immunologiczna, zależna od limfocytów T i B, która skierowana jest pierwotnie przeciwko antygenom patogenu, może objąć także w reakcji krzyżowej podobne autoantygeny gospodarza.

Infekcję wirusem SARS-CoV-2 łączy się z indukowaniem wielu różnych chorób o podłożu autoimmunizacyjnym, takich jak reaktywne zapalenie stawów, zapalenie naczyń, małopłytkowość immunologiczna, autoimmunizacyjne zapalenie tarczycy, łuszczyca oraz zespół Guillain-Barré'ego (tabela III, str. 36). W przebiegu infekcji wirusem SARS-CoV-2 obserwuje się, opisane w rozdziale 6.5 (str. 29-34), aktywację i uszkodzenia komórek śródbłonka związane z bezpośrednim działaniem wirusa, a także odpowiedzią immunologiczną. Prowadzą one do uwalniania autoantygenów, co w sytuacji silnej odpowiedzi zapalnej i stymulacji układu immunologicznego sprzyja aktywacji autoreaktywnych limfocytów T [188]. W przebiegu zakażenia wirusem SARS-CoV-2 dochodzi także do nasilonego uwalniania zewnątrzkomórkowych sieci neutrofilowych, a wraz z nimi dużych ilości autoantygenów [189]. Wykazano też podobieństwa sekwencji aminokwasowych białek ludzkich (m.in. białek szoku cieplnego, tkanek płuc oraz układu nerwowego) i białek wirusa SARS-CoV-2 [190–195]. Dodatkowo, przeciwciała skierowane przeciwko białkom wirusa SARS-CoV-2 mogą krzyżowo rozpoznawać autoantygeny gospodarza [188]. Przemawia to za możliwym udziałem mechanizmów mimikry molekularnej w indukowaniu odpowiedzi autoimmunizacyjnej w wyniku zakażenia wirusem SARS-CoV-2.

Tabela III. Choroby i stany autoimmunizacyjne związane z infekcją wirusem SARS-CoV-2.

Autoimmunizacyjne zapalenie tarczycy [183,196,197]
Choroba Kawasakiego [183,197–199]
Choroby pęcherzowe skóry [196]
Łuszczyca i łuszczycowe zapalenie stawów (183, 192)
Miopatie zapalne [201]
Niedokrwistość Addisona-Biermera [196]
Niedokrwistość autoimmunohemolityczna [197]
Obecność przeciwciał antyfosfolipidowych [117,196,202–205]
Pierwotna małopłytkowość immunologiczna [183,196,197,206]
Reaktywne zapalenie stawów [196,201,207]
Reumatoidalne zapalenie stawów [196,201]
Stwardnienie rozsiane [196]
Toczeń rumieniowaty układowy [201]
Zapalenie naczyń [196,201]
Zespół Guillain-Barré [183,197,208–211]

W praktyce klinicznej wykrywanie autoprzeciwciał i ocena ich swoistości są podstawowymi i najłatwiej dostępnymi badaniami laboratoryjnymi wykorzystywanymi w diagnostyce chorób autoimmunizacyjnych. Zaznaczyć jednak należy, iż sama obecność autoprzeciwciał bez towarzyszących objawów klinicznych nie jest wystarczająca do postawienia rozpoznania. Rozpatrywać ją można jako dysfunkcję układu immunologicznego i wykształcanie się fenotypu autoimmunizacyjnego, które poprzedzać może pojawienie się objawów choroby (tzw. bezobjawowa autoimmunizacja) [212].

7. Założenia i cel pracy

Zakażenie wirusem SARS-CoV-2 należy rozpatrywać bardziej jako ogólnoustrojową chorobę o potencjalnie istotnych odległych powikłaniach ze strony wielu układów i narządów, niż tylko zakażenie układu oddechowego. Istotną rolę w rozwoju tych powikłań odgrywa uszkodzenie komórek śródbłonka oraz indukowanie odpowiedzi autoimmunizacyjnej przez wirus SARS-CoV-2. Do tej pory ocenie poddawano stężenie różnych markerów uszkodzenia endotelium zarówno u chorych w ostrej fazie choroby, jak i u ozdrowieńców (tabela II, str. 34). Opisano też występowanie u chorych na COVID-19 wielu rodzajów autoprzeciwciał, zarówno klasycznych przeciwciał przeciwjądrowych (ANA, ang. antinuclear antibodies) [203,213,214], przeciwciał związanych z zespołem antyfosfolipidowym (tabela III, str. 36), jak i przeciwciał skierowanych przeciwko poszczególnym cytokinom [215], których oznaczanie wykracza jak do tej pory poza rutynową praktykę kliniczną. Do grup badanych włączano najczęściej osoby, u których choroba COVID-19 miała przebieg od umiarkowanego aż po skrajnie ciężki, wymagający leczenia w oddziałach intensywnej terapii. Często były to osoby obciążone innymi chorobami związanymi z uszkodzeniem śródbłonka (cukrzyca, nadciśnienie tętnicze, otyłość, przewlekła choroba nerek), a ich status serologiczny, w kontekście autoprzeciwciał, sprzed włączenia do badań, nie był znany. Pamiętać należy, że zakażeniu wirusem SARS-CoV-2 ulegają także osoby zdrowe, nieobciążone czynnikami ryzyka. Znajomość biologii wirusa SARS-CoV-2 pozwala przypuszczać, że u tych osób także mogą zachodzić niekorzystne procesy związane z zakażeniem, a one same mogą być narażone na rozwój odległych powikłań związanych z uszkodzeniem i zaburzeniem funkcjonowania endotelium oraz rozwojem autoimmunizacji.

Celem pracy jest pogłębienie wiedzy na temat wpływu zakażenia wirusem SARS-CoV-2 na komórki śródbłonka oraz rozwój autoimmunizacji u osób, które nie są obciążone dodatkowymi czynnikami ryzyka.

Wyniki przeprowadzonych badań mogą być przydatne w ustaleniu, czy przebyte zakażenie wirusem SARS-CoV-2 powoduje długotrwałe uszkodzenie endotelium oraz zwiększa ryzyko indukcji fenotypu autoimmunizacyjnego z obecnością ANA u osób, u których nie stwierdza się innych istotnych czynników ryzyka wymienionych stanów. Otrzymane wyniki mogą pomóc w szacowaniu prawdopodobieństwa wystąpienia odległych następstw choroby COVID-19 w tej grupie osób.

Cele szczegółowe pracy to:

1. ocena związanego z zakażeniem wirusem SARS-CoV-2 uszkodzenia komórek śródbłonka,
2. ocena uszkodzenia śródbłonkowego glikokaliksu,
3. ocena występowania fenotypu autoimmunizacyjnego u ozdrowieńców,
4. ocena częstości występowania przeciwciał antyfosfolipidowych u ozdrowieńców.

8. Kopie opublikowanych prac

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Article

Mild-to-Moderate COVID-19 Convalescents May Present Pro-Longed Endothelium Injury

Paweł Kozłowski [1], Marcin Śmiarowski [2], Wiktoria Przyborska [2], Karolina Zemlik [2], Milena Małecka-Giełdowska [1,3], Aleksandra Leszczyńska [1], Marzena Garley [4] and Olga Ciepiela [1,3,*]

[1] Central Laboratory, University Clinical Centre, Medical University of Warsaw, 02-097 Warsaw, Poland
[2] Students Scientific Group of Laboratory Medicine, Medical University of Warsaw, 02-097 Warsaw, Poland
[3] Department of Laboratory Medicine, Medical University of Warsaw, 02-097 Warsaw, Poland
[4] Department of Immunology, Medical University of Bialystok, 15-269 Bialystok, Poland
* Correspondence: olga.ciepiela@wum.edu.pl; Tel.: +48-225992405

Abstract: Background: The SARS-CoV-2 pandemic posed a great threat to public health, healthcare systems and the economy worldwide. It became clear that, in addition to COVID-19 and acute disease, the condition that develops after recovery may also negatively impact survivors' health and quality of life. The damage inflicted by the viral infection on endothelial cells was identified quite early on as a possible mechanism underlying the so-called post-COVID syndrome. It became an urgent matter to establish whether convalescents present chronic endothelial impairment, which could result in an increased risk of cardiovascular and thrombotic complications. Methods: In this study, we measured the levels of CRP, ICAM-1, VCAM-1, E-selectin and syndecan-1 as markers of inflammation and endothelial injury in generally healthy convalescents selected from blood donors and compared these to a healthy control group. Results: We found higher concentrations of E-selectin and a lower level of syndecan-1 in convalescents in comparison to those of the control group. Conclusion: Based on our results, it can be concluded that, at least 6 months after infection, there is only slight evidence of endothelial dysfunction in COVID-19 convalescents who do not suffer from other comorbidities related to endothelial impairment.

Keywords: COVID-19; endothelium; ICAM-1; VCAM-1; E-selectin; syndecan-1

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1. Introduction

In May 2020, the World Health Organization (WHO) declared a pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 infection presentations may vary from asymptomatic or mild flu-like symptoms, e.g., fever, headache, muscle pain, anorexia, fatigue and (characteristic for COVID-19) anosmia, up to severe life-threatening conditions, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), general hyperinflammation and multiorgan failure, which require hospitalisation and intensive treatment [2]. The virus' high rate of spread in society, the severity of clinical symptoms and rapidly collected scientific data about the nature of this infection raised concerns about the possible consequences on survivors' health in the future. Over time, some convalescents experienced a broad variety of persistent symptoms, e.g., cough, dyspnoea, radiological lesions, pulmonary fibrosis, chest pain, heart arrythmia, an increased risk of venous and arterial thromboembolism, chronic diarrhoea, depression, mood change, impaired cognitive function, fatigue, general muscle and joint pain, and weakness. In September 2020, the WHO established the term "long COVID" or "post-COVID" to describe health issues caused by past SARS-CoV-2 infections in all groups of COVID-19 adult survivors—those who required intensive treatment as well as asymptomatic cases [3]. It has been defined as the presence of symptoms in individuals with a history of confirmed or probable SARS-CoV-2 infection that show up usually 3 months after infection, last for at least 2 months and cannot

be explained by any other reasonable cause [4–9]. Endothelial damage ("endothelitis") caused by a broad spectrum of inflammatory mechanisms (e.g., an increase in proinflammatory cytokines, complement system and leukocyte activation) and the depletion of natural endothelial regulatory properties seem to be associated with the development of acute infection and post-COVID symptoms [10]. Comorbidities, such as obesity, diabetes and hypertension, which are associated with endothelial impairment, increase the risk of severe COVID-19 with a poor outcome [11]. The specific protein angiotensin converting enzyme 2 (ACE2), called the ACE2 receptor, was established to be an entry point for the virus to infect human cells [12]. This protein is expressed on various types of tissues, e.g., airway and alveoli epithelial cells, the endothelium, enterocytes and smooth muscle cells, as well as in the kidneys and heart [13,14]. The SARS-CoV-2 S protein (spike protein) interaction with the ACE2 receptor on the endothelial surface acts as a trigger to start the process of virus–receptor complex internalisation and cell infection. Due to massive use under these conditions, endothelial ACE2 expression is depleted, which profoundly distorts the balance in the renin–angiotensin system (RAS) [15]. It promotes vasoconstriction, inflammation and tissue remodelling with fibrosis [16]. Via the depletion of ACE2, SARS-CoV-2 can shift the renin–angiotensin system towards a proinflammatory and profibrotic state [17].

The significant decrease in nitric oxide (NO) synthesis, as well as low arachidonic acid (AA) release and prostacyclin I2 (PGI2) production, result in the inhibition of antiadhesive mechanisms. This allows platelets and leukocytes to adhere more easily, and thus promotes clot formation and neutrophil extracellular trap (NET) release [18]. An infection with SARS-CoV-2 can stimulate excessive proinflammatory cytokine release called the "cytokine storm" (e.g., IL-1α, IL-1β, IL-2, IL-6, IL-8, TNF-α and IFN-γ) [19,20]. These cytokines affect the endothelium and change its anti-coagulation properties into pro-coagulation effects [21]. Natural endothelial profibrinolytic activity is also distorted by the cytokine storm, with an increased release of the fibrinolysis inhibitor PAI-1, and a decreased synthesis of tissue plasminogen activator (t-PA) [19]. Endothelial cell activation results in the release of high-molecular-weight von Willebrand factor (vWF) multimers, while the synthesis of the ADAMTS13 enzyme, responsible for their cleavage, is decreased. This leads to the presence of huge vWF multimers in the circulation, which promotes platelet aggregation. Due to this, the activity of factor VIII is increased, thus facilitating fibrin formation [22,23]. The immune response towards coronavirus is also responsible for destroying the glycocalyx layer on the endothelial surface, which not only regulates vessel permeability and prevents platelet and leukocyte adhesion, but also activates the highly potent natural coagulation inhibitor antithrombin (AT) [24,25]. With glycocalyx impairment, all these functions are lost [26,27]. The increased expression of endothelial adhesion molecules, e.g., E-selectin (CD62E) and P-selectin (CD62P), as well as intercellular adhesion molecule 1 (ICAM-1; CD54) and vascular cell adhesion protein 1 (VCAM-1;CD106), together with the changes described above, facilitate platelet and leukocyte adhesion and fibrin formation [19]. The mechanisms briefly described above can lead to severe endothelial dysfunction, causing an increase in endothelial permeability, which greatly contributes to developing ARDS [28]. The aim of this study was to evaluate whether COVID-19 convalescents, without any additional comorbidities, develop chronic endothelial damage, which can be a trigger for further complications (e.g., thrombotic and cardiovascular incidents) in the future. For this purpose, we examined serum samples from healthy individuals and convalescents to measure the concentrations of inflammation and endothelial damage markers: C-reactive protein (CRP), VCAM-1, ICAM-1, E-selectin and syndecan-1.

2. Materials and Methods

2.1. Control Group and Study Group

There were 294 adult participants recruited to this study among volunteer blood donors at Warsaw's Blood Centre from August 2021 to April 2022. All of them were examined by a physician and qualified as healthy and able to donate blood in accordance with the guidelines of the Polish Minister of Health (Table 1). Additionally, they were all

tested for SARS-CoV-2 infection and received a negative PCR result on the day of admission. Among all enrolled subjects, 147 of them declared previous mild to moderate SARS-CoV-2 infection at least 6 months before blood donation (the minimal period of time required between the disease and blood donation, which was confirmed by a positive PCR test result). Mild disease was defined as a lack of symptoms of lower respiratory disease (shortness of breath (dyspnoea) and abnormal chest imaging) and oxygen saturation measured by pulse oximetry (SpO_2) $\geq$94%. Moderate COVID-19 was defined as symptoms of lower respiratory disease with SpO_2 $\geq$94%. Another 147 individuals declared no SARS-CoV-2 infection. In the next step, all the participants were tested for antibodies against the SARS-CoV-2 N protein in their blood. Based on the anti-N SARS-CoV-2 antibody evaluation, among the 147 subjects who declared no contact with SARS-CoV-2 and no signs of respiratory tract infection, 68 had a positive result for these antibodies and were eventually classified as asymptomatic convalescents. Thus, the study group (convalescents) consisted of 215 subjects, and the control group consisted of 79 subjects. All the participants were 18–65 years old; specific data, including age and sex, as well as physical characteristics (body weight, blood pressure, etc.) were not provided.

Table 1. Exclusion criteria for the study.

increased bleeding risk.
acute or chronic disease of: the cardiovascular system, central nervous system, gastrointestinal tract, airways and lungs, genitourinary system, immune system, endocrine system, skin
acute or chronic connective tissue disease
diabetes
any type of cancer, now or in the past
infectious diseases: positive PCR for SARS-CoV-2 at the day of admission HBV, HCV, any hepatitis of undetermined cause HIV-1/2, HTLV any parasitosis patients with any risk of transmissible spongiform encephalopathies (TSE) syphilis at any time in life history of drug or alcohol abuse history of risky sexual behaviour sex workers any sexually transmitted diseases (STD) in the past allotransplant recipients increased body temperature in the last several weeks any vaccination in the last 4 weeks any medical or non-medical procedure with risk of infection (e.g., surgery, tattoo, etc.) in the last 6 months travelling in the last 6 months to countries in which there is an increased risk of infectious diseases

2.2. Materials and Methods

A serum sample was obtained from every participant at the day of admission to the study and stored at −70 °C until analysis. Each sample was tested and the concentrations of CRP, VCAM-1, ICAM-1, E-selectin and syndecan 1 were measured. The CRP concentrations were measured on a Dimension Exl (Siemens, Munich, Germany) analyser. For CRP measurements, a high-sensitivity particle-enhanced immunoturbidimetric assay (PETIA) was used, and the normal reference value of $\leq$3.0 mg/L was established. The concentrations of VCAM-1, ICAM-1, E-selectin and syndecan 1 were measured using enzyme-linked immunosorbent assays (BOSTER PicoKine^{TM} ELISA kit, catalogue numbers: VCAM-1 EK0537, ICAM-1 EK0370, E-selectin EK0501 and Syndecan-1 EK1339). For the detection and titre measurement of anti-SARS-CoV-2 N protein antibodies, expressed as COI, an

electrochemiluminescence immunoassay (ECLIA) on the Cobas e801 apparatus (Roche Diagnostics, Basel, Switzerland) was used.

2.3. Statistical Analysis

The statistical analysis was performed with GraphPad Prism 9 software. The results of all parameters had a non-normal distribution according to the Shapiro–Wilk, Anderson–Darling, Kolmogorov–Smirnov–Lillefors and D'Agostino–Pearson tests. The Mann–Whitney U test was used for the statistical analysis of the results. The relationship between the presence of anti-SARSCoV-2 N protein antibodies and the expression of endothelial damage markers was tested with the Spearman correlation test. The results of the tested molecules and antibodies titres are expressed as the median (M), first quartile (Q1) and third (Q3) quartile [M(Q1;Q3)]. The probability value $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Anti-SARS-CoV-2 N Protein Antibody Titres

All participants were divided into two groups according to the anti-SARS-CoV-2 N protein antibody results: the control group had the negative results, and the study group had a positive result or a previous positive PCR test for SARS-CoV-2 infection. The results were defined as negative for COI <1.0 and positive for COI ≥1.0. There was a statistically significant difference in antibody titres between the two groups with $p < 0.0001$ (Figure 1). The comparison between symptomatic and asymptomatic participants within the study group showed no statistically significant difference in the median antibody titres, i.e., 23.60 (6.82; 63.60) and 15.30 (3.88; 87.98), respectively. This observation confirms that anti-SARS-CoV-2 N protein antibodies can be useful in distinguishing between convalescents and individuals who were never infected; however, it does not depend on the presence or severity of infection symptoms.

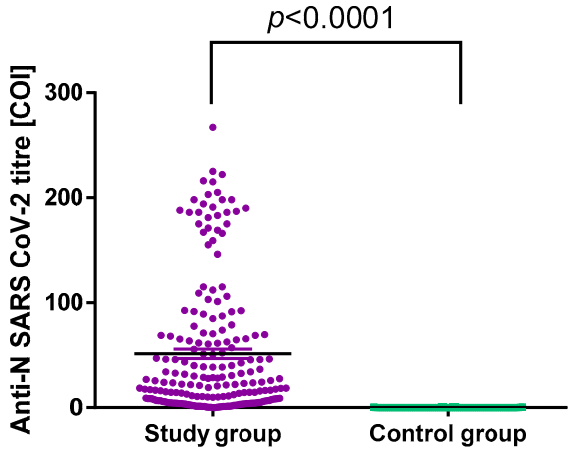

Figure 1. Anti-SARS-CoV-2 N protein antibodies titres in the control and study groups.

3.2. Comparison of CRP Concentrations

CRP, measured with a high-sensitivity method, was chosen as a possible marker of a sustained post-COVID-19 inflammatory state resulting in endothelial activation and damage. However, no statistically significant differences between the control and study groups were found for this analyte (Table 2).

Table 2. Median concentrations of soluble adhesion molecules and CRP in the study and control groups. There were significant differences in E-selectin and syndecan-1 between the two groups.

	E-Selectin [pg/mL]	ICAM-1 [pg/mL]	VCAM-1 [pg/mL]	Syndecan-1 [pg/mL]	CRP [mg/L]
Control group	1633 (1272; 1918)	1465 (1034; 2065)	40,341 (23,578; 54,718)	934 (466; 1944)	2.4 (2.0; 3.2)
Study group	1754 (1422; 2520)	1738 (1338; 2157)	41,959 (24,738; 50,490)	692 (342; 1138)	2.5 (1.9; 3.0)
	$p = 0.0135$	$p = 0.73$	$p = 0.59$	$p = 0.0082$	$p = 0.36$

3.3. Comparison of Adhesion Molecules Concentrations

The median E-selectin values between the control and study groups showed a significant difference ($p = 0.0135$), with higher values in the study group. A statistically significant difference was also shown for the concentration of syndecan-1 between the analysed groups; however, a higher concentration was found in the group of non-infected subjects ($p = 0.0082$). No differences between the study and control groups were observed for VCAM-1 and ICAM-1 (Table 2, Figure 2).

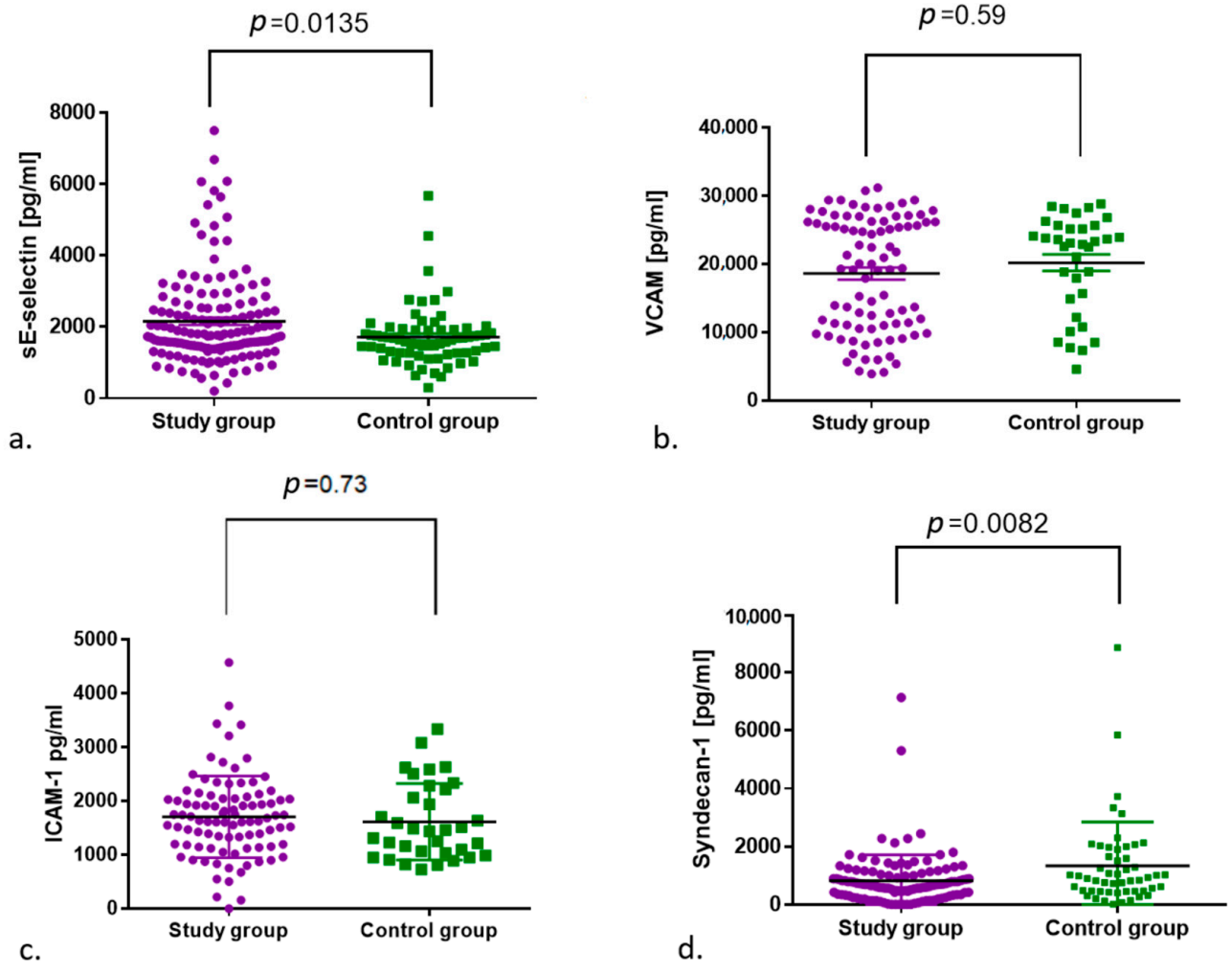

Figure 2. Comparison of (**a**) E-selectin and (**b**) VCAM-1, (**c**) ICAM-1 and (**d**) syndecan-1 concentrations between the control and study groups.

4. Discussion

The COVID-19 pandemic has had a huge influence on people's lives and placed new challenges on healthcare systems worldwide. Researchers all over the world have launched new investigations to understand the nature of SARS-CoV-2 virus infection, its impact on the human body and the pattern of the immune response to the virus, as well as possible long-term side effects. As the number of new COVID-19 cases increased rapidly, so did the amount of data about the role of endothelial damage in the pathogenesis of the disease. Many authors have investigated various markers related to endothelial cell activation and impairment in different groups of COVID-19 patients, divided according to the severity of the disease. Birnhuber et al. showed increased serum concentrations of E-selectin, VCAM-1, ICAM-1 and platelet endothelial cell adhesion molecule (PECAM-1; CD31) in critically ill COVID-19 patients compared to healthy controls [29]. Tong et al. tested serum concentrations of endothelial adhesion molecules (ICAM-1 and VCAM-1), proinflammatory cytokines (IL-1, IL-6, IL-18) and CRP as inflammation markers in groups of COVID-19 patients with mild and severe disease in comparison to healthy controls. They indicated that the concentration of all the aforementioned markers were positively correlated with the severity of the disease, with the highest values in the patients with a severe course of COVID-19 and lower values in the mild-disease group. The healthy control group had significantly lower values in comparison to both COVID-19 groups [30]. The same results were presented by Liu et al. [31] for the markers mentioned above and by Oliva et al. for E-selectin [32].

The increasing number of convalescents has created an opportunity to study the role of chronic endothelial activation and damage in developing post-COVID-19 syndrome. Haffke et al. assessed endothelial dysfunction using the reactive hyperaemia index (RHI) in patients with post-COVID-19 chronic fatigue symptoms and general weakness [33]. The RHI was found to be decreased in convalescents compared to healthy controls, indicating possible endothelium cell impairment in the first group. The same conclusion was made by Ambrosino et al. They used the measurement of brachial artery flow-mediated dilation (FMD) in COVID-19 convalescents compared to sex- and age-matched healthy controls. The authors found that lower FMD values indicated endothelial dysfunction in a group of men, whereas in women no significant differences in FMD were observed in comparison to healthy controls. The authors suggested that the protective role of sex hormones may explain these results [34]. FMD was also used by Lambadiar et al. and Oikonomou et al., who found lower values in COVID-19 patients compared to control groups [35,36]. Additional findings, such as an increased erythrocyte sedimentation rate, microparticles, homocysteine and interleukin-6 concentrations, strongly support the thesis of the destructive influence of SARS-CoV-2 infection on the endothelium. Another approach in COVID-19-related endothelial injury testing was presented by Chioh et al. [10]. In this study, the number of circulating endothelial cells (CEC) was used to assess the degree of endothelial damage. The authors found increased numbers of CEC in convalescents and a positive correlation between CEC and endothelial impairment markers, e.g., ICAM-1 and P-selectin. Since endothelial impairment triggers a subsequent reparative process, Poyatos et al. investigated the number of endothelial colony-forming cells (ECFC) as a tool to measure the intensity of this process [37]. The authors found higher numbers of ECFC in convalescents 3 months after the onset of COVID-19. Additionally, an increase in ECFC was correlated with the degree of hypoxia and high haemoglobin concentrations, as well as the male gender. These results confirm an increase in endothelial repair in COVID-19 survivors. Unfortunately, the described parameters, like many others, cannot be used in routine laboratory testing, which is why there is a constant pressure to look for widely available markers that could be easily measured to evaluate endothelial dysfunction in COVID-19 survivors. For our study, we chose CRP as a basic and routinely measured inflammation marker, which plays a role in determining the disease severity and is well established in COVID-19 patients [38,39]. Based on the fact that their endothelial expression is upregulated by the proinflammatory cytokine storm, the adhesion molecules E-selectin,

ICAM-1 and VCAM-1 were selected as endothelial activation markers. Additionally, we evaluated the concentration of syndecan-1 in serum, since this proteoglycan component of the glycocalyx is shed as a result of endothelial stimulation by pro-inflammatory cytokines. The participant enrolment procedure provided the possibility of comparing the chosen endothelial marker concentrations between people who have not suffered from SARS-CoV-2 infection up to the day of enrolment and COVID-19 survivors without additional comorbidities, which could greatly influence the results. We did not observe significant differences in CRP as well as ICAM-1 and VCAM-1 concentrations between the healthy control and study groups. A possible explanation may be the time that had passed since infection. It was stated by Fogarty et al. that, during the first 10 weeks after acute SARS-CoV-2 infection, endothelial activation is common in all individuals [40]. This period of time may be extended in those who develop a severe form of the disease and suffer from other comorbidities. In our research, we tested healthy blood donors who were enrolled to the study after complete recovery. This could have provided sufficient time to diminish inflammation and re-establish the balance in endothelial metabolism, thus decreasing CRP concentrations and ICAM-1 and VCAM-1 expression and release into the bloodstream. Similar results to our observations were obtained by Tong et al. in a study performed on 345 COVID-19 survivors one year after infection. After comparing the concentrations of ICAM-1, VCAM-1, P-selectin and fractalkine in convalescents to those in healthy controls, the authors concluded that SARS-CoV-2 infection in the past does not impose an increased risk of cardiovascular events, not only for patients with a mild form of infection, but also for those who were severely ill [41].

Similar results showing positive correlations between increased CRP, ICAM-1 and VCAM-1 concentrations and disease severity were described by Liu et al. [31], Tong et al. [30] and Karampoor et al. [42]. However, the long-COVID phenomenon has raised concerns about persistent post-disease endothelial injury. This was tested in the aforementioned study by Haffke et al. in a group of patients with post-COVID-19 syndrome, who presented chronic fatigue and exertion intolerance [33]. The authors found increased concentrations of endothelin-1 in convalescents compared to healthy controls, a discovery that confirms the role of endothelial damage in post-COVID-19 syndrome.

In contrast to the results on adhesion molecules, we found significant differences in the concentrations of the soluble form of E-selectin between the healthy control and study groups, with higher values in the latter. This finding may indicate post-COVID-19 endothelial injury; however, it was not correlated with the VCAM-1 concentration, although the expression of both of them was enhanced by pro-inflammatory cytokines. A possible explanation may be the fact that VCAM-1 can be found on a broad repertoire of cells, whereas E-selectin is more specific for endothelial cells and better reflects changes in endothelial homeostasis induced by SARS-CoV-2 infection [43]. In a study by Oliva et al., the concentration of soluble E-selectin was compared between COVID-19 patients with varying clinical disease severity: those admitted or not admitted to intensive care units, those who survived or died, and those who developed thrombosis or those who did not [32]. The authors indicated that E-selectin concentrations were significantly higher in critically ill patients who required admission to the intensive care unit, which makes E-selectin a possible predictor of disease severity. The difference between the E-selectin median concentration values in this study and our research (26.1 ng/mL vs. 1.75 ng/mL vs. 1.63 ng/mL in hospitalised patients, the study group and the control group, respectively) is noteworthy. It shows that the more severe the disease with endothelial injuries caused by the pro-inflammatory cytokine storm, the higher the E-selectin concentration. In our study, while testing generally healthy convalescents and the control group, we found low E-selectin concentrations, but these were still significantly higher in COVID-19 survivors. This finding may highlight the possible role of E-selectin as a marker of endothelial cell recovery and balance restoration. Due to scarce data about the changes in E-selectin concentrations in COVID-19 convalescents, especially in those who suffer from sustained endotheliopathy, more research is required.

Another interesting finding is the difference in syndecan-1 concentrations between the control group and the study group with surprisingly lower values in the latter. Many studies have shown the role of syndecan-1 and its increased release from the endothelium as a marker of disease severity and poor prognosis in the acute phase of the disease. Suzuki et al. presented the case of a patient with acute COVID-19 with severe lungs involvement, whose elevated syndecan-1 concentration was correlated with their worsening clinical condition and finally decreased as the healing process progressed [44]. In the research by Zhang et al., COVID-19 patients admitted to an ICU were divided into groups of survivors and non-survivors, and the change in syndecan-1 concentration was established. The authors indicated that an increased level of syndecan-1 was a predictor of a poor prognosis since it reflected deeper endothelial injury and a more severe pro-inflammatory cytokine storm [25]. The same conclusion was made by Ogawa et al. based on their study on critically ill patients who required intensive care. These authors also concluded that the more severe clinical presentation of SARS-CoV-2 infection, the higher the syndecan-1 concentration. According to them, this is due to the inflammation process, which through many mechanisms is directly responsible for destroying glycocalyx and thus releasing syndecan-1 [45]. Additionally, Lambadiari et al. found significantly reduced glycocalyx thickness in COVID-19 as well as hypertensive patients in comparison to those of a healthy control group. The glycocalyx reduction in these groups was not correlated with the disease severity [35]. In our study the results we obtained are opposite to previously cited articles. First of all, the concentration values in the control and study groups were significantly lower compared to those in the aforementioned studies (approx. 100–1000 ng/mL versus 0.6–1.0 ng/mL). The most reasonable explanation is the type of study group. We focused not on critically ill patients admitted to intensive care units, but on generally healthy convalescents in comparison to the control group. Our participants were free from other comorbidities which could be related to chronic endothelium activation and injury. This fact can explain the much lower syndecan-1 level that we obtained in our study. However, the difference in the concentration of syndecan-1, with a surprisingly lower level in convalescents, needs to be explained. The role of the heparan sulphate proteoglycans group in different cells' biology, including in wound healing, angiogenesis and malignancy, has been studied for many years. A syndecan-1 molecule expression on endothelial cells has been linked especially to regenerative and angiogenesis processes. In 1996, Kainulainen et al. published their study, in which they were testing the influence of different pro-inflammatory cytokines on syndecan-1 endothelial expression [46]. The authors concluded that among many tested cytokines, only TNF-α was able to inhibit syndecan-1 gene expression, thus decreasing this protein quantity on the endothelial cells' surface. This influence was observed especially during regeneration. As syndecan-1 is believed to take part in the healing process, its downregulation might have a negative impact on restoring homeostasis in the endothelium. However, Javadi et al. indicated that syndecan-1 overexpression can also result in the inhibition of endothelial cell proliferation and the restoration of physiologic balance in the endothelium [47]. Despite some sparse reports that may support such a hypothesis, we still believe that the observed lower syndecan-1 concentrations in convalescents need to be explained and the possible mechanisms of its downregulation need to be established.

Knowledge about different pro- and anti-inflammatory cytokines changes and the effects they have on cells seems to be crucial in understanding the mechanism underlying post-COVID-19 endotheliopathy with possible complications. One limitation of our study was the inclusion of patients who suffered from COVID-19 only; it has to be mentioned that other acute systemic infectious conditions may induce endothelial injury as well. However, our aim was to assess the possible effect of SARS-CoV-2 infection on endothelial function in generally healthy subjects. Nonetheless, it might be interesting to compare how different types of systemic inflammation may affect endothelial function. Another limitation of our study is the lack of data about participants' sex and BMI; however, we believe that both the

study and control groups can be regarded as homogenous due to the very strict criteria for blood donation.

5. Conclusions

Based on our results, it can be concluded that, at least 6 months after infection, there is only slight evidence of endothelial dysfunction in COVID-19 convalescents who do not suffer from other comorbidities related to endothelial impairment. The increased E-selectin concentrations in convalescents, which may be associated with prolonged endothelial injury, require further investigation and confirmation.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study when donating blood to the Regional Blood Centre.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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ORIGINAL ARTICLE

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Mild-to-moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis

Paweł Kozłowski[1] | **Michalina Lulek**[2] | **Agata Skwarek**[2] | **Marcin Śmiarowski**[2] | **Milena Małecka-Giełdowska**[1,3] | **Olga Ciepiela**[1,3]

[1]Central Laboratory, University Clinical Centre of Medical University of Warsaw, Warsaw, Poland

[2]Students Scientific Group of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland

[3]Department of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland

Correspondence
Dr Olga Ciepiela, Department of Laboratory Medicine, Medical University of Warsaw; ul. Banacha 1a; 02-097 Warszawa, Warsaw, Poland.
Email: olga.ciepiela@wum.edu.pl

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Abstract

An infection with severe acute respiratory syndrome coronavirus (SARS-CoV-2) may have a significant impact on the human immune system. Interactions between the virus and defence mechanisms may promote the development of autoimmune processes which manifest as antinuclear antibody (ANA) synthesis. Since many different viruses are suspected to take part in the pathogenesis of different systemic autoimmune rheumatic diseases (SARDs), we examined whether coronavirus disease 2019 convalescents who suffer from mild to moderate disease have a higher risk of developing ANA and anti-β_2-glicoprotein I IgG antibodies (β_2 GPI). In a retrospective study, we examined 294 adults among volunteer blood donors divided into convalescents (N = 215) and healthy controls (N = 79). For ANA detection, we used line-blotting, a type of indirect immunofluorescence assay (IF), to determine antigenic specificity and ELISA for β_2 GPI. We found a lower incidence of ANA in convalescents than in healthy controls, with the majority of these antibodies directed to antigens with no known clinical significance. Additionally, no participants were positive for β_2 GPI in either group. Our results show that COVID-19 with mild to moderate symptoms in the generally healthy population does not induce the development of ANA or anti-β2 GPI antibodies for at least 6 months following the disease.

1 | INTRODUCTION

A high number of respiratory tract infections with a novel coronavirus was observed in December 2019 in Wuhan, China. The new pathogen called severe acute respiratory syndrome coronavirus, has spread quickly around the world, forcing the World Health Organization (WHO) to declare the coronavirus disease 2019 pandemic in May 2020.[1] The clinical symptoms of SARS-CoV-2 infection may vary from mild ones, such as fever, cough, myalgia, headache etc., to life-threatening systemic conditions, for example, disseminated intravascular coagulation (DIC), pneumonia, acute respiratory distress syndrome (ARDS) and hyperinflammation, which may have a negative impact on many vital organs and systems, including the renal, cardiovascular and nervous systems, as well as on haematopoiesis.[2,3] There are many lines of evidence supporting the thesis that interactions between the viral and host

immune systems may give rise to an autoimmune phenotype.[4–9] The infection triggers an immune response, both innate and adaptive, the purpose of which is to limit viral spread; nevertheless, it must be precisely orchestrated. A broad spectrum of viral infections has been recognized as a prominent factor which can trigger an autoimmune response in genetically susceptible individuals.[10–14] There are several mechanisms involved in this process, such as molecular mimicry and tissue impairment caused by hyperinflammation related to an excessive antiviral immune response. Damaged cells release self-antigens, which are taken up by aantigen-presentingcells (APCs) and presented to autoreactive T lymphocytes to induce a cell-mediated autoimmune response towards host tissues. A growing amount of evidence shows common features in immune system dysregulation between COVID-19 and autoimmune diseases, for example, overactivation of macrophages, monocytes and neutrophils[15–17]; alteration in the number and activity of lymphocyte subpopulations[18] and hyperinflammation caused by increased release of proinflammatory cytokines[19,20] as well as the presence of increased levels of damage-associated molecular patterns (DAMPs).[21] Over time, it has been noted that some COVID-19 convalescents present symptoms (for example, chronic fatigue, dyspnoea, myalgia, cough, cognitive dysfunction) which can only be attributed to past SARS-CoV-2 infection. This phenomenon was called post-COVID (long COVID).[22–25] Whether some of these long-term consequences may be caused by autoimmunity development following COVID-19 still needs clarification. Since SARS-CoV-2 has spread widely in the human population, the question of long-term public health consequences remains open. It is important to understand the possible impact the virus may have in the future to develop proper procedures for more accurate diagnosis and treatment. Whereas many scientific and clinical efforts have been focused on patients with severe COVID-19, those individuals with mild and moderate infection still comprise the majority of convalescents who may develop possible complications, such as autoimmunization, in the future. The aim of our study was to investigate whether generally healthy individuals (with no additional comorbidities) who have recovered from mild and moderate COVID-19 have a higher risk of developing the autoimmune phenotype found in systemic autoimmune rheumatic diseases (SARDs), as well as antibodies related to thrombosis.

2 | PATIENTS AND METHODS

A total of 294 adult (18–65 years old) participants among volunteer blood donors at Warsaw's Blood Centre were enrolled in the study from August 2021 to April 2022. All of them underwent an examination and were qualified by a physician as healthy and able to donate blood according to the guidelines of the PolishMinistryr of Health (Table 1). All participants were tested on the day of enrolment for SARS-CoV-2 infection, and a negative PCR result was obtained. Specific data such as age, sex and additional comorbidities (not listed in the guidelines) were not provided.

A serum sample was collected from every participant on the admission day and stored at −70°C until analysis. Each sample was tested for anti-SARS-CoV-2 protein N antibodies by means of electrochemiluminescence immunoassay (ECLIA) on the Cobas e801 apparatus (Roche Diagnostics, Basel, Switzerland). These results were expressed as COI, defined as negative when COI <1.0 and positive when COI ≥1.0. In the next step, samples were tested for ANA using an indirect immunofluorescence assay (IF) with HEp-2 cells (Inova Diagnostics). The initial 1:80 dilution was used to classify samples as ANA negative (no fluorescence observed) or ANA positive (fluorescent pattern observed). The microscopic evaluation of fluorescence was performed by an experienced laboratory specialist. Detected ANA patterns were described according to the standards of the International Consensus on ANA

TABLE 1 Exclusion criteria for the study.

Increased bleeding risk
Acute or chronic disease of the cardiovascular system, central nervous system, gastrointestinal tract, airways and lungs, genitourinary system, immune system, endocrine system, skin
Acute or chronic connective tissue disease
Diabetes
Any type of cancer now or in the past
Infectious diseases:
Positive PCR for SARS-CoV-2 on the day of admission
HBV, HCV, or any hepatitis of undetermined cause
HIV-1/2, HTLV
Any parasitosis
Patients with any risk of TSE
Syphilis at any time in life
History of drug abuse
History of risky sexual behaviour
Sex workers
Any STD in the past
Allotransplant recipients
Increased body temperature in the last several weeks
Any vaccination in the last 4 weeks
Any medical or non-medical procedure with a risk of infection in the last 6 months

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV, human T-cell lymphoma virus; PCR, polymerase chain reaction; STD, sexually transmitted diseases; TSE, transmissible spongiform encephalopathies.

Patterns (ICAP).[26] All ANA-positive samples underwent serial dilutions to establish the final result as a fluorescence pattern and titre. To determine antigen specificity of detected ANA, blotting was performed on ANA IF-positive samples by means of Euroline ANA Profil 3 plus DFS line-blot system (Euroimmun), which consists of 16 purified antigens (nRNP/Sm, Sm, SS-A (Ro), Ro-52, SS-B (La), Scl-70, PM/Scl-100, JO-1, CENP B, PCNA, dsDNA, nucleosomes, histones, Rib-P, AMA-M2, DFS70) and uses an anti-IgG conjugate. The line blot strips were scanned on a EUROLineScan device (Euroimmun, Lübeck, Germany), and the results were expressed as the reaction intensity (0 – negative; +, ++ and +++ as positive) for every antigen. Additionally, all samples were tested for the presence of IgG anti-β_2-glycoprotein antibodies with the QUANTA Lite® β_2 GPI IgG ELISA kit (Inova Diagnostics). The results were reported semi-quantitatively using a standard IgG anti-β_2-glycoprotein unit (SGU). According to the manufacturer's guidelines, samples with a result from 0 to 20 SGU were considered as negative, whereas samples with a result greater than 20 SGU were described as positive for anti-β_2-glycoprotein IgG antibodies. Statistical analysis was performed with GraphPad Prism 6 software. The non-normal distribution of all parameters was proven by Shapiro–Wilk, Anderson-Darling, Kolmogorov–Smirnov-Lillefors and D'Agostino-Pearson tests. The difference in anti-SARS-CoV-2 protein N antibody titre was tested by means of the Mann–Whitney U test. Fisher's exact test for frequency comparison was used to compare ANA IF results and positive versus negative blotting results between the convalescent and control groups. The presence of autoantibodies against specific antigens between the convalescent and control groups was compared using a chi-square test. A probability value $p < .05$ was considered as statistically significant in all comparisons.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethical Committee of the Medical University of Warsaw (protocol code AKBE/136/2021, date of approval 6.09.2021). Informed consent was obtained from all subjects involved in the study when donating blood to Regional Blood Centre.

3 | RESULTS

Anti-SARS-CoV-2 protein N antibodies in the convalescent and control groups.

Among all participants, 215 (73%) were positive for anti-protein N antibodies, thus they were classified as those who had been infected with SARS-CoV-2 in the past (convalescent group). Seventy-nine (27%) individuals negative for anti-protein N antibodies were described as those who had never been infected with SARS-CoV-2 (control group). The difference in antibody titers between the convalescent and control groups was statistically significant $p < .0001$ (Figure 1).

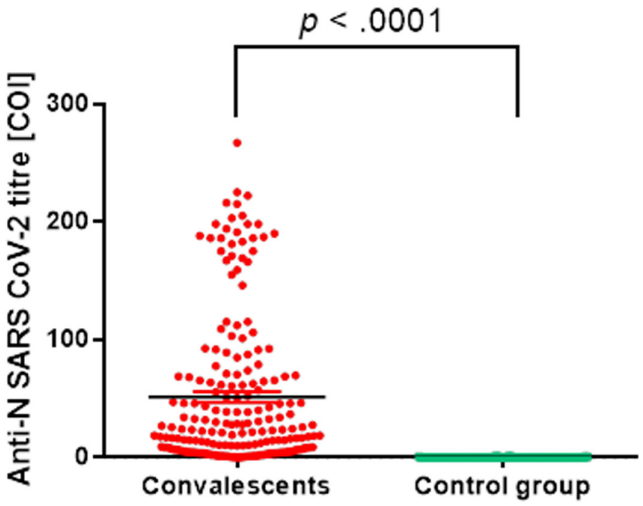

FIGURE 1 The difference in antibody titers between the convalescent and control groups.

Among the convalescent group, 174 (81%) participants declared symptomatic mild to moderate COVID-19 in the past, at least 6 months prior to study enrolment (symptomatic convalescents). The mild disease was defined as a lack of symptoms of lower respiratory disease (shortness of breath [dyspnoea] and abnormal chest imaging) and oxygen saturation measured by pulse oximetry (SpO_2) ≥94%. Moderate COVID-19 was defined as symptoms of lower respiratory disease with $SpO_2 \geq 94\%$. Forty-one (19%) individuals in the convalescent group declared no symptoms of COVID-19 in the past (asymptomatic convalescents). There were no significant differences in antibody titers between symptomatic and asymptomatic convalescents, with results of 23.60 (6.82; 63.60) and 15.30 (3.88; 87.98), respectively.

3.1 | Comparison of ANA presence in the convalescent and study groups

Antinuclear antibodies detected by indirect immunofluorescence assay (IF) were found in 23 (11%) convalescents and 17 (22%) individuals from the control group. No ANA were found in 192 (89%) and 62 (78%) samples in the convalescent and control groups, respectively (Table 2). A statistically significant difference was observed for these results with $p = .02$ (Figure 2). Fifteen ANA-positive samples from convalescents and 16 samples from the control group with the highest ANA titers were tested by line blot to determine the ANA antigenic specificity (Table 3). No significant difference was found between convalescents and the control group in the number of positive and negative blotting results (Figure 3) or in ANA antigen specificity

TABLE 2 ANA patterns and median titers in the convalescent and control groups.

	Homogenous (AC-1)	Dense fine speckled (AC-2)	Speckled (AC-5)	Nucleolar (AC-8)	Nuclear envelope (AC-11)	PCNA (AC-13)	Cytoplasmic speckled (AC-20)
Convalescents	1	1	11	2	0	1	7
(N = 23)	(1:320)	(1:640)	(1:80)	(1:80)		(NA)	(1:80)
Control group	0	1	8	1	2	2	3
(N = 17)		(1:1280)	(1:160)	(1:160)	(1:160)	(NA)	(1:160)

Abbreviations: AC, number of ANA patterns according to ICAP[26]; NA, non-applicable.

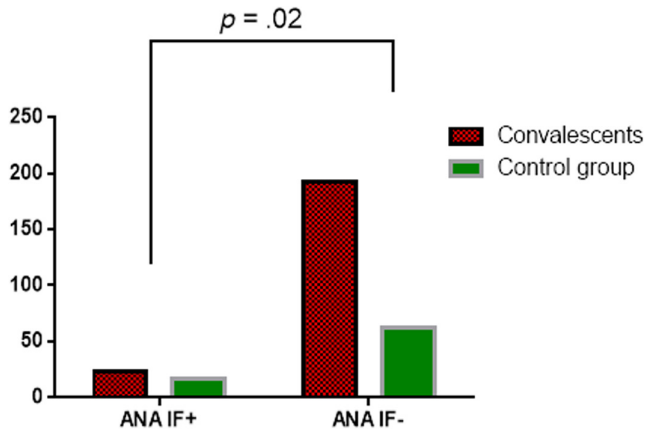

FIGURE 2 The difference in ANA presence between the convalescent and control groups.

(Figure 4). For all samples from the convalescent and control groups, negative results for anti-β_2-glicoprotein I IgG antibodies were obtained.

4 | DISCUSSION

The results of our study show that SARS-CoV-2 infection with a mild to moderate clinical presentation does not increase the risk of developing ANA in generally healthy individuals in a time period of several months following the disease. We chose ANA as a general marker for autoimmunity due to its established role in diagnosing and monitoring SARDs. We used the IF technique since it is a recommended and widely used method for ANA detection which allows for the detection of a very broad spectrum of autoantibodies against different components of cells. We found that there were statistically significantly more ANA-negative individuals in convalescents than in the control group. There are many studies describing a strong connection between acute COVID-19 and the presence of autoantibodies. Many of them focus on the relation between disease severity, the type and titre of specific autoantibodies and their use in predicting disease severity and outcome. In their study, Peker et al.[27] tested 50 patients with acute COVID-19 for ANA using the IF technique and compared the results to those of a healthy control group. The study indicated a higher rate of ANA in SARS-CoV-2-infected individuals. The result may be contrary to our findings since it is expected that COVID-19 patients remain ANA-positive after recovery. However, there is a great difference between these two studies in terms of the composition of the study and control groups. Generally, healthy individuals who were COVID-19 convalescents for at least 6 months (with asymptomatic, mild-to-moderate disease) were enrolled in our research, while Peker et al. focused on patients with acute COVID-19 with radiological signs of pneumonia who were hospitalized due to the illness, some of them in the intensive care unit. A second difference was the time of serum sampling for testing. We obtained samples from COVID-19 convalescents and healthy controls while they were in generally good health, whereas Peker et al. focused on patients with ongoing SARS-CoV-2 infection. Similar discrepancies in cohort structure, sampling time and results may be found between our research and the work of Gazzaruso et al.,[28] Chang et al.[29] and Pascolini et al.[30] Those authors found a high prevalence of ANA in hospitalized patients with radiologically confirmed pneumonia due to SARS-CoV-2 infection (35.6%, 21.3% and 33.3%, respectively). Vlachoyiannopoulos et a.[31] and Lerma et al.[32] described similar results in severely ill COVID-19 patients, in whom they detected ANA in 34.5% and 25% of individuals, respectively. These results indicate that pre-existing autoimmunity may be a risk factor for a severe clinical course of COVID-19; however, they do not support the thesis that SARS-CoV-2 infection inevitably induces autoimmunity. An interesting observation was made by Trahtemberg et al.,[33] who compared ANA and other types of autoantibodies in critically ill COVID-19 and non-COVID-19 patients with acute respiratory failure. Although the authors found a surprisingly high prevalence of ANA in COVID-19 patients, they also discovered a high prevalence of ANA in non-COVID-19 individuals (68% vs. 60%, respectively), with no statistical significance. According to the authors, this phenomenon may indicate that the presence of autoantibodies may contribute to a severe course of respiratory failure with no connection to COVID-19 status. This finding may support the assumption that, in at least some

TABLE 3 Antigen specificity of ANA in selected positive samples from the convalescent and control groups.

	Positive					Negative
	DFS70	PM/Scl-100 (PM100)	SS-A (Ro)	Scl-70 (SCL)	JO-1	Undetermined antigen
Convalescents (N = 15)	5 (33%)	2 (13%)	1 (7%)	2 (13%)	0	5 (33%)
Control group (N = 16)	5 (31%)	2 (13%)	1 (6%)	0	1 (6%)	7 (44%)

Abbreviations: DFS70, dense fine speckled (70-kDa protein); Jo-1, histidyl-tRNA synthetase; PM/Scl-100; polymyositis/systemic sclerosis; Scl-70; DNA topoisomerase 1; SS-A (Ro), Sjögren syndrome antigen A.

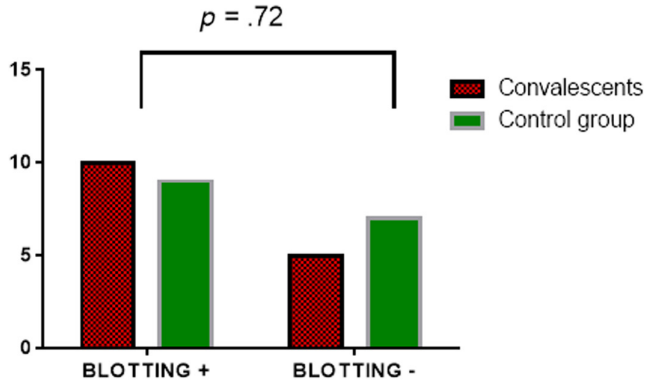

FIGURE 3 Positive and negative results of blotting in the convalescent and control groups.

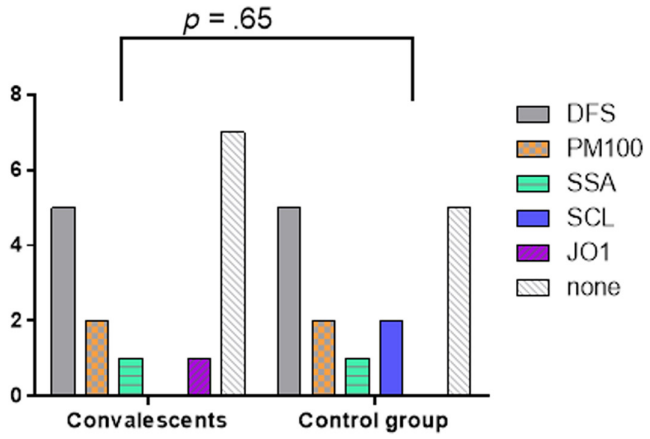

FIGURE 4 Antigen specificity of ANA in the convalescent and control groups.

cases, SARS-CoV-2 infection is not a trigger of ANA production, and thus, detecting ANA in COVID-19 convalescents, especially those with a mild clinical course, may be a simple, unrelated coincidence. Our results also seem to support this hypothesis. Interesting studies performed by Liu et al.[34] and Rojas et al.[4] on the presence of autoantibodies in convalescents showed that SARS-CoV-2 infection may promote the synthesis of different autoantibodies, thus promoting a post-COVID autoimmune phenotype. These results, however, do not contradict ours since both authors used sophisticated detection assays that allow for the detection of autoantibodies against a very broad set of distinct antigens, which do not, however, belong to classic ANA. This finding may be proof of COVID-19-related induction of an autoimmune phenotype in convalescents; however, it does not directly confirm a systemic autoimmune response leading to the development of SARD and expressed by classic ANA production. An interesting observation came from the results of ANA antigen specificity. Watanabe et al.,[35] Muro et al.,[36] Mahler et al.[37] and Shovman et al.[38] independently indicated that single presence of anti-DFS70 antibodies is significantly more frequent in healthy individuals than in patients with SARD, and thus their detection makes a systemic autoimmune disease diagnosis less likely. In our study, approximately one-third of ANA tested in line-blot, in both the convalescent and control groups, was detected as only anti-DFS70. This finding, according to the studies mentioned above, supports the main conclusion of our research, that COVID-19 does not have to induce systemic autoimmunity. Moreover, it is highly probable that many individuals in the convalescent group were positive for ANA, including anti-DFS70, prior to the disease. The lack of information about their pre-COVID-19 serologic status is one of the limitations of our study. The next interesting observation is the relevant number of samples with undetermined antigen specificity in both groups identified by use of a set of purified autoantigens with established significance in routine diagnostics of SARDs. This may be explained by the fact that ANA, especially in low titers, may be found using the IF method in a significantly high percentage of the healthy population,[39] with these autoantibodies having no connection to any systemic autoimmune disease. It is thus possible that at least some convalescents in our study were ANA positive prior to COVID-19, and SARS-CoV-2 infection played no role in autoantibody development. The fact that we actually found a higher number of ANA-positive individuals in the healthy control group than among convalescents supports our conclusion. The individual cases of ANA against antigens specific for SARDs (for example SS-A, PM/Scl-100, Scl-70, Jo1) are not sufficient to make any conclusion due to their low number.

One of the important effects of COVID-19 is a hypercoagulable state. A possible mechanism is the presence of

autoantibodies typically found in antiphospholipid syndrome (APS), like lupus anticoagulant (LA) and antibodies against cardiolipin (aCL) as well as β_2-glicoprotein I (β_2 GPI).[40] For the comparison between convalescents and the healthy control group in our study, we chose anti β_2 GPI IgG antibodies for two reasons. First of all, we tested COVID-19 convalescents who recovered at least 6 months before enrolment in this study, and thus there was sufficient time for possible development of IgG-class antibodies. Second of all, we chose anti-β_2 GPI as an antigen, since it is the most prominent target protein for autoantibodies responsible for increasing the risk of thrombosis.[41] We found, however, no positive results in either of the studied groups, which may indicate, together with our findings, that COVID-19 may not initiate auto-response to self-antigens, including β_2 GPI, and thus it does not increase the risk of thrombosis via this mechanism. The consistent observation about these autoantibodies, though based on a study involving a small number of patients suffering from acute COVID-19 pneumonia with concomitant thrombosis, was made by Galeano-Valle et al.[42] The authors did not find an increased incidence of not only anti-β_2 GPI in M and G class but also aCL antibodies in both isotypes. The same result was obtained by Trahtemberg et al.[43] In their prospective study on 42 patients with acute respiratory failure due to COVID-19 and other than COVID-19 causes, they found no case of either IgG or IgM anti-β_2 GPI positivity. The connection between COVID-19 and an increased risk of thrombosis seems to be undeniable, though its understanding still requires further studies.

We recognize some limitations of our work. First, we focused on a group of voluntary blood donors, individuals in generally good health without any serious comorbidities, who had mild to moderate COVID-19 in the past. None of the convalescents enrolled in our study required intensive treatment or hospitalization due to the disease. This fact limits our findings to this type of COVID-19 survivor, and hence they cannot be extrapolated to the general population. The other limitation is the lack of any information participants' ANA status prior to enrolment in the study, as well as precise data about their sex and age.

In conclusion, our results show that COVID-19 with mild to moderate symptoms in a generally healthy population does not induce the development of ANA and anti-β2 GPI antibodies for at least 6 months following the disease. Based on our results, we can also conclude that it may not be necessary to monitor mild-to-moderate COVID-19 convalescents who do not present with long COVID symptoms for autoimmune development.

AUTHOR CONTRIBUTIONS

OC conceived the concept of the study. PK, MM-G and OC were responsible for the design and methodology of the study. All authors were involved in data collection. PK, ML, AS and MS performed the formal analysis; PK was responsible for writing and original draft preparation, P.K.; writing—review and editing, ML, AS, MS, M.M-G., O.C.; PK, ML, AS and OC worked on visualization, OC coordinated funding of the project. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

ORCID

Olga Ciepiela https://orcid.org/0000-0002-3694-4076

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Pawe l Koz lowski , Aleksandra Leszczyńska , Olga Ciepiela

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Review Article

Long COVID definition, symptoms, risk factors, epidemiology and autoimmunity – A narrative review

Paweł Kozłowski[1], Aleksandra Leszczyńska[1], Olga Ciepiela[1,2,*]

[1]Central Laboratory, University Clinical Centre of the Medical University of Warsaw;
1a Stefana Banacha street, 02-097 Warsaw, Poland
pawel.kozlowski@uckwum.pl (PK)

aleksandra.leszczynska1@uckwum.pl (AL)

[2]Department of Laboratory Medicine, Medical University of Warsaw;
1a Stefana Banacha street, 02-097 Warsaw, Poland

olga.ciepiela@wum.edu.pl (OC)

*Correspondence: olga.ciepiela@wum.edu.pl; Tel.: +48225992405

Authors contribution:

Paweł Kozłowski: Conceptualization, Investigation, Methodology, Formal Analysis, Writing- Original draft preparation, Software, Visualisation, Validation. **Aleksandra Leszczyńska:** Investigation, Data Curation, Visualisation. **Olga Ciepiela:** Conceptualization, Investigation, Project Administration, Supervision, Writing- Reviewing and Editing, Validation.

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Abstract

The virus called SARS-CoV-2 emerged in 2019 and quickly spread worldwide, causing COVID-19. It has greatly impacted on everyday life, healthcare systems, and the global economy. In order to save as many lives as possible, precautions such as social distancing, quarantine, and testing policies were implemented, and effective vaccines were developed. A growing amount of data collected worldwide allowed the characterization of this new disease, which turned out to be more complex than other common respiratory tract infections. An increasing number of convalescents presented with a variety of non-specific symptoms emerging after the acute infection. This possible new global health problem was identified and labelled as long COVID. Since then, a great effort has been made by clinicians and the scientific community to understand the underlying mechanisms and to develop preventive measures and effective treatment. The role of autoimmunity induced by SARS-CoV-2 infection in the development of long COVID is discussed in this review. We aim to deliver a description of several conditions with an autoimmune background observed in COVID-19 convalescents, including Guillain-Barré syndrome, antiphospholipid syndrome and related thrombosis, and Kawasaki disease highlighting a relationship between SARS-CoV-2 infection and the development of autoimmunity. However, further studies are required to determine its true clinical significance.

Keywords: Antiphospholipid syndrome, Autoimmunity, Guillain-Barré syndrome, Kawasaki disease, Long COVID

Introduction

An increasing number of respiratory tract acute infections caused by a novel coronavirus was observed in Wuhan, China, in November 2019 [1]. The pathogen was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease as coronavirus disease 2019 (COVID-19) [2]. Due to the rapid spreading of the disease, the World Health Organization (WHO) declared COVID-19 a pandemic in May 2020 [3]. According to the WHO report, from the first COVID-19 cases in November 2019 to March 2023, approximately 759 million SARS-CoV-2 infections and 6.9 million deaths due to COVID-19 have been confirmed worldwide. In the same document, the WHO estimated the number of COVID-19-related excess deaths was approximately 14.9 million by the end of 2021, and nearly 337 million years of life were lost in 2020-2021 due to the pandemic [4]. Respiratory symptoms range from asymptomatic or mild coryza to severe pneumonia and damage to alveoli and

capillaries caused by hyperinflammation resulting in impaired gas exchange and, in turn, hypoxia, a state called acute respiratory distress syndrome (ARDS). Despite the name of the pathogen indicating respiratory involvement, COVID-19 may impair many other systems and organs, and should be considered a systemic multiorgan disease [5]. Data collected from clinical practice and observation, as well as scientific studies, showed an unquestionable negative impact of SARS-CoV-2 infection on the cardiovascular [6–8], renal [9–12], gastrointestinal [13–16], nervous [17–20], and skin systems [21–23]. The clinical presentation and severity of COVID-19 symptoms have changed over time, with approximately 15% of infected individuals requiring hospitalization and oxygen supply at the beginning of the pandemic, to only 3% of cases in 2023 [24]. This drop in the number of individuals requiring intensive therapy is caused by changes to SARS-CoV-2 itself, ongoing general immunization, development of vaccination protocols with the first messenger (mRNA)-based vaccine approved in 2020 [25], the emergence of new variants causing milder infections, and changes to testing policies worldwide. Although most COVID-19 convalescents make a full recovery, some experience protracted symptoms that may be associated with past SARS-CoV-2 infection. This phenomenon has been called "long COVID," "post COVID-19," and "post-acute sequelae of COVID-19 (PASC)". Among many mechanisms indicated as those responsible for sustained symptoms, autoimmunity draws attention. As has been shown by many researchers, SARS-CoV-2, like other viruses with a confirmed role in developing autoimmunity, can modulate an immune response to facilitate the weakening tolerance to the host's self-antigens, induce autoantibody production due to molecular mimicry, and sustain inflammation which impairs tissues and results in the release of the host's self-antigens as possible targets for an autoimmune response [26,27]. It is important to emphasize that among many possible long-lasting complications of SARS-CoV-2 infection, autoimmunity is of great importance and can contribute to developing long COVID in many individuals (Figure 1).

The interaction between SARS-CoV-2 and the immune system, together with several possible side effects of an autoimmune nature, are described. The number of patients who have recovered from COVID-19 is still growing, and new SARS-CoV-2 variants with altered virulence and transmissibility are still emerging worldwide. This predictably will lead to an increase in the number of individuals suffering from long-lasting complications, including those of an autoimmune nature. Due to this, there is a need for concise description of the subject, which could present the most important aspects of long COVID. The literature about

this is very rich, with many excellent research studies and analyses performed by experienced teams that describe the subject in great detail. This narrative review aims to deliver general information about long COVID and related autoimmunity, highlighting selected conditions (antiphospholipid syndrome, Guillain-Barré syndrome and Kawasaki disease). For this purpose, the PubMed/MEDLINE database was searched from 2019 to August 2023 for original articles, systematic reviews, narrative reviews, and meta-analyses (all published in English) with the following combinations of keywords: long COVID AND autoimmunity, long COVID AND epidemiology, long COVID AND symptoms, long COVID AND review, SARS-CoV-2 AND autoimmunity, SARS-CoV-2 AND Guillain-Barré, SARS-CoV-2 AND Kawasaki. Additionally, the PubMed/MEDLINE database was searched for literature describing the biology of SARS-CoV-2 with keywords: SARS-CoV-2 AND genome, SARS-CoV-2 AND infection, SARS-CoV-2 AND (immune OR response), SARS-CoV-2 AND variants. Some information was also gathered from the WHO reports and websites.

Long COVID definition, symptoms, and epidemiology

The term "long COVID" describes the persistent presence of diverse symptoms related to past infection with SARS-CoV-2, often weeks and months after the acute phase of the disease. The symptoms may include those typical for acute COVID-19, as well as entirely new symptoms that emerge following recovery. Since there are no distinct criteria for the diagnosis of long COVID, several definitions have been proposed to establish the indications and time frames for better distinction between acute and long COVID. One definition of long COVID is that of the presence of symptoms beyond 3 weeks after the initial signs of acute SARS-CoV-2 infection, distinguishing it from post-acute COVID-19 (4^{th} to 11^{th} week) and chronic COVID-19 (12^{th} week and beyond) [28]. A similar classification was proposed by the UK National Institute for Health and Care Excellence (NICE), the UK Scottish Intercollegiate Guidelines Network (SIGN), and the UK Royal College of General Practitioners (RCGP), with the first 4 weeks of SARS-CoV-2 infection described as acute COVID-19; from 5 to 12 weeks as "ongoing symptomatic COVID-19"; and beyond 12 weeks as "post-COVID-19" in the case of persistent symptoms [29]. A complex study resulting in the creation of the clinical definition of long COVID was performed by the WHO using the Delphi consensus-based methodology [30]. The term "long COVID" was defined as the development of symptoms in individuals with confirmed or probable infection with SARS-CoV-2, while the time between recovery and 3 months has been defined as "post-COVID-19". The symptoms should appear or be sustained during the 3 months following recovery

from the acute phase of infection and last for at least 2 months with persistent or fluctuating severity. They also negatively impact on everyday life and have no connection to other known medical conditions. The long COVID diagnosis does not depend on the individuals' viral status, with most of the patients being PCR-negative at the time of diagnosis [31,32]. An effort is still being made to evaluate the prevalence of long COVID. However, the heterogeneity of study groups and methodology makes assessing this difficult. The persistence of symptoms following infection is described in some studies in relation to age, sex, disease severity, and follow-up time. Among COVID-19 patients discharged from hospitals in Michigan in the United States, almost 33% declared persistent symptoms in a 60-day follow-up study [33]. In comparison, in another study in France with a 60 day follow-up time, around 66% of convalescents with non-critical COVID-19 declared persistent symptoms, and almost 33% reported feeling worse than during the acute phase of infection [34]. The studies showed a significant difference in the number of individuals with long COVID between those treated as outpatients (10-30%) and those who required hospitalization (50-80%), as well as vaccinated individuals [35–41]. The clinical symptoms related to long COVID are incredibly heterogeneous and include the respiratory and gastrointestinal tracts, joints, central and peripheral nervous system, bone marrow, endocrine system, etc. [42]. General symptoms like fatigue are also broadly reported. This heterogeneity comes from a vast number of reports engaging many individuals, thus, it is important to point out the most common symptoms. The incidence of the most frequent symptoms described in systematic reviews is listed in Table 1 [43–49].

Risk factors of long COVID

There are several risk factors of long COVID described in the literature. Data collected from 4,182 COVID-19 convalescents showed a positive correlation between the risk of developing long COVID and female sex, increasing age, increased body mass index (BMI), and the occurrence of over five symptoms in the acute phase of the disease [50]. There is no single explanation of why female sex is a risk factor. However, the role of genetic, hormonal, and environmental differences in innate and adaptive immune responses between females and males is likely to be relevant [51]. Due to these variations, females maintain stronger inflammatory responses and are generally more susceptible to the development of autoimmune diseases, with both phenomena playing an important role in the development of symptoms of long COVID [52]. However, due to the same differences, males are more likely to develop the severe form of acute COVID-19, which is described as an independent risk factor

of long COVID [53]. Increasing age and obesity (increased BMI and waist-hip ratio) are often accompanied by other comorbidities such as hypertension and type 2 diabetes, which have a negative health impact prior to the onset of COVID-19, thus worsening the disease outcome and increasing the prevalence of long COVID [54–57]. Other diseases, particularly those of the respiratory tract such as bronchial asthma, and conditions treated with immunosuppressive drugs may also put patients at increased risk of long COVID [53]. Since many symptoms of long COVID are related to an exaggerated inflammatory response, the severity of the acute phase of COVID-19 is associated with a higher risk of developing long COVID, especially for patients who required intensive care and mechanical ventilation [58]. Additionally, increased viral load during the acute phase of infection may increase the incidence of persistent symptoms due to stimulation of the immune system, resulting in extensive tissue damage and viral persistence [55]. Some studies have reported the persistent presence of viral RNA in feces and the nasopharynx in COVID-19 convalescents who developed a humoral response with sufficient production of neutralizing antibodies [59,60]. Additionally, autopsies performed on COVID-19 patients several weeks after the disease outcome showed SARS-CoV-2 RNA in pneumocytes and endothelial cells [61]. One study with 203 post-symptomatic COVID-19 convalescents tested for the presence of viral RNA in the nasopharynx at two time points after their recovery found that 12.8% and 5.3% were positive for the coronavirus RNA at the 23rd and 90th day, respectively [62]. Moreover, there were no differences in neutralizing antibody levels between patients with and without viral RNA. However, the former presented a strengthened $CD8^+$ T lymphocyte-dependent antiviral response. These findings indicate that in some individuals persistent viral replication is maintained, which in turn leads to sustained activation of the immune system. This results in chronic hyperinflammation, causing subsequent tissue damage [63]. There is evidence that among patients in the acute phase of COVID-19 infection, latent viruses, including Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Herpes simplex virus type 1 (HSV-1), and Human herpesvirus (HHV-6 and HHV-7) can be reactivated. This has a potentially negative impact on disease severity and the risk of developing long COVID [55,64–66]. For the list of long COVID risk factors, see Table 2.

COVID-19 and the innate immune system

SARS-CoV-2, like other human viruses, is an obligate intracellular pathogen that uses the host's own cells to replicate and subsequently spread in the environment. It belongs to the group of coronaviruses, which also includes SARS-CoV-1 and Middle East respiratory

syndrome coronavirus (MERS-CoV), that are responsible for mild to severe infections in humans [67,68]. The SARS-CoV-2 virion contains several surface proteins. The spike protein (S protein) with the receptor binding domain (RBD) is used to infect cells via the hosts' angiotensin-converting enzyme 2 receptor (ACE2) [69–71]. The viral envelope (E) and membrane (M) structural proteins are essential to the process of assembling new virions. An effective entry to the cell requires the host cell surface transmembrane serine protease 2 (TMPRSS2), responsible for proteolytic cleavage of the S protein, to permit fusion between the virus and the cell [72,73]. Expression of ACE2 and TMPRSS2, therefore, makes host cells prone to coronavirus infection and has been described in many tissues and organs in the body, including the epithelial cells of the respiratory system, type II pneumocytes in pulmonary alveoli, endothelium, liver, kidney, enterocytes, placenta, glial cells, and platelets [74–85]. The SARS-CoV-2 virus binds ACE2 with higher affinity than SARS-CoV. This phenomenon may explain its high infectiousness and ability to cause a pandemic [86,87].

Viral entry to the cells triggers different pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs), which are responsible for triggering the complex cascade of different proteins to induce an innate antiviral response. The Toll-like receptor 3 (TLR3), TLR7, and TLR 8 are located in the membrane of endosomes', while RIG-like receptors (RLR), RIG-I, and melanoma-associated differentiation-associated gene 5 (MDA5) can be found in the cytosol in most of the tissues including the epithelium [88]. After binding viral RNA, endosomal TLRs polymerize their cytoplasmic tails, thus activating the whole chain of reactions involving various protein kinases, which results in the activation of transcription factors: nuclear factor kB (NF-kB), interferon response factor 3 (IRF3), and IRF7. NF-κB is responsible for activating inflammatory responses by enhancing the expression of genes for tumor necrosis factor (TNF), interleukin (IL)-1, chemokines (CCL2 and CXCL8), and the adhesion molecule E-selectin [88–90]. The crucial interferon-mediated response is triggered by cytosolic RLRs through IRF3 and IRF7. After binding viral RNA RIG-I and MDA5 are bonded by the mitochondrial antiviral-signaling protein (MAVS) to the outer layer of the mitochondrial membrane. By doing so, they initiate the recruitment of TNF receptor-associated factor 3 (TRAF3), TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1), and inhibitor of nuclear factor κB (IκB) kinase-ε (IKKε), an activator of different transcription factors (for example IRF3, IRF7, and NF-κB), thus, promoting expression of the type I interferons (IFN-α and IFN-β) and a group of early interferon-stimulated genes (early ISGs) [91,92] (see Figure 2). Type I IFNs stimulate the

expression of hundreds of genes that encode products for creating potent antiviral reactions. This process is a cascade consisting of different factors and signal transmitters. The Jak tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1) are activated by the complex of IFN-I and IFN-alpha and beta receptor (IFNAR) [93]. An active TYK2 and JAK1 then phosphorylate the signal transducer and activator of transcription 1 (STAT1) and STAT2. These transducers are responsible for forming a complex called IFN-stimulated growth factor 3 (ISGF3) consisting of STAT heterodimers and IRF9. Subsequently, the ISGF3 binds to particular sequences in the ISG promoters called IFN-stimulated response elements (ISREs). This leads to the activation of ISG transcription. One of these genes encodes protein kinase receptor (PKR), which can detect viral dsRNA synthesized during viral replication and completely halt all translational processes in the cell [94]. Another antiviral mechanism induced by IFN-I includes the expression of cytosolic enzymes, which can degrade viral nucleic acids or cause mutations in their sequence, as well as production of proteins that can interfere with releasing new viral particles from infected cells, thus restricting infection in nearby cells.

IFN in SARS-CoV-2 infection

Since viral-induced IFN-dependent responses engage hundreds of genes, some of which are responsible for cell survival processes, its overreaction can be dangerous to a host. Due to this, the whole mechanism must remain under strict host control. Distinct viruses can induce IFN synthesis with various strengths and evolve many different mechanisms to counteract it. Studies evaluating the level of IFN-I response triggered by SARS-CoV-2 on cell and animal models showed, that the coronavirus induces a milder response in comparison to other respiratory viruses [95]. At the same time the virus can evade this response using distinct mechanisms.

Inhibition of IFN pathway

Viral strategies to counteract IFN-dependent responses are focused on inhibiting of IFN-I production and preventing signal transduction to surrounding cells. To do so, the virus inhibits all proteins and signal pathways leading to IFN-I synthesis in host cells. Several SARS-CoV-2 proteins engaged in this process have been identified. The recognition of viral RNA by RIG-I and MDA5 can be inhibited by several viral non-structural proteins (NSP10, NSP14, NSP15, NSP16) [96–98], while NSP1 can enhance the degradation of the host's mRNA encoding the IFN molecule [99]. The viral open reading frame (ORF) proteins interfere with RLRs and TLRs signal transduction by blocking MAVS or MAVS together with TRAF3 and

TRAF6 proteins (ORF3b and ORF9b, respectively) and IRF3 signal transducer (ORF6) [100,101]. The ORF3a can enforce IFNAR degradation, while proteins NSP1 and ORF6 interact with STAT-dependent signal transduction, thus blocking IFN-I induced ISG expression [102]. The virus can also use the host's own regulatory mechanism involved in IFN pathway control to counteract its activation. For example, the expression of the suppressor of cytokine signaling 3 (SOCS3) can be intensified by viral S protein in B lymphocytes, leading to degradation of IRF7 as well as inhibition of JAK-STAT signal transduction [103] (see Figure 2 and 3).

Cells in the innate immune response in COVID-19

The chemokines secreted in response to infection initiate the recruitment of innate immune cells: dendritic cells (DCs), macrophages, and neutrophils, which cooperate hand in hand with both IFNs and adaptive immunity in fighting the infection [104]. These cells react to the presence of soluble factors by releasing a broad set of molecules to orchestrate the immune response. The NF-κB-dependent mediators can attract macrophages and neutrophils to the site of infection [105]. Therefore, the evidence shows an increased number of $CD169^{+}$ macrophages, with high ACE2 expression, containing viral N protein in the respiratory tract, spleen, and lymph nodes of infected individuals [106,107]. These macrophages, together with mast cells, epithelial, and endothelial cells, are the prominent but not the only source of pro-inflammatory mediators and chemokines (for example, IL-1, IL-2, IL-4, IL-6, IL-17, TNF, IFNγ, CCL2, CCL3, CCL5, CXCL8, CXCL9) found in both: bronchoalveolar lavage fluid (BALF) as well as in blood [108–113].

Alteration in nonclassical monocytes

An acute phase of SARS-CoV-2 infection is related to an expansion of intermediate monocytes ($CD14^{+}$ $CD16^{+}$), which are a source of pro-inflammatory IL-6 and, as a result, can create a self-perpetuating loop enhancing inflammation [114,115]. This cell type seems to have a negative impact on the disease severity since its highest expansion was observed in patients who required intensive care treatment [116]. Together with the recovery, however, there is a shift in monocyte subpopulation in favor of nonclassical monocytes ($CD14^{lo}$ $CD16^{+}$). These cells, also described as vascular sentinels, caretakers, or patrolling monocytes, are responsible for anti-inflammatory responses and for maintaining vascular homeostasis. They constantly crawl on endothelial surfaces using a lymphocyte function-associated antigen/intracellular adhesion molecule 1 (LFA/ICAM-1)-dependent mechanism and, by means of TLR7,

recognize damaged endothelial cells and contribute to cellular debris removal [117–121]. Many studies performed on mice models showed their protective role in conditions related to endothelial injury. Studies showed an interesting behavior of nonclassical monocytes in atherosclerosis. While classical monocytes are essential to start an atherosclerotic plaque formation, nonclassical cells, although recruited to the site of atherogenesis, remained inside the vessel, patrolling the surface of the endothelium [118,119,122–126]. Additionally, in several studies, nonclassical monocytes-deprived mice fed an atherogenic Western diet showed accelerated plaque formation [127–129]. However, there are reports suggesting the opposite conclusion [130,131], thus, further investigation referring to humans is needed. The protective role of nonclassical monocytes was also suggested in diminishing vasculature damage in kidneys [132,133] and heart [134,135]. An increased number of this specific subpopulation was found in patients with long COVID in comparison to healthy controls [129]. Although they are generally considered anti-inflammatory and vascular protective cells, there is some evidence suggesting their potentially ambiguous role in certain autoimmune-mediated diseases such as lupus erythematosus [136–139], rheumatoid arthritis [140–142] and demyelination in the central nervous system [143,144]. It was also proven that nonclassical monocytes, which migrate to tertiary lymphoid organs (TLOs) and express programmed cell death protein 1 (PD-L1) molecules, may intensify T lymphocytes apoptosis through a PD-1/PD-L1 (programmed cell death ligand 1) mechanism [145]. This may have a negative impact, especially when it accompanies inflammation or oncogenesis.

Cytokine storm in COVID-19

Acute COVID-19 infection is characterized by a significant release of pro-inflammatory cytokines, particularly IL-6. This is not only one of the main pro-inflammatory mediators but also the cytokine responsible for the amplification of the inflammatory process [107,146]. This phenomenon, described as a "cytokine storm," is more common in critically ill COVID-19 patients compared to those with milder symptoms, and is a poor prognostic indicator [147–152]. The ability of SARS-CoV-2 to induce excessive cytokine release is attributable to the interaction of the viral S protein with ACE2 receptors on host macrophages and endothelial cells, which induces the intracellular NOD (nucleotide-binding oligomerization domain)-like receptor family pyrin domain containing 3 (NLRP3) inflammasome [153]. This happens in different ways: for example, the virus impairs the mitochondrial respiratory chain inside cells, thus causing increased production of reactive oxygen species (ROS) [154,155]. The viral N protein causes activation of the complement system, which releases the anaphylatoxins C3a

and C5a and forms the membrane attack complex (MAC) [154]. Additionally, the use of the cellular ACE2 receptor results in an alteration to the synthesis of specific forms of angiotensin [156]. As a result of this activation of the NLRP3 inflammasome, pro-inflammatory cytokines (IL-1β, IL-18) are released, leading to cell injury and death via pyroptosis [157]. An inflammasome is a complex composed of different proteins, which also contain NOD-like receptors for PAMPs. This complex can activate caspase enzymes. The formation of the NLRP3 inflammasome results in the activation of caspase-1 via the proteolysis of IL-1β and IL-18 [158] (see Figure 5). As inflammasomes can detect PAMPs and form larger aggregates, they are an important player in the innate immune response. By inducing inflammation, they are often able to eliminate or at least limit the spread of an invading pathogen [159]. Inflammasome activity needs to be strictly regulated, as uncontrolled activation may facilitate the development of conditions such as Alzheimer's disease, gout, atherosclerosis, and autoinflammatory diseases [160].

The source of the first wave of pro-inflammatory cytokines in COVID-19 is still uncertain. Studies have excluded classical DC, M1, and M2 macrophages as a relevant source of pro-inflammatory mediators in the acute phase of infection with SARS-CoV-2, even though these cells were prone to infection [161–164]. There is evidence that neutrophils can play an important role in generating pro-inflammatory cytokines. Aymonnier et al. [165] reported that during the acute phase of COVID-19, an NLRP3 inflammasome can be effectively activated in neutrophils. The role of IL-1 in inducing the cytokine storm is noteworthy as it may enhance the transcription of its own genes as well as those of other pro-inflammatory cytokines [166–169]. This would amplify the production of inflammatory mediators. Excessive pro-inflammatory cytokine release, particularly IL-1, IL-6, TNF, and IFNs, may result in endothelial and epithelial cell damage. This can cause multiorgan injury, as well as disseminated intravascular coagulation (DIC) and thrombosis [170–178]. A significant number of deaths among COVID-19 patients was due to ARDS, which is also a common feature of infection with SARS-CoV and MERS-CoV [110,179]. This pulmonary involvement can be at least partially explained by the inflammation-related impairment of the membrane between alveoli and capillaries resulting in pulmonary edema and respiratory failure [180,181]. The direct viral injury of the endothelium and myocardium, together with a severe pro-inflammatory shift in the immune system, may also lead to a variety of cardiovascular symptoms and worsen disease outcomes [182,183]. COVID-19 patients were developing direct myocardial damage (ischemia, myocardial infarction, myocarditis) as well as a broad spectrum of arrhythmias with possible

cardiogenic shock [6,184–189]. There are reports indicating renal involvement, including acute kidney injury (AKI) and secondary complications (electrolyte and acid-base imbalance, hypertension), with laboratory findings such as proteinuria and hematuria [10,11,190,191]. The ability of SARS-CoV-2 to infect neurons, astrocytes, and microglia and, as a consequence, to activate inflammasomes in the central nervous system may play a role in neurological complications due to ongoing inflammation and related tissue injury [192,193].

Adaptive immunity in COVID-19

An adaptive immune response with its cytotoxic effect and neutralizing antibody production is required for efficient and complete elimination of the virus. A cytotoxic effect is provided by $CD8^+$ lymphocytes, whereas different subclasses of $CD4^+$ lymphocytes act as enhancers or regulators as well as coordinate the adaptive humoral response against SARS-CoV-2, which leads to the presence of IgM, IgG and IgA class antibodies in patient's serum [194,195]. The research of Tarke et al. [196] performed on COVID-19 convalescents indicated immunodominance of several SARS-CoV-2 proteins (including NSP3, NSP4, NSP12, S, M, N, and ORF3a) which were responsible for the majority of $CD4^+$ and $CD8^+$ T cell responses. In the humoral response, the neutralizing antibodies are directed against the viral S protein (and its RBD) as well as against proteins forming the nucleocapsid [197–202]. It has been indicated by Suthar et al. [203] that the production of neutralizing antibodies in the acute phase of COVID-19 is rapid, with the presence of detectable titers of specific antibodies on average on the 6^{th} day after PCR confirmation of the infection. Additionally, the authors showed quick antibody class switching into IgG with a predominance of IgG1 and IgG3 subclasses in serum [203]. An important role of SARS-CoV-2 specific IgA neutralizing antibodies, present on the mucosal membranes in the respiratory tract, has been shown by Quinti et al. [204], who describe its role in preventing the virus from infecting epithelial cells. Except for interrupting the process of infecting new cells by blocking crucial viral proteins, antibodies also coordinate antibody-dependent cell-mediated cytotoxic effect (ADCC) and cellular phagocytosis (ADCP), as well as complement-dependent cytotoxicity (CDC) [205].

T cells exhaustion and T_{CM} decrease

An antiviral response relies, among others, on the activity of $CD8^+$ T lymphocytes and natural killer (NK) cells, which act as the effector and process-regulating cells. The decreased count of $CD4^+$, $CD8^+$, and NK lymphocytes, and their functional exhaustion related to the disease severity is caused by the inflammation-related acceleration of apoptosis [206–212].

Additionally, the cells continued to present exhaustion features after the patients' recovery and restoration of their amount. This observation may indicate the potential ability of SARS-CoV-2 to weaken the antiviral immune response [106,209,213,214]. Among patients presenting symptoms of long COVID, an elevated number of exhausted $CD4^+$ and $CD8^+$ T cells was described in comparison to the disease convalescents and healthy controls, whereas the number of $CD4^+$ T central memory cells (T_{CM}) was decreased [129,215]. However, the production of the intracellular pro-inflammatory cytokines (IL-2, IL6, and IL-4) was found to be increased in both $CD4^+$ and $CD8^+$ cells of long COVID patients compared to healthy controls and convalescents without persistent symptoms [129].

SARS-CoV-2 variants and immune response

An emergence of virus variants is caused by errors occurring during viral genome replication [216] and due to editing and modification of viral RNA done by the host's own cellular enzymes: apolipoprotein B mRNA editing catalytic polypeptide-like enzyme (APOBEC) and adenosine deaminase RNA specific 1 enzyme (ADAR1) [217] (see Figure 4). APOBEC and ADAR1 are responsible for cytosine-to-uracil and adenosine-to-inosine substitution in viral RNA, respectively [218,219]. They are a part of the host's antiviral innate immune defense system. However, they can be used by a virus for the creation of new variants [220]. This results in nucleotide sequence changes, which sometimes may either facilitate or impede viral replication and transmission, as well as the virus' ability to escape from the host's immune response [221]. SARS-CoV-2 variants may be described as variants of concern (VOC) and variants of interest (VOI) defined by the WHO in the report published in 2021 [222]. According to this document, VOC is defined as a variant with increased transmissibility and virulence, which negatively influences epidemiology and clinical presentation. Another feature defining VOC is the decreased effectiveness of preventive measures, therapy, and vaccination against it. In comparison, VOI is described as a variant that differs phenotypically from the standard isolate and has been found to cause multiple cases of COVID-19 in clusters or worldwide. A viral S protein is a main target for neutralizing antibodies that have been elicited by either SARS-CoV-2 infection or vaccination [199,223,224]. Thus, genetic mutations leading to changes in its structure are responsible for the virus escaping from the humoral immune response. The details of VOCs are presented in Table 3.

Autoimmunity and COVID-19

Infection with SARS-CoV-2 seems to promote the autoimmune phenotype, particularly in genetically predisposed individuals. There are several mechanisms involved in this process, which are typical not only for the coronaviruses but also for other viral infections, including EBV, CMV, rubella virus, hepatitis B and C viruses (HBV, HCV), human immunodeficiency virus (HIV), and human T lymphotropic virus (HTLV) [27,229–232]. One of these mechanisms is the loss of tolerance to self-antigens due to infection-related lymphopenia, a state that consequently facilitates the development of autoimmune processes by enhancing the proliferation of T lymphocytes that recognize self-antigens [233]. Additionally, a decrease in suppressor T lymphocytes ($CD3^+$, $CD8^+$, $CD28^+$) as well as increased regulatory T lymphocytes ($CD3^+$, $CD4^+$, $CD25^+$, $CD45^+$, $CD127^{lo}$) was noted in severe cases of COVID-19 [234]. Another mechanism is hyperinflammation and its consequences of tissue damage with an exposition of new antigens and bystander activation of autoreactive T lymphocytes by activated dendritic cells [235]. Molecular mimicry also plays a significant role in the induction of autoreactivity [236]. Over 30 protein SARS-CoV-2 antigens have been described so far, which share parts of their linear sequence with humans. There is a high probability that antibodies against the viral antigens may interact with the host's self-antigens, thus triggering the autoimmune process [237]. An excessive release of cytokines in the acute phase of COVID-19 leads to the release of neutrophil extracellular traps (NETs) and intracellular enzymes [238]. The released traps are a prominent source of self-antigens (DNA, histones, and other chromatin proteins), while enzymes may induce modifications of the host's proteins, thus turning them into targets for an autoreactive humoral response [239]. The hyperinflammation related to COVID-19 and the use of different drugs, including antibiotics as treatment, leads to dysbiosis or changes to the human gut microbiome. These changes to the microbiome may, in turn, enhance hyperinflammation due to the altered release of gut bacteria-derived mediators, which negatively modulate the host's immune response [240,241]. Studies have found similarities in qualitative and quantitative gut microbiome changes in COVID-19 patients and those suffering from autoimmune diseases (for example, systemic lupus erythematosus [SLE]) compared to healthy controls. For example, decreased microbiome diversity and a shift in dominant bacteria have been reported to have a negative impact on COVID-19 patients by favoring severe forms of the disease [242–247].

The detection of distinct autoantibodies is the most commonly used method to discern an autoimmune phenotype. As SARS-CoV-2 can induce hyperinflammation with subsequent

immune system alterations and tissue damage, followed by a release of self-antigens, the development of various autoantibodies is likely. Studies were performed for the detection of autoantibodies and for establishing their specificity. Rojas et al. [248] tested 100 COVID-19 adult convalescents and 30 control healthy individuals for a broad spectrum of autoantibodies in both the IgG and IgM isotypes. Autoantibodies against thyroglobulin, classic antinuclear antibodies ANA (anti-centromere, anti-La/SS-B, anti-histone, anti-PL7, anti-U1snRNP), and anti-GAD65 (glutamic acid decarboxylase 65) were found, as well autoantibodies against a broad spectrum of IFNs.

Antiphospholipid antibodies in COVID-19

There is evidence that infection with SARS-CoV-2 may result in the production of pro-thrombotic antiphospholipid antibodies (APLAs). Pascolini et al. [249] tested unwell COVID-19 patients and non-COVID-19 patients for the presence of APLAs. This included anti-β2-glycoprotein I (β2-GPI) and anti-cardiolipin (aCL). In the group of patients with COVID-19, the authors reported an incidence of 9.1% and 24.2%, respectively, while in the control group there was only one participant (4%) positive for IgG aCL autoantibodies. Other studies, including COVID-19 patients who were admitted to hospitals due to the severity of the disease, have reported similar findings [250,251]. They all found a higher prevalence of APLAs, particularly lupus anticoagulant (LA) and aCL, in COVID-19 patients compared to control groups or to the prevalence of APLAs in the general population. The APLAs were more often IgA rather than IgM or IgG isotypes. There was a correlation between the presence of APLAs and the severity of COVID-19 disease, although no association was found between APLAs and the risk of thromboembolism. Additionally, Galeano-Valle et al. [252] tested a group of 24 COVID-19 patients with confirmed pulmonary embolism or deep vein thrombosis and found that only two (8%) were weakly positive for aCL and β2-GPI. This finding supports the other findings that APLAs do not contribute to thromboembolic complications during the acute phase of COVID-19. All these studies used small cohorts. However, a meta-analysis including 1,591 adult COVID-19 patients enrolled in 21 studies reported similar findings [251]. The prevalence of APLAs among participants was high (46.8%), with LA being the most frequent antibody (50.7% of all APLAs), while no association between APLA positivity and COVID-19 severity or thromboembolism occurrence was found. Also of interest is the persistence of APLAs following recovery, as according to revised antiphospholipid syndrome (APS) diagnostic guidelines from a conference in Sapporo, Japan only the persistent presence of APLAs can be considered to be a serologic marker of APS [253]. Ghilardi et al. [254] performed

a prospective study including COVID-19 patients admitted to the intensive care unit due to disease severity. At admission, the prevalence of LA, aCL, and β2-GPI was 36.5%, 12.5%, and 15.6% respectively, which is comparable to previously described conclusions. As in previous reports, the authors did not report an association between APLAs and COVID-19 disease severity. All patients positive for APLAs were re-evaluated for antibody presence after at least 12 weeks following recovery. The titers and prevalence of APLAs were generally lower. Another report from Blickstein et al. [255] estimated APLA prevalence in convalescents from mild COVID-19 to be around 6%. Additional research and studies are required to reach a conclusion however, the evidence currently available suggests that APLAs induced by COVID-19 may be transient in most patients. However, some types of APLAs, especially anticardiolipin antibodies, may be expressed after other viral or bacterial infections [256]. Therefore, their presence cannot always be considered to be a marker of possible APS development, making their contribution to the development of long COVID uncertain.

Vasculitis and Kawasaki disease in children

It has been postulated that infection with SARS-CoV-2 may cause some forms of vasculitis as a result of the direct cytotoxic effect of the virus on endothelial cells, as well as an intense immune response and possible involvement of an autoimmune response against vessel walls [257]. Among many case reports describing single individuals with different forms of vasculitis possibly induced by SARS-CoV-2, Kawasaki disease draws special attention. This is an autoimmune-mediated inflammatory disease of medium and small vessels. The highest incidence is in Japan, where there are an estimated 240 cases per 100,000 children under 5 years old [258]. Kawasaki disease has a seasonal pattern in Japan, the highest incidence is in January, June, and July, and the lowest incidence is in October [259]. Differences in prevalence between ethnicities suggest an important role of genetic factors, with the highest prevalence in the Asian population [260]. Viral infections such as EBV, HIV, adenovirus, and parvovirus are known to be triggers of the disease, as they activate the antiviral immune response which engages IFN-dependent pathways and the recruitment of immune cells, thus leading to the development of an inflammatory process within vessels walls [261,262]. An exaggerated immune response, with different cells releasing a broad spectrum of cytokines, may result not only in breaking immune self-tolerance with subsequent autoimmune response against self-antigens but also in weakening the structure of vessel walls. This can lead to the development of an aneurysm, particularly in the coronary arteries in Kawasaki disease [262–266]. Kawasaki disease

is one of the most prominent causes of acquired heart disease in developed countries [267]. Ouldali et al. [268] reported an increase in Kawasaki disease incidence among patients under 18 years hospitalized in a pediatric center in the Paris, France region. There were one to two cases per month in the 15 years before the SARS-CoV-2 pandemic, compared to six cases per month in April 2020 after the peak of COVID-19 incidence in their region. An increase in Kawasaki disease incidence was also reported by Verdoni et al. [269] in the Italian province of Bergamo. There were 19 cases among children during the 5 years prior to the COVID-19 pandemic, compared to 10 cases in 3 months of the first half of 2020, 8 of which had a confirmed SARS-CoV-2 infection. There was not only an increased incidence but also differences in the clinical picture: they reported an older age at the time of diagnosis, a higher rate of coronary artery involvement, and signs of macrophage activation syndrome (MAS). In COVID-19, the symptoms typical of Kawasaki disease are similar to those of hyperinflammation caused by SARS-CoV-2 and are considered to be a part of a bigger clinical picture, where endothelial injury is mediated by the COVID-19-related mechanisms described above. For this reason, there are other names used to describe this phenomenon: multisystem inflammatory syndrome in children (MIS-C) and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS) [263]. This new and potentially life-threatening syndrome may lead to the impairment of vital organs and systems, and it was, therefore, crucial to establish guidelines to help clinicians in the diagnosis and treatment of the condition. Algarni et al. [270] compared previously published guidelines from the WHO, American Centers for Disease Control and Prevention, American Academy of Pediatrics, and American College of Rheumatology in an attempt to unify their recommendations. All guidelines included were consistently specified (below 21 years of age) and fever (documented fever $\geq 38^{o}$C for $\geq$ 24 hours; or subjective fever for $\geq$ 24 hours). As signs of endothelial injury, the following laboratory findings were proposed: elevated C-reactive protein (CRP) and ferritin concentration; increased erythrocyte sedimentation rate (ESR); indicators of myocardial injury and cardiac insufficiency (elevated troponin and brain natriuretic peptide [BNP]), and indicators of thrombin generation with consecutive fibrin formation and fibrinolysis (elevated D-dimer and low platelet count). The authors suggested a 4 to 6-week period between SARS-CoV-2 infection and the beginning of MIS-C symptoms, and the presence of symptoms typical of Kawasaki disease with involvement of more than two organs, as all guidelines were consistent on this. Knowledge and understanding of the mechanisms underlying the development of MIS-C influenced on recommended therapies, including anti-inflammatory and anti-platelet drugs, which reflect the important role of the

inflammatory process and endothelial injury in the pathogenesis of MIS-C. Kawasaki disease related to SARS-CoV-2 infection is more severe compared to classic form of the disease [271].

Guillain-Barré syndrome after COVID-19

Guillain-Barré syndrome (GBS) is an acute post-infectious polyradiculoneuropathy caused by the autoimmune-mediated demyelination of nerves. Clinically, it is characterized by symmetrical ascending motor weakness, paresthesia, reduced or absent deep tendon reflexes, and sensory distortions. There are classification systems of GBS based on clinical characteristics (sensorimotor; paraparetic; pure motor; pure sensory; Miller-Fisher syndrome [MFS]; bilateral facial palsy with paresthesia; pharyngeal-cervical-branchial variant; and Bickerstaff brainstem encephalitis) or results of electromyography (acute inflammatory demyelinating polyneuropathy [AIDP]; acute motor axonal neuropathy [AMAN]; and acute motor-sensory and axonal neuropathy [AMSAN]) [272,273]. The incidence of GBS has been estimated to be between 1.1 and 1.8 per 100,000 per year. There is a lower incidence in children and an increasing incidence with age up to an estimated 3.3 per 100,000 per year after 50 years of age [274]. In two-thirds of cases, GBS is preceded by either a respiratory or gastrointestinal tract infection [275]. The identified infectious triggers include *Campylobacter jejuni* [276], *Haemophilus influenzae*, *Mycoplasma pneumonia*, EBV, CMV, influenza A virus, HSV, HIV, Zika virus [277,278], and hepatitis E virus [279,280]. Both cellular and humoral immune mechanisms are involved in GBS development. The molecular patterns of infectious agents may resemble gangliosides located in the myelin sheath of nerves [281]. Specific anti-ganglioside antibodies corresponding to distinct types of the disease can be found in patients with GBS [275,281]. There have been reports of GBS secondary to MERS-CoV infection [282], a coronavirus that shares genetic homology with SARS-CoV-2. As such, the possible role of SARS-CoV-2 in causing GBS during the pandemic has been taken into account. A systematic review by Aladawi et al. [283] includes 109 individuals with confirmed or suspected SARS-CoV-2 infection prior to the onset of GBS. A meta-analysis by Palaiodimou et al. [284] reported that the incidence of GBS among COVID-19 patients is higher than in non-infected individuals in both contemporary and historical data. The same conclusion was drawn by Filosto et al. [285] based on a comparison of GBS incidence in 14 hospitals in Northern Italy during the years preceding the COVID-19 pandemic with data during the pandemic, from March 2020 to March 2021. Both studies [284,285] concluded that GBS tends to be more severe among COVID-19 patients compared to cases not related to COVID-19, and there is a predominance of demyelinating forms of the disease in COVID-19-related cases. They also

indicated, as did Aladawi et al. [283] and Rahimi [286], that the majority of patients with COVID-19-related GBS do not have anti-ganglioside antibodies. In a systematic review, including 436 patients who developed COVID-19-related GBS, Pimentel et al. described clinical and laboratory findings [287]. The mean age of patients was 61 years. Additionally, a distinct predominance of men (67.2%) was found. The clinical symptoms of COVID-19-related GBS did not differ from the cases of GBS caused by other factors, with general weakness and weakness of both upper and lower limbs being the most prominent symptom. The laboratory findings were also similar to those found in patients with GBS of other causes with typical protein-cytological dissociation in cerebrospinal fluid. The low incidence of anti-ganglioside antibodies in COVID-19-related GBS was also corroborated in this review. Although these antibodies play an important role in the diagnosis of GBS, the mechanism leading to demyelination and impairment of nerves is complex and involves both the complement system and immune cells. T lymphocytes and macrophages can infiltrate nerves from neural veins and reside in very close contact with myelin. Due to molecular mimicry, T lymphocytes activate macrophages after recognizing antigens, which subsequently release pro-inflammatory cytokines. This damages the myelin sheath and causes local increased vascular permeability, thus facilitating the migration of immune cells and intensification of inflammation [288]. The concept of intense macrophage stimulation resulting in a release of pro-inflammatory cytokines during COVID-19 was proposed by McGonagle et al. [289]. This process, together with endothelial injury, may play an important role in damaging nerves and causing the resulting GBS symptoms in COVID-19 patients without the presence of typical autoantibodies [290].

Autoimmunity following vaccination

In 2020, two mRNA vaccines against SARS-CoV-2 were authorized by the U.S. Food and Drug Administration for use in adults [25]. In the following year, permission was extended to younger individuals and to other vaccines based on adenovirus vectors and recombinant proteins [25]. All of these vaccines, whether based on mRNA, replicating or non-replicating viral vectors, or S protein subunits, were designed to induce a humoral response against the SARS-CoV-2 S protein. According to “Our World in Data,” the non-profit organization based in the United Kingdom that gathers information from official sources worldwide, by August 20, 2023, 70.5% (5,620,582,121 individuals) of the world’s population had received at least one dose of a COVID-19 vaccine, and 64.8% (5,168,306,076 individuals) had received the full vaccination protocol [291]. Universal vaccination has played an important role

in reducing the number of severe cases of COVID-19, which require hospitalization and intensive treatment, thus improving survival rates [292,293]. Vaccination against SARS-CoV-2 may, however, lead to the development of various adverse effects. Common side effects include headache, temporary pyrexia, and muscle pain, particularly at the site of injection [294], as well as less common autoimmune-mediated events. In a review by Chen et al. [295], clinical cases included vaccine-induced thrombotic thrombocytopenia (VITT), immune thrombocytopenic purpura (ITP), autoimmune hepatitis (AIH), GBS, IgA nephropathy, rheumatoid arthritis, endocrinological diseases including Grave's disease and diabetes mellitus type 1, and SLE. Most of these conditions were related to mRNA vaccines, although VITT and SLE were also described in patients who had received adenovirus vector-based vaccines. Among the reported VITT cases, antibodies against platelet factor 4 (PF4) were found despite the patients having had no previous exposure to any type of heparin [296,297]. ITP was reported in several distinct cases in adults who had received courses of different vaccines. A report by Simpson et al. [298] described an association between adenovirus-based vaccines and a small increased risk of developing ITP and thromboembolic events. On the contrary, no link between these phenomena and the mRNA-based vaccine had been reported. Jara et al. [299] described reports of adverse autoimmune effects of vaccination against COVID-19 in a review with a total number of 36 participants. Among neurologic, rheumatologic, endocrinologic, and hematologic diseases associated with different vaccines, the most numerous belong to the neurologic domain: GBS (10 participants vaccinated mostly with an adenovirus vector vaccine), optical neuromyelitis (5 participants vaccinated with different vaccines), and transverse myelitis (4 participants vaccinated mostly with an adenovirus vector vaccine) were the most common. Other conditions in individual cases included autoimmune encephalitis, Kawasaki vasculitis, ANCA-associated vasculitis, Graves' disease, thyroiditis, and VITT. There are also reports [300–303] describing cases of adult-onset Still's disease (AOSD) as a complication after vaccination in patients who had not been previously diagnosed with this disease. Additionally, other reports [304,305] describe COVID-19 vaccination as a potential factor causing new flares of AOSD in patients previously diagnosed and treated for this condition. A rich review was given by Camacho-Domínguez et al. [306]. The report included 34 case reports documenting the development of autoimmune-related diseases after exposure to different vaccines, including thrombocytopenia with thrombosis, hemolytic anemia, Graves' disease, vasculitis, hepatitis, thyroiditis, GBS, and arthritis. On the contrary a population-based study by Peng et al. [307] on a vast group of patients reports that vaccination against SARS-CoV-2, with mRNA-based inactivated virus

vaccines may, in fact, play a protective role against the development of several SARS-CoV-2-induced autoimmune conditions like autoimmune arthritis, APS, and immune-mediated thrombocytopenia.

There are a number of mechanisms proposed to explain the development of autoimmune phenotypes following vaccination against COVID-19. Molecular mimicry, for example, is mutual to autoimmunity development after vaccination and an infection with SARS-CoV-2. In both cases, viral antigens presented to an immune system mimic the host's own antigens. A second explanation is the role of adjuvants, which are included in vaccines to strengthen the immune response and make it last longer. There is evidence that some adjuvants may promote the formation of inflammasomes, which in turn facilitate autoimmune mechanisms [308]. The incidence of autoimmune conditions triggered by COVID-19 vaccines is difficult to establish as there are no reliable worldwide registers documenting these phenomena. Furthermore, distinguishing between autoimmunity caused by vaccines and flares of pre-existing autoimmunity is very difficult. Nevertheless, Jara et al. [299] concluded that the incidence of these adverse effects seems to be very low, and they should not discourage people from being vaccinated, as the expected benefits outweigh the risks of these side effects.

Pre-existing autoimmunity and COVID-19

The impact of pre-existing autoimmunity on the severity of COVID-19 has been widely described. However, it is important to make a distinction between testing positive for ANA and suffering from autoimmune systemic disease. In the first case, the presence of ANA is considered to be an autoimmune phenotype, which may potentially lead to the development of autoimmune disease in the future. In contrast, a diagnosis of a particular systemic autoimmune disease is based on serologic patterns and clinical symptoms, followed by immunosuppressive therapy. Additionally, systemic autoimmune diseases can provoke significant damage to vital organs such as the kidneys, lungs, and heart. This may have an influence on COVID-19 severity and outcome. For this reason, this probable influence should be considered separately for these two groups of patients. There are studies describing the incidence of ANA among COVID-19 patients [249,309–315]. They present results that indicate that ANA incidence in COVID-19 individuals is high. Gazzaruso et al. [309] and Chang et al. [310] tested 45 and 47 patients with SARS-CoV-2-related pneumonia for ANA, respectively. There was a high prevalence in the groups (35.6% and 21.3%, respectively). Furthermore,

Gazzaruso et al. [309] estimated the incidence of LA to be 11.1% in the study group. The lack of a control group is a limitation of these findings, therefore, the relationship between ANA presence and COVID-19 outcome requires further investigation. Pascolini et al. [249] performed a study including 33 COVID-19 patients and 25 individuals with pneumonia not related to COVID-19. The authors found that 11% of patients were positive for ANA compared to 8% in the non-COVID-19 group. The prevalence of COVID-19 among adult and pediatric patients with autoimmune systemic diseases who have been treated with immunosuppressive drugs has been described by Michelena et al. [311] in a retrospective study of 959 participants with diagnosed rheumatic diseases, including rheumatoid arthritis, psoriatic arthritis, axial spondylarthritis, juvenile arthritis, and systemic autoimmune diseases, and treated with disease-modifying antirheumatic drugs (DMARDs) including anti-TNF-alpha inhibitors, and IL-1, IL-6, and IL-17 inhibitors. The authors did not report a higher risk of contracting COVID-19 nor of having a more severe disease outcome compared to the general population. There were similar findings in the pediatric population only Filocamo et al. [312] collected data from 123 participants who had been diagnosed with rheumatic diseases and treated with DMARDs. No participant developed a severe form of COVID-19, and there had been no need to withdraw immunosuppressive therapy for any child. However, a definitive conclusion regarding COVID-19 incidence in this specific group could not be made due to the lack of a control group. A prospective study by Haberman et al. [313] included patients with COVID-19 and autoimmune inflammatory diseases treated with DMARD therapy based on anti-cytokine drugs. They found that the use of these medications in the treatment of rheumatic and inflammatory diseases did not worsen the outcomes of COVID-19. Similar conclusions about the safety of DMARDs in COVID-19 were reached by Monti et al. [314] and Favalli et al. [315].

Management of long COVID and future perspectives

At the start of the COVID-19 pandemic the main task was to limit the spread of the virus and deliver treatment for a constantly increasing number of infected individuals. Over time the long-lasting consequences of COVID-19 have become an important factor affecting the lives of many convalescents and therefore there is a need for preventive measures and appropriate treatment to overcome them. According to the review by Koc et al. [53] the approach to that has been divided into three parts: prevention of infection, treatment of acute phase of the disease and finally management of long-COVID symptoms. Prevention is based on a healthy lifestyle (balanced diet, physical activity, good sleeping habits), which is important in reducing co-morbidity and maintaining the proper activity of the immune system, personal protection

(wearing face masks, social distancing, washing hands) to decrease the risk of infection, and finally vaccination to reduce the risk of severe course of the disease and the development of long COVID. The treatment of acute COVID-19 is important in diminishing inflammation and therefore reducing tissue damage and consequently the risk of the development of long-term side effects, including autoimmunity. The third part is focused on individuals who have developed long COVID. All convalescents require clinical assessment to detect those who present conditions related to past SARS-CoV-2 infection. The diagnosis and treatment of long COVID may require the involvement of different specialties to cover as many clinical presentations of long COVID as possible. An approach to establishing treatment based on either identifying symptoms or interfering in the mechanisms responsible for long-COVID development (for example: hypercoagulability, neuroinflammation, development of autoimmunity) is described in the review by Davis et al. [316]. However, the authors emphasize the fact that the described treatment options are based on either trials performed on small groups of patients, or on knowledge and experience gained from treating similar conditions. According to the authors extensive research and education of medical professionals are needed to counteract the long-lasting effects of the COVID-19 pandemic.

Summary

SARS-CoV-2 has spread worldwide and infected hundreds of millions of individuals. It is regularly developing novel mutations resulting in the emergence of new variants with increased virulence and transmissibility, and as such, will likely remain circulating in the human population as a pathogen of great importance. The recent pandemic showed that COVID-19 is not a simple respiratory tract infection but, in many cases, develops into a systemic disease affecting vital organs and causing long-lasting health deterioration. Due to the large number of infected individuals, convalescents, and those at risk of infection, delayed COVID-19-related conditions are presenting challenges to healthcare systems and social care in many countries. Among a broad spectrum of diseases and symptoms reported in convalescents we focused on several specific conditions with autoimmune backgrounds. In all of them there is a connection between the virus and the host's immune response with subsequent hyperinflammation, endothelial injury, and development of autoimmunity. The first two processes are very well described, whereas the relationship between autoimmunity and COVID-19 is still not fully understood. Although the link between other viral infections and autoimmunity has been well studied and described, it is still unclear if SARS-CoV-2 is able to independently induce the development of autoimmunity or whether this process

requires additional triggers. The growing volume of published data delivers descriptions of a broad collection of different autoimmune-related diseases diagnosed in COVID-19 convalescents, but cannot fully explain the contribution of SARS-CoV-2 to the development of autoimmunity. In many cases, there is no available information about patients' health status, especially pre-existing autoimmunity prior to the COVID-19 onset. Furthermore, defining autoimmunity itself is difficult. The group of conditions with an autoimmune background is heterogeneous: there are both systemic and organ-specific autoimmune diseases, and subsequent categories within these two groups include different risk factors, immunological mechanisms, and various diagnostic approaches. Although an increasing amount of data suggests that infection with SARS-CoV-2 leads to the development of a variety of autoantibodies against a broad spectrum of host antigens, the clinical implication of these phenomena still needs to be established. The presence of autoantibodies without clinical symptoms is usually not sufficient to make a diagnosis of an autoimmune disorder. On the other hand, there is a possibility of developing autoimmune conditions due to past SARS-CoV-2 infection without the presence of specific autoantibodies, as was described for COVID-19 convalescents with Guillain-Barré syndrome. Another conundrum is the observation that although COVID-19 generally results in an increased antiphospholipid antibodies prevalence, these antibodies are not necessarily associated with disease severity or risk of thrombosis. This shows that the testing for antiphospholipid autoantibodies alone may not be a sufficient assessment of the risk of thrombosis after recovery from COVID-19. All these factors make attempts at establishing and explaining the true nature of the relationship between COVID-19 and autoimmunity a challenging task. This, however, should be done to provide better healthcare and treatment to huge number of patients with COVID-19-associated autoimmunity in the future. Some lessons may be learned from autoimmunity induced by anti-SARS-CoV-2 vaccination, especially regarding the specific molecular features of the virus, which play an important role in inducing autoimmunity. This may be an important matter in the future as the number of vaccinated people is high and is expected to grow at various rates worldwide. As a result, so is the number of possible autoimmune conditions related to the vaccination. Along with the large number of COVID-19 convalescents, as time goes by, there will be an increase in the number of people affected by complications, including those with backgrounds of autoimmune disease. This will pose new challenges for healthcare systems and professionals around the world.

There are some limitations of our narrative review. First of all our work covers a very broad subject hence some of its aspects are only discussed in general and others may not be addressed. Additionally some of the information that we decided to include in this review comes from case reports and trials on small groups and thus the objectivity of our work is limited. Finally, we are fully aware that our literature search may not be complete due to the ever-increasing amount of scientific literature on the subject.

Conflict of interest

Authors declare no conflict of interest.

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				Respiratory tract [%]			Neurology [%]		Other [%]		
	Number of participants	Median of age [years]	% of women	Dyspnea	Cough	Reduced lung capacity	Concentration disorder	Anosmia dysgeusia	Joints pain	Chronic fatigue	Chest pain
Groff D [43]	250,351	54	44	30	13	30	24	13	10	37	13
Sanchez-Ramirez D [44]	5,323	55	44	32	13	39	NR	NR	38	16	NR
Nalbandian A [45]	3,398	57	47	34	15	NR	NR	13	16	53	13
Michelen M [46]	10,951	56	48	25	NR	26	26	NR	NR	31	NR
Lopez-Leon S. [47]	48,009	52	55	24	19	10	27	23	19	58	16
Garg M. [48]	6,924	52	77	43	20	NR	NR	24	27	66	17
Kessel S. [49]	3,000	46	NR	29	36	NR	NR	16	NR	47	22

Table 1 The incidence of the most frequent long COVID symptoms described in systematic reviews.

There is a broad spectrum of different symptoms related to long COVID. In this table, we gathered the most common symptoms reported by a total of 327,956 COVID-19 convalescents in seven systematic reviews.

NR = not reported

Female sex
Increasing age
Increased BMI, comorbidities related to obesity: diabetes, hypertension
Respiratory tract disease (e.g., bronchial asthma)
Immunosuppression
Severe form of the acute phase of COVID-19 with the presence of more than 5 symptoms
Increased viral load during the acute phase of COVID-19
Persistent SARS-CoV-2 replication
Reactivation of latent viral infections (e.g. EBV, CMV, HSV-1, HHV-6, HHV-7)

Table 2 Risk factors of developing long COVID.

The table summarizes the risk factors of long COVID, described in detail in the text.

BMI = body mass index; CMV = Cytomegalovirus; EBV = Epstein-Barr virus; HHV = Human herpesvirus; HSV = Herpes simplex virus

WHO label	PANGO Lineage [225]	Viral proteins (its domains) with mutations	Effect on transmissibility	Effect on virulence	Vaccines efficacy
Alpha	B.1.1.7	S (RBD and NTD), NSP6, N	increased	increased	decreased
Beta	B.1.351	S (RBD and NTD)	increased	increased	decreased
Delta	B.1.617.2	S (RBD and NTD), ORF1a/b, ORF3, ORF7a, N	increased	increased	decreased
Gamma	P.1	S (RBD and NTD)	increased	increased	decreased
Kappa	B.1.617.1	S (RBD and NTD), ORF1a/b, ORF3, ORF7a, N	increased	unknown	decreased
Omicron	B.1.1.529	S (RBD and NTD)	increased	decreased	decreased

Table 3 The VOCs characteristic.

The mechanism responsible for an increase of transmissibility, virulence, and a decrease of the efficacy of the different types vaccines is the change of the virus' S protein (in its RBD and NTD domains) and additional mutations in ORF, NSP, and N proteins.

NSP = non-structural protein; NTD – N-terminal domain of protein S; ORF = open reading frame protein; RBD = receptor binding domain of protein S; S = spike protein

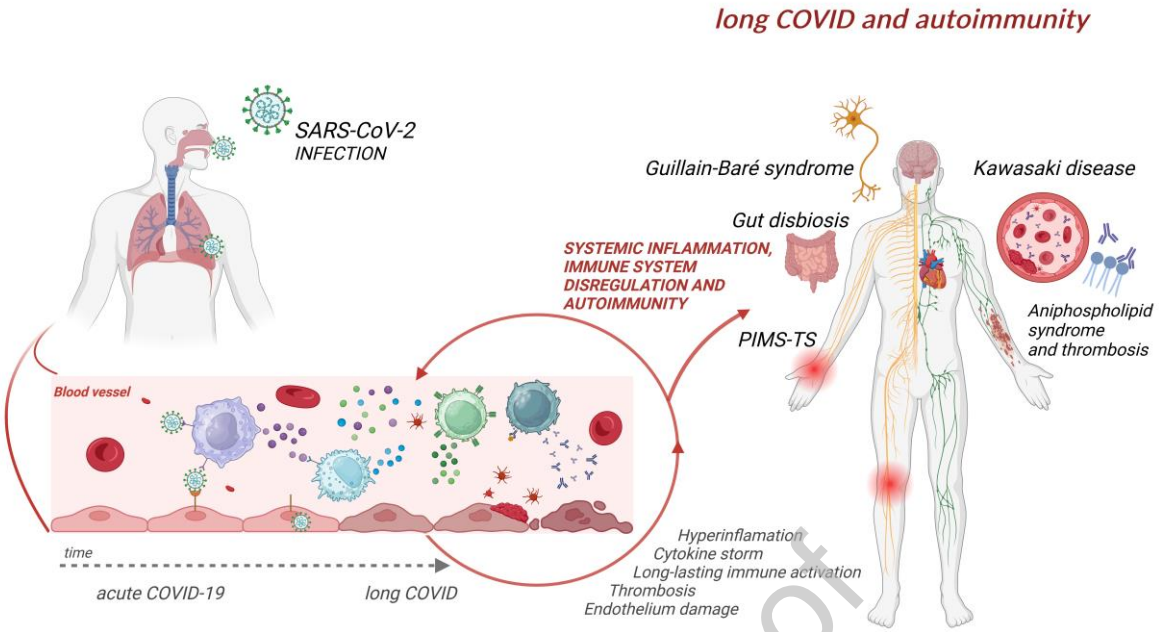

Figure 1 The connection between SARS-CoV-2 infection and the development of autoimmunity (Created with BioRender.com).

SARS-CoV-2 infection may cause long-lasting activation of the immune system and consecutive hyperinflammation and an excessive release of pro-inflammatory cytokines. This, in turn, together with additional factors (the release of self-antigens from damaged tissues, gut dysbiosis and molecular mimicry) facilitates the development of autoimmunity.

PIMS-TS = pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

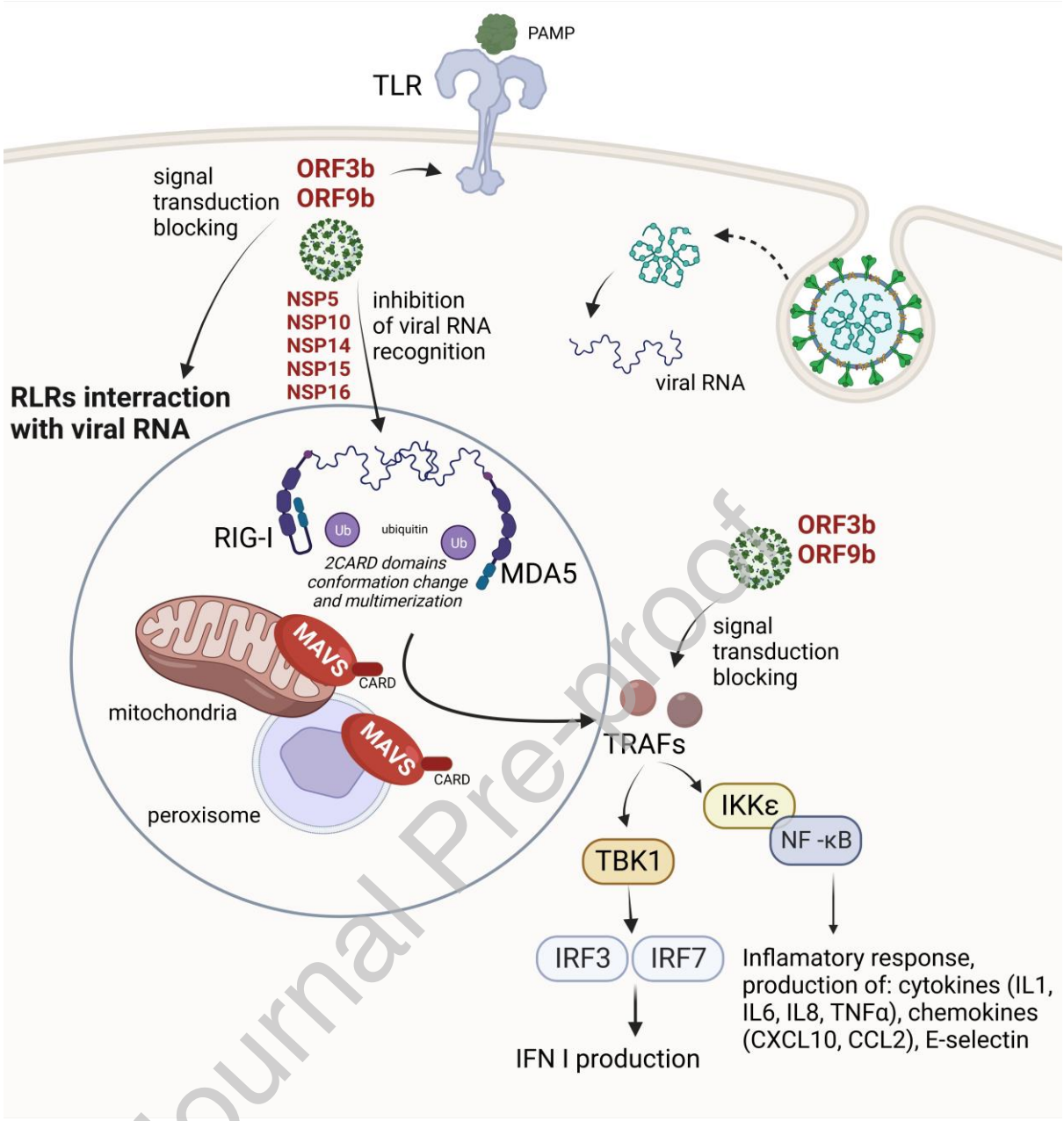

Figure 2 The pathways of the inflammatory response activation triggered by the entry of SARS-CoV-2 to the cells (Created with BioRender.com).

Interaction between SARS-CoV-2 proteins and PRRs triggers the production of IFNs and proinflammatory cytokines. This is an important part of antiviral innate immune response. However, SARS-CoV-2 has the ability to evade this response by blocking its antigens recognition and signal transduction in the cascades (see text for details).

CARD = caspase activation and recruitment domain; CCL2 = monocyte chemoattractant protein 1; CXCL10 = interferon-γ-induced protein of 10 kDa; IKKε = inhibitor of nuclear factor κB (IκB) kinase-ε; IL=interleukin; IFN = interferon; IRF = interferon response factor;

MAVS = mitochondrial antiviral-signaling protein; MDA5 = melanoma-associated differentiation-associated gene 5; NF-kB = nuclear factor kB; NSP = non-structural protein; ORF = open reading frame protein; PAMP = pathogen-associated molecular pattern; PRRs = pattern recognition receptors; RIG-I = retinoic acid-inducible gene-I; RLR = RIG-like receptor; TBK1 = TRAF family member-associated NF-κB activator (TANK)-binding kinase 1; TLR = Toll-like receptor; TNFα = tumor necrosis factor α; TRAF = TNF receptor-associated factor; Ub = ubiquitin

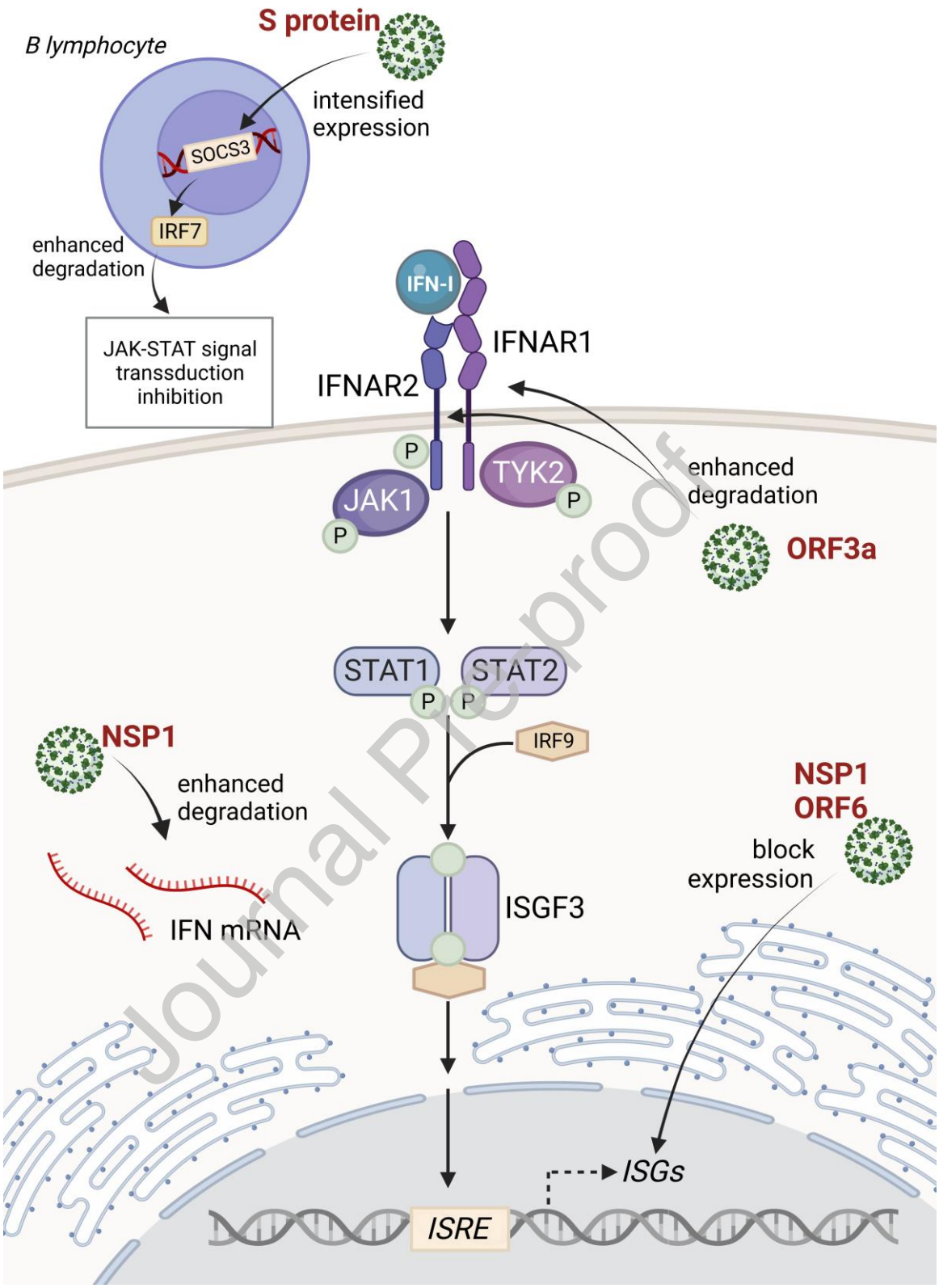

Figure 3 SARS-CoV-2 can block the expression of ISGs and weaken antiviral innate immune response (Created with BioRender.com).

Viral proteins can interfere in the transduction of the signal from IFNARs to the nucleus and directly block the expression of ISGs.

IFN-I = interferon type I; IFNAR = complex of IFN-I and IFN-alpha and beta receptor; IRF7 = interferon response factor 7; ISGF3 = IFN-stimulated growth factor 3; ISGs = interferon-stimulated genes; ISRE = IFN-stimulated response element; JAK1 = Janus kinase 1; mRNA = messenger RNA; NSP = non-structural protein; ORF = open reading frame; SOCS3 = suppressor of cytokine signaling 3; STAT = signal transducer and activator of transcription; TYK2 = Jak tyrosine kinase 2

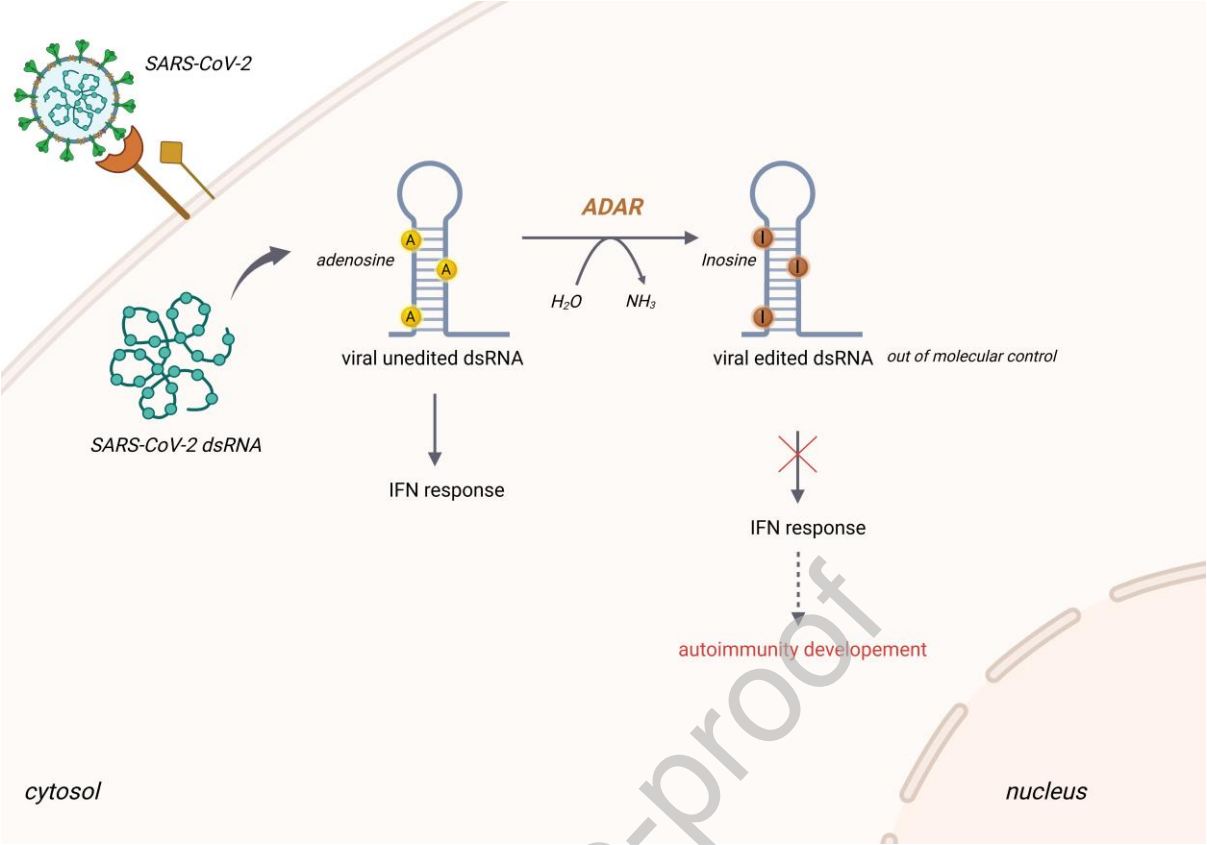

Figure 4 The role of ADAR in changing nucleotide sequence in viral RNA (Created with BioRender.com).

ADAR is responsible for changing adenosine into inosine in viral RNA. This, in turn, weakens the IFN production and facilitates the development of new SARS-CoV-2 variants.

ADAR = adenosine deaminase RNA specific 1 enzyme; dsRNA = double-stranded RNA; IFN = interferon

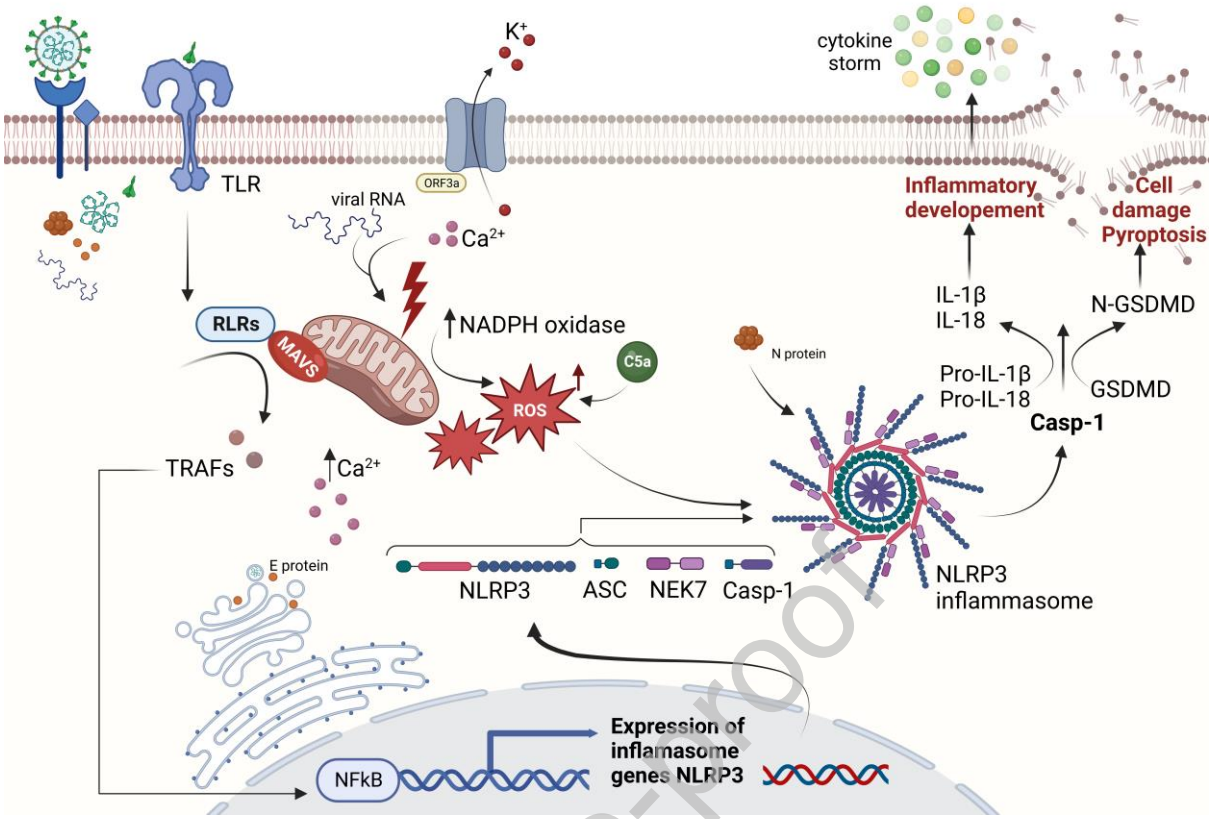

Figure 5 SARS-CoV-2 induces the formation of NLRP3 inflammasome (Created with BioRender.com).

An activation of inflammasome by SARS-CoV-2 in different mechanisms is responsible for an excessive release of proinflammatory cytokines, tissue damage, and consequently autoimmunity development.

ASC = apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; C5a = complement component 5a; Casp-1 = caspase 1; GSDMD = gasdermin D; IL – interleukin; MAVS = mitochondrial antiviral-signaling protein; NADPH = nicotinamide adenine dinucleotide phosphate; NEK7 = NIMA-related kinase 7; NF-kB = nuclear factor kB; N-GSDMD = N-terminal fragment of gasdermin D; NIMA = non-inherited maternal antigen; NLRP3 = NOD-like receptor family pyrin domain containing 3; ORF = open reading frame protein; RLR = RIG-like receptor; ROS = reactive oxygen species; TLR = Toll-like receptor

List of abbreviations:

ACE2	angiotensin-converting enzyme 2 receptor
aCL	anti-cardiolipin antibodies
ADAR1	adenosine deaminase RNA specific 1 enzyme
ADCC	antibody-dependent cell-mediated cytotoxic effect
ADCP	antibody-dependent cellular phagocytosis
AIDP	acute inflammatory demyelinating polyneuropathy
AIH	autoimmune hepatitis
AKI	acute kidney injury
AMAN	acute motor axonal neuropathy
AMSAN	acute motor-sensory and axonal neuropathy
ANA	antinuclear antibodies
AOSD	adult-onset Still's disease
APLAs	antiphospholipid antibodies
APOBEC	apolipoprotein B mRNA editing catalytic polypeptide-like enzyme
APS	antiphospholipid syndrome
ARDS	acute respiratory distress syndrome
BALF	bronchoalveolar lavage fluid
BMI	body mass index
BNP	brain natriuretic peptide
β2-GPI	anti-β2-glycoprotein I antibodies
CDC	complement-dependent cytotoxicity
cDC1	cross-presenting dendritic cells
CMV	Cytomegalovirus
COVID-19	coronavirus disease 2019
CRP	C-reactive protein
DC	dendritic cell
DIC	disseminated intravascular coagulation
DMARDs	disease-modifying antirheumatic drugs
E protein	envelope protein
EBV	Epstein-Barr virus

ESR	erythrocyte sedimentation rate
GAD65	glutamic acid decarboxylase 65
GBS	Guillain-Barré syndrome
HBV	hepatitis B virus
HCV	hepatitis C virus
HHV	Human herpesvirus
HIV	human immunodeficiency virus
HSV	Herpes simplex virus
HTLV	human T lymphotropic virus
ICAM-1	intracellular adhesion molecule 1
IFN	interferon
IFNAR	complex of IFN-I and IFN-alpha and beta receptor
IKKε	inhibitor of nuclear factor κB (IκB) kinase-ε
IL	interleukin
IRF	interferon response factor
ISGF3	IFN-stimulated growth factor 3
ISGs	interferon-stimulated genes
ISRE	IFN-stimulated response element
ITP	immune thrombocytopenic purpura
IκB	inhibitor of nuclear factor κB
JAK1	Janus kinase 1
LA	lupus anticoagulant
LFA	lymphocyte function-associated antigen
M protein	membrane protein
MAC	membrane attack complex
MAVS	mitochondrial antiviral-signaling protein
MDA5	melanoma-associated differentiation-associated gene 5
MERS-CoV	Middle East respiratory syndrome coronavirus
MFS	Miller-Fisher syndrome
MHC	major histocompatibility complex
MIS-C	multisystem inflammatory syndrome in children
mRNA	messenger RNA
NETs	neutrophil extracellular traps

NF-kB	nuclear factor kB
NICE	UK National Institute for Health and Care Excellence
NLRP3	NOD-like receptor family pyrin domain containing 3
NOD	nucleotide-binding oligomerization domain
NSP	non-structural protein
NTD	amino-terminal domain
ORF	open reading frame protein
PAMPs	pathogen-associated molecular patterns
PASC	post-acute sequelae of COVID-19
PD-1	programmed cell death protein 1
PD-L1	programmed cell death ligand 1
PF4	platelet factor 4
PIMS-TS	pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2
PKR	protein kinase receptor
PRRs	pattern recognition receptors
RBD	receptor binding domain
RCGP	UK Royal College of General Practitioners
RLR	RIG-like receptor
ROS	reactive oxygen species
S protein	spike protein
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SIGN	UK Scottish Intercollegiate Guidelines Network
SOCS3	suppressor of cytokine signaling 3
SLE	systemic lupus erythematosus
STAT	signal transducer and activator of transcription
TANK	TRAF family member-associated NF-κB activator
TBK1	TRAF family member-associated NF-κB activator (TANK)-binding kinase 1
TCM	T central memory cells
TLOs	tertiary lymphoid organs
TLR	Toll-like receptor
TMPRSS2	cell surface transmembrane serine protease 2
TNF	tumor necrosis factor
TRAF	TNF receptor-associated factor

TYK2	Jak tyrosine kinase 2
VITT	vaccine-induced thrombotic thrombocytopenia
VOC	variant of concern
VOI	variant of interest
WHO	World Health Organization

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

9. Podsumowanie

W niniejszej pracy, której celem było pogłębienie wiedzy na temat wpływu zakażenia wirusem SARS-CoV-2 na komórki śródbłonka oraz rozwój autoimmunizacji, uwagę skupiono na osobach nieobciążonych dodatkowymi czynnikami ryzyka. Badaniu poddano grupę 294 honorowych dawców krwi, których włączono do badania pomiędzy sierpniem 2021 r. a kwietniem 2022 roku. Uczestników badania podzielono ze względu na obecność przeciwciał przeciwko białku N wirusa SARS-CoV-2: na ozdrowieńców (grupa badana, n = 215) oraz osoby, które nie były do momentu włączenia do badania zakażone (grupa kontrola, n = 79). Minimalny czas od ustąpienia objawów choroby COVID-19 do włączenia do badania wynosił 6 miesięcy. Dane dotyczące wieku i płci chorych nie były dostępne, jednakże podana przez Narodowe Centrum Krwi struktura demograficzna honorowych dawców krwi w roku 2022 wskazuje na znaczącą w tej grupie przewagę osób w wieku 25-44 lat oraz przewagę liczby mężczyzn nad liczbą kobiet [216]. Każdy dawca przeszedł przed donacją krwi ocenę stanu zdrowia, w której choroby stanowiące czynniki ryzyka ciężkiego przebiegu COVID-19 i „long COVID" (a tym samym czynniki ryzyka uszkodzenia śródbłonka i autoimmunizacji) stanowiły podstawę do wykluczenia z dawstwa krwi (m.in. choroby sercowo-naczyniowe, cukrzyca, układowe choroby tkanki łącznej, choroba nowotworowa, immunosupresja, zakażenia).

W pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro-Longed Endothelium Injury", opublikowanej w czasopiśmie „Journal of Clinical Medicine", oceniono nasilenie stanu zapalnego oraz aktywację komórek śródbłonka i uszkodzenie glikokaliksu u ozdrowieńców. W grupie badanej oraz w grupie kontrolnej otrzymano stężenie białka C-reaktywnego poniżej wartości 3,0 mg/l (przy użyciu metody ultraczułej), a pomiędzy tymi dwiema grupami nie zaobserwowano istotnej statystycznie różnicy. Ocena stężenia markerów aktywacji śródbłonka w surowicy wykazała brak istotnych statystycznie różnic w stężeniu ICAM-1 i VCAM-1. Zaobserwowano wyższe stężenie selektyny E w grupie ozdrowieńców – w porównaniu do grupy kontrolnej (p=0,0135). Obserwacja ta jest zbieżna z wynikami autorów, którzy porównywali stężenia selektyny E u chorych na COVID- i wskazywali na dodatnią korelację pomiędzy jej stężeniem a ciężkością choroby [139,141–143]. Zaznaczyć trzeba jednak, że stężenia selektyny E w grupie badanej i grupie kontrolnej były znacząco niższe w porównaniu do stężeń raportowanych we wspomnianych wyżej pracach,

na potrzeby których badano osoby chore na ciężką postać COVID-19. Różnica ta wynika z doboru osób do badania (ozdrowieńcy nieobciążeni dodatkowymi czynnikami ryzyka, którzy przeszli postać choroby łagodną do umiarkowanej *vs* osoby wymagające hospitalizacji i intensywnej terapii). W celu oszacowania uszkodzenia glikokaliksu oceniono w surowicy ozdrowieńców i osób z grupy kontrolnej stężenie syndekanu-1. W pracach oceniających stężenie syndekanu-1 u chorych na COVID-19 wykazano dodatnią korelację pomiędzy jego stężeniem a ciężkością przebiegu choroby i niekorzystnym rokowaniem [173,175,217,218]. Wzrost stężenia cząsteczki w przebiegu ciężkiej postaci COVID-19 związany był z nasilonym przez stan zapalny niszczeniem śródbłonkowego glikokaliksu. W niniejszej pracy stężenie syndekanu-1 w grupie ozdrowieńców okazało się niższe w porównaniu do grupy kontrolnej (p=0,0082). Wynik ten – w połączeniu z obserwowanym u chorych na COVID-19 ścieńczeniem glikokaliksu niezależnym od ciężkości choroby [219] – może być przesłanką za tym, iż przebyte zakażenie i związane z nim uszkodzenie komórek śródbłonka może u osób nieobciążonych dodatkowymi czynnikami powodować zaburzenie struktury glikokaliksu i zmniejszenie jego grubości. Zaznaczyć należy jednak, że stwierdzenie to oparte jest na pojedynczych przesłankach i wymaga potwierdzenia w dalszych badaniach. Podsumowując, otrzymane wyniki pozwoliły na sformułowanie wniosku, iż u osób nieobciążonych dodatkowymi czynnikami ryzyka, po minimum 6 miesiącach od ustąpienia objawów choroby COVID-19, obserwuje się cechy przetrwałej aktywacji i uszkodzenia śródbłonka z prawdopodobnie utrzymującym się zaburzeniem struktury glikokaliksu.

W pracy pt. „Mild-to-moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis", która ukazała się w czasopiśmie „Scandinavian Journal of Immunology", analizie poddano częstość występowania przeciwciał ANA oraz przeciwciał przeciwko β2-glikoproteinie I (β2-GPI, ang. β2-glicoprotein I) w klasie IgG. Obecność przeciwciał ANA bez objawów klinicznych może wskazywać na rozregulowanie odpowiedzi immunologicznej i wytworzenie fenotypu autoimmunizacyjnego. Stan taki nazwany jest bezobjawową autoimmunizacją, którą rozpatruje się jako jeden z pierwszych etapów prowadzących do rozwoju wielu układowych chorób reumatycznych o podłożu autoimmunizacyjnym SARDs (ang. systemic autoimmune rheumatic diseases) [212]. Przeciwciała β2-GPI są związane ze zwiększonym ryzykiem wystąpienia incydentów zakrzepowych

i są włączone do kryteriów klasyfikacyjnych zespołu antyfosfolipidowego (APS, ang. antiphospholipid syndrome) [220]. W niniejszej pracy częstość występowania przeciwciał ANA była niższa w grupie ozdrowieńców w porównaniu do grupy kontrolnej. W grupie ozdrowieńców (n = 215) wykryto przeciwciała ANA u 23 osób (11%), w grupie kontrolnej (n = 79) u 17 osób (22%). W obydwu grupach miano przeciwciał ANA było niskie, z medianą wynoszącą 1:80. Dominującym wzorem fluorescencji był wzór ziarnisty (AC-5) oraz cytoplazmatyczny ziarnisty (AC-20) (nomenklatura wzorów fluorescencji ANA wg międzynarodowego konsensusu ICAP [221]). Badania nad ustaleniem swoistości antygenowej wykazały, iż wykryte autoprzeciwciała skierowane były najczęściej przeciwko antygenowi LEDGF (ang. lens epithelium derived growth factor). W praktyce laboratoryjnej określane są one jako DFS-70 (ang. dense fine speckled 70). W badaniach nie wykazano obecności przeciwciał β2-GPI u żadnego dawcy w grupie badanej ani w grupie kontrolnej. Przeciwciała ANA w niewysokim mianie są obecne u pewnego odsetka zdrowej populacji, co uważane jest za objaw prawidłowej regulacji działania układu odpornościowego [222]. Trwa dyskusja na temat konieczności zrewidowania pojęcia istotnego klinicznie miana przeciwciał ANA, uważa się jednak, iż miano 1:80 ma bardzo niską dodatnią wartość predykcyjną dla wszystkich SARDs (≤4%) [223]. Rozpatrywane w tym kontekście wyniki uzyskane w pracy pozwoliły na sformułowanie wniosku, iż infekcja wirusem SARS-CoV-2 nie spowodowała w czasie nie krótszym niż 6 miesięcy od ustąpienia objawów powstania fenotypu autoimmunizacyjnego u osób zdrowych, nieobciążonych dodatkowymi czynnikami ryzyka. Za wnioskiem tym przemawia także wykazana swoistość autoprzeciwciał (DFS-70), którą wiąże się ze zmniejszonym ryzykiem rozwoju SARDs [224–227]. Wykryte w badaniu pojedyncze przypadki swoistości wobec antygenów swoistych dla wybranych SARDs uznano za nieistotne z klinicznego punktu widzenia ze względu na niskie miano wykrytych autoprzeciwciał. W pracy wykazano brak obecności autoprzeciwciał β2-GPI w klasie IgG u wszystkich uczestników badania.Wynik ten jest zgodny z obserwacjami innych autorów badających chorych na ciężką postać choroby COVID-19 [205,228] i przemawia za tym, iż w grupie osób nieobciążonych dodatkowymi czynnikami ryzyka, w okresie obserwacji, nie istniało zwiększone ryzyko powikłań zakrzepowo-zatorowych spowodowanych autoimmunizacyjną odpowiedzią ustroju.

W pracy poglądowej pt. „Long COVID definition, symptoms, risk factors, epidemiology and autoimmunity – A narrative review”, opublikowanej w czasopiśmie

„American Journal of Medicine Open", zebrano informacje na temat wzajemnego oddziaływania wirusa SARS-CoV-2 z układem odpornościowym człowieka oraz wpływu zakażenia na rozwój fenotypu autoimmunizacyjnego z uwzględnieniem przeciwciał charakterystycznych dla APS. Podsumowane w pracy dane pozwalają wnioskować, iż ciężki przebieg choroby COVID-19 zwiększa prawdopodobieństwo pojawienia się przeciwciał typowych dla APS, które jednak nie zwiększają ryzyka incydentu zakrzepowo-zatorowego. Zaznaczyć należy, że w obecnie obowiązujących kryteriach klasyfikacyjnych APS podkreśla się konieczność trwałej obecności przeciwciał, która zdefiniowana jest jako uzyskany dwukrotnie wynik dodatni w odstępie co najmniej 12 tygodni. Zastrzeżenie to wynika z faktu, iż przeciwciała antyfosfolipidowe mogą pojawiać się jako epifenomen na skutek infekcji bakteryjnej lub wirusowej i stopniowo zanikać po ustąpieniu choroby. Fakt ten może tłumaczyć opisywaną w literaturze wyższą częstość przeciwciał antyfosfolipidowych u chorych na ciężką postać choroby COVID-19 oraz uzyskane w niniejszej pracy ujemne wyniki u wszystkich ozdrowieńców po minimum 6 miesiącach od wyzdrowienia. W opisywanej publikacji zebrano także informacje na temat wpływu zakażenia wirusem SARS-CoV-2 na rozwój odległych powikłań, składających się na „long COVID", które będąc czasem bardzo nozologicznie odległymi od siebie jednostkami chorobowymi, mają w swojej patogenezie elementy wspólne: uszkodzenie śródbłonka oraz procesy autoimmunizacyjne. Ze względu na fakt, iż zakażenie SARS-CoV-2 dotyka nie tylko osób obciążonych czynnikami ryzyka, które zwiększają prawdopodobieństwo wystąpienia ciężkiej postaci choroby COVID-19 oraz jej odległych powikłań, lecz także ogromnej liczby ludzi ogólnie zdrowych, badanie wpływu zakażenia na uszkodzenie śródbłonka i rozwój procesów autoimmunizacyjnych jest nie tylko zasadne, ale nawet konieczne.

Zwiększająca się stale w grupie osób nieobciążonych dodatkowymi czynnikami ryzyka liczba ozdrowieńców oraz pojawiające się nowe warianty wirusa mogą potencjalnie mieć istotne konsekwencje dla zdrowia tej populacji w przyszłości. Z przeprowadzonych w niniejszej pracy badań wynika, iż w okresie nie krótszym niż 6 miesięcy od ustąpienia choroby COVID-19, u ozdrowieńców nieobciążonych dodatkowymi czynnikami ryzyka, utrzymuje się uszkodzenie komórek śródbłonka, nie dochodzi jednak do rozwoju fenotypu autoimmunizacyjnego wyrażonego jako obecność przeciwciał ANA oraz β2-GPI. Pogłębione badania są jednak niezbędne w celu lepszego poznania omawianego tematu.

W prezentowanej pracy należy wskazać kilka ograniczeń. Pierwszym z nich jest brak dokładnych informacji demograficznych o osobach włączonych do badania. Kolejnym jest brak danych na temat masy ciała uczestników, a także wartości ciśnienia tętniczego krwi, jednak biorąc pod uwagę kryteria kwalifikacji dla osób oddających krew i produkty krwiopochodne, można podejrzewać, że w grupie badanej nie było osób z nadciśnieniem tętniczym. Istotnym ograniczeniem pracy jest brak informacji na temat obecności badanych autoprzeciwciał u uczestników przed zachorowaniem na COVID-19 i włączeniem do badania. Przeprowadzenie takich badań wymagałoby zaplanowania eksperymentu medycznego prospektywnie. Czas włączania pacjentów do badania musiałby przypadać na okres przed pandemią, której jednak nie można było przewidzieć. Pomimo przedstawionych tu ograniczeń, wielkość grupy badanej i stan zdrowia jej uczestników przy włączaniu do badania w opinii autora pozwala uznać wyniki za wysoce wiarygodne, a ich opublikowanie w wysoko punktowanych czasopismach naukowych wydaje się potwierdzać tę tezę.

WNIOSKI

1. Nieobciążeni dodatkowymi czynnikami ryzyka ozdrowieńcy, po przebyciu łagodnego do umiarkowanego COVID-19 mogą być narażeni na ryzyko rozwoju chorób związanych z przetrwałym uszkodzeniem śródbłonka, powinni więc być poddawani regularnym badaniom profilaktycznym.
2. Łagodny do umiarkowanego przebieg COVID-19 u osób nieobciążonych dodatkowymi czynnikami ryzyka nie zwiększa ryzyka incydentów zakrzepowo-zatorowych wynikających z aktywacji procesów autoimmunizacyjnych.
3. Zakażenie wirusem SARS-CoV-2 może mieć negatywny wpływ na zdrowie populacji w czasie odległym, dlatego z perspektywy zdrowia publicznego ważne jest propagowanie wiedzy na temat możliwych odległych powikłań zakażenia.

10. Piśmiennictwo

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11. Opinia Komisji Bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

Warszawa, dnia 06 września 2021r.

AKBE/136 / 2021

Dr hab. Olga Ciepiela
Zakład Medycyny Laboratoryjnej
ul. Banacha 1a,
02-097 Warszawa

OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 06 września 2021 r. przyjęła do wiadomości informację na temat badania pt.: „Ocena odległych powikłań COVID-19 wyrażonych obecnością markerów uszkodzenia narządowego u ozdrowieńców." Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21 ust. 1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty(Dz.U. z 2018 r. poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

12. Oświadczenia współautorów publikacji

Warszawa 06 września 2023
(miejscowość, data)

mgr Marcin Śmiarowski
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wykonanie badań, analiza wyników, opracowanie wyników w formie graficznej, przygotowanie manuskryptu, pozyskiwanie funduszy.

Mój udział procentowy w przygotowaniu publikacji określam jako 20 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)


.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

Wiktoria Przyborska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
analiza wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Wiktoria Przyborska
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

Karolina Zemlik
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
analiza wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Karolina Zemlik
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

mgr Milena Małecka-Giełdowska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przygotowanie metodyki, analiza wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Milena Małecka-Giełdowska
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

mgr Aleksandra Leszczyńska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
analiza wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Aleksandra Leszczyńska
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

dr n. med. Marzena Garley
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
analiza wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Marzena Garley
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

prof. dr. hab. n. med. i n. o zdr. Olga Ciepiela
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 Convalescents May Present Pro Longed Endothelium Injury"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
opracowanie założeń projektu, opracowanie graficzne wyników, analiza wyników, przygotowanie manuskryptu, ocena merytoryczna manuskryptu, pozyskiwanie funduszy.

Mój udział procentowy w przygotowaniu publikacji określam jako 15 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

mgr Michalina Lulek
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based throbosis"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wykonywanie badań, analiza wyników.

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 65%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Michalina Lulek
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

mgr Agata Skwarek
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wykonywanie badań, analiza wyników.

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 65%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Agata Skwarek
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 06 września 2023
(miejscowość, data)

mgr Marcin Śmiarowski
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wyszukiwanie informacji naukowej, analiza wyników.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 65%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

mgr Milena Małecka-Giełdowska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wyszukiwanie informacji naukowej, przygotowanie materiału biologicznego, analiza wyników.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 65%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

Milena Małecka-Giełdowska
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 06 września 2023
(miejscowość, data)

prof. dr hab. n. med. i n. o zdr. Olga Ciepiela
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Mild-to-Moderate COVID-19 does not predispose to the development of autoimmune rheumatic diseases or autoimmune-based thrombosis"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przygotowanie merytoryczne projektu, analiza wyników, przygotowanie manuskryptu, pozyskiwanie funduszy.

Mój udział procentowy w przygotowaniu publikacji określam jako 20 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 65%,
(imię i nazwisko kandydata do stopnia)
obejmował on: przygotowanie metodyki, wyszukiwanie informacji naukowej, wykonanie badań, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 12 lutego 2024
(miejscowość, data)

mgr Aleksandra Leszczyńska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Long COVID definition, symptoms, risk factors, epidemiology and autoimmunity – A narrative review"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wyszukiwanie informacji naukowej, analizę wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 10 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55 %,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie metodyki, wyszukiwanie informacji naukowej, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)


(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 12 lutego 2024
(miejscowość, data)

prof. dr hab. n. med. i n. o zdr. Olga Ciepiela
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Long COVID definition, symptoms, risk factors, epidemiology and autoimmunity – A narrative review"

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przygotowanie metodyki, wyszukiwanie informacji naukowej, analizę wyników, przygotowanie manuskryptu.

Mój udział procentowy w przygotowaniu publikacji określam jako 35 %.
Wkład mgr. Pawła Kozłowskiego w powstawanie publikacji określam jako 55 %,
(imię i nazwisko kandydata do stopnia)
obejmował on przygotowanie metodyki, wyszukiwanie informacji naukowej, analizę wyników, przygotowanie manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr. Pawła Kozłowskiego
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

13. Spis rycin

14. Spis tabel