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Ocena odporności humoralnej oraz komórkowej w odpowiedzi na zakażenie SARS-CoV-2 u dzieci z młodzieńczym idiopatycznym zapaleniem stawów

The assessment of humoral and cellular immunity in children with juvenile idiopathic arthritis in response to SARS-CoV-2 infection

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

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Rozprawę doktorską dedykuję mojemu Mężowi – Idanowi, ta praca nie powstałaby bez Twojego nieustającego wsparcia.

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1. Wykaz prac wchodzących w skład cyklu

Rozprawa doktorska stanowi spójny tematycznie zbiór trzech publikacji naukowych w skład których wchodzą dwie prace oryginalne oraz jedna praca poglądowa. Łączna punktacja cyklu wynosi **IF 7,9** oraz **320 punktów MNiSW**.

W skład zbioru wchodzą następujące prace:

- **Kapten K.**, Orczyk K., Smolewska E. Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic. Arch. Immunol. Ther. Exp. 71, 7 (2023). https://doi.org/10.1007/s00005-023-00673-0 **IF 2,9, 140 pkt. MNiSW**

- **Kapten K.**, Orczyk K., Smolewska E. Application of Interferon-γ Release Assay in the Assessment of T-Cell Immunity to SARS-CoV-2 Antigens in the Cohort of Pediatric Patients with Juvenile Idiopathic Arthritis. Children 2024, 11, 736. https://doi.org/10.3390/children11060736 – **IF 2,0, 40 pkt. MNiSW**

- **Kapten K.**, Orczyk K., Maeser A., Smolewska E. Interferon-γ Release Assay in the Assessment of Cellular Immunity—A Single-Centre Experience with mRNA SARS-CoV-2 Vaccine in Patients with Juvenile Idiopathic Arthritis. J. Clin. Med. 2024, 13, 2523. https://doi.org/10.3390/jcm13092523 **IF 3,0, 140 pkt. MNiSW**

2. Wprowadzenie

Badania naukowe dotyczące reumatologii dziecięcej w dużej mierze obejmują pacjentów z najczęstszą artropatią zapalną wieku rozwojowego, jaką jest młodzieńcze idiopatyczne zapalenie stawów (MIZS). [1] Jest to klinicznie heterogenna jednostka chorobowa obejmująca zapalenia stawów o nieznanej etiologii, o początku przed 16 rż. i czasie trwania co najmniej 6 tygodni, po wykluczeniu innych przyczyn obserwowanych objawów. [2] Aktualnie trwają prace nad nową klasyfikacją MIZS, jednak na ten moment obowiązuje klasyfikacja przyjęta przez International League of Associations for Rheumatology (ILAR) w 1997 r. w Durban, zmodyfikowana w 2001 r. w Edmonton, na jej podstawie wyróżniamy 7 podtypów MIZS: MIZS o początku uogólnionym, MIZS o początku skąpostawowym, MIZS o początku wielostawowym bez obecności czynnika reumatoidalnego (RF–), MIZS o początku wielostawowym z obecnością czynnika reumatoidalnego (RF+), łuszczycowe MIZS, MIZS z towarzyszącym zapaleniem przyczepów ścięgien oraz niezróżnicowane MIZS, niespełniające kryteriów żadnego z powyższych zapaleń lub spełniające kryteria więcej niż jednego podtypu MIZS. [3] Wskaźniki zapadalności i chorobowości różnią się znacznie w zależności od rejonu geograficznego, z fragmentarycznych danych dla Polski liczba nowych przypadków oscyluje w granicach 5-6,5/100 000/rok. [4] Etiopatogeneza tej jednostki chorobowej pozostaje nie w pełni poznana, na jej rozwój wpływają złożone interakcje między predysponującymi czynnikami genetycznymi, środowiskowymi i zaburzeniami w układzie immunologicznym, co ma odzwierciedlenie w zróżnicowaniu poszczególnych podtypów MIZS. Mimo braku leczenia przyczynowego, klasyczne leki modyfikujące przebieg choroby (LMPCh), takie jak metotreksat czy sulfasalazyna, pozostają pierwszą i w większości przypadków skuteczną linią leczenia. Dzięki postępowi w rozwoju oraz coraz większej dostępności szeregu leków biologicznych skierowanych przeciwko cytokinom zapalnym, których nadmierna aktywacja leży u podłoża chorób autoimmunizacyjnych, osiągnięto znaczne polepszenie rokowania i szansę na osiągnięcie remisji nawet u pacjentów z ciężkimi postaciami choroby, opornymi na LMPCh. [5] Rozwój celowanych terapii ograniczył również późne powikłania MIZS prowadzące do pogorszenia jakości życia, sprawności fizycznej, a w ciężkich przypadkach nawet do kalectwa. [6] Mimo tego, dzieci z artropatiami zapalnymi pozostają grupą pacjentów wymagającą szczególnej uwagi ze strony lekarzy i opiekunów, szczególnie w sytuacjach dużego narażenia na infekcję, jak podczas zagrożenia epidemiologicznego w trakcie pandemii SARS-CoV-2. Według większości dostępnych badań leki stosowane u pacjentów cierpiących na MIZS, poza glikokortykosteroidami systemowymi, nie powinny mieć istotnego wpływu na częstość występowania, czy cięższy przebieg infekcji górnych dróg oddechowych w tej grupie chorych. [7-9] Nadal jednak wystąpienie jakiejkolwiek infekcji u pacjenta reumatologicznego jest czynnikiem ryzyka zaostrzenia choroby podstawowej, które może prowadzić do konieczności intensyfikacji lub zmiany leczenia, czy nawet hospitalizacji. [10]

Pandemia SARS-CoV-2 od swojego początku na przełomie 2019 i 2020r. stanowiła wyzwanie zarówno dla systemów opieki zdrowotnej na całym świecie, jak i dla naukowców, którzy stanęli przed trudnym zadaniem jak najszybszego znalezienia efektywnych metod diagnostycznych, terapeutycznych oraz prewencyjnych, mających na celu zatrzymanie lub przynajmniej zwolnienie tempa rozprzestrzeniania się epidemii nowego patogenu. Jednym z głównych kierunków badań naukowych w dobie pandemii SARS-CoV-2 była weryfikacja dostępnych metod potwierdzających przebycie zakażenia, w tym metod oceny wytworzonej odporności komórkowej i humoralnej oraz jej wpływu na układ immunologiczny. [11] Osoby dotknięte chorobami autoimmunizacyjnymi stanowią szczególną grupę pacjentów, ze względu na odrębności w funkcjonowaniu ich układu odpornościowego i stanowią tym samym dodatkowe wyzwanie zarówno diagnostyczne, jak i terapeutyczne w przypadku zakażenia SARS-CoV-2. [12] Co więcej, szeroko opisywana w literaturze rola wirusów, a nawet bakterii, takich jak wirusa Epsteina-Barr, wirusa różyczki

czy bakterii Mycoplasma pneumoniae jako czynników spustowych kaskady immunologicznej, prowadzącej do rozwoju chorób autoimmunizacyjnych, czy nowotworów nasuwa pytanie o istnienie podobnych mechanizmów w przypadku infekcji nowym koronawirusem. [13-15] Pomimo licznych doniesień w literaturze dotyczących istotnego rozregulowania układu odpornościowego w reakcji na zakażenie SARS-CoV-2, indukowanie konkretnych chorób autoimmunizacyjnych, czy zaostrzanie istniejących procesów nadal pozostaje przedmiotem badań. [16, 17] Wraz z wprowadzeniem programów szczepień przeciwko COVID-19 pojawiła się potrzeba wprowadzenia wytycznych dla chorych z chorobami autoimmunizacyjnymi, zarówno ze względu na proces chorobowy mogący wpływać na reakcję na szczepienie, jego skuteczność i wystąpienie ewentualnych powikłań, jak i na przyjmowane leki immunomodulujące [18, 19]. Dzieci cierpiące na choroby autoimmunizacyjne, takie jak MIZS, są szczególną podgrupą pacjentów, dla której istnieje ciągła potrzeba doprecyzowania wytycznych postępowania w przypadku zakażenia SARS-CoV-2 i przyjmowania szczepień ochronnych przeciwko zakażeniu koronawirusem. Takie rekomendacje powinny być oparte na jak największej ilości dobrze przeprowadzonych badań naukowych i muszą uwzględniać zróżnicowanie terapii stosowanych przez dzieci cierpiące na MIZS. [20, 21]

Od początku pandemii na szeroką skalę wykonywane były ilościowe badania pozwalające określić stężenia przeciwciał przeciwko antygenowi S1 wirusa SARS-CoV-2 w klasie IgG oraz IgM przy użyciu testów immunoenzymatycznych (ELISA). [22] Badania te mają jednak swoje ograniczenia, istnieje grupa pacjentów, która mimo przebycia zakażenia pozostaje seronegatywna. Są to zarówno pacjenci, którzy przebyli zakażenie bezobjawowo, mieli objawy infekcji, jak i został u nich zdiagnozowany tzw. „long Covid". [23-25] Ze względu na zwiększone ryzyko reinfekcji w porównaniu do pacjentów seropozytywnych, istotna jest ich identyfikacja [24] i możliwość weryfikacji przebycia zakażenia poprzez ocenę odpowiedzi komórkowej. Dzięki od dawna znanej i szeroko wykorzystywanej w diagnostyce zakażenia Mycobacterium tuberculosis metodzie badającej komórkową odpowiedź immunologiczną przy wykorzystaniu testów uwalniania interferonu gamma (IGRA), określających zdolność rozpoznawania antygenów wirusa przez limfocyty T CD4+ po przebytym zakażeniu, mamy możliwość zastosowania badania IGRA również w diagnostyce zakażenia SARS-CoV-2. Dostępna literatura dotycząca oceny odpowiedzi limfocytów T pokazuje skuteczność tej metody zarówno w ocenie wytworzonej odporności po przebytym zakażeniu, jak i po szczepieniu przeciwko SARS-CoV-2. [26-28] Ocena odporności komórkowej może być szczególnie przydatna w przypadku pacjentów obciążonych chorobami autoimmunizacyjnymi, u których zaburzenia immunologiczne oraz zastosowane leczenie mogą wpływać na odpowiedź układu odpornościowego. Mimo pojawiania się pojedynczych badań dotyczących przebiegu zakażenia SARS-CoV-2 u pacjentów z chorobami autoimmunizacyjnymi oraz jego wpływu na chorobę podstawową, ciągle brakuje publikacji dotyczących pacjentów pediatrycznych, w tym uwzględniających zalecenia dotyczące szczepień przeciwko SARS-CoV-2 w tej szczególnej grupie chorych. [29, 30]

3. Cel pracy:

- ocena wytworzonej odpowiedzi immunologicznej, zarówno komórkowej, jak i humoralnej, na zakażenie wirusem SARS-CoV-2 u pacjentów cierpiących na MIZS, w tym u pacjentów, którzy otrzymali szczepienie przeciwko COVID-19, uwzględniając czynniki takie jak: wiek, płeć, różne podtypy choroby podstawowej, czas od rozpoznania choroby podstawowej, trwające zaostrzenie (objawy kliniczne, wartości markerów stanu zapalnego),
- poszukiwanie zależności między stopniem wytworzonej odporności, a stosowanym leczeniem immunomodulującym, ze szczególnym uwzględnieniem pacjentów leczonych biologicznie,
- określenie przydatności testu IGRA jako wiarygodnego narzędzia do oceny wytworzonej odpowiedzi komórkowej po kontakcie z wirusem SARS-CoV-2 i ustalenie znaczenia reaktywności limfocytów T w praktyce klinicznej,
- dalsza obserwacja kliniczna pacjentów uwzględniająca występowanie zakażenia SARS-CoV-2 po zakończeniu badania i ocena jego zależności z wcześniej oznaczonymi wartościami wytworzonej odpowiedzi immunologicznej.

4. Omówienie publikacji wchodzących w skład cyklu

4.1. Publikacja I

Publikacja „Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic" jest pracą poglądową. Jej celem był przegląd istniejącego piśmiennictwa dotyczącego odpowiedzi immunologicznej zarówno na zakażenie SARS-CoV-2, jak i na szczepienie przeciwko nowemu koronawirusowi. Publikacja uwzględniała mechanizmy immunologiczne odpowiadające za wytworzoną odporność wraz z aspektami klinicznymi, w różnych kohortach, w tym pacjentów z zaburzeniami odpowiedzi immunologicznej.

We wstępie pracy odniesiono się do wpływu trzech lat trwania pandemii na bezprecedensową skalę badań naukowych mających na celu zatrzymanie rozprzestrzeniania się wirusa SARS-CoV-2. Zwrócono również uwagę na dwie poprzednie epidemie beta-koronawirusów i możliwe korzyści płynące z dokonanych odkryć i osiągnięć na polu chorób zakaźnych, nie tylko w kontekście ostatniej pandemii, ale również ewentualnych przyszłych zagrożeń epidemiologicznych.

Praca obejmuje mechanizmy odpowiedzi immunologicznej od pierwszej, nieswoistej linii obrony po swoistą odporność humoralną i komórkową. Publikacja miała na celu uzupełnienie istniejącej literatury o kompleksowy przegląd aktualnego stanu wiedzy na temat wirusa SARS-CoV-2, dostępnych metod diagnostycznych oraz skuteczności szczepień ochronnych, uwzględniający pacjentów immunoniekompetentnych.

Publikacja przedstawia ogólną charakterystykę wirusa SARS-CoV-2. Autorzy omówili budowę nowego koronawirusa, która ma bezpośredni wpływ na jego zdolność wiązania się z receptorami komórek człowieka, możliwości replikacji oraz patogenność. Podkreślono powinowactwo SARS-CoV-2 do konkretnych komórek, głównie nabłonka dróg oddechowych, ale również przewodu pokarmowego i miokardium. Omówiono również, jak wirus wiąże się z komórkami gospodarza, podkreślając, że różnorodność mechanizmów i dróg, którymi SARS-CoV-2 infekuje tkanki ludzkie, razem ze zdolnością do częstych mutacji, ma istotny wpływ na szybkość rozprzestrzeniania się zakażenia w populacji.

Przegląd obejmuje również omówienie wrodzonych mechanizmów odpowiedzi immunologicznej, które odgrywają istotną rolę w obronie przed wirusem SARS-CoV-2, a często są wystarczającą barierą ochronną i hamują rozwój zakażenia, jeszcze zanim dołączy do nich odpowiedź swoista. W pracy omówiono poszczególne mechanizmy odporności nieswoistej, dzieląc je na komórkowe oraz humoralne. Podkreślono znaczenie cytokin prozapalnych, takich jak Interleukina-6 (IL-6), której nadmierna produkcja powoduje burzę cytokinową, uszkodzenie komórek śródbłonka, nieszczelność kapilar i w rezultacie prowadzi do rozwoju zespołu niewydolności oddechowej. Omówiono również znaczenie nadmiernej aktywacji makrofagów i wtórnego zespołu hemofagocytarnego jako zagrażającego życiu procesu, prowadzącego do rozległej mikrozakrzepicy płuc. Kolejnym istotnym mechanizmem podjętym w pracy były zaburzenia w wydzielaniu Interferonów (IFN), prowadzące do nieadekwatnej reakcji zapalnej w odpowiedzi na zakażenie SARS-CoV-2. W publikacji podkreślono również rolę neutrofilów odpowiedzialnych za rozwój procesu nazywanego NET-ozą, w którym wytwarzane są zewnątrzkomórkowe sieci neutrofilowe promujące uszkodzenia narządowe oraz koagulopatie. Jeszcze innym procesem obserwowanym w trakcie zakażenia SARS-CoV-2 jest wyczerpanie komórek NK (ang. Natural Killers), jednak ze względu na istnienie sprzecznych informacji dotyczących tych mechanizmów, jest to temat wymagający dalszych badań.

Sekcja pracy dotycząca swoistych mechanizmów odpowiedzi immunologicznej została podzielona na część traktującą o odporności humoralnej oraz na segment poświęcony odpowiedzi komórkowej. Publikacja podkreśla, że jedną z pierwszych i podstawowych metod diagnostyki zakażenia SARS-CoV-2 jest ocena miana wytworzonych przeciwciał, głównie w klasie IgM oraz IgG, ale również IgA. Autorzy omówili wpływ nasilenia odpowiedzi humoralnej na przebieg zakażenia, poruszając problem potencjalnego ułatwiania przedostawania się cząstek patogenu przez przeciwciała, skutkującego cięższą odpowiedzią zapalną. Jednak ze względu na brak udowodnionej korelacji między wysokimi stężeniami przeciwciał a zgonami z powodu COVID-19, zależności te pozostają tematem badań. Ponadto poruszono istotny wpływ dynamiki reakcji organizmu i opóźnionej produkcji przeciwciał, jako kolejnego czynnika negatywnie wpływającego na obronę przed zakażeniem. Omówiono również dostępną literaturę dotyczącą przebiegu infekcji SARS-CoV-2 u pacjentów z niedoborami odporności humoralnej, zwracając uwagę na rolę innych mechanizmów immunologicznych, w tym odpowiedzi komórkowej. Ponadto przedstawiono aktualny stan wiedzy dotyczący czasu utrzymywania się dodatniego miana przeciwciał, i tym samym trwałości odpowiedzi humoralnej oraz istotności serologii w klasie IgA, w kontekście powinowactwa wirusa SARS-CoV-2 do błon śluzowych.

Omawiając znaczenie odporności komórkowej w odpowiedzi na zakażenie nowym koronawirusem, przedstawiono publikacje odnoszące się do poprzednich epidemii koronawirusowych i trwałości odporności wytworzonej przez limfocyty T. Dostępna literatura przedstawiona w aktualnej publikacji pokazuje zależność sprawnej aktywacji limfocytów T CD8+ ze skutecznym usuwaniem wirusa oraz złe rokowanie co do przebiegu zakażenia przy niskiej reaktywności limfocytów T CD8+. Omówiono również możliwe mechanizmy wytwarzania skutecznej odpowiedzi komórkowej przy braku serokonwersji, a również przypadki pacjentów niewytwarzających żadnej wykrywalnej swoistej odpowiedzi na zakażenie SARS-CoV-2. Przedstawiono ponadto wykorzystanie testu IGRA w diagnostyce odpowiedzi T-komórkowej.

Publikacja zawiera także najważniejsze informacje dotyczące szczepień przeciwko zakażeniu SARS-CoV-2, ich bezpieczeństwa oraz skuteczności. Autorzy omówili możliwe działania niepożądane szczepionek przeciwko COVID-19. Ukazano wpływ programów szczepień na zmniejszenie liczby zachorowań, podkreślając jednak trudności płynące z częstych mutacji, którym podlegają koronawirusy i które mogą ograniczać efektywność szczepień ochronnych. Omówiono również skuteczność i długotrwałość wytworzonej w odpowiedzi na szczepienie odporności humoralnej oraz komórkowej. Podsumowując, podkreślono znaczenie odporności hybrydowej (zarówno naturalnej, jak i poszczepiennej) jako najskuteczniejszej i najtrwalszej.

Ostatnim poruszanym w pracy zagadnieniem była odpowiedź na szczepienia u pacjentów z zaburzeniami odporności. Przedstawiono badania wykonane u biorców przeszczepów, ukazujące osłabienie reakcji na szczepienia. Podobne badania wykonano też w grupie pacjentów cierpiących na stwardnienie rozsiane, w którym podkreślano znaczenie otrzymywanego leczenia immunosupresyjnego.

Podsumowując przegląd piśmiennictwa, autorzy znaleźli kilka obszarów wymagających dalszych badań. Jednym z nich była dokładna ocena czasu utrzymywania się odporności, zarówno poszczepiennej, jak i po zakażeniu SARS-CoV-2, szczególnie w kontekście pojawiania się nowych mutacji wirusa i tym samym dostosowanie liczby i czasu podawania dawek przypominających szczepionki. Ponadto, istotnym problemem jest poprawna identyfikacja grup pacjentów mogących wymagać dodatkowej ochrony przed zakażeniem i tym samym dostosowanie do nich programów szczepień. Przedstawienie pełnej złożoności mechanizmów immunologicznych składających się na odporność na zakażenie SARS-CoV-2 jest niemal niemożliwe, autorzy starali się jednak omówić te najbardziej aktualne i o największej wartości klinicznej. Na zakończenie publikacji podkreślono znaczenie

wrodzonych mechanizmów odporności oraz rolę odpowiedzi komórkowej, jako niezwykle istotnej komponenty swoistej odpowiedzi układu odpornościowego, zwracając również uwagę na potrzebę dalszych badań mających na celu ustalenie konkretnych wytycznych dotyczących szczepień przeciwko SARS-CoV-2 u pacjentów z zaburzeniami odporności.

4.1.1. Załącznik I - kopia publikacji I

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REVIEW

Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic

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Abstract
Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and its mechanisms have been thoroughly studied by researchers all over the world with the hope of finding answers that may aid the discovery of new treatment options or effective means of prevention. Still, over 2 years into the pandemic that is an immense burden on health care and economic systems, there seem to be more questions than answers. The character and multitude of immune responses elicited in coronavirus disease 2019 (COVID-19) vary from uncontrollable activation of the inflammatory system, causing extensive tissue damage and consequently leading to severe or even fatal disease, to mild or asymptomatic infections in the majority of patients, resulting in the unpredictability of the current pandemic. The aim of the study was to systematize the available data regarding the immune response to SARS-CoV-2, to provide some clarification among the abundance of the knowledge available. The review contains concise and current information on the most significant immune reactions to COVID-19, including components of both innate and adaptive immunity, with an additional focus on utilizing humoral and cellular responses as effective diagnostic tools. Moreover, the authors discussed the present state of knowledge on SARS-CoV-2 vaccines and their efficacy in cases of immunodeficiency.

Keywords SARS-CoV-2 · Innate immunity · Humoral response · Cellular response · Vaccinations · Immunosuppression

Introduction

Since the end of 2019, when the novel coronavirus emerged in Wuhan, China, there has been an unprecedented incentive of researchers, doctors, and scientists from all over the world attempting to get the full picture of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Soon it became clear that only a complete and comprehensive understanding of the new virus could provide the healthcare systems and governments with the means to not only limit the spread of the disease but also provide necessary data for drugs and vaccine development. Even though, during the course of the pandemic, these aims proved to be more challenging than initially suspected, with the complexity of the coronavirus disease 2019 (COVID-19) and its various clinical presentations (Dong et al. 2020). Nearly three years into the pandemic we ended up with more detailed knowledge of SARS-CoV-2 than possibly any other virus throughout history. As of today, the current pandemic is the third serious epidemic caused by beta-coronavirus since 2002 preceded by severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (Gusev et al. 2022). Since these pathogens bear high resemblance in their capability of infecting multiple cell types in several organ systems (Gu et al. 2005), the discoveries made regarding SARS-CoV-2 may not only benefit us in the current epidemiological situation but also in the years to come, as new challenges may arise for medical professionals.

In this narrative review, the authors attempt to systematize the data on both innate and adaptive immunity to the SARS-CoV-2 infection (Table 1). The scope of this paper is to cover immune mechanisms, from the most indispensable in the first line of defense against pathogens, like the role of combined influx of cytokines, macrophages and interferons (IFNs), which significance was well noted by Silva et al.

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Table 1 Main immunity mechanism in SARS-CoV-2 infection mentioned in the paper

Main immunity mechanisms in SARS-CoV-2 infection		
	Innate immunity	Adaptive immunity
Humoral components	Complement	B-cell produced antibodies mediated immunity
	Coagulation-fibrinolysis cascades	
	Proteins	
	Cytokines: chemokines, ILs, IFNs, TNF	
	Naturally occurring antibodies	
	Macrophages and monocytes	
Cellular components	NK cells	T cells mediated immunity (mainly $CD8^+$)
	Nonspecific leucocytes	IL (IL-6, IL-17) produced by T cells

IL interleukin, *IFN* interferons, *TNF* tumor necrosis factor, *NK* natural killer

(2022), to a more comprehensive insight into both humoral and cellular immunity. As stated by Vályi-Nagy et al. (2022) in a review on adaptive immunity in SARS-CoV-2, only a coordinated and balanced work of both immune systems guarantee overcoming the infection. Additionally, the article explores the immune reactions to the newly developed and widely used vaccines, pointing out the limitations of the sole assessment of antibodies titers and significance of cellular immunity as was signaled in similar reviews concentrating solely on vaccinations to SARS-CoV-2 (Laidlaw and Ellebedy 2022; Sadarangani et al. 2021). The authors aimed to add to the current literature a comprehensive overview of a current state of knowledge regarding SARS-CoV-2 virus with its implications on available testing methods and vaccination efficacy, with an additional focus on immunodeficient patients.

General Characteristics of SARS-CoV-2

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus. It shares main structural and molecular characteristics with other coronaviruses, such as the presence of four structural proteins: S (spike), E (envelope), M (membrane), and N (nucleocapsid) that are critical for binding with cellular receptors, viral replication, and pathogenicity (Huang and Wang 2021). The M glycoprotein is responsible for the formation and stability of the viral envelope and the N protein interacts with the genomic RNA. Communication of the virus with the host cell is facilitated by angiotensin converting enzyme 2 (ACE2) receptors and is mediated by the S protein of the virus. ACE2 receptors are highly expressed on the cell surface of many tissues and organs, mainly the respiratory tract mucosa, but also the myocardial surface and digestive system mucosa (Lei et al. 2021; Rizzo et al. 2020). The S1 region of the S protein is responsible for binding to the host cell receptor, while the S2 region is responsible for the fusion of the viral particles and genome with the host cell (Gadanec et al. 2021). Binding of the viral S glycoprotein to the ACE2 receptor on the cell's surface must be followed by the proteolytic cut at the S1/S2 site of the S glycoprotein by the host protease furin. S protein has to be cleaved by the host factor, transmembrane serine protease 2 (TMPRSS2) at the S2 site in order to expose a fusion peptide which is an essential step for viral fusion with the host cell. These processes occur sequentially, with the cleavage at the S1/S2 site occurring first followed by the subsequent cleavage at S2′. TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 (Bestle et al. 2020). ACE2 presence on the plasma membranes is regulated by A disintegrin and metalloprotease 17 (ADAM17) which promotes the shedding of the protein (Rizzo et al. 2020) (Fig. 1). However, as the newest research shows, ACE2 receptors are not imperative for SARS-CoV-2 infection. Not only does the presence of specific co-receptors enables the virus to infect cells with low ACE2 expression on membranes, but there is also growing evidence of the existence of alternative ACE2 pathways for target cell infection, utilizing immune receptors like neuropilin-1, C-lectin type receptors, Toll-like receptors and the non-immune receptor glucose regulated protein 78 (Amraei et al. 2021; Choudhury and Mukherjee 2020; Gadanec et al. 2021; Gao et al. 2020; Ibrahim et al. 2020). The variety of pathways in which SARS-CoV-2 infects human tissues may explain its high affinity and robust spread through the population.

As a result of replication errors mediated by RNA polymerase and reverse transcriptase enzymes, SARS-CoV-2 as an RNA virus has a substantially higher mutation rate than DNA viruses. Thus, the continuous transmission and the high rate of replication errors of the virus have led to the emergence of many mutations across geographical regions, mainly observed in the receptor-binding domain in the S-glycoprotein. Therefore, the identification of all SARS-CoV-2 variants and specification of their pathophysiology is merely impossible (Gusev et al. 2022; Huang and Wang 2021; Santacroce et al. 2021).

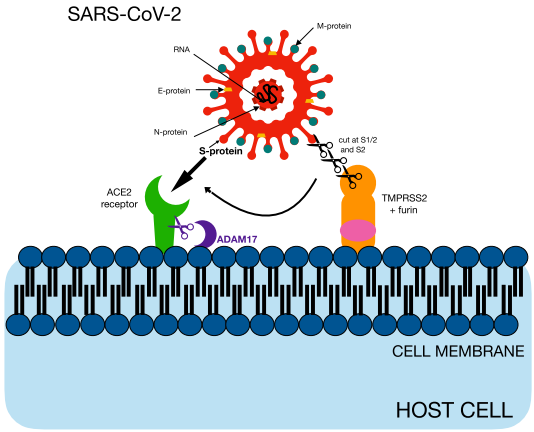

Fig. 1 Structure and cell entry mechanism of SARS-CoV-2. The figure illustrates the architecture of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It is characterized by the presence of four structural proteins: S (spike), E (envelope), M (membrane), and N (nucleocapsid), which interacts with the genomic RNA. Virus communication with the host cell is mediated by S protein and facilitated mainly by angiotensin-converting enzyme 2 (ACE2) receptors, which are highly expressed on the cell membranes of many tissues and organs. The binding of the S glycoprotein to the ACE2 receptor is followed by the proteolytic cut at the S1/S2 site of the S glycoprotein by the host protease furin and cleavage at the S2 site by transmembrane serine protease 2 (TMPRSS2), subsequently. ACE2 presence on the plasma membranes is regulated by the shedding of the protein, promoted by A disintegrin and metalloprotease 17 (ADAM17)

Innate Immunity

As an effective adaptive immune response to SARS-CoV-2 could not be expected to occur until at least 2–3 weeks after the initial exposure, very efficient control of the infection was observed in the vast majority of first-time infected population which shows that the added value of the innate immune response cannot be overlooked. Antiviral innate immunity reaction includes both humoral components, such as complement, coagulation-fibrinolysis cascades, proteins, chemokines, and naturally occurring antibodies, as well as cellular components like natural killer (NK) cells and other nonspecific phagocytic and cytolytic leukocytes (Boechat et al. 2021; Weber 2021).

Interleukin (IL)-6 was identified as one of the first potentially pathogenic factors in the development of acute respiratory distress syndrome (ARDS) in the course of COVID-19. Being a part of both innate and adaptive immunity, IL-6 has a crucial role in the initial response to pathogens and ischemic injury by producing acute phase proteins. Additionally, it directs immune cell differentiation and takes part in immunoglobulin production, by having stimulatory effects on both B cells and T cells, thus, promoting chronic inflammation (Gabay 2006). The uncontrolled production of IL-6 is a common characteristic of autoimmune and autoinflammatory diseases. The introduction of anti-IL-6 receptor monoclonal antibodies has resulted in great therapeutical success in rheumatic diseases (Jordan et al. 2020; Jordan 2021). The excessive IL-6 production in SARS-CoV-2 infection is reported to cause a cytokine storm, leading to endothelial cell damage, capillary leak, and eventually ARDS (Jordan 2021). Additionally, it was proved to be a risk factor for the requirement of mechanical ventilation in COVID-19 patients (Herold et al. 2020). For this reason, preventing excessive IL-6 production or targeting IL-6 receptors, was considered a viable treatment approach that could potentially limit morbidity and mortality in the context of SARS-CoV-2 infection. However, while initial results from observational studies were very promising (Guaraldi et al. 2020; Price et al. 2020; Somers et al. 2021; Wise 2020), they were not always followed by comparable results in clinical trials (García-Lledó et al. 2022; Stone et al. 2020).

Furthermore, patients with a severe course of SARS-CoV-2 infection were found to have significantly elevated serum levels of not only IL-6 but also several other proinflammatory cytokines, including IL-1β, IL-2, IL-8, IL-17, as well as granulocyte and granulocyte–macrophage colony-stimulating factors, IFN-γ-induced protein 10, monocyte chemoattractant protein-1 and tumor necrosis factor. The newest studies stress the role of IL-17, which high levels in both nasal swabs and lung autopsies of patients with fatal SARS-CoV-2 infection were associated with higher levels of proinflammatory cytokines. Hence, creating a positive feedback loop intensifies the impact of IL-17 and causes a possibly self-sustaining process of IL-17 secretion (Sharif-Askari et al. 2022). Furthermore, it can lead to a cytokine storm, which, through a further positive feedback circuit can cause multiple organ failure with extensive tissue damage to the heart, liver, and kidneys, as well as substantial pulmonary pathology with neutrophils and macrophages infiltration, leading to diffused alveolar damage. Autopsies done on patients who died from COVID-19 revealed a high infiltration of macrophages within the areas of bronchopneumonia (Barton et al. 2020). In addition, laboratory parameters found in patients with severe SARS-CoV-2 infection, such as abnormally high levels of C-reactive protein, D-dimers, and ferritin, together with coagulopathy and hypoproteinemia are characteristic features of hyperinflammation known under the umbrella term of macrophage activation syndrome (MAS) or secondary hemophagocytic lymphohistiocytosis (HLH) (Cao 2020; McGonagle et al. 2020). It is a life-threatening condition characterized by pancytopenia, liver failure, hyperferritinemia, coagulopathy, and neurologic symptoms due to uncontrolled proliferation of well-differentiated macrophages, leading to cytokine overproduction and hemophagocytosis. Macrophages which are, essentially, the tissue analogues of monocytic cells, are classified according

to their activation pathways. While both classically (M1), and alternatively polarized macrophages (M2) can suppress SARS-CoV-2 infection, M1 activated mainly by IFN-γ or lipopolysaccharides, and non-activated macrophages (M0) have been found to overstimulate the inflammatory response and lead to lung cells apoptosis. Adversely, M2 activated by IL-3 or IL-13, can be generally characterized as anti-inflammatory agents (Lian et al. 2022; Świdrowska-Jaros et al. 2016). Secondary HLH has been already observed during lethal influenza pandemics and previous SARS and MERS coronavirus outbreaks (Gómez-Rial et al. 2020). The MAS-like disease which may develop in the course of SARS-CoV-2 infection is mainly limited to the lungs and characterized by extensive pulmonary microthrombosis rather than disseminated intravascular coagulation that typically follows, making it difficult to discern from ARDS (McGonagle et al. 2020).

As we already know, monocytes and macrophages fuel the cytokine storm observed in COVID-19 patients, therefore they are one of the key elements leading to ARDS and subsequently poor prognosis (Schiuma et al. 2022). The newest research on the topic indicates, that the replication of SARS-CoV-2 in human lung macrophages activates inflammasomes which initiate an inflammatory cascade, eventually resulting in pyroptosis of macrophages and contributing to the downstream type-I-IFN response. While the inflammasome activation stops the virus replication, the excessive inflammation that occurs through this mechanism alongside the dysregulated IFN response may lead to an over-exuberant inflammatory reaction that we observe in COVID-19 (Sefik et al. 2022).

Neutrophils may as well play an important role in the inflammatory response to SARS-CoV-2, by promoting organ injury and coagulopathy (immunothrombosis) via direct tissue infiltration and formation of neutrophil extracellular traps (NETs) in a process known as NETosis (Middleton et al. 2020; Zuo et al. 2020). Activated through inflammasome pathways, $CD14^+$ monocytes accomplish phagocytosis of dead neutrophils and promote NETosis in the lungs, leading to decreased lymphocyte/neutrophils ratio and therefore, as ample pieces of evidence suggest, a higher risk of death (Roy et al. 2021).

Lymphopenia was found to be one of the hallmarks of SARS-CoV-2 infection, with lower lymphocyte counts, including three main populations, T, B, and NK cells, closely linked to bad prognosis (Antonioli et al. 2020; Moss 2022; Tan et al. 2020; Wang et al. 2020). NK cells are yet another component of the dysregulated immune system that proved to play a pivotal role in the pathogenesis of COVID-19. The evidence shows that SARS-CoV-2 infection might compromise the innate antiviral immunity by exhaustion of NK cells functions (Antonioli et al. 2020; Market et al. 2020). Simultaneously, an increase in NK cells count and a decrease in NK cell receptor (NKG2A) expression were observed (Bortolotti et al. 2020; Market et al. 2020; Yaqinuddin and Kashir 2020). Interestingly, a notable reduction of NK cells activation and their ability to degranulate was observed while no direct effect of the viral proteins on NK cells activation was proved in vivo if the same process was evaluated in lung epithelial cells (Bortolotti et al. 2020). It has also been postulated that SARS-CoV-2 infection can compromise innate immunity even after the patient's recovery.

A delayed and inadequate IFN response to COVID-19 contributed further to the unrestrained viral replication and therefore tissue damage. Upon SARS-CoV-2 infection, signaling cascades are activated, which results in IFN production by epithelial and endothelial cells, alveolar macrophages, NK cells, dendritic cells, and inflammatory monocyte-macrophages. SARS-CoV-2 developed mechanisms to weaken the IFN response, such as proteins that interspersed between the structural genes of the virus, antagonizing or evading the IFN response, contributing to its delayed expression. As IFNs are a wide group of cytokines that are divided into three main groups IFN-I, IFN-II, and IFN-III, a multitude of research was performed attempting to correlate the release of specific IFNs to the severity of SARS-CoV-2 infection. However, with contradicting data on that matter, an inter-patient variability in IFN response should be assumed. A severe course of the disease was found to be accompanied by both prolonged or insufficient IFN-I and IFN-3 production depending on the individual, while other data suggests an upregulation of IFN-II production in critically ill patients (Galani et al. 2021; Hadjadj et al. 2020; Huang et al. 2020; Lowery et al. 2021; Lucas et al. 2020b). What remains evident is that early IFN response can be protective in the acute phase of the infection while a disrupted IFN production is a risk factor for severe COVID-19 (Bastard et al. 2020; Lowery et al. 2021).

Adaptive Immunity

Humoral Immunity

While the humoral response to SARS-CoV-2 has been one of the most thoroughly investigated components of the immunity against the virus, there is still some controversy over its role in the defense against subsequent COVID-19, as well as its place in the assessment of both post-vaccination and post-exposure immunity.

The seroconversion of IgM and IgA antibodies can vary between 4 and 6 or even 3–12 days after the onset of the disease, for IgG antibodies between 5 and 18 days, depending on the source and individual. With a substantially high positive detection rate of SARS-CoV-2 utilizing IgM antibody ELISA assays and the possibility of achieving even

higher sensitivity by combining antibodies detection with polymerase chain reaction, IgM antibodies testing has become a viable accessory in the diagnosis of COVID-19 in its acute phase (Choteau et al. 2022; Guo et al. 2020; Zhao et al. 2020).

Numerous research show early appearance and higher titers of anti-SARS-CoV-2 antibodies in patients suffering from severe forms of the disease compared with milder cases, suggesting not only the relation between the magnitude of IgG response to both viral load and disease severity but also raising the possibility of a pathological role of antibody response (Boechat et al. 2021; Choteau et al. 2022; Lynch et al. 2021; Zhang et al. 2020; Zhao et al. 2020). As has been observed in other infections, there is a potential antibody-dependent enhancement mechanism of SARS-CoV-2, which is characterized as antibody-mediated augmentation of viral entry and initiation of a severe inflammatory response (Arvin et al. 2020; Cao 2020; Lee and Oh 2021). However, Lucas et al. (2021) found that deceased patients did not have overall higher titers of antibodies than recovered individuals, suggesting that in severe cases, there is a robust, but short-lived immune response. Therefore, slow kinetics and delayed production of neutralizing antibody may be the key to the impaired viral control in fatal cases of SARS-CoV-2 (Lucas et al. 2020a).

While studies on COVID-19 patients suffering from primary humoral immunodeficiencies (such as common variable immunodeficiency) confirm that B-cell response plays an important role in the course of the infection (Quinti et al. 2020), a full recovery in patients with immune deficits have been observed. Cases of patients with agammaglobulinemia show that even with a higher risk of developing pneumonia in the course of SARS-CoV-2 (Soresina et al. 2020), mild symptoms and eventually favorable outcome of the disease was attained (Quinti et al. 2020). Similarly, the report on patients with common variable immunodeficiencies suggested that they are at a standard risk for developing severe disease (Cohen et al. 2021). Moreover, studies suggest that in cases of limited humoral responses, the role of T-cell immunity should not be overlooked (Bange et al. 2021).

A further concern is the longevity of humoral immunity and its critical role in protection from pathogen re-infection, since the existence of controversial data concerning the persistence of antibodies titers after SARS-CoV-2 exposure (Isho et al. 2020). While some studies have shown long-lasting and stable levels of neutralizing antibodies (Al-Naamani et al. 2021; Choteau et al. 2022) others have described a rapid decline of anti-SARS-CoV-2 IgG titers in a few months after the disease has resolved. A prompt drop in antibody levels is mostly associated with mild or asymptomatic disease, which cannot be ignored since they account for the majority of COVID-19 cases (Ibarrondo et al. 2020; Long et al. 2020).

While most of the standard serological testing in SARS-CoV-2 focuses on IgM and IgG antibodies, IgA response to coronavirus infection was found to be stronger and more persistent than IgM does (Padoan et al. 2020). According to Sterlin et al. (2021), IgA contributed to virus neutralization to a greater extent than IgG. IgA antibodies measured in serum, saliva, and bronchoalveolar lavage fluid dominated in the early response to the virus. However, whilst specific neutralizing antibodies remained detectable in saliva for a long time, IgA titers in serum decreased notably a month after the onset of symptoms, keeping the long-term efficacy of this first wave response still in question. As we know, IgA is critical in the protection of mucosal surfaces against pathogens. As showed in the previous studies conducted both on influenza and parainfluenza viruses, IgA neutralizing antibodies are not only blocking the attachment of virions to the host epithelial cells but also inhibit intracellular viral replication (Mazanec et al. 1995; Sterlin et al. 2021). Likewise, the IgA response to pathogens has been widely investigated in a vast array of infections, ranging from rotavirus to human immunodeficiency virus (Blutt et al. 2012; Planque et al. 2010). Therefore, specific IgA antibodies may provide effective immunity against SARS-CoV-2 within the respiratory system, in a similar manner, that was already observed in other infectious diseases (Ejemel et al. 2020).

Cell-Mediated Immunity

Despite the initial underestimation, there has been growing evidence indicating a critical role of T-cell adaptive immune response in the control of SARS-CoV-2. Studies on SARS-CoV-1 clearly showed the durability of cellular immunity, which was found to prevail 17 years after the infection, while antibodies titers proved to be considerably short-lived and undetectable after approximately 3–6 years post-exposure (Hellerstein 2020; Le Bert et al. 2020; Ng et al. 2016; Tang et al. 2011; Wu et al. 2007). Evidence gathered during both SARS-CoV-1 and MERS outbreaks confirm the findings regarding the novel coronavirus, suggesting that high antibody titers are associated with impaired clinical outcomes, presumably due to the extensive and uncontrollable inflammation (Hellerstein 2020; Liu et al. 2019; Lynch et al. 2021; Zhang et al. 2020), Additionally, low lymphocyte count, along with high levels of specific cytokines, were one of the first immunological discoveries in moderate and severe SARS-CoV-2 cases, suggesting a missing key element in the disease control, beyond the sole role of the humoral response (Cao 2020; Neidleman et al. 2021; Zhao et al. 2017).

Early and potent cellular response which rises seven days after exposure and peaks around 14th day, more precisely the activation of bystander $CD8^+$ T cells was found to correlate with efficient viral clearance and therefore a mild or asymptomatic disease. Meanwhile, delayed bystander responses,

together with systemic inflammation, were characteristic for subjects that required hospitalization (Bergamaschi et al. 2021; Moss 2022). More severe cases were also observed in delayed humoral response and poor antibody kinetics (Lucas et al. 2020a, 2021). Low $CD8^+$ T cell count was found to be an independent mortality-related risk factor in SARS-CoV-2 infection while higher proportions of specific $CD8^+$ lymphocytes were associated with milder cases, suggesting their role in mitigating the disease severity (Kared et al. 2021; Luo et al. 2020; Neidleman et al. 2021; Peng et al. 2020; Schulien et al. 2021; Sekine et al. 2020). Overall, an effective and sustainable neutralizing antibody protection along with broad and functional $CD8^+$ T-cell response is associated with low inflammation and early recovery. What is more, $CD8^+$ lymphocytes developed during COVID-19 were found to specifically differentiate into stem cells or transitional memory states, which may be crucial in forming durable protection (Kared et al. 2021). Individuals with the asymptomatic disease were found to have a highly balanced secretion of IFN-γ, IL-2, IL-10, and other inflammatory mediators, while a disproportionate secretion of pro-inflammatory cytokines was typical for symptomatic patients (Le Bert et al. 2021; Mathew et al. 2020). Numerous studies showed that $CD4^+$ and $CD8^+$ T cells in patients with a severe course of disease present a disrupted status of activation and function, with high concentrations of genes encoding pro-inflammatory cytokines (de Candia et al. 2021; Xu et al. 2020). Dysregulation of T helper 17 cells was found to enhance the expression of IL-17 in the lungs and thus promote the production of pro-inflammatory cytokines, which elevated levels correlated positively with the severity of COVID-19 symptoms (Sharif-Askari et al. 2022).

Interestingly, T-cell responses have been found in the majority of convalescent patients, including those with undetectable serology (Schulien et al. 2021; Sekine et al. 2020). In most human infections the presence of antibodies against a pathogen is typically regarded as a "gold standard" of immune response since antibodies are known to provide protection in the first stage of infection. However, an important concept of "cellular sensitization without seroconversion" in SARS-CoV-2 infection has emerged. Growing evidence shows that there are individuals who fail to produce virus-specific antibodies, regardless of significant exposure. However, they do develop a specific T-cell response, suggesting that a cell-mediated immune response has the potential of eliminating an infection before it fully develops (Moss 2022; Sekine et al. 2020). In addition, early studies on seronegative unexposed to SARS-CoV-2 individuals have shown that S and N-protein-specific T-cells can be found in healthy humans with no contact with the COVID-19 virus as a result of cross-reactivity with human endemic coronaviruses that cause the common cold (Le Bert et al. 2020; Pia 2020). Another theory that underpins the value of T-cell response and requires a more thorough investigation is the cell-to-cell transmission potential of SARS-CoV-2, an evasion mechanism that can explain the limitation of antibodies and complement inhibition in sera (Zeng et al. 2022).

Even though the majority of individuals develop immune response after exposure to SARS-CoV-2, whether it is humoral, cellular or both, there are cases of patients that failed to mount any immunological reaction, despite the lack of any immunodeficiencies and regardless of the severity of the disease. Therefore, providing a plausible cause for re-infections observed in some patients (Mohn et al. 2022; Nielsen et al. 2021).

While major progress has been done regarding the development of immunoassays detecting antibody responses to SARS-CoV-2, the means of assessing cell-mediated immune response are considerably less explored. However, since the IFN-γ release assay (IGRA), a well-tested and widely used tool for diagnosis of latent *Mycobacterium tuberculosis* infection, has been applied to measure IFN-γ release by antigen-specific T cells that have been developed during SARS-CoV-2 infection, the evaluation of cellular immune response has become more accessible for clinical use. As the research shows, IGRA can detect a cellular immune response to SARS-CoV-2 and therefore distinguish between convalescents and uninfected healthy blood donors with high sensitivity and accuracy. Thus, serology alone may not be sufficient in assessing the individual's protection after infection, while IGRA can serve as an invaluable diagnostic tool in the current and possibly future epidemics, especially in the assessment of patients with mild infections (Fernández-González et al. 2021; Murugesan et al. 2021; Wyllie et al. 2020).

Vaccinations

Since the end of 2020 and the beginning of pro-vaccination campaigns all over the world, an abundance of research has emerged trying not only to assess their effectiveness but also their safety, taking into consideration the very limited timescale in which the vaccines were produced. As for today, approximately 2 years since the launch of large-scale vaccination programs, first further-reaching conclusions can be drawn. Most studies confirm the statistical reliability of the relatively high safety of currently used COVID-19 vaccines, concluding that the risk of the administration is commensurate and acceptable. The majority of adverse effects that have been observed after the first dose of the vaccine included symptoms like fever, headache and joint pain. Less frequently, cases of myocarditis or pericarditis have been reported. Among the more serious reactions that have occurred, death cases were not related to an anaphylactic or allergic reaction to the vaccine but to an aggravation

of pre-existing chronic diseases (Castells et al. 2009; Fazio et al. 2022; Prakash 2022). The Pfizer/BioNTech and Moderna vaccines, both being based on the new technology of mRNA molecules, have been available on the market since the very beginning of the mass inoculation campaigns. Their efficacy in disease prevention of 95–87.5% and protection from a severe course of the infection on the level of 94.5–100%, showed the indisputable benefit of inoculation for an individual (Mascellino et al. 2021). Population wise, as far as the effectiveness of rapid, mass vaccinations to SARS-CoV-2 is concerned, the high rate of inoculation within the community was proved to be a competent tool to curb the spread of the virus (Paetzold et al. 2022). However, the decline of vaccine efficacy in preventing viral transmission over time, is one of the leading concerns regarding vaccine protection in the long term (Daković et al. 2022; Thomas et al. 2021). Moreover, the occurrence of new variants of SARS-CoV-2, such as Delta, is yet another factor that was reported to lower the vaccine-induced immunity against SARS-CoV-2 infection (Keehner et al. 2021; Nanduri et al. 2021; Rivasi et al. 2022).

According to the data available, the vast majority of individuals develop a serological response to vaccine, which was found to be stronger in the younger population and previously infected subjects. The levels of immune response to one dose of vaccination in pre-exposed subjects were comparable to two doses of vaccinations in naïve individuals (Bradley et al. 2021; Dan et al. 2021; Krammer et al. 2021; Prendecki et al. 2021; Sariol et al. 2021; Visci et al. 2022). The rapid decrease in antibody titers that were observed after SARS-CoV-2 infection, has been reported after vaccination, likewise (Visci et al. 2022). Nonetheless, it remains to be determined to what extent the antibody titers correspond with the efficacy of one's immune response. Sariol et al. (2021) state that a decline in specific antibody titers is not commensurate to the neutralizing activity, suggesting the relevance of functional neutralizing antibody testing. Heterogeneous findings are assessing the potency of immune response elicited by vaccination in comparison with natural infection, with some reports of lower titers of antibodies after mRNA vaccine and others stating that vaccinations elicit stronger and broader immune responses than those after natural SARS-CoV-2 exposure (Altawalah 2021; Greaney et al. 2021; Richardson et al. 2022; Sariol et al. 2021). Furthermore, just as the role of cellular response to COVID-19 infection has been recently acknowledged and thoroughly investigated, so has the T-cell reaction to the vaccination. As confirmed with IGRA testing, which is slowly becoming a more popular and most importantly reliable method of quantifying T-cell response after SARS-CoV-2 infection or vaccination, cellular response is elicited in nearly all vaccinated individuals, including immunosuppressed patients, adding value to the sole serological testing (Fernández-González et al. 2021; Huzly et al. 2022; Sahin et al. 2020). Moreover, researchers state that T-cell responses are not affected by the mutations of SARS-CoV-2 and thus can provide effective protection against the new variants of the virus that escape humoral responses (Geers et al. 2021; Tarke et al. 2022).

As we approach the end of the third year of the pandemic, with possibly half of the global population infected with SARS-CoV-2 by early 2022, the promising data regarding the natural protection against the virus has emerged. Fatality rates of SARS-CoV-2 infection in 2022 have been very low, when compared to the previous years. It is yet to be determined to which extent: whether it was due to less immunogenic potential of Omicron and its subvariants that caused milder course of the disease (Pilz and Ioannidis 2022), or it was the result of a growing virus resistance after vaccination and prior infections, or both. Nonetheless, the epidemiological data confirms, that previous infections generated immunity against any SARS-CoV-2 infection that relatively waned over time (Isho et al. 2020). However, the protection against a severe course of disease remained strong. As studies show, the natural immunity may offer greater or at least equal defense against COVID-19 infection compared to one after the mRNA vaccine. The hybrid immunity, which is the combination of a previous infection and a vaccination still seems to confer the greatest protection against the disease, the benefit–cost ratio of future vaccination recommendations, especially regarding numerous vaccine boosters, should be carefully evaluated (Flacco et al. 2022; Pilz et al. 2022).

Immune Response to Vaccine Antigens in Immunocompromised Patients

Immune response after SARS-CoV-2 vaccination among immunosuppressed patients has been of great concern to healthcare professionals in a variety of medical fields. Not only are the immunocompromised individuals a considerable number of patients, but they are also the frailest and are most likely to have a severe course of COVID-19 infection. Hence, the benefit of an effective vaccination in this cohort of patients is invaluable. Among the liver transplantation recipients that were under immunosuppressive regimens, almost half of the patients failed to develop a neither humoral nor a cellular response to the vaccines. The adjustment of drug regimens, together with the administration of calcineurin inhibitors have provided the best immune response potential, in contrast to multi-drug immunosuppressive regimens containing mycophenolate mofetil, and possibly implementing additional vaccine boosters may lead to better post-vaccination results (D'Offizi et al. 2022; Ruether et al. 2022). Correspondingly, studies performed on a group of immunocompromised kidney transplant patients

showed a weakened immune response. Nonetheless, a number of seronegative patients did develop T-cell responses, suggesting the development of partial immunity, that may limit the infection, if not prevent it (Zhang et al. 2022). As far as immunomodulatory medications are concerned, studies conducted among patients with multiple sclerosis emphasized the radical difference in developing an immune reaction to vaccination depending on drug regimens, from intact to strongly reduced immune responses. Interestingly, as far as B-cell depleting therapies are concerned, the advantage of T-cell response testing over serological assays has been proved within the scope of different studies (Bock et al. 2022; Marty et al. 2022).

Limitations and Gaps Found by the Study

While immense progress was made in our understanding of COVID-19 infection since the beginning of the pandemic, some knowledge gaps remain to be filled. The authors revealed some visible limitations in the accuracy of the assessment of protective immunity waning and duration, whether on previous infection or vaccination. Identifying the individuals in need of extra protection against the virus is still not clear and needs further investigation. Another, and possibly the most crucial issue at the moment, is the lack of a consensus on SARS-CoV-2 vaccination programs, specifically with the constant occurrence of new variants of the virus and the benefit–cost ratio of recommending multiple vaccine boosters within the population. Possibly, more research concerning individual needs for vaccinations could be valuable at the moment.

Closing Remarks

As presenting the full complexity of the SARS-CoV-2 mechanisms and the immune responses it elicits is nearly impossible, the authors tried to outline the most current and clinically valuable data. As far as the COVID-19 infection is concerned, the value of innate immunity components must not be overlooked, as they directly affect the outcome of the disease. However, one of the main points of focus of this study was to present the role of cellular immunity as it has proved to play a vital role in the immune defense against the novel coronavirus. New available methods of marking T-cell responses, such as applying IGRA testing, should be thoroughly explored as an effective tool in the assessment of immunity post-SARS-CoV-2 infection and vaccination. Additionally, with the development of new vaccines against SARS-CoV-2, there is a great need for elaboration on effective vaccination programs in immunocompromised patients.

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Declarations

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References

Al-Naamani K, Al-Jahdhami I, Al-Tamtami W et al (2021) Prevalence and persistence of SARS-CoV-2 antibodies among healthcare workers in Oman. J Infect Public Health 4:1578–1584. https://doi.org/10.1016/j.jiph.2021.09.00

Altawalah H (2021) Antibody responses to natural SARS-CoV-2 infection or after COVID-19 vaccination. Vaccines 9:910. https://doi.org/10.3390/vaccines9080910

Amraei R, Yin W, Napoleon MA et al (2021) CD209L/L-SIGN and CD209/DC-SIGN act as receptors for SARS-CoV-2. ACS Cent Sci 7:1156–1165. https://doi.org/10.1021/acscentsci.0c01537

Antonioli L, Fornai M, Pellegrini C et al (2020) NKG2A and COVID-19: another brick in the wall. Cell Mol Immunol 17:672–674. https://doi.org/10.1038/s41423-020-0450-7

Arvin AM, Fink K, Schmid MA et al (2020) A perspective on potential antibody-dependent enhancement of SARS-CoV-2. Nature 584:353–363. https://doi.org/10.1038/s41586-020-2538-8

Bange EM, Han NA, Wileyto P et al (2021) CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. Nat Med 27:1280–1289. https://doi.org/10.1038/s41591-021-01386-7

Barton LM, Duval EJ, Stroberg E (2020) COVID-19 autopsies, Oklahoma, USA. Am J Clin Pathol 153:725–733. https://doi.org/10.1093/ajcp/aqaa062. (**Erratum in: Am J Clin Pathol 153:852**)

Bastard P, Rosen LB, Zhang Q et al (2020) Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 370:eabd4585. https://doi.org/10.1126/science.abd4585

Bergamaschi L, Mescia F, Turner L et al (2021) Longitudinal analysis reveals that delayed bystander CD8+ T cell activation and early immune pathology distinguish severe COVID-19 from mild

disease. Immunity 54:1257-1275.e8. https://doi.org/10.1016/j.immuni.2021.05.010
Bestle D, Heindl MR, Limburg H et al (2020) TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. Life Sci Alliance 3:e202000786. https://doi.org/10.26508/lsa.202000786
Blutt SE, Miller AD, Salmon SL et al (2012) IgA is important for clearance and critical for protection from rotavirus infection. Mucosal Immunol 5:712–719. https://doi.org/10.1038/mi.2012.51
Bock H, Juretzek T, Handreka R et al (2022) Humoral and cellular immune responses to SARS CoV-2 vaccination in people with multiple sclerosis and NMOSD patients receiving immunomodulatory treatments. Mult Scler Relat Disord 59:103554. https://doi.org/10.1016/j.msard.2022.103554
Boechat JL, Chora I, Morais A et al (2021) The immune response to SARS-CoV-2 and COVID-19 immunopathology—current perspectives. Pulmonology 27:423–437. https://doi.org/10.1016/j.pulmoe.2021.03.008
Bortolotti D, Gentili V, Rizzo S et al (2020) SARS-CoV-2 Spike 1 protein controls natural killer cell activation via the HLA-E/NKG2A pathway. Cells 9:1975. https://doi.org/10.3390/cells9091975
Bradley T, Grundberg E, Selvarangan R et al (2021) Antibody responses after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med 384:1959–1961. https://doi.org/10.1056/NEJMc2102051
Cao X (2020) COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol 20:269–270. https://doi.org/10.1038/s41577-020-0308-3
Castells M, Demoly P, Tanno LK (2009) Anaphylaxis and COVID-19 vaccines. Rev Fr Allergol 61:8S30-8S35. https://doi.org/10.1016/S1877-0320(21)00439-5
Choteau M, Scohy A, Messe S et al (2022) Development of SARS-CoV-2 humoral response including neutralizing antibodies is not sufficient to protect patients against fatal infection. Sci Rep 12:2077. https://doi.org/10.1038/s41598-022-06038-5
Choudhury A, Mukherjee S (2020) In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. J Med Virol 92:2105–2113. https://doi.org/10.1002/jmv.25987
Cohen B, Rubinstein R, Gans MD et al (2021) COVID-19 infection in 10 common variable immunodeficiency patients in New York City. J Allergy Clin Immunol Pract 9:504-507.e1. https://doi.org/10.1016/j.jaip.2020.11.006
Daković Rode O, Bodulić K, Zember S et al (2022) Decline of anti-SARS-CoV-2 IgG antibody levels 6 months after complete BNT162b2 vaccination in healthcare workers to levels observed following the first vaccine dose. Vaccines 10:153. https://doi.org/10.3390/vaccines10020153
Dan JM, Mateus J, Kato Y et al (2021) Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 371:eabf4063. https://doi.org/10.1126/science.abf4063
de Candia P, Prattichizzo F, Garavelli S et al (2021) T cells: warriors of SARS-CoV-2 infection. Trends Immunol 42:18–30. https://doi.org/10.1016/j.it.2020.11.002
D'Offizi G, Agrati C, Visco-Comandini U et al (2022) Coordinated cellular and humoral immune responses after two-dose SARS-CoV-2 mRNA vaccination in liver transplant recipients. Liver Int 42:180–186. https://doi.org/10.1111/liv.15089
Dong X, Cao YY, Lu XX et al (2020) Eleven faces of coronavirus disease 2019. Allergy 75:1699–1709. https://doi.org/10.1111/all.14289
Ejemel M, Li Q, Hou S et al (2020) A cross-reactive human IgA monoclonal antibody blocks SARS-CoV-2 spike-ACE2 interaction. Nat Commun 11:4198. https://doi.org/10.1038/s41467-020-18058-8
Fazio ND, Delogu G, Bertozzi G et al (2022) SARS-CoV-2 vaccination adverse events trend in Italy: a retrospective interpretation of the last year (December 2020–September 2021). Vaccines 10:216. https://doi.org/10.3390/vaccines10020216
Fernández-González M, Agulló V, Padilla S et al (2021) Clinical performance of a standardized SARS-CoV-2 interferon-γ release assay for simple detection of T-cell responses after infection or vaccination. Clin Infect Dis 10:ciab1021. https://doi.org/10.1093/cid/ciab1021
Flacco ME, Acuti Martellucci C, Baccolini V et al (2022) Risk of reinfection and disease after SARS-CoV-2 primary infection: meta-analysis. Eur J Clin Investig 52:e13845. https://doi.org/10.1111/eci.13845
Gabay C (2006) Interleukin-6 and chronic inflammation. Arthritis Res Ther 8(Suppl 2):S3. https://doi.org/10.1186/ar1917
Gadanec LK, McSweeney KR, Qaradakhi T et al (2021) Can SARS-CoV-2 virus use multiple receptors to enter host cells? Int J Mol Sci 22:992. https://doi.org/10.3390/ijms22030992
Galani IE, Rovina N, Lampropoulou V et al (2021) Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. Nat Immunol 22:32–40. https://doi.org/10.1038/s41590-020-00840-x
Gao C, Zeng J, Jia N et al (2020) SARS-CoV-2 spike protein interacts with multiple innate immune receptors. BioRxiv. https://doi.org/10.1101/2020.07.29.227462(Preprint)
García-Lledó A, Gómez-Pavón J, González Del Castillo J et al (2022) Pharmacological treatment of COVID-19: an opinion paper. Rev Esp Quimioter 35:115–130. https://doi.org/10.37201/req/158.2021
Geers D, Shamier MC, Bogers S et al (2021) SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol 6:eabj1750. https://doi.org/10.1126/sciimmunol.abj1750
Gómez-Rial J, Rivero-Calle I, Salas A et al (2020) Role of monocytes/macrophages in COVID-19 pathogenesis: implications for therapy. Infect Drug Resist 13:2485–2493. https://doi.org/10.2147/IDR.S258639
Greaney AJ, Loes AN, Gentles LE et al (2021) Antibodies elicited by mRNA-1273 vaccination bind more broadly to the receptor binding domain than do those from SARS-CoV-2 infection. Sci Transl Med 13:eabi9915. https://doi.org/10.1126/scitranslmed.abi9915
Gu J, Gong E, Zhang B et al (2005) Multiple organ infection and the pathogenesis of SARS. J Exp Med 202:415–424. https://doi.org/10.1084/jem.20050828
Guaraldi G, Meschiari M, Cozzi-Lepri A et al (2020) Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol 2:e474–e484. https://doi.org/10.1016/S2665-9913(20)30173-9
Guo L, Ren L, Yang S et al (2020) Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 71:778–785. https://doi.org/10.1093/cid/ciaa310
Gusev E, Sarapultsev A, Solomatina L et al (2022) SARS-CoV-2-specific immune response and the pathogenesis of COVID-19. Int J Mol Sci 23:1716. https://doi.org/10.3390/ijms23031716
Hadjadj J, Yatim N, Barnabei L et al (2020) Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 369:718–724. https://doi.org/10.1126/science.abc6027
Hellerstein M (2020) What are the roles of antibodies versus a durable, high quality T-cell response in protective immunity against SARS-CoV-2? Vaccine X 6:100076. https://doi.org/10.1016/j.jvacx.2020.100076
Herold T, Jurinovic V, Arnreich C et al (2020) Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in

COVID-19. J Allergy Clin Immunol 146:128-136.e4. https://doi.org/10.1016/j.jaci.2020.05.008
Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5
Huang SW, Wang SF (2021) SARS-CoV-2 entry related viral and host genetic variations: implications on COVID-19 severity, immune escape, and infectivity. Int J Mol Sci 22:3060. https://doi.org/10.3390/ijms22063060
Huzly D, Panning M, Smely F et al (2022) Accuracy and real life performance of a novel interferon-γ release assay for the detection of SARS-CoV-2 specific T cell response. J Clin Virol 148:105098. https://doi.org/10.1016/j.jcv.2022.105098
Ibarrondo FJ, Fulcher JA, Goodman-Meza D et al (2020) Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild COVID-19. N Engl J Med 383:1085–1087. https://doi.org/10.1056/NEJMc2025179
Ibrahim IM, Abdelmalek DH, Elshahat ME et al (2020) COVID-19 spike-host cell receptor GRP78 binding site prediction. J Infect 80:554–562. https://doi.org/10.1016/j.jinf.2020.02.026
Isho B, Abe KT, Zuo M et al (2020) Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol 5:eabe5511. https://doi.org/10.1126/sciimmunol.abe5511
Jordan SC (2021) Innate and adaptive immune responses to SARS-CoV-2 in humans: relevance to acquired immunity and vaccine responses. Clin Exp Immunol 204:310–320. https://doi.org/10.1111/cei.13582
Jordan SC, Ammerman N, Choi J et al (2020) Interleukin-6: an important mediator of allograft injury. Transplantation 104:2497–2506. https://doi.org/10.1097/TP.0000000000003249
Kared H, Redd AD, Bloch EM et al (2021) SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. J Clin Investig 131:e145476. https://doi.org/10.1172/JCI145476
Keehner J, Horton LE, Binkin NJ et al (2021) Resurgence of SARS-CoV-2 infection in a highly vaccinated health system workforce. N Engl J Med 385:1330–1332. https://doi.org/10.1056/NEJMc2112981
Krammer F, Srivastava K, Alshammary H et al (2021) Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med 384:1372–1374. https://doi.org/10.1056/NEJMc2101667
Laidlaw BJ, Ellebedy AH (2022) The germinal centre B cell response to SARS-CoV-2. Nat Rev Immunol 22:7–18. https://doi.org/10.1038/s41577-021-00657-1
Le Bert N, Tan AT, Kunasegaran K et al (2020) SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature 584:457–462. https://doi.org/10.1038/s41586-020-2550-z
Le Bert N, Clapham HE, Tan AT et al (2021) Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. J Exp Med 218:e20202617. https://doi.org/10.1084/jem.20202617
Lee E, Oh JE (2021) Humoral immunity against SARS-CoV-2 and the impact on COVID-19 pathogenesis. Mol Cells 44:392–400. https://doi.org/10.14348/molcells.2021.0075
Lei HY, Ding YH, Nie K et al (2021) Potential effects of SARS-CoV-2 on the gastrointestinal tract and liver. Biomed Pharmacother 133:111064. https://doi.org/10.1016/j.biopha.2020.111064
Lian Q, Zhang K, Zhang Z et al (2022) Differential effects of macrophage subtypes on SARS-CoV-2 infection in a human pluripotent stem cell-derived model. Nat Commun 13:2028. https://doi.org/10.1038/s41467-022-29731-5
Liu L, Wei Q, Lin Q et al (2019) Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight 4:e123158. https://doi.org/10.1172/jci.insight.123158
Long QX, Tang XJ, Shi QL et al (2020) Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 26:1200–1204. https://doi.org/10.1038/s41591-020-0965-6
Lowery SA, Sariol A, Perlman S (2021) Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. Cell Host Microbe 29:1052–1062. https://doi.org/10.1016/j.chom.2021.05.004
Lucas C, Klein J, Sundaram M et al (2020a) Kinetics of antibody responses dictate COVID-19 outcome. medRxiv. https://doi.org/10.1101/2020.12.18.20248331
Lucas C, Wong P, Klein J et al (2020b) Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 584:463–469. https://doi.org/10.1038/s41586-020-2588-y
Lucas C, Klein J, Sundaram ME et al (2021) Delayed production of neutralizing antibodies correlates with fatal COVID-19. Nat Med 27:1178–1186. https://doi.org/10.1038/s41591-021-01355-0
Luo M, Liu J, Jiang W et al (2020) IL-6 and CD8+ T cell counts combined are an early predictor of in-hospital mortality of patients with COVID-19. JCI Insight 5:e139024. https://doi.org/10.1172/jci.insight.139024
Lynch KL, Whitman JD, Lacanienta NP et al (2021) Magnitude and kinetics of anti-severe acute respiratory syndrome coronavirus 2 antibody responses and their relationship to disease severity. Clin Infect Dis 72:301–308. https://doi.org/10.1093/cid/ciaa979
Market M, Angka L, Martel AB et al (2020) Flattening the COVID-19 curve with natural killer cell based immunotherapies. Front Immunol 11:1512. https://doi.org/10.3389/fimmu.2020.01512
Marty PK, Van Keulen VP, Erskine CL et al (2022) Antigen specific humoral and cellular immunity following SARS-CoV-2 vaccination in ANCA-associated vasculitis patients receiving B-cell depleting therapy. Front Immunol 13:834981. https://doi.org/10.3389/fimmu.2022.834981
Mascellino MT, Di Timoteo F, De Angelis M, Oliva A (2021) Overview of the main anti-SARS-CoV-2 vaccines: mechanism of action, efficacy and safety. Infect Drug Resist 14:3459–3476. https://doi.org/10.2147/IDR.S315727
Mathew D, Giles JR, Baxter AE et al (2020) Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science 369:eabc8511. https://doi.org/10.1126/science.abc8511
Mazanec MB, Coudret CL, Fletcher DR (1995) Intracellular neutralization of influenza virus by immunoglobulin A anti-hemagglutinin monoclonal antibodies. J Virol 69:1339–1343. https://doi.org/10.1128/JVI.69.2.1339-1343.1995
McGonagle D, Sharif K, O'Regan A et al (2020) The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun Rev 19:102537. https://doi.org/10.1016/j.autrev.2020.102537
Middleton EA, He XY, Denorme F et al (2020) Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 136:1169–1179. https://doi.org/10.1182/blood.2020007008
Mohn KG, Bredholt G, Zhou F et al (2022) Durable T-cellular and humoral responses in SARS-CoV-2 hospitalized and community patients. PLoS ONE 17:e0261979. https://doi.org/10.1371/journal.pone.0261979
Moss P (2022) The T cell immune response against SARS-CoV-2. Nat Immunol 23:186–193. https://doi.org/10.1038/s41590-021-01122-w
Murugesan K, Jagannathan P, Pham TD et al (2021) Interferon-γ release assay for accurate detection of severe acute respiratory syndrome coronavirus 2 T-cell response. Clin Infect Dis 73:e3130–e3132. https://doi.org/10.1093/cid/ciaa1537

Nanduri S, Pilishvili T, Derado G et al (2021) Effectiveness of Pfizer-BioNTech and Moderna vaccines in preventing SARS-CoV-2 infection among nursing home residents before and during widespread circulation of the SARS-CoV-2 B.1.617.2 (delta) variant—National Healthcare Safety Network, March 1-August 1, 2021. MMWR Morb Mortal Wkly Rep 70:1163–1166. https://doi.org/10.15585/mmwr.mm7034e3

Neidleman J, Luo X, George AF et al (2021) Distinctive features of SARS-CoV-2-specific T cells predict recovery from severe COVID-19. Cell Rep 36:109414. https://doi.org/10.1016/j.celrep.2021.109414

Ng OW, Chia A, Tan AT et al (2016) Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. Vaccine 34:2008–2014. https://doi.org/10.1016/j.vaccine.2016.02.063

Nielsen SS, Vibholm LK, Monrad I et al (2021) SARS-CoV-2 elicits robust adaptive immune responses regardless of disease severity. EBioMedicine 68:103410. https://doi.org/10.1016/j.ebiom.2021.103410

Padoan A, Sciacovelli L, Basso D et al (2020) IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study. Clin Chim Acta 507:164–166. https://doi.org/10.1016/j.cca.2020.04.026

Paetzold J, Kimpel J, Bates K et al (2022) Impacts of rapid mass vaccination against SARS-CoV-2 in an early variant of concern hotspot. Nat Commun 13:612. https://doi.org/10.1038/s41467-022-28233-8

Peng Y, Mentzer AJ, Liu G et al (2020) Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol 21:1336–1345. https://doi.org/10.1038/s41590-020-0782-6

Pia L (2020) SARS-CoV-2-reactive T cells in patients and healthy donors. Nat Rev Immunol 20:353. https://doi.org/10.1038/s41577-020-0333-2

Pilz S, Ioannidis JPA (2022) Does natural and hybrid immunity obviate the need for frequent vaccine boosters against SARS-CoV-2 in the endemic phase? Eur J Clin Investig 11:e13906. https://doi.org/10.1111/eci.13906

Pilz S, Theiler-Schwetz V, Trummer C et al (2022) SARS-CoV-2 reinfections: overview of efficacy and duration of natural and hybrid immunity. Environ Res 209:112911. https://doi.org/10.1016/j.envres.2022.112911

Planque S, Salas M, Mitsuda Y et al (2010) Neutralization of genetically diverse HIV-1 strains by IgA antibodies to the gp120-CD4-binding site from long-term survivors of HIV infection. AIDS 24:875–884. https://doi.org/10.1097/QAD.0b013e3283376e88. Erratum.In:AIDS201125:1345-1346

Prakash S (2022) Development of COVID 19 vaccine: a summarized review on global trials, efficacy, and effectiveness on variants. Diabetes Metab Syndr 16:102482. https://doi.org/10.1016/j.dsx.2022.102482

Prendecki M, Clarke C, Brown J et al (2021) Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. Lancet 397:1178–1181. https://doi.org/10.1016/S0140-6736(21)00502-X

Price CC, Altice FL, Shyr Y et al (2020) Tocilizumab treatment for cytokine release syndrome in hospitalized patients with coronavirus disease 2019: survival and clinical outcomes. Chest 158:1397–1408. https://doi.org/10.1016/j.chest.2020.06.006

Quinti I, Lougaris V, Milito C et al (2020) A possible role for B cells in COVID-19? Lesson from patients with agammaglobulinemia. J Allergy Clin Immunol 146:211-213.e4. https://doi.org/10.1016/j.jaci.2020.04.013

Richardson JR, Götz R, Mayr V et al (2022) SARS-CoV2 wild type and mutant specific humoral and T cell immunity is superior after vaccination than after natural infection. PLoS ONE 17:e0266701. https://doi.org/10.1371/journal.pone.0266701

Rivasi G, Bulgaresi M, Bandinelli C et al (2022) Long-term effects of SARS-CoV-2 vaccination in the nursing home setting. J Am Geriatr Soc 70:1336–1341. https://doi.org/10.1111/jgs.17773

Rizzo P, Vieceli Dalla Sega F, Fortini F et al (2020) COVID-19 in the heart and the lungs: could we "Notch" the inflammatory storm? Basic Res Cardiol 115:31. https://doi.org/10.1007/s00395-020-0791-5

Roy RK, Sharma U, Wasson MK et al (2021) Macrophage activation syndrome and COVID 19: impact of MAPK driven immune-epigenetic programming by SARS-Cov-2. Front Immunol 12:763313. https://doi.org/10.3389/fimmu.2021.763313

Ruether DF, Schaub GM, Duengelhoef PM et al (2022) SARS-CoV-2-specific humoral and T-cell immune response after second vaccination in liver cirrhosis and transplant patients. Clin Gastroenterol Hepatol 20:162-172.e9. https://doi.org/10.1016/j.cgh.2021.09.003

Sadarangani M, Marchant A, Kollmann TR (2021) Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol 21:475–484. https://doi.org/10.1038/s41577-021-00578-z

Sahin U, Muik A, Derhovanessian E et al (2020) COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 586:594–599. https://doi.org/10.1038/s41586-020-2814-7

Santacroce L, Charitos IA, Carretta DM et al (2021) The human coronaviruses (HCoVs) and the molecular mechanisms of SARS-CoV-2 infection. J Mol Med 99:93–106. https://doi.org/10.1007/s00109-020-02012-8

Sariol CAA, Pantoja P, Serrano-Collazo C et al (2021) Function is more reliable than quantity to follow up the humoral response to the receptor-binding domain of SARS-CoV-2-spike protein after natural infection or COVID-19 vaccination. Viruses 13:1972. https://doi.org/10.3390/v13101972

Schiuma G, Beltrami S, Bortolotti D et al (2022) Innate immune response in SARS-CoV-2 infection. Microorganisms 10:501. https://doi.org/10.3390/microorganisms10030501

Schulien I, Kemming J, Oberhardt V et al (2021) Characterization of pre-existing and induced SARS-CoV-2-specific CD8+ T cells. Nat Med 27:78–85. https://doi.org/10.1038/s41591-020-01143-2

Sefik E, Qu R, Junqueira C et al (2022) Inflammasome activation in infected macrophages drives COVID-19 pathology. Nature 606:585–593. https://doi.org/10.1038/s41586-022-04802-1

Sekine T, Perez-Potti A, Rivera-Ballesteros O et al (2020) Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 183:158-168.e14. https://doi.org/10.1016/j.cell.2020.08.017

Sharif-Askari FS, Sharif-Askari NS, Hafezi S et al (2022) Interleukin-17, a salivary biomarker for COVID-19 severity. PLoS ONE 17:e0274841. https://doi.org/10.1371/journal.pone.0274841

Silva MJA, Rodrigues YC, Lima KVB et al (2022) Innate immunity to SARS-CoV-2 infection: a review. Epidemiol Infect 150:e142. https://doi.org/10.1017/S095026882200125X

Somers EC, Eschenauer GA, Troost JP et al (2021) Tocilizumab for treatment of mechanically ventilated patients with COVID-19. Clin Infect Dis 73:e445–e454. https://doi.org/10.1093/cid/ciaa954

Soresina A, Moratto D, Chiarini M et al (2020) Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover. Pediatr Allergy Immunol 31:565–569. https://doi.org/10.1111/pai.13263

Sterlin D, Mathian A, Miyara M et al (2021) IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med 13:eabd2223. https://doi.org/10.1126/scitranslmed.abd2223

Stone JH, Frigault MJ, Serling-Boyd NJ et al (2020) BACC Bay Tocilizumab Trial Investigators. Efficacy of tocilizumab in patients hospitalized with Covid-19. N Engl J Med 383:2333–2344. https://doi.org/10.1056/NEJMoa2028836

Świdrowska-Jaros J, Orczyk K, Smolewska E (2016) Macrophages—silent enemies in juvenile idiopathic arthritis. Postepy Hig Med Dosw 70:743–750. https://doi.org/10.5604/17322693.1208887

Tan L, Wang Q, Zhang D et al (2020) Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 5:33. https://doi.org/10.1038/s41392-020-0148-4.Erratum.In:SignalTransductTargetTher5:61

Tang F, Quan Y, Xin ZT et al (2011) Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol 186:7264–7268. https://doi.org/10.4049/jimmunol.0903490

Tarke A, Coelho CH, Zhang Z et al (2022) SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell 185:847-859.e11. https://doi.org/10.1016/j.cell.2022.01.015

Thomas SJ, Moreira ED Jr, Kitchin N et al (2021) C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine through 6 months. N Engl J Med 385:1761–1773. https://doi.org/10.1056/NEJMoa2110345

Vályi-Nagy I, Uher F, Rákóczi É et al (2022) Adaptive immunity to viruses: what did we learn from SARS-CoV-2 infection? Int J Mol Sci 23:13951. https://doi.org/10.3390/ijms232213951

Visci G, Zunarelli C, Mansour I et al (2022) Serological response after SARS-CoV2 vaccination in healthcare workers: a multicenter study. Med Lav 113:e2022022. https://doi.org/10.23749/mdl.v113i2.13017

Wang F, Nie J, Wang H et al (2020) Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis 221:1762–1769. https://doi.org/10.1093/infdis/jiaa150

Weber F (2021) Antiviral innate immunity: introduction. Encyclopedia of Virology 2021:577–583. https://doi.org/10.1016/B978-0-12-809633-8.21290-9

Wise J (2020) COVID-19: Critically ill patients treated with arthritis drug tocilizumab show improved outcomes, researchers report. BMJ 371:m4530. https://doi.org/10.1136/bmj.m4530

Wu LP, Wang NC, Chang YH et al (2007) Duration of antibody responses after severe acute respiratory syndrome. Emerg Infect Dis 13:1562–1564. https://doi.org/10.3201/eid1310.070576

Wyllie D, Jones HE, Mulchandani R et al (2020) SARS-CoV-2 responsive T cell numbers and anti-Spike IgG levels are both associated with protection from COVID-19: a prospective cohort study in keyworkers. medRxiv. https://doi.org/10.1101/2020.11.02.20222778

Xu Z, Shi L, Wang Y et al (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8:420–422. https://doi.org/10.1016/S2213-2600(20)30076-X

Yaqinuddin A, Kashir J (2020) Innate immunity in COVID-19 patients mediated by NKG2A receptors, and potential treatment using monalizumab, cholroquine, and antiviral agents. Med Hypotheses 140:109777. https://doi.org/10.1016/j.mehy.2020.109777

Zeng C, Evans JP, King T et al (2022) SARS-CoV-2 spreads through cell-to-cell transmission. Proc Natl Acad Sci USA 119:e2111400119. https://doi.org/10.1073/pnas.2111400119

Zhang B, Zhou X, Zhu C et al (2020) Immune phenotyping based on the neutrophil-to-lymphocyte ratio and IgG level predicts disease severity and outcome for patients with COVID-19. Front Mol Biosci 7:157. https://doi.org/10.3389/fmolb.2020.00157

Zhang R, Shin BH, Gadsden TM et al (2022) Assessment of humoral and cellular immune responses to SARS CoV-2 vaccination (BNT162b2) in immunocompromised renal allograft recipients. Transpl Infect Dis 24:e13813. https://doi.org/10.1111/tid.13813

Zhao J, Alshukairi AN, Baharoon SA et al (2017) Recovery from the Middle East respiratory syndrome is associated with antibody and T-cell responses. Sci Immunol 2:eaan5393. https://doi.org/10.1126/sciimmunol.aan5393

Zhao J, Yuan Q, Wang H et al (2020) Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis 71:2027–2034. https://doi.org/10.1093/cid/ciaa344

Zuo Y, Yalavarthi S, Shi H et al (2020) Neutrophil extracellular traps in COVID-19. JCI Insight 5:e138999. https://doi.org/10.1172/jci.insight.138999

4.2. Publikacja II

Publikacja II „Application of Interferon-γ Release Assay in the Assessment of T-Cell Immunity to SARS-CoV-2 Antigens in the Cohort of Pediatric Patients with Juvenile Idiopathic Arthritis" jest pracą oryginalną. Celem pracy była ocena użyteczności oznaczania odpowiedzi komórkowej na zakażenie SARS-CoV-2 przy pomocy testu IGRA w grupie pacjentów chorujących na MIZS.

We wstępie opisano aktualny rozwój epidemii SARS-CoV-2 zaznaczając, że zakażenia nowym koronawirusem w niedalekiej przyszłości z ogólnoświatowej pandemii przekształcą się w problem endemiczny i najprawdopodobniej w takiej formie zostaną z nami na dłuższy czas. Obecnie, gdy większość populacji została w sposób naturalny narażona na kontakt z wirusem i/lub została zaszczepiona, istotna wydaje się ocena wytworzonej odporności i jej bezpośrednie przełożenie na ochronę przed zakażeniem SARS-CoV-2. Prezentując złożoność odpowiedzi immunologicznej na infekcję COVID-19, podkreślono możliwe kierunki dalszych badań celem lepszego zrozumienia mechanizmów odpowiedzialnych za wytwarzanie odporności swoistej na infekcję koronawirusową i ich istotność kliniczną. Ponadto wyjaśniono zastosowanie testu IGRA w ocenie odpowiedzi immunologicznej na zakażenie SARS-CoV-2.

Badanie przeprowadzono na grupie 55 dzieci w wieku od 2 do 16 lat, chorujących na MIZS i hospitalizowanych w Klinice Kardiologii i Reumatologii Dziecięcej Uniwersytetu Medycznego w Łodzi w okresie od czerwca 2021 r. do lutego 2023 r. Zakwalifikowani do badania pacjenci byli w różnych stadiach choroby, od nowo rozpoznanych przypadków, po pacjentów chorujących od wielu lat, będących na różnych etapach leczenia, zarówno w stadiach remisji, jak i zaostrzenia choroby podstawowej. Ponadto do kohorty włączono zarówno pacjentów z zakażeniem SARS-CoV-2 potwierdzonym metodą PCR (n = 8), jak również dzieci, które nie przebyły takiej infekcji lub nie uzyskały pozytywnego wyniku testu PCR (n = 47). Wśród uczestników badania 8 pacjentów otrzymało szczepionkę mRNA przed jego rozpoczęciem. Żaden z pacjentów zaszczepionych przeciwko COVID-19 nie przebył w przeszłości zakażenia SARS-CoV-2. Próbki krwi potrzebne do przeprowadzenia eksperymentu zostały pobrane podczas rutynowych badań wykonywanych w trakcie hospitalizacji. Odpowiedź limfocytów T na antygeny SARS-CoV-2 mierzono za pomocą ilościowego testu IGRA w pełnej krwi (EUROIMMUN Quan-T-Cell ELISA). Po stymulacji białkiem S oraz odwirowaniu otrzymane osocze analizowano ilościowym testem ELISA w celu określenia stężenia uwolnionego IFN-γ. Następnie u wszystkich pacjentów wykonano badanie na obecność przeciwciał anty-SARS-CoV-2 testem EUROIMMUN Anty-SARS-CoV-2 ELISA w klasach IgA, IgG oraz IgM. Dodatkowo w celu dokładniejszego oznaczenia przeciwciał zastosowano czułą detekcję IgG przy użyciu białka nukleokapsydu (NCP). Oprócz wyników testów immunologicznych analizowano także następujące parametry pobierane podczas hospitalizacji niezależnie od przeprowadzanego eksperymentu medycznego: całkowitą morfologię krwi, białko C-reaktywne (CRP) i Odczyn Biernackiego (OB).

Analizowane zmienne ciągłe nie miały rozkładu normalnego na podstawie testu Shapiro – Wilka. Dlatego też wszystkie porównania grupowe obliczono przy użyciu testu U Manna – Whitneya oraz testu H Kruskala – Wallisa. Do oceny zależności pomiędzy zmiennymi ilościowymi wykorzystano współczynniki korelacji rang Spearmana. Różnice uznawano za istotne statystycznie, gdy współczynnik p wynosił poniżej 0,05. Wszystkie obliczenia statystyczne wykonano przy użyciu programu Statistica 13.1.

Badanie wykazało istotną statystycznie korelację pomiędzy odpowiedzią limfocytów T na zakażenie SARS-CoV-2 mierzoną testem IGRA, a odpowiedzią humoralną mierzoną mianem

przeciwciał w klasie IgA ($p < 0,00003$, $R = 0,537$) oraz IgG ($p < 0,0001$, $R = 0,668$), łącznie z wysoce czułym IgG NCP ($p < 0,003$, $R = 0,0399$). Nie stwierdzono korelacji z mianami IgM w surowicy. U pięciu pacjentów seronegatywnych (we wszystkich klasach przeciwciał) stwierdzono wytworzenie odpowiedzi komórkowej. Podobnie u kilku pacjentów, u których nie stwierdzono odpowiedzi limfocytów T, oznaczono dodatnie wartości przeciwciał w klasie IgG (n=2) i IgA (n=3).

Ponadto po przeanalizowaniu różnych leków stosowanych u uczestników badania (metotreksat, sulfasalazyna, cyklosporyna, hydroksychlorochina, azatiopryna oraz glikokortykosteroidy stosowane krótkotrwale w małych lub średnich dawkach) nie wykazano istotnego obniżenia odpowiedzi immunologicznej podczas żadnej z tych terapii. Pacjenci otrzymujący leczenie biologiczne, w tym blokery czynnika martwicy nowotworu (TNF) (adalimumab, etanercept), inhibitor IL-6 (tocilizumab) i inhibitor kinazy janusowej (baricytynib), mieli istotnie niższe ($p = 0,0369$) miano przeciwciał SARS-CoV-2 w porównaniu z pacjentami, którzy nie otrzymywali takiego leczenia. Autorzy zaznaczyli, że nie zaobserwowano podobnego wpływu leków biologicznych na odporność komórkową, ani nie odnotowano znaczących zmian w liczbie limfocytów T u pacjentów stosujących taką terapię. Nie stwierdzono korelacji odpowiedzi immunologicznej ze standardowymi badaniami krwi, ani ze wskaźnikami stanu zapalnego (CRP czy OB).

W dyskusji porównano uzyskane wyniki z danymi przedstawionymi w innych artykułach naukowych i badaniach populacyjnych.

Niniejszym badaniem potwierdzono, że test IGRA może stać się przydatnym narzędziem do oceny odporności jednostki na zakażenie SARS-CoV-2. Odpowiedź limfocytów T nie tylko korelowała z wynikami badań serologicznych, ale w niektórych przypadkach wskazywała na obecność odporności komórkowej przy braku odpowiedzi humoralnej. Bardzo możliwe, że w pewnych przypadkach oznaczanie wyłącznie humoralnej odpowiedzi na wirusa może prowadzić do niepełnej oceny odporności jednostki, a nawet negatywnie wpływać na dalsze postępowanie kliniczne. Patrząc w przyszłość, istnieje potrzeba przeprowadzenia bardziej kompleksowych badań w celu ustalenia, jaki stopień i rodzaj odporności resztkowej jest niezbędny, aby uniknąć ciężkiej choroby w razie ponownej infekcji. Co więcej, potrzeba dużych badań prospektywnych z udziałem populacji pediatrycznej celem dokładnej oceny wpływu leków biologicznych na odpowiedź humoralną.

children

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Article

Application of Interferon-γ Release Assay in the Assessment of T-Cell Immunity to SARS-CoV-2 Antigens in the Cohort of Pediatric Patients with Juvenile Idiopathic Arthritis

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Abstract: Background: an accurate assessment of the immunity against SARS-CoV-2 can facilitate a better understanding and management of not only the recent coronavirus but similar pathogens as well. **Objective:** the aim of this study was to evaluate T-cell immunity with reference to antibody titers in a group of pediatric patients with autoimmune arthritides utilizing the widely known Interferon-γ Release Assay (IGRA). **Materials and Methods:** This study was conducted in the cohort of 55 children suffering from Juvenile Idiopathic Arthritis (JIA). This research analyzed the SARS-CoV-2 T-cell response measured by a specific quantitative IGRA, followed by a serological ELISA test measuring the presence and quantity of IgG, IgM, and IgA antibodies in serum. **Results:** The cellular response to SARS-CoV-2 measured by the IGRA test significantly correlated with the antibody titers, IgA ($p < 0.00003$, R = 0.537), IgG ($p < 0.0001$, R = 0.668), and IgG nucleocapsid protein (NCP) ($p < 0.003$, R = 0.0399), with no correlation with IgM levels. The antibody levels in patients receiving biological agents were significantly lower compared to the rest of the cohort ($p = 0.0369$), while traditional disease-modifying antirheumatic drugs had no such effect. **Limitations:** the main limitation of the research is the small sample size, mostly due to the specific cohort of patients and the lack of a healthy control. **Conclusions:** IGRA appears to be a viable tool in the accurate evaluation of T-cell responses to SARS-CoV-2, and serodiagnostics alone is not always sufficient in the assessment of immune responses.

Keywords: JIA; SARS-CoV-2; COVID-19; cellular immunity; T-cells

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1. Introduction

1.1. Current State of the Pandemic

Despite the substantially diminishing morbidity rate of SARS-CoV-2, with more than 700 million confirmed cases and almost 7 million fatalities worldwide in over 3 years, COVID-19 continues to be a global health concern [1]. As for today, scientists predict that it will remain an endemic issue for the foreseeable future [2]. In the course of the pandemic that was announced on 11 March 2020 by the World Health Organization, the scientists and healthcare professionals encountered a great many obstacles, showing the level of challenges that public health had to face [3]. While the rapid identification and isolation of infected individuals became the main objective at the beginning of the pandemic, currently, after most of the population was naturally exposed to the virus or/and vaccinated, the researchers' attention shifted to accurately assessing one's immunity which directly translates to the protection against SARS-CoV-2.

1.2. Humoral Immunity

Viruses such as SARS-CoV-2 initiate the infection with the viral antigen, activating adaptive immune responses through the antigen-presenting cells or B-cell receptors, inducing defense mechanisms against the pathogen. Following the infection, immunological memory is developed [4]. Due to the sterilizing qualities of antibodies, they were the first target for vaccine development and therefore the primary interest of the majority of the research. However, it was not long before it became clear that the antibody responses to COVID-19 were far more complex than simply marking the past infection. Early studies showed that higher antibody titers in SARS-CoV-2 infection are associated with more severe clinical manifestations of the disease [5,6], while a weak IgG response correlated with a significantly higher viral clearance, suggesting a pathological role of antibodies [7]. Interestingly, further research proved this correlation to be far more complex, and various factors, including the kinetics of seroconversion, antibody isotypes, and antigen specificity, should be considered to determine the effect of humoral response on disease severity. While a correlation between the longevity of antibody titers in serum and protection against reinfection was confirmed in numerous studies [8,9], factors like the severity of the infection or different variants of the virus may affect patients' seropositivity [10,11].

1.3. Cellular Immunity

As adaptive immunity consists both of humoral and cellular components, the assessment of the T-cell response to COVID-19 appears to be no less relevant. The research on cellular immunity after SARS-CoV-1 infection indicated the high durability of T-cells, prevailing even 17 years after exposure to the virus, while a considerable drop in antibody titers was observed in the same patients just after 3–6 years [12,13]. Furthermore, it was noted that the cellular immunity gained by exposure to SARS-CoV-1 exhibited robust and expanding cross-reactivity to the N protein of SARS-CoV-2 and, even more interestingly, SARS-CoV-2-specific Interferon-γ (IFNγ) responses were found in donors previously unexposed to neither SARS-CoV-1 nor SARS-CoV-2 and thus are believed to come from exposure to unknown coronaviruses [13]. Similarly, the newest data on cellular memory against SARS-CoV-2 indicate a high durability of CD4+ and CD8+ T-cells [14], proposing its assessment, as they are essential for viral clearance and long-term, sustainable antiviral immunity. Moreover, the diagnostic T-cell assays for SARS-CoV-2 can be utilized in the cohorts of immunodeficient patients, who fail to produce antibodies or were given antibodies passively through immunoglobulins, but can still develop a cellular response to the virus [15,16]. While T-cells have a major protective role in the early stages of the disease, they can potentially contribute to the onset of fatal comorbidities, through T-cell exhaustion and hyperinflammation. As these new findings present a potential for new treatment options, further research on T-cell responses is essential [17,18].

1.4. Interferon-γ Release Assay (IGRA)

The Interferon-γ Release Assay (IGRA) is a widely known and validated test, utilized not only in the diagnostic process of diseases like tuberculosis [19] but also in other, mostly viral, infections [20,21]. Today, we can use the IGRA to determine the activity of SARS-CoV-2-reactive T-cells. Its high specificity, as well as sensitivity, has been confirmed in numerous research, emphasizing the potential in marking a long-lasting, durable cellular immunity, both shortly after exposure to the virus and weeks or months after the infection or vaccination against COVID-19 [22–24]. It becomes more apparent that reinfections with SARS-CoV-2 will remain a medical issue for quite some time. Therefore, the proper assessment of post-exposure immunity to SARS-CoV-2 can give us information on a person's residual immunity and therefore the likelihood of a serious infection.

The objective of this study was to validate IGRA as a feasible tool to test cellular immunity, mediated by both CD4+ and CD8+ cells in possibly immunocompromised individuals exposed to SARS-CoV-2.

2. Materials and Methods

This prospective study was performed in the cohort of 55 pediatric patients diagnosed with Juvenile Idiopathic Arthritis (JIA) during their hospitalization in the Department of Pediatric Cardiology and Rheumatology, Medical University of Lodz. The recruitment for this study and all the laboratory testing that was part of this research took place between June 2021 and February 2023. The study group consisted of children between the ages of 2 and 16, in different stages of the disease, receiving various treatment regimes. The cohort included patients with both a negative (n = 47) and positive, PCR-confirmed, history of COVID-19 infection (n = 8), before this study commenced. Only 8 patients received the mRNA vaccine before being included in this study, while the rest of the group (n = 47) had not received the SARS-CoV-2 inoculation before this study commenced. None of the patients vaccinated against COVID-19 had a past history of SARS-CoV2 infection. The inclusion criteria for this study were confirmed JIA, in compliance with the International League of Associations for Rheumatology (ILAR) classification, and an age below 16 years old, with no lower age limit. All the patients in the cohort were previously immunized with the Bacillus Calmette–Guérin (BCG) vaccine against tuberculosis (TB), as it is an obligatory inoculation for newborns in Poland. None of the subjects reported having contact with TB. Additionally, all the patients who were qualified for biological treatment were tested for TB as part of the qualification procedure before receiving biological agents. The exclusion criteria for this study were a severe flare of JIA that required high immunosuppressive doses of steroids and the presence of concomitant autoimmune diseases, including diabetes mellitus, that could affect the results of this research.

SARS-CoV-2 T-cell response was measured using the EUROIMMUN Quan-T-Cell ELISA, catalog No. EQ 6841-9601 assay, which is a specific quantitative IGRA in whole blood. About 1.5 mL of blood was sampled during routine testing. The heparinized blood was then incubated in a set of three tubes (EUROIMMUN SARS-CoV-2 IGRA stimulation tube set, catalog No. ET 2606–3003): first tube—IGRA BLANK with no activating components, marking the individual's Interferon as background; second tube—IGRA TUBE for specific T-cell stimulation by SARS-CoV-2 antigen spike protein; and third tube—IGRA STIM for unspecific T-cell stimulation with mitogen, for determining stimulation ability. Then, the obtained plasma was analyzed by the quantitative enzyme-linked immunosorbent assay (ELISA) to determine the concentration of released IFN-γ. All the patients were then tested for anti-SARS-CoV-2 antibodies, with EUROIMMUN Anty-SARS-CoV-2 ELISA, catalog No. EI 2606-9601 A (IgA); EUROIMMUN Anty-SARS-CoV-2 QuantiVac ELISA, catalog No. EI 2602-9601-10 G (IgG); and EUROIMMUN Anty-SARS-CoV-2-NCP, catalog No. EI 2606-9601-2 M (IgM). Additionally, the sensitive detection of IgG using the nucleocapsid protein (NCP) was used for the more accurate marking of antibodies [25] (EUROIMMUN Anty-SARS-CoV-2-NCP, catalog No. EI 2606-9601-2 G).

One of the prerogatives of this study was collecting blood samples needed for the tests mentioned above, during the standard blood drawing performed upon every hospitalization. Thus, in addition to the results of immune assays, the following parameters were analyzed as well: Total Blood Count, including leucocytes and platelets, as possible markers of ongoing inflammation and the most common inflammatory markers; C-reactive Protein (CRP); and Erythrocytes Sedimentation Rate (ESR).

All continuous variables were non-normally distributed based on the Shapiro–Wilk test. Therefore, all group comparisons were calculated utilizing the Mann–Whitney U test and Kruskal–Wallis H test. Spearman's rank correlation coefficients were used to assess dependencies between quantitative variables. All statistical calculations were performed using Statistica 13.1 software (Statsoft Polska, Krakow, Poland). All the diagnostic tools were ordered from EUROIMMUN POLSKA, Wroclaw, Poland. This study was approved by the local Bioethics Committee, with approval number RNN/117/21/KE.

3. Results

The specifics of the cohort included in this study are presented in Table 1.

Table 1. General characteristics of the study group.

	n = 55
Male/Female	14/41
Age on examination (years)	10.31 ± 4.16
Positive IgA antibodies	33
Positive IgG antibodies	40
Positive IgG NCP antibodies	23
Positive IgM antibodies	6
Positive IGRA (>200 mIU/mL)	41
History of confirmed SARS-CoV-2 infection	8
Received SARS-CoV-2 vaccination	8 *
COVID-19-like symptoms in the history	32
Treatment protocol:	
Biological agents:	22
adalimumab	13
tocilizumab	6
etanercept	2
baricitinib	1
Methotrexate	32
Sulfasalazine	8
Hydroxychloroquine	7
Cyclosporine	3
Azathioprine	1
Glucocorticoids	4

Values presented as mean ± standard deviation (SD). NCP—nucleocapsid protein; IGRA—Interferon-γ Release Assay. * None of the vaccinated subjects had a history of PCR-confirmed SARS-CoV-2 infection.

This research confirmed a significant correlation between the T-cell response to SARS-CoV-2 infection measured by the IGRA test and the humoral response measured by antibody titers. While IGRA positively correlated with the levels of IgA ($p < 0.00003$, R = 0.537) (Figure 1) and IgG antibodies, both in the test utilizing the modified S1 domain of the spike protein as an antigen ($p < 0.0001$, R = 0.668) (Figure 2), and in the highly sensitive NCP ($p < 0.003$, R = 0.0399), no correlation with IgM titers in serum was found.

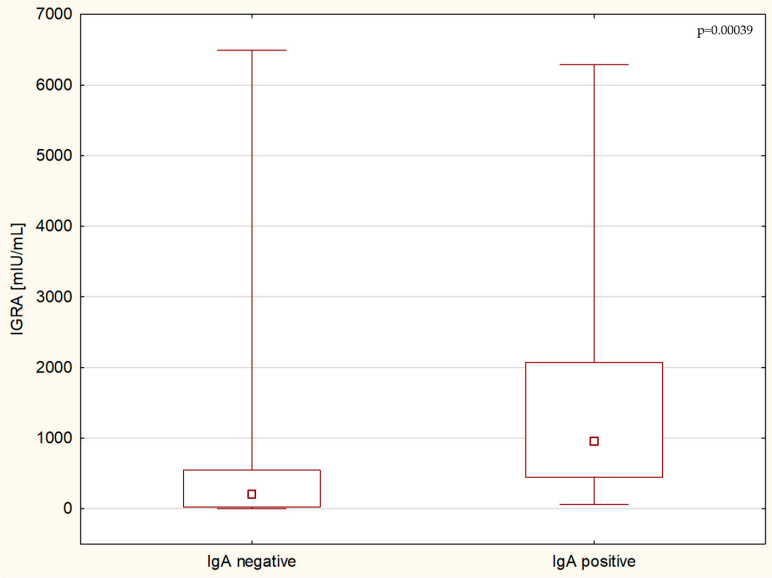

Figure 1. Group analysis of IGRA levels between IgA-negative and IgA-positive patients.

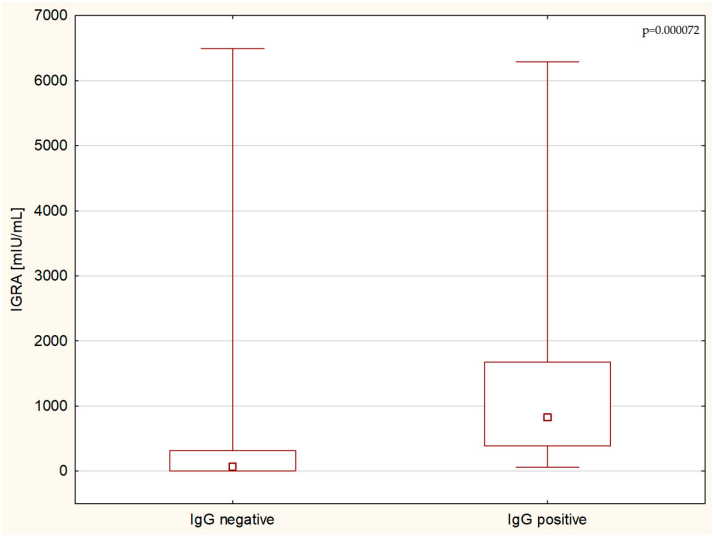

Figure 2. Group analysis of IGRA levels between IgG-negative and IgG-positive patients.

Within the study group, five seronegative patients (in all antibodies classes), which constitute over 9% of the cohort, presented a SARS-CoV-2 T-cell response. While in patients with positive IgG titers, two had negative IGRA, and two had a cellular response in the grey area (100–200 mIU/mL), similarly, in patients with positive IgA titers, three had negative IGRA results. Regarding IgM response, one patient had positive IgM titers with no T-cell response; however, this was a patient with no IgG and no IgA response as well. This study analyzed the effects of different drug protocols on patients' immunity. The investigated disease-modifying antirheumatic drugs (DMARDs) included methotrexate, sulfasalazine, cyclosporine, hydroxychloroquine, and azathioprine. None of these therapies significantly lowered immune responses, neither humoral nor cellular. Moreover, no correlation was found between children receiving glucocorticoids (GCs) in the study group and their immunity levels. However, it needs to be noted that none of the patients were on high doses of the GCs, none of the patients reached 2 mg of prednisone per kilogram of body weight, and none were receiving a prolonged GC therapy.

Patients receiving biological treatment, including Tumor Necrosis Factor (TNF) blockers (adalimumab, etanercept), interleukin 6 inhibitor (tocilizumab), and Janus kinase inhibitor (baricitinib), had significantly lower (p = 0.0369) SARS-CoV-2 antibody titers when compared to the patients not receiving such treatment (Figure 3). The most promising results were noted with baricitinib; however, there was only one patient under this regime. Notably, no effects of biological agents were observed regarding cellular immunity, and no significant changes in T-cell levels of patients receiving such therapy were noted.

The age and sex of the patients did not correspond with immune responses. No correlation with standard blood tests was found, neither with inflammatory markers like CRP or ESR nor with leukocytosis or levels of platelets. Additionally, neither the SARS-CoV-2 antibody titers nor the specific T-cells levels correlated with the confirmed COVID-19 cases. However, it should be noted that only eight patients had tested positive for SARS-CoV-2 in Real-Time Polymerase Chain Reaction (RT-PCR) before this research commenced. Also, the history of COVID-like symptoms reported by the children's parents did not correspond with the markers of the immune response.

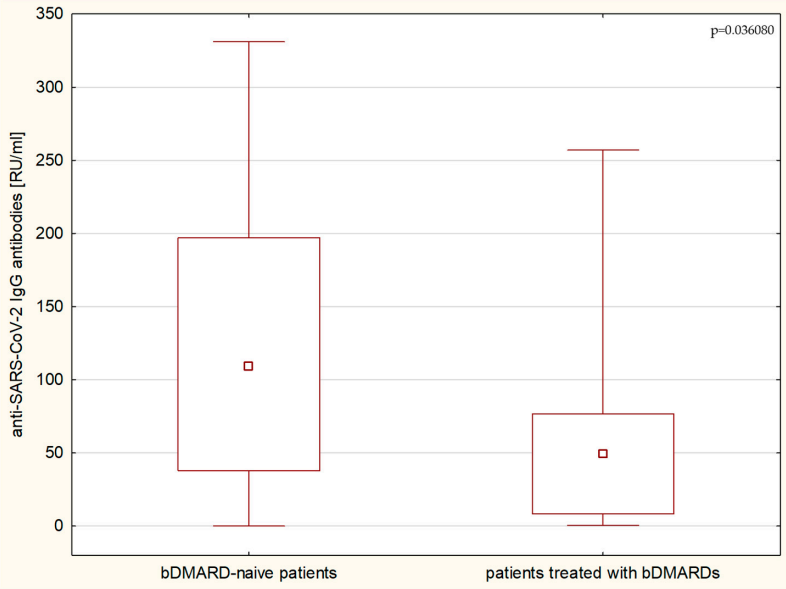

Figure 3. Group analysis of received biological treatment between IgG-negative and IgG-positive patients. bDMARDS—biological disease-modifying anti-rheumatic drugs.

4. Discussion

Cellular immunity is an invaluable component of the immune response to intracellular pathogens; thus, it is critical for the control of viruses, such as SARS-CoV-2 [17,26]. As randomized controlled trials have shown, the virus-specific cellular immune response prevents the spread of the virus within the host and eradicates the pathogen [27].

Evaluating cellular immunity as well as humoral responses to COVID-19 has proven to be an added value to standard serological testing [28]. A recent study confirmed a positive correlation between T-cell responses and SARS-CoV-2 antibody titers. These results are consistent with multiple studies, including one by Brand et al. [29] who evaluated T-cell responses against multiple SARS-CoV-2 structural proteins in a large number of patients, both exposed and unexposed to COVID-19, as well as with the results of Björkander et al. [30] who conducted a large population study on humoral and cellular immunity to SARS-CoV-2 in young adults. Moreover, Oja et al. postulated that antibody titers significantly correlate with S-specific CD4+ T-cell responses in patients with mild coronavirus disease, which remains in accordance with the results from the current study, as no patients within the cohort were reported to suffer from severe SARS-CoV-2 infection or required hospitalization [31]. In asymptomatic and mild cases of COVID-19, cellular immunity has been proven to be a better predictor of long-term immune memory than antibody titers, since T-cell responses remain at a detectable level when humoral memory starts to wane [32–34]. Interestingly, Wang et al. noted the importance of the coordination of cellular and humoral immunity for long-term protection, as he unraveled that high levels of virus-specific CD4+ T cells were associated with a slower decline in antibody titers [35]. However, the lack of consistent cut-off values for IGRA across different studies, with proposed values ranging from 25 IU/mL to 200 IU/mL, is notably a point that requires further validation. Applying universal values to this assay will benefit its utility both in clinical practice and research [22–24].

While the recent study proved the correlation between humoral and cellular responses, the lack of seroconversion in several patients who still mounted T-cell immunity should be noted. These results are concordant with multiple studies [36,37] postulating that exposure to SARS-CoV-2 can induce virus-specific T-cell responses without eliciting specific antibody production. In the study of Reynolds et al. on healthcare workers exposed to COVID-19,

some seronegative subjects had undetectable T cell responses to spike protein, as well as T cells reactive to other SARS-CoV-2 antigens [38]. Thus, it can be speculated that pre-existing, cross-reactive memory T-cells can support the rapid clearance of infection in previously unexposed individuals [37,39].

Positive IgA antibodies titers that were found in 33 out of 55 patients within the study group correlating with cellular responses to the virus support the previous findings, stating that an early SARS-CoV-2-specific humoral response is dominated by IgA antibodies and is crucial in virus neutralization [40,41]. IgA is produced by B lymphocytes with T-independent and T-dependent mechanisms and its subclass is the primary immunoglobulin in the respiratory tract, protecting the epithelial barriers from pathogens. While the IgA response to COVID-19 was proved to be highly effective and contributed significantly to virus neutralization, it is predominantly associated with early viral response as its titers in serum decreases notably as soon as a month after the infection, and while it has been found that specific local neutralizing IgA remains detectable in the saliva and airways for a longer period of time, it remains to be discovered whether these secretory antibodies may contribute to a long-term immunity against reinfection [40–42]. As SARS-CoV-2 is a mucosal-targeted virus (Figure 4), IgA plays a vital role in its management and should not be overlooked when assessing immunological responses in respiratory tract infections [43]. According to Infantile et al., the assessment of IgA antibodies in patients with early COVID-19 infection could help with closing the gap in serological testing, as they appear early, in high concentrations, and persist up to over 25 days from the disease onset [44]. However, there are some conflicting results regarding the correlation between the severity of COVID-19 and IgA levels. While Hennings et al. postulated that IgA-dominated responses was more likely to occur in asymptomatic infection with asymptomatic infection, Zervou et al. stated that high IgA titers positively correlated with the severity of the disease and were the highest in critically ill patients [42,45].

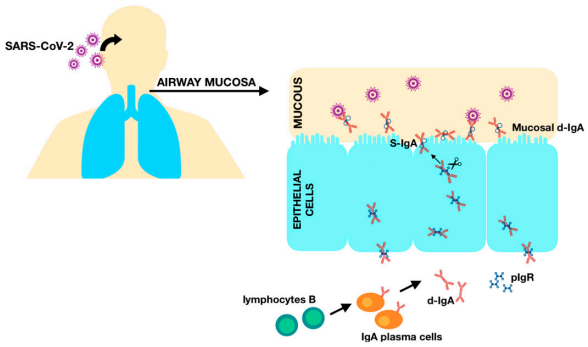

Figure 4. IgA eliminating SARS-CoV-2 on mucous membranes.

The figure illustrates the pathway of IgA from the blood circulation to mucous membranes in the human respiratory system. IgA is secreted by plasma cells, which are differentiated B cells after class switch recombination. IgA in secretions like mucus is found mainly in its dimeric form (d-IgA). Polymeric immunoglobulin receptor (pIgR) is a transmembrane protein that transports d-IgA across the mucosal epithelium. In order to cross the mucous layer by diffusion, the secretory component of pIgR (SC) undergoes endoproteolytic cleavage. Lastly, S-IgA consisting of d-IgA and SC can bind and neutralize pathogens, including SARS-CoV-2.

The data on immune responses to SARS-CoV-2 in patients suffering from immune-related rheumatic diseases (RD) are quite scarce; however, the available literature suggests that both cellular and humoral responses elicited in patients suffering from RD are comparable to healthy control groups and not impaired by standard immunomodulating therapies [46–48]. The recent study, for the most part, confirms these findings, where

patients receiving DMARDs did not have impaired immunological mechanisms. However, the weakening of the humoral response was noted in patients receiving biological treatment. These results are consistent with the study conducted by Simon et al., who noted lower IgG titers, less frequent seroconversion, and reduced longevity of the humoral response in adult patients with RD receiving TNF-blockers and cytokine inhibitors [49]. Nonetheless, the data on clinical implications of the usage of biological agents in patients with RD suggest that the therapies should be continued; hence, they do not lead to more severe manifestations of COVID-19, including in the pediatric population [50,51]. In addition, many studies suggest that the usage of biological agents may have a protective effect against SARS-CoV-2 infection, since some of the drugs, like tocilizumab, have been successfully used in the management of COVID-19 [52–55]. Nevertheless, we should still be wary of rheumatic patients during this viral pandemic, as they may be at risk of poor outcomes of the infection; in particular, if the RD activity is high, they suffer from comorbidities or receive specific treatments like high doses of GCs or rituximab [55,56].

We acknowledge that our study has several limitations, such as a relatively small sample size and the lack of a control group. Adding a healthy control of children without RD may have yielded more solid results in the assessment of immunological responses to SARS-CoV-2 in patients with autoimmune disease when compared to their healthy peers. Additionally, due to a dynamic course of the pandemic, it has been nearly impossible to pinpoint the immunization stage of each and every patient from the onset of this study. Due to a low rate of PCR testing for SARS-CoV-2 within the pediatric population [57], including the cohort in the recent study, an undocumented or unrealized contact with the virus cannot be excluded. Moreover, a heterogeneous group with a relatively small number of patients being subjected to specific drug regimes allowed us to find trends rather than draw highly detailed conclusions regarding therapeutic recommendations. As the utilization of biological agents becomes more and more common in immune diseases, as well as in children, expanding the sample size to a larger cohort of pediatric patients receiving such treatment and focusing on specific agents may lead to the most reliable results and seems to be the best way forward for this research.

5. Conclusions

IGRA appears to be a valid tool in the assessment of individuals' immunity to SARS-CoV-2 infection. T-cell responses proved to not only statistically correlate with the patient's serological results, but in some cases indicate immunity in the absence of a humoral response. Thus, it is possible that, in some circumstances, marking solely the humoral responses to the virus can lead to an incomplete assessment of individual immunity and even negatively affect further clinical management. Looking into the future, there is a need for more comprehensive research to establish what level and type of residual immunity are needed to avoid severe disease after reinfection. Moreover, while, according to a recent study, the usage of DMARDs by patients with RD does not affect their immunity, a larger cohort study involving the pediatric population may help in investigating the effect of biological agents on humoral responses.

Author Contributions: K.K.: Investigation, Writing—Original Draft Preparation, Visualization; K.O.: Formal Analysis, Data Curation, Writing—Review and Editing; E.S.: Conceptualization, Validation, Supervision, Project Administration. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was performed in line with the principles of the Declaration of Helsinki and approved by the Bioethics Committee on 11 May 2021, approval number RNN/117/21/KE.

Informed Consent Statement: Since the patients were underaged (16 and younger), informed consent was obtained from all the children's parents. Moreover, participants who were 13 years old and above were also asked for written consent after being provided with an age-appropriate explanation of the study.

Data Availability Statement: The data used to support the findings of this study are included in the article. The supplementary data are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cascella, M.; Rajnik, M.; Aleem, A.; Dulebohn, S.C.; Di Napoli, R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2023.
2. Arduini, A.; Laprise, F.; Liang, C. SARS-CoV-2 ORF8: A Rapidly Evolving Immune and Viral Modulator in COVID-19. *Viruses* **2023**, *15*, 871. [CrossRef]
3. Fernandes, Q.; Inchakalody, V.P.; Merhi, M.; Mestiri, S.; Taib, N.; Moustafa Abo El-Ella, D.; Bedhiafi, T.; Raza, A.; Al-Zaidan, L.; Mohsen, M.O.; et al. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Ann. Med.* **2022**, *54*, 524–540. [CrossRef]
4. Primorac, D.; Vrdoljak, K.; Brlek, P.; Pavelić, E.; Molnar, V.; Matišić, V.; Erceg Ivkošić, I.; Parčina, M. Adaptive Immune Responses and Immunity to SARS-CoV-2. *Front. Immunol.* **2022**, *13*, 848582. [CrossRef]
5. Garcia-Beltran, W.F.; Lam, E.C.; Denis, K.S.; Nitido, A.D.; Garcia, Z.H.; Hauser, B.M.; Feldman, J.; Pavlovic, M.N.; Gregory, D.J.; Poznansky, M.C.; et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* **2021**, *184*, 2372–2383.e9. [CrossRef]
6. Hashem, A.M.; Algaissi, A.; Almahboub, S.A.; Alfaleh, M.A.; Abujamel, T.S.; Alamri, S.S.; Alluhaybi, K.A.; Hobani, H.I.; AlHarbi, R.H.; Alsulaiman, R.M.; et al. Early Humoral Response Correlates with Disease Severity and Outcomes in COVID-19 Patients. *Viruses* **2020**, *12*, 1390. [CrossRef] [PubMed]
7. Lee, E.; Oh, J.E. Humoral Immunity against SARS-CoV-2 and the Impact on COVID-19 Pathogenesis. *Mol. Cells* **2021**, *44*, 392–400. [CrossRef] [PubMed]
8. Islamoglu, M.S.; Cengiz, M.; Uysal, B.B.; Ikitimur, H.; Ozdemir, Z.; Karamehmetoglu, A.; Akbulut, A.E.; Bakdur, A.N.; Ozdemir, A.; Kayıkcıoglu, H.; et al. Relationship between Antibody Levels and SARS-CoV-2 Reinfection. *Ann. Clin. Lab. Sci.* **2021**, *51*, 750–755. [PubMed]
9. Sullivan, A.; Alfego, D.; Hu, P.; Gillim, L.; Grover, A.; Garcia, C.; Cohen, O.; Letovsky, S. Antibody titer levels and the effect on subsequent SARS-CoV-2 infection in a large US-based cohort. *Heliyon* **2023**, *9*, e13103. [CrossRef]
10. Ibarrondo, F.J.; Fulcher, J.A.; Goodman-Meza, D.; Elliott, J.; Hofmann, C.; Hausner, M.A.; Ferbas, K.G.; Tobin, N.H.; Aldrovandi, G.M.; Yang, O.O. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild COVID-19. *N. Engl. J. Med.* **2020**, *383*, 1085–1087. [CrossRef]
11. Błaszczuk, A.; Michalski, A.; Sikora, D.; Malm, M.; Drop, B.; Polz-Dacewicz, M. Antibody Response after SARS-CoV-2 Infection with the Delta and Omicron Variant. *Vaccines* **2022**, *10*, 1728. [CrossRef]
12. Ng, O.W.; Chia, A.; Tan, A.T.; Jadi, R.S.; Leong, H.N.; Bertoletti, A.; Tan, Y.J. Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. *Vaccine* **2016**, *34*, 2008–2014. [CrossRef] [PubMed]
13. Le Bert, N.; Tan, A.T.; Kunasegaran, K.; Tham, C.Y.; Hafezi, M.; Chia, A.; Chng, M.H.Y.; Lin, M.; Tan, N.; Linster, M.; et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* **2020**, *584*, 457–462. [CrossRef] [PubMed]
14. Zhang, J.; Lin, H.; Ye, B.; Zhao, M.; Zhan, J.; Dong, S.; Guo, Y.; Zhao, Y.; Li, M.; Liu, S.; et al. One-Year Sustained Cellular and Humoral Immunities in Coronavirus Disease 2019 (COVID-19) Convalescents. *Clin. Infect. Dis.* **2022**, *75*, e1072–e1081. [CrossRef] [PubMed]
15. Ameratunga, R.; Woon, S.T.; Steele, R.; Lehnert, K.; Leung, E.; Brooks, A.E.S. Critical role of diagnostic SARS-CoV-2 T cell assays for immunodeficient patients. *J. Clin. Pathol.* **2022**, *75*, 793–797. [CrossRef] [PubMed]
16. Steiner, S.; Schwarz, T.; Corman, V.M.; Gebert, L.; Kleinschmidt, M.C.; Wald, A.; Gläser, S.; Kruse, J.M.; Zickler, D.; Peric, A.; et al. SARS-CoV-2 T Cell Response in Severe and Fatal COVID-19 in Primary Antibody Deficiency Patients without Specific Humoral Immunity. *Front. Immunol.* **2022**, *13*, 840126. [CrossRef] [PubMed]
17. Toor, S.M.; Saleh, R.; Sasidharan Nair, V.; Taha, R.Z.; Elkord, E. T-cell responses and therapies against SARS-CoV-2 infection. *Immunology* **2021**, *162*, 30–43. [CrossRef]
18. Heitmann, J.S.; Bilich, T.; Tandler, C.; Nelde, A.; Maringer, Y.; Marconato, M.; Reusch, J.; Jäger, S.; Denk, M.; Richter, M.; et al. A COVID-19 peptide vaccine for the induction of SARS-CoV-2 T cell immunity. *Nature* **2022**, *601*, 617–622. [CrossRef]
19. Sester, M.; Sotgiu, G.; Lange, C.; Giehl, C.; Girardi, E.; Migliori, G.B.; Bossink, A.; Dheda, K.; Diel, R.; Dominguez, J.; et al. Interferon-γ release assays for the diagnosis of active tuberculosis: A systematic review and meta-analysis. *Eur. Respir. J.* **2011**, *37*, 100–111. [CrossRef]
20. Kim, S.H. Interferon-γ Release Assay for Cytomegalovirus (IGRA-CMV) for Risk Stratification of Posttransplant CMV Infection: Is It Time to Apply IGRA-CMV in Routine Clinical Practice? *Clin. Infect. Dis.* **2020**, *71*, 2386–2388. [CrossRef]

21. Terada, K.; Itoh, Y.; Fujii, A.; Kitagawa, S.; Ogita, S.; Ouchi, K. Varicella-zoster virus-specific, cell-mediated immunity with interferon-gamma release assay after vaccination of college students with no or intermediate IgG antibody response. *J. Med. Virol.* **2015**, *87*, 350–356. [CrossRef]
22. Huzly, D.; Panning, M.; Smely, F.; Enders, M.; Komp, J.; Falcone, V.; Steinmann, D. Accuracy and real life performance of a novel interferon-γ release assay for the detection of SARS-CoV2 specific T cell response. *J. Clin. Virol.* **2022**, *148*, 105098. [CrossRef] [PubMed]
23. Barreiro, P.; Sanz, J.C.; San Román, J.; Pérez-Abeledo, M.; Carretero, M.; Megías, G.; Viñuela-Prieto, J.M.; Ramos, B.; Canora, J.; Martínez-Peromingo, F.J.; et al. A Pilot Study for the Evaluation of an Interferon Gamma Release Assay (IGRA) To Measure T-Cell Immune Responses after SARS-CoV-2 Infection or Vaccination in a Unique Cloistered Cohort. *J. Clin. Microbiol.* **2022**, *60*, e0219921. [CrossRef] [PubMed]
24. Ham, J.Y.; Lee, N.Y.; Hwang, N.; Song, K.E. Diagnostic Performance of Interferon Gamma Release Assay (IGRA) for Cell-Mediated Immune Responses to SARS-CoV-2. *Clin. Lab.* **2023**, *69*, 604. [CrossRef] [PubMed]
25. Asti, A.L.; Lilleri, D.; Zelini, P.; Gregorini, M.; Morosini, M.; Pattonieri, E.F.; Grignano, M.A.; Libetta, C.; Sepe, V.; Di Toro, A.; et al. SARS-CoV-2-specific IgG and NCP in vulnerable patients without symptoms. *New Microbiol.* **2022**, *45*, 213–218. [PubMed]
26. Moss, P. The T cell immune response against SARS-CoV-2. *Nat. Immunol.* **2022**, *23*, 186–193. [CrossRef] [PubMed]
27. Le Bert, N.; Clapham, H.E.; Tan, A.T.; Chia, W.N.; Tham, C.Y.; Lim, J.M.; Kunasegaran, K.; Tan, L.W.L.; Dutertre, C.A.; Shankar, N.; et al. Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. *J. Exp. Med.* **2021**, *218*, e20202617. [CrossRef] [PubMed]
28. Kapten, K.; Orczyk, K.; Smolewska, E. Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic. *Arch. Immunol. Ther. Exp.* **2023**, *71*, 7. [CrossRef] [PubMed]
29. Brand, I.; Gilberg, L.; Bruger, J.; Garí, M.; Wieser, A.; Eser, T.M.; Frese, J.; Ahmed, M.I.; Rubio-Acero, R.; Guggenbuehl Noller, J.M.; et al. Broad T Cell Targeting of Structural Proteins After SARS-CoV-2 Infection: High Throughput Assessment of T Cell Reactivity Using an Automated Interferon Gamma Release Assay. *Front. Immunol.* **2021**, *12*, 688436. [CrossRef]
30. Björkander, S.; Du, L.; Zuo, F.; Ekström, S.; Wang, Y.; Wan, H.; Sherina, N.; Schoutens, L.; Andréll, J.; Andersson, N.; et al. BAMSE COVID-19 study group. SARS-CoV-2-specific B- and T-cell immunity in a population-based study of young Swedish adults. *J. Allergy Clin. Immunol.* **2022**, *149*, 65–75. [CrossRef]
31. Oja, A.E.; Saris, A.; Ghandour, C.A.; Kragten, N.A.; Hogema, B.M.; Nossent, E.J.; Heunks, L.M.; Cuvalay, S.; Slot, E.; Linty, F.; et al. Divergent SARS-CoV-2-specific T- and B-cell responses in severe but not mild COVID-19 patients. *Eur. J. Immunol.* **2020**, *50*, 1998–2012. [CrossRef]
32. Chen, J.; Liu, X.; Zhang, X.; Lin, Y.; Liu, D.; Xun, J.; Wang, Z.; Gu, L.; Li, Q.; Yin, D.; et al. Decline in neutralising antibody responses, but sustained T-cell immunity, in COVID-19 patients at 7 months post-infection. *Clin. Transl. Immunol.* **2021**, *10*, e1319. [CrossRef] [PubMed]
33. Schnizer, C.; Andreas, N.; Vivas, W.; Kamradt, T.; Baier, M.; Kiehntopf, M.; Glöckner, S.; Scherag, A.; Löffler, B.; Kolanos, S.; et al. Persistent humoral and CD4+ TH cell immunity after mild SARS-CoV-2 infection—The CoNAN long-term study. *Front. Immunol.* **2023**, *13*, 1095129. [CrossRef] [PubMed]
34. Guo, L.; Wang, G.; Wang, Y.; Zhang, Q.; Ren, L.; Gu, X.; Huang, T.; Zhong, J.; Wang, Y.; Wang, X.; et al. SARS-CoV-2-specific antibody and T-cell responses 1 year after infection in people recovered from COVID-19: A longitudinal cohort study. *Lancet Microbe* **2022**, *3*, e348–e356. [CrossRef] [PubMed]
35. Wang, Z.; Yang, X.; Mei, X.; Zhou, Y.; Tang, Z.; Li, G.; Zhong, J.; Yu, M.; Huang, M.; Su, X.; et al. SARS-CoV-2-specific CD4+ T cells are associated with long-term persistence of neutralizing antibodies. *Signal Transduct. Target. Ther.* **2022**, *7*, 132. [CrossRef] [PubMed]
36. Swadling, L.; Diniz, M.O.; Schmidt, N.M.; Amin, O.E.; Chandran, A.; Shaw, E.; Pade, C.; Gibbons, J.M.; Le Bert, N.; Tan, A.T.; et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. *Nature* **2022**, *601*, 110–117. [CrossRef] [PubMed]
37. Gallais, F.; Velay, A.; Nazon, C.; Wendling, M.J.; Partisani, M.; Sibilia, J.; Candon, S.; Fafi-Kremer, S. Intrafamilial Exposure to SARS-CoV-2 Associated with Cellular Immune Response without Seroconversion, France. *Emerg. Infect. Dis.* **2021**, *27*, 113–121. [CrossRef] [PubMed]
38. Reynolds, C.J.; Swadling, L.; Gibbons, J.M.; Pade, C.; Jensen, M.P.; Diniz, M.O.; Schmidt, N.M.; Butler, D.K.; Amin, O.E.; Bailey, S.N.; et al. Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection. *Sci. Immunol.* **2020**, *5*, eabf3698. [CrossRef] [PubMed]
39. Schmidt, K.G.; Nganou-Makamdop, K.; Tenbusch, M.; El Kenz, B.; Maier, C.; Lapuente, D.; Überla, K.; Spriewald, B.; Bergmann, S.; Harrer, E.G.; et al. SARS-CoV-2-Seronegative Subjects Target CTL Epitopes in the SARS-CoV-2 Nucleoprotein Cross-Reactive to Common Cold Coronaviruses. *Front. Immunol.* **2021**, *12*, 627568. [CrossRef] [PubMed]
40. Quinti, I.; Mortari, E.P.; Fernandez Salinas, A.; Milito, C.; Carsetti, R. IgA Antibodies and IgA Deficiency in SARS-CoV-2 Infection. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 655896. [CrossRef]
41. Sterlin, D.; Mathian, A.; Miyara, M.; Mohr, A.; Anna, F.; Claër, L.; Quentric, P.; Fadlallah, J.; Devilliers, H.; Ghillani, P.; et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci. Transl. Med.* **2021**, *13*, eabd2223. [CrossRef]
42. Zervou, F.N.; Louie, P.; Stachel, A.; Zacharioudakis, I.M.; Ortiz-Mendez, Y.; Thomas, K.; Aguero-Rosenfeld, M.E. SARS-CoV-2 antibodies: IgA correlates with severity of disease in early COVID-19 infection. *J. Med. Virol.* **2021**, *93*, 5409–5415. [CrossRef]

43. de Fays, C.; Carlier, F.M.; Gohy, S.; Pilette, C. Secretory Immunoglobulin A Immunity in Chronic Obstructive Respiratory Diseases. *Cells* **2022**, *11*, 1324. [CrossRef] [PubMed]
44. Infantino, M.; Manfredi, M.; Grossi, V.; Lari, B.; Fabbri, S.; Benucci, M.; Fortini, A.; Damiani, A.; Mobilia, E.M.; Panciroli, M.; et al. Closing the serological gap in the diagnostic testing for COVID-19: The value of anti-SARS-CoV-2 IgA antibodies. *J. Med. Virol.* **2021**, *93*, 1436–1442. [CrossRef] [PubMed]
45. Hennings, V.; Thörn, K.; Albinsson, S.; Lingblom, C.; Andersson, K.; Andersson, C.; Järbur, K.; Pullerits, R.; Idorn, M.; Paludan, S.R.; et al. The presence of serum anti-SARS-CoV-2 IgA appears to protect primary health care workers from COVID-19. *Eur. J. Immunol.* **2022**, *52*, 800–809. [CrossRef] [PubMed]
46. Lledó, A.; Retuerto, M.; Almendro-Vázquez, P.; Fernández-Ruiz, M.; Galindo, M.; Laguna-Goya, R.; Paz-Artal, E.; Lalueza, A.; Aguado, J.M.; Pablos, J.L. SARS-CoV-2-specific T-cell responses after COVID-19 recovery in patients with rheumatic diseases on immunosuppressive therapy. *Semin. Arthritis Rheum.* **2021**, *51*, 1258–1262. [CrossRef] [PubMed]
47. Shenoy, P.; Ahmed, S.; Shanoj, K.C.; Shenoy, V.; Damodaran, D.; Menon, A.R.; Alias, B.; SanjoSaijan; Devakumar, D.; Babu, A.S. Antibody responses after documented COVID-19 disease in patients with autoimmune rheumatic disease. *Clin. Rheumatol.* **2021**, *40*, 4665–4670. [CrossRef] [PubMed]
48. Deakin, C.T.; Cornish, G.H.; Ng, K.W.; Faulkner, N.; Bolland, W.; Hope, J.; Rosa, A.; Harvey, R.; Hussain, S.; Earl, C.; et al. Favorable antibody responses to human coronaviruses in children and adolescents with autoimmune rheumatic diseases. *Med* **2021**, *2*, 1093–1109. [CrossRef] [PubMed]
49. Simon, D.; Tascilar, K.; Kleyer, A.; Fagni, F.; Krönke, G.; Meder, C.; Dietrich, P.; Orlemann, T.; Kliem, T.; Mößner, J.; et al. Impact of Cytokine Inhibitor Therapy on the Prevalence, Seroconversion Rate, and Longevity of the Humoral Immune Response against SARS-CoV-2 in an Unvaccinated Cohort. *Arthritis Rheumatol.* **2022**, *74*, 783–790. [CrossRef] [PubMed]
50. Filocamo, G.; Minoia, F.; Carbogno, S.; Costi, S.; Romano, M.; Cimaz, R. Pediatric Rheumatology Group of the Milan Area. Absence of Severe Complications from SARS-CoV-2 Infection in Children with Rheumatic Diseases Treated with Biologic Drugs. *J. Rheumatol.* **2021**, *48*, 1343–1344. [CrossRef] [PubMed]
51. Haslak, F.; Yildiz, M.; Adrovic, A.; Sahin, S.; Koker, O.; Aliyeva, A.; Barut, K.; Kasapcopur, O. Management of childhood-onset autoinflammatory diseases during the COVID-19 pandemic. *Rheumatol. Int.* **2020**, *40*, 1423–1431. [CrossRef]
52. Santos, C.S.; Férnandez, X.C.; Morales, C.M.; Álvarez, E.D.; Castro, C.Á.; Robles, A.L.; Sandoval, T.P. Biological agents for rheumatic diseases in the outbreak of COVID-19: Friend or foe? *RMD Open* **2021**, *7*, e001439. [CrossRef] [PubMed]
53. Ceribelli, A.; Motta, F.; De Santis, M.; Ansari, A.A.; Ridgway, W.M.; Gershwin, M.E.; Selmi, C. Recommendations for coronavirus infection in rheumatic diseases treated with biologic therapy. *J. Autoimmun.* **2020**, *109*, 102442. [CrossRef] [PubMed]
54. Esatoglu, S.N.; Tascilar, K.; Babaoğlu, H.; Bes, C.; Yurttas, B.; Akar, S.; Pehlivan, O.; Akleylek, C.; Tecer, D.; Seyahi, E.; et al. Turkish Society for Rheumatology COVID-19 Registry Investigators. COVID-19 among Patients with Inflammatory Rheumatic Diseases. *Front. Immunol.* **2021**, *12*, 651715. [CrossRef] [PubMed]
55. Zanetti, A.; Carrara, G.; Landolfi, G.; Rozza, D.; Chighizola, C.B.; Alunno, A.; Andreoli, L.; Caporali, R.; Gerli, R.; Sebastiani, G.D.; et al. CONTROL-19 study by the Italian Society for Rheumatology. Increased COVID-19 mortality in patients with rheumatic diseases: Results from the CONTROL-19 study by the Italian Society for Rheumatology. *Clin. Exp. Rheumatol.* **2022**, *40*, 2038–2043.
56. Grainger, R.; Kim, A.H.J.; Conway, R.; Yazdany, J.; Robinson, P.C. COVID-19 in people with rheumatic diseases: Risks, outcomes, treatment considerations. *Nat. Rev. Rheumatol.* **2022**, *18*, 191–204. [CrossRef]
57. Liguoro, I.; Pilotto, C.; Bonanni, M.; Ferrari, M.E.; Pusiol, A.; Nocerino, A.; Vidal, E.; Cogo, P. SARS-CoV-2 infection in children and newborns: A systematic review. *Eur. J. Pediatr.* **2020**, *179*, 1029–1046; Erratum in: *Eur. J. Pediatr.* **2021**, *180*, 2343. [CrossRef] [PubMed]

4.3. Publikacja III

Publikacja III „ Interferon-γ Release Assay in the Assessment of Cellular Immunity—A Single-Centre Experience with mRNA SARS-CoV-2 Vaccine in Patients with Juvenile Idiopathic Arthritis.” jest pracą oryginalną. Jej celem była ocena przydatności testu IGRA w ocenie odpowiedzi immunologicznej na szczepienie przeciwko zakażeniu SARS-CoV-2 wśród pacjentów z MIZS.

We wstępie przedstawiono najnowsze, dostępne w czasie publikacji artykułu, dane dotyczące wyszczepialności populacji przeciwko zakażeniu nowym koronawirusem oraz zalecenia United States Food and Drug Administration (FDA) na jesień 2023r. dotyczące przyjmowania dodatkowych dawek szczepionki przeciwko zakażeniu SARS-CoV-2. Następnie opisano sprzeczne doniesienia dotyczące występowania zwiększonego ryzyka zakażenia i cięższego przebiegu COVID-19 w populacji osób cierpiących na choroby autoimmunizacyjne i autozapalne oraz lukę w wiedzy dotyczącą szczególnej grupy pacjentów, jaką są pacjenci pediatryczni z powyższymi zaburzeniami. Skuteczność i bezpieczeństwo programów szczepień przeciwko SARS-CoV-2 u dorosłych pozwoliły na stopniowe rozszerzanie grup wiekowych mogących przyjąć szczepienie, włączając w nie osoby małoletnie. Niestety ciągle brakuje dużych wieloośrodkowych badań prospektywnych dotyczących efektywności oraz bezpieczeństwa szczepień przeciwko koronawirusowi u dzieci z chorobami reumatycznymi. Dostępne wyniki, i tym samym wydawane zalecenia, opierają się głównie na retrospektywnych analizach i ocenie miana wytworzonych przeciwciał z pominięciem oceny odpowiedzi komórkowej czy dalszej obserwacji klinicznej pacjentów w kontekście reinfekcji. Ponadto opisano aktualny stan wiedzy dotyczący wykorzystania testu IGRA do diagnostyki odpowiedzi komórkowej po zakażeniu lub szczepieniu przeciwko SARS-CoV-2.

Badanie przeprowadzono na grupie 55 pacjentów (41 dziewczynek i 14 chłopców) z medianą wieku 10,31 lat, u których rozpoznano MIZS zgodnie z kryteriami ILAR. Kwalifikacja do badania odbywała się podczas hospitalizacji w Klinice Kardiologii i Reumatologii Dziecięcej Uniwersytetu Medycznego w Łodzi. U piętnastu pacjentów było to świeże rozpoznanie choroby, reszta zgłosiła się do Oddziału z powodu zaostrzenia objawów lub celem podaży leku biologicznego. Jedynymi kryteriami wyłączenia z badania były: wiek pacjenta >16 r.ż. oraz zaostrzenie choroby wymagające podaży wysokich dawek glikokortykosteroidów systemowych. Dwudziestu dziewięciu pacjentów było zdiagnozowanych jako typ skąpostawowy zapalenia stawów (co jest najczęstszym podtypem choroby), dwudziestu jako typ wielostawowy, a pozostałych sześciu jako MIZS o początku uogólnionym. Uczestnicy badania znajdowali się na różnych etapach leczenia, w tym biologicznego. Ośmioro dzieci otrzymało co najmniej jedną dawkę szczepionki mRNA Comirnaty w okresie od 1 do 18 miesięcy przed kwalifikacją do badania, pozostali pacjenci nie zostali uodpornieni przeciwko SARS-CoV-2. Wśród uczestników badania byli zarówno pacjenci, którzy przebyli COVID-19, jak i ci, którzy negowali zakażenie SARS-CoV-2.

Zmiennymi ilościowymi analizowanymi w badaniu była odpowiedź humoralna, mierzona na podstawie miana wytworzonych przeciwciał w klasie IgM, IgG i IgA oraz odporność komórkowa limfocytów T po kontakcie z antygenem SARS-CoV-2. Próbki krwi pobierane były wraz z rutynowymi badaniami laboratoryjnymi wykonywanymi w trakcie hospitalizacji. Specyficzną odpowiedź komórek T na antygeny SARS-CoV-2 mierzono za pomocą ilościowego testu IGRA w pełnej krwi przy użyciu testu Quan-T-Cell SARS-CoV-2 EUROIMMUN. Heparynizowaną krew inkubowano w zestawie trzech probówek: (1) probówka bez składników aktywujących; (2) probówka IGRA do specyficznej stymulacji komórek T białkiem S antygenu SARS-CoV-2; oraz (3) probówka do nieswoistej stymulacji komórek T, celem określenia zdolności do stymulacji. Po usunięciu komórek podczas wirowania, otrzymane osocze analizowano za pomocą testu ELISA w celu określenia stężenia uwolnionego IFN-γ. Dodatkowo u wszystkich pacjentów wykonano badanie ELISA

anty-SARS-CoV-2 w celu oznaczenia stężeń przeciwciał IgA, IgM i IgG. Zarówno testy IGRA, jak i ELISA, przeprowadzono przy użyciu próbek pobranych w tym samym momencie, zaraz po włączeniu do badania.

Ponadto przeprowadzono ankietę telefoniczną z rodzicami uczestników dotyczącą wystąpienia infekcji COVID-19 lub jej podejrzenia u ich dzieci w miesiącach po zakończeniu badania. Celem zachowania bezstronności, rozmowę z rodzicami przeprowadził lekarz niezaangażowany wcześniej w eksperyment i niemający wglądu w jego wyniki.

Analizę statystyczną przeprowadzono za pomocą oprogramowania Statistica 13.3. Porównania grupowe przeprowadzono za pomocą testu U Manna–Whitneya. Wartości p poniżej 0,05 uznawano za istotne statystycznie. Aby obliczyć najdokładniejszą wartość odcięcia dla testu IGRA, autorzy wykorzystali indeks Youdena. Czułość i swoistość badania IGRA jako markera podatności poszczególnych osób na zakażenie SARS-CoV-2 analizowano za pomocą krzywej ROC (ang. Receiver Operating Characteristic curve).

Wszyscy pacjenci biorący udział w badaniu, którzy otrzymali szczepienie przeciwko SARS-CoV-2, wytworzyli zarówno specyficzną odpowiedź limfocytów T mierzoną testem IGRA ($p = 0,0016$), jak i odpowiedź humoralną ocenianą na podstawie miana przeciwciał, istotną w klasie IgA ($p = 0,001$) i IgG ($p = 0,008$). Nie stwierdzono zależności z przeciwciałami w klasie IgM. Ponadto stwierdzono różnice w odporności wytworzonej po szczepieniu oraz odporności „dzikiej" po przechorowaniu infekcji SARS-CoV-2. Zarówno odpowiedź limfocytów T, jak i wartości przeciwciał w klasach IgG i IgA, były wyższe po uodpornieniu biernym niż po przebyciu choroby. Według przeprowadzonej ankiety jedynie szóstka dzieci zgłosiła potwierdzoną w PCR infekcję COVID-19 po zakończeniu badania. Dziesięcioro dzieci miało objawy sugerujące zakażenie SARS-CoV-2, jednak bez potwierdzenia laboratoryjnego. Ze względu na brak uniwersalnej normy laboratoryjnej dla badania IGRA, autorzy ustalili wartość punktu odcięcia na 1022,15, przy 60% czułości i 80% swoistości. Wśród osób zaszczepionych, pacjenci, którzy zgłosili zakażenie SARS-CoV-2 w dalszej obserwacji mieli istotnie niższe miano przeciwciał IgA ($p = 0,0102$), IgG ($p = 0,058$) i wysokoczułego IgG NCP ($p = 0,0029$) niż dzieci, u których nie zgłaszano wystąpienia COVID-19 po zakończeniu badania.

W dyskusji porównano uzyskane wyniki z danymi z innych badań nad odpornością komórkową oraz publikacji zawierających badania uwzględniające pacjentów z chorobami autoimmunizacyjnymi. Odniesiono się również do ograniczeń badania związanych z relatywnie małą liczebnością grupy badanej oraz braku grupy kontrolnej.

Aktualnie dostępne szczepienia przeciwko zakażeniu SARS-CoV-2 powodują efektywne wytworzenie zarówno odporności komórkowej, jak i humoralnej u pacjentów pediatrycznych z MIZS, niezależnie od przyjmowanego przez nich leczenia. Test IGRA służący do oceny odpowiedzi komórek T na kontakt z antygenem może stanowić wartościowe narzędzie w ocenie wytworzonej odpowiedzi immunologicznej, samodzielnie lub jako uzupełnienie serologii. Dalsza obserwacja po zakończeniu badania wskazuje na niski odsetek zakażeń SARS-CoV-2, mimo spodziewanych zaburzeń odporności spowodowanych chorobą podstawową lub przyjmowanym leczeniem. Konieczne są jednak większe badania kohortowe w celu ustalenia kompleksowych wytycznych dotyczących programów szczepień przeciwko SARS-CoV-2 u dzieci chorych z chorobami autoimmunizacyjnymi.

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Interferon-γ Release Assay in the Assessment of Cellular Immunity—A Single-Centre Experience with mRNA SARS-CoV-2 Vaccine in Patients with Juvenile Idiopathic Arthritis

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Abstract: Background: As the SARS-CoV-2 virus remains one of the main causes of severe respiratory system infections, the Food and Drug Administration strongly advises the continuation of current vaccination programs, including the distribution of updated boosters, especially in high-risk groups of patients. Therefore, there is an unceasing need for further research on the safety and, no less importantly, the clinical effectivity of the vaccines, with an extra focus on cohorts of patients with underlying health problems. This study aimed to assess the efficacy of the SARS-CoV-2 vaccine in possibly immunocompromised children with rheumatic disease while utilizing the interferon-gamma release assay (IGRA) as a marker for COVID-19 immunity in the study follow-up. **Methods**: This prospective study was performed in a group of 55 pediatric patients diagnosed with juvenile idiopathic arthritis. Eight participants were immunized with the Comirnaty mRNA vaccine before the research commenced, while the rest of the group (n = 47) had not been vaccinated against SARS-CoV-2. At the study baseline, the cellular response to the virus antigen was measured using a specific quantitative IGRA in whole blood; subsequently, the anti-SARS-CoV-2 test was performed, marking the antibodies' levels in serum. Around four months after the enrollment of the last patient in the study, a follow-up survey regarding the events of COVID-19 infection within the cohort was conducted. **Results**: The study confirmed that all the vaccinated children developed specific T-cell (p = 0.0016) and humoral (p = 0.001 for IgA antibodies, p = 0.008 for IgG antibodies) responses to the inoculation, including those receiving biological treatment and those on conventional disease-modifying anti-rheumatic drugs. The study also showed the different patterns of immunity elicited both after infection and post-vaccination, with higher levels of antibodies and T-cell response after inoculation than after natural exposure to the pathogen. According to the follow-up survey, six children developed PCR-confirmed SARS-CoV-2 infection, whereas the additional 10 patients admitted to having COVID-like symptoms with no laboratory verification. **Conclusions**: SARS-CoV-2 vaccinations elicit valid immune responses in pediatric rheumatic patients. Including the assessment of T-cell immunity in the evaluation of inoculation-induced immunization can enhance the accuracy of sole humoral response assays.

Keywords: SARS-CoV-2; mRNA vaccine; cellular immunity; juvenile idiopathic arthritis

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1. Introduction

As of July 2023, more than 70% of the world population had received at least one dose of the SARS-CoV-2 vaccine, which accounts for over 13 billion doses administered globally

and more than 40 thousand shots administered every single day [1]. A variety of advanced vaccine candidates, including i. a. mRNA, inactivated virus, protein subunit and virus-like particles, entered different phases of clinical trials [2], while more than 20 have met approval to be used [3]. The current guidelines of the United States Food and Drug Administration (FDA) for fall 2023 advise that everyone, including children older than six months, should receive at least one dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine updated with an XBB-lineage of the Omicron variant [4]. Moreover, moderately and severely immunocompromised patients may require additional boosters [5]. That being said, there is an urgent and unfaltering need for continuous monitoring of both the safety and efficacy of vaccines against SARS-CoV-2.

There is still a lack of consensus regarding the risk of more severe or fatal COVID-19 infection in patients suffering from autoimmune and inflammatory disorders, including rheumatic diseases (RDs). While initial studies suggested a similar or just slightly higher risk of contracting the virus or worse prognosis for infection in patients with RDs compared to healthy individuals [6,7], a more recent and extensive meta-analysis states that RDs predispose to substantially higher rates of SARS-CoV-2 infection and increased mortality [8]. Notably, most of the data available are obtained from the adult population, leaving a knowledge gap in the management of pediatric patients. While the studies that evaluated the course of COVID-19 in children with RDs did not prove a significantly higher risk of poor disease outcomes compared to the previously healthy population, the infection can still lead to a flare of the underlying condition and a need for therapy escalation. In some cases, it can result in a reduced health-related quality of life [9,10]. Thus, pediatric patients with RDs or other autoimmune diseases seem to represent a special group of interest in which protective measures taken to prevent the severe course of COVID-19 should remain significant.

After a successful trial of the BNT162b2 mRNA vaccine in adults, it was also approved for the inoculation of children between the ages of 12 and 18. Regarding the safety of vaccination in the population suffering from RDs, it may be important to acknowledge the dependencies between the SARS-CoV-2 vaccine and the onset of autoimmune diseases mentioned in several studies. In their extensive systematic reviews of new cases of arthritis or the worsening of the already existing condition as a result of the SARS-CoV-2 vaccine, researchers stressed the need for awareness of joint-related inoculation side effects. However, the lack of data from well-controlled trials questions the significance of these findings. Additionally, in most of the patients, the clinical symptoms subsided after the use of nonsteroidal anti-inflammatory drugs or glucocorticoids without the need to introduce disease-modifying antirheumatic drugs (DMARDs) [11]. A similar response to steroid therapy, with a tendency to resolve spontaneously in some cases, was observed in SARS-CoV-2 vaccine-induced vasculitides [12]. However, there are also reports of more severe, life-threatening cases of autoimmune and autoinflammatory syndromes after the new vaccination, including dermatomyositis complicated by lung disease [13] and adult-onset Still disease [14]. Markedly, these complications were predominantly found in adults, seemingly sparing the pediatric population.

The safety and presence of humoral response to the vaccination in the cohort of adolescents with RDs have been confirmed in the prospective studies [15,16]. However, while the most accurate assessment of inoculation efficacy should be performed in randomized control trials (RCTs) with the evaluation of mild to severe infections, the assessment may be problematic in trials with smaller sample sizes [17,18]. Therefore, the majority of available data on the assessment of vaccine potency in children with RDs are based primarily on the sole analysis of antibody titers.

However, during the pandemic, numerous studies confirmed the utility of marking cellular immunity after COVID-19 infection or inoculation. The currently available mRNA vaccines are found to elicit long-lasting T-cell responses across variants of the virus, detectable even after the decrease in antibody titers [19]. Interestingly, with the new mutations of COVID-19, like Omicron, it was suggested that the T-cell response was retained in vac-

cinated individuals, even with the lack of neutralizing antibodies [20]. The SARS-CoV-2 interferon-gamma release assay (IGRA), which was previously used mainly in the diagnostics of tuberculosis [21], is a useful tool for the assessment of immune responses after inoculation. The test measures the levels of interferon gamma (IFN-γ) secreted by T-helper 1 and T-cytotoxic cells that were previously primed and activated by SARS-CoV-2-specific S protein produced upon vaccination [22]. The authors present the more detailed mechanism of the mRNA vaccine in Figure 1. Moreover, the assessment of vaccine-elicited cellular response has been successfully applied to cohorts of immunocompromised individuals, including patients suffering from RDs [23,24]. IGRA has already been utilized in the monitoring of cellular response and therefore the efficacy of vaccination in pediatric patients suffering from inflammatory bowel disease [25].

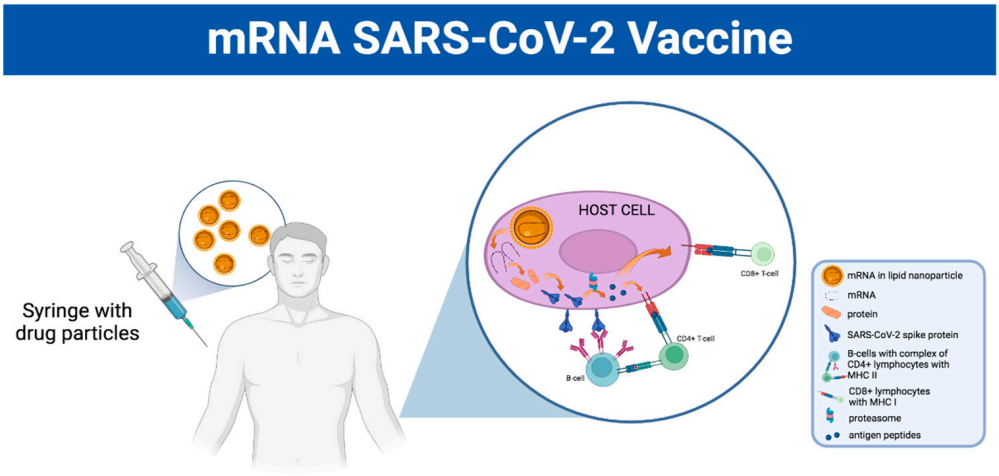


Figure 1. Mechanism of messenger ribonucleic acid (mRNA) vaccine. The mRNA molecules encapsulated in lipid nanoparticles facilitating the release of mRNA into the cells are injected into the body. Once the mRNA molecules are inside the cytosol of the host cell, they are translated into viral protein to elicit a specific immune response. Once inside host cells, the mRNA is translated into SARS-CoV-2 spike protein, which is expressed on the surface of the host cells and is exposed to the extracellular space. The transient expression of this spike antigen induces neutralizing antibodies and cellular immune responses against it. After proteasomal processing, antigen peptides join the major histocompatibility complex class I molecule (MHC I) and are transferred to the antigen-presenting cell surface, activating CD8+ T cells. Protein antigens can activate CD4+ T cells via the MHC Class II presentation pathway.

The current research aimed to conduct a comprehensive assessment of both humoral and cellular immunity mounted by the SARS-CoV-2 mRNA vaccine in patients with JIA and to assess IGRA as a marker of immunity against COVID-19 in the study follow-up.

2. Materials and Methods

The design of this prospective study included the assessment of humoral and cellular responses to SARS-CoV-2 in a group of vaccinated and unvaccinated pediatric patients with JIA. After the enrollment of all the participants, a follow-up survey regarding breakthrough COVID-19 cases was conducted.

The research was performed in a cohort of 55 children with juvenile idiopathic arthritis (JIA) during their hospitalization in the Department of Pediatric Cardiology and Rheumatology, Medical University of Lodz, Poland, between June 2021 and February 2023 with the follow-up survey 2 years after the recruitment of the first patient and 4 months after including the last participant. All the patients were diagnosed with JIA according to the

International League of Associations for Rheumatology (ILAR) classification criteria [26], with onset before the 16th birthday and arthritis persisting for at least 6 weeks. Among the causes of hospitalization were a newly diagnosed JIA, a flare of the disease demanding modification of the treatment, or routine day-case hospitalizations that are a part of biological agent protocols. Eight children within the study group received the Comirnaty mRNA vaccine between 1 and 18 months prior to their enrollment in the study, whereas the rest of the group (n = 47) had not been vaccinated against SARS-CoV-2 when the tests were performed. Patients who qualified for the study had both positive and negative histories of COVID-19. The inclusion criteria for the study were a confirmed diagnosis of JIA and a maximum age of 16 years on the day of recruitment. The cohort consisted of both newly diagnosed cases (n = 15) and patients with years-long treatments; participants were selected consecutively. None of the patients were in a flare of the rheumatic process severe enough to require high doses of steroids. The results of the follow-up questionnaire were acquired from 53 out of the 55 parents of the patients included in the research. The questionnaire was designed to include all the participants, although two patients could not be reached by phone and were therefore not considered in this final part of the research.

The quantitative variables measured in this study were the cellular and humoral responses to SARS-CoV-2 antigens. The endpoint of the study in the follow-up was COVID-19 (whether confirmed or assumed). While the cohort of the study was not homogenous regarding the age of the children, social status and the received immunomodulating treatment, the study was conducted during the peak of the pandemic in Poland, so exposure to the virus should remain fairly comparable for all the participants. The specific T-cell response to SARS-CoV-2 antigens was measured using quantitative IGRA in whole blood with a Quan-T-Cell SARS-CoV-2 EUROIMMUN assay. Blood samples with 1.5 mL nominal volume were drawn simultaneously with routine laboratory tests during the patients' hospitalization. The heparinized blood was then incubated in a set of three tubes: (1) IGRA BLANK with no activating components for the individual's IFN-γ background; (2) IGRA TUBE for specific T-cell stimulation by SARS-CoV-2 antigen spike protein; and (3) IGRA STIM for unspecific T-cell stimulation with mitogen for determining stimulation ability. After the removal of cells during centrifugation, the obtained plasma was analyzed by a quantitative enzyme-linked immunosorbent assay (ELISA) to determine the concentration of released IFN-γ. Additionally, the anti-SARS-CoV-2 ELISA was performed in all patients to mark the levels of IgA, IgM and IgG antibodies. Both IGRA and ELISA assays were performed using the samples gathered at the same time point during the initial enrollment of each participant in the study.

During the follow-up survey, the parents of the children who participated in the study were asked questions regarding confirmed or suspected SARS-CoV-2 infection in the months following the trial. To keep the follow-up survey unbiased, it was conducted by a doctor not previously involved in the study and with no access to the acquired data. The achieved sample size was the result of a two-year timeframe established by the authors at the beginning of the research.

Group comparisons were performed using the Mann–Whitney U test. p values below 0.05 were considered significant. To calculate the most accurate cutoff value for IGRA, the authors utilized the Youden index. The sensitivity and specificity of IGRA as a marker for individuals' susceptibility to SARS-CoV-2 infection were analyzed using the receiver operating characteristic (ROC) curve. All statistical calculations were performed using Statistica 13.1 software (Statsoft Polska, Krakow, Poland). The reporting in this study conforms to STROBE [27].

The study was approved by the local Bioethics Committee, with approval number RNN/117/21/KE. All the diagnostic tools were ordered from EUROIMMUN POLSKA, Wroclaw, Poland.

3. Results

The general characteristics of the study group are presented in Table 1. Patient age, sex, subtype of JIA (oligoarthritis, polyarthritis or systemic-onset arthritis) and time from the initial diagnosis to study onset or rheumatic disease flare were all found to have no statistical significance for the patients' immune responses.

Table 1. General characteristics of the study group.

	Total Number of Patients	**Received SARS-CoV-2 Vaccine**
	***n* = 55**	***n* = 8**
Male/Female	14/41	2/6
Age on examination (years)	10.31 ± 4.16	13.75 ± 1.47
Type of JIA:		
systemic	6	2
oligoarticular	29	4
polyarticular	20	2
Newly diagnosed JIA	15	2
Flare of JIA	8	0
History of confirmed SARS-CoV-2 infection	8	0
Treatment regime:		
Biological agents specifically:	22	3
adalimumab	13	3
tocilizumab	6	0
etanercept	2	0
baricitinib	1	0
Methotrexate	32	4
Sulfasalazine	8	0
Hydroxychloroquine	7	2
Cyclosporine	3	0
Azathioprine	1	1
Glucocorticoids	4	0

Values presented as mean ± standard deviation (SD), JIA—Juvenile Idiopathic Arthritis.

Participants in the study received various immunomodulating treatments, including biologic and oral disease-modifying antirheumatic drugs; a few children were on moderate or small doses of prednisone. Among the vaccinated individuals, three were receiving biological treatment with tumor necrosis factor (TNF) inhibitor, four were treated with methotrexate, two with hydroxychloroquine and one with azathioprine.

The research confirmed that all the vaccinated children developed specific T-cell responses measured by IGRA ($p = 0.0016$) (Figure 2A) and humoral responses assessed by antibody titers, significant in IgA ($p = 0.001$) (Figure 2B) and IgG ($p = 0.008$) class (Figure 2C). No dependencies with IgM antibody levels were found. When analyzing and comparing humoral and cellular immunity after vaccination with the naturally induced immune responses, there was a notable difference between the immunity elicited by vaccination and post-exposure immunity. The study indicated higher levels of IGRA (Figure 3A) and higher titers of IgG (Figure 3B) and IgA (Figure 3C) antibodies after inoculation in comparison with natural exposure to the virus.

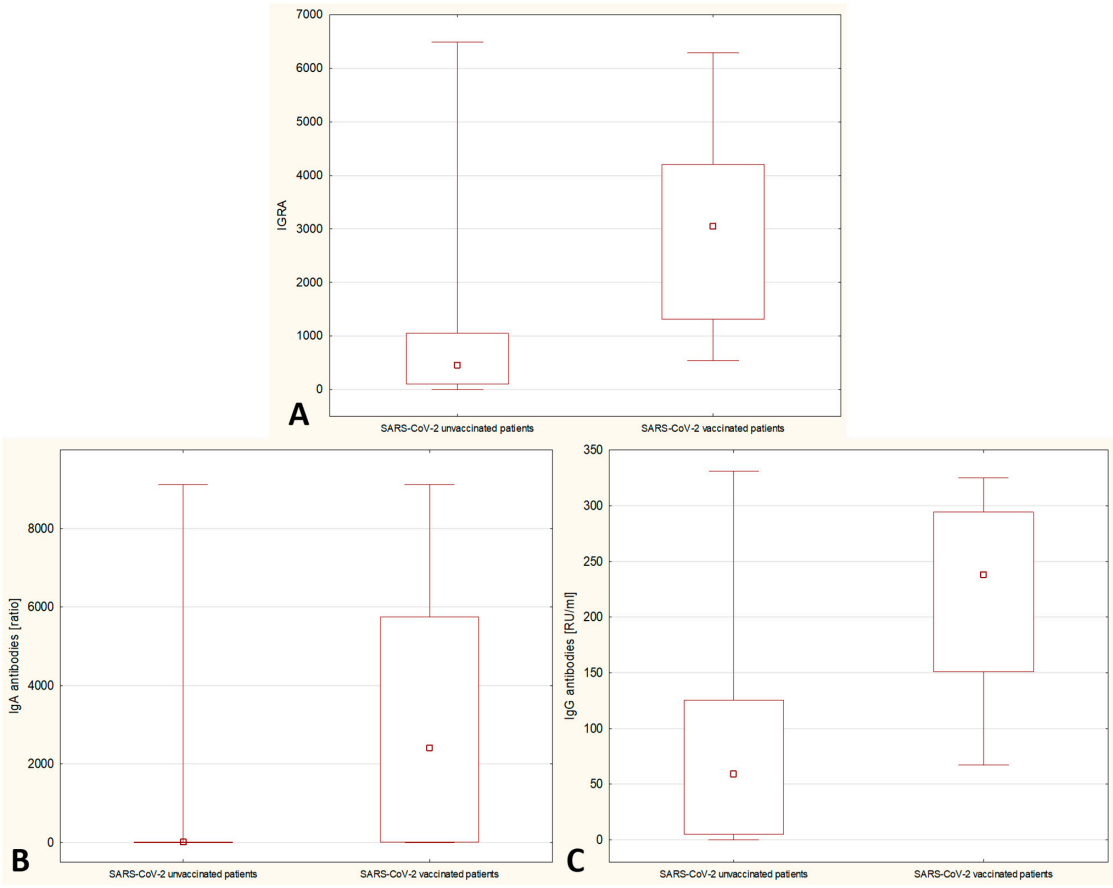


Figure 2. (**A**) Association between Interferon-γ Release Assay (IGRA) and SARS-CoV-2 vaccination. (**B**) Dependency between IgA antibodies and SARS-CoV-2 vaccination. (**C**) Dependency between IgG antibodies and SARS-CoV-2 vaccination.

Details of the survey are presented in Table 2. Among the participants, 37 patients denied contracting SARS-CoV-2 in the months following their participation in the study. Only six children had SARS-CoV-2 infection confirmed by PCR, while 10 participants admitted to having COVID-like symptoms with no PCR confirmation (Scheme 1). None of the cases of SARS-CoV-2 within the study group were severe. As there is no well-established cutoff value for IGRA, the authors attempted to calculate one utilizing the follow-up survey results. IGRA showed higher specificity and sensitivity when the clinical symptoms were supplemented with laboratory confirmation. The proposed cutoff value for positive IGRA was 1022.15 (Figure 4), with 60% sensitivity and 80% specificity, as calculated

with the ROC curve (Figure 5). The results obtained after the follow-up survey indicated that, among the vaccinated patients, significantly lower IgA ($p = 0.0102$), IgG ($p = 0.058$), and IgG NCP ($p = 0.0029$) antibody titers were found in participants with breakthrough SARS-CoV-2 infections.

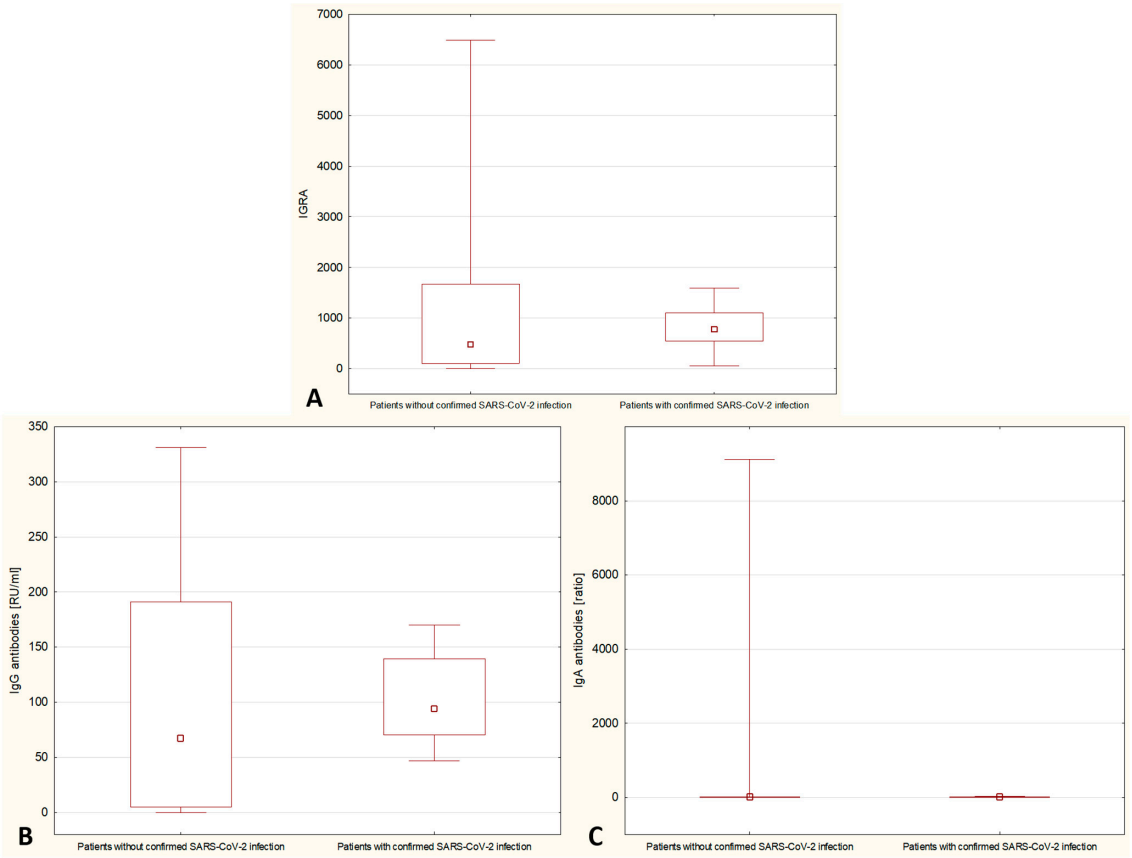
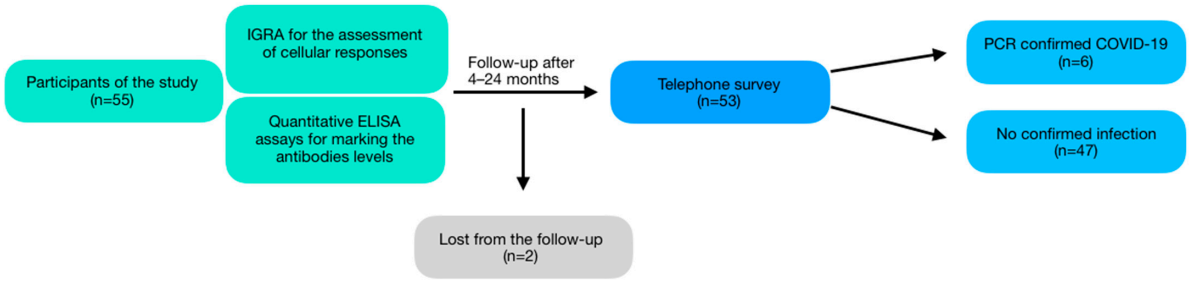


Figure 3. (**A**) Association between Interferon-γ Release Assay (IGRA) and postinfection natural immunity. (**B**) Dependency between IgA antibodies and postinfection natural immunity. (**C**) Dependency between IgG antibodies and postinfection natural immunity.

Table 2. A follow-up telephone survey concerning SARS-CoV-2 infection events.

Questions	**Did Your Child Have Confirmed SARS-CoV-2 Infection or COVID-like Symptoms since Being Included in the Clinical Trial?**
Answers:	Number of patients
No	37
Possible, present some of COVID-like symptoms, no PCR confirmation	8
Highly possible, many COVID-like symptoms, no PCR confirmation	2
Yes, infection confirmed in PCR	6
Did not answer the phone	2

Question and answers have been translated into English. PCR-polymerase chain reaction.



Scheme 1. A flow diagram showing the steps of the study and the results of the follow-up survey. IGRA-interferon-gamma release assay, ELISA-enzyme-linked immunosorbent assay, PCR-polymerase chain reaction.

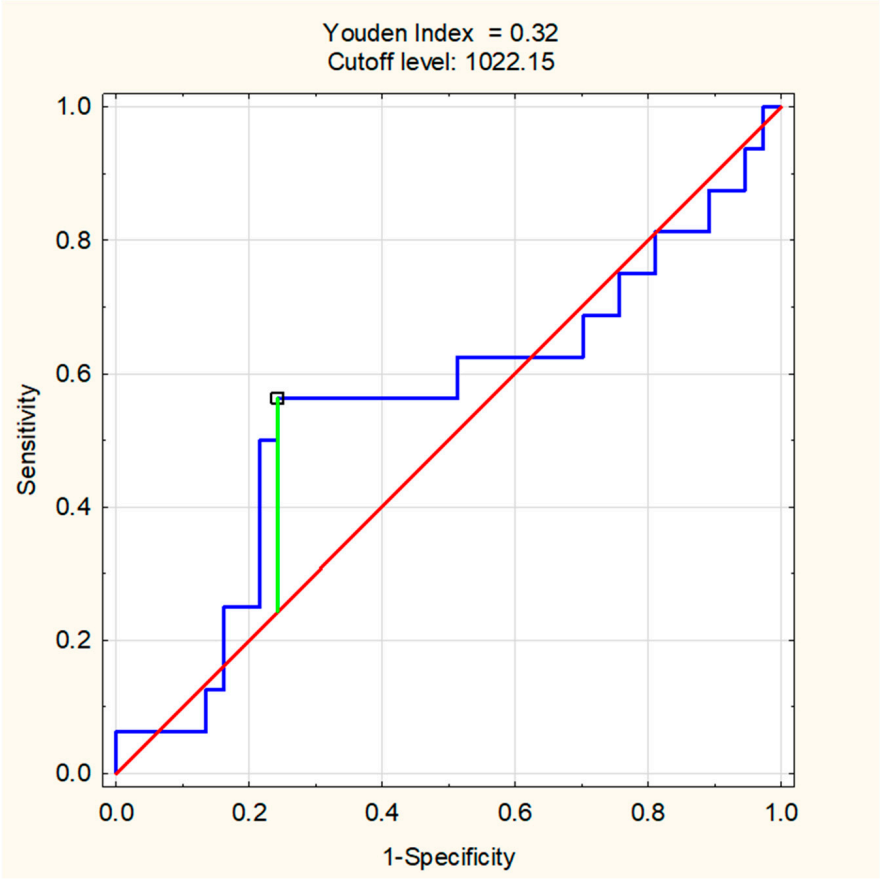


Figure 4. The receiver operating characteristic curve and the cutoff value for a positive IGRA result.

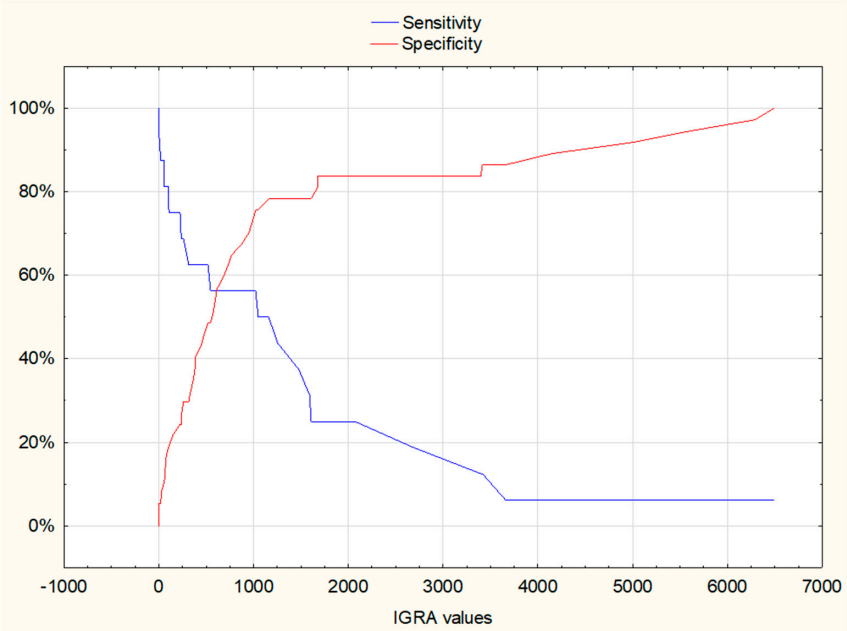


Figure 5. The specificity and sensitivity of IGRA in terms of assessing a patient's susceptibility to SARS-CoV-2 infection.

4. Discussion

As far as the main goals of the current research are concerned, the study confirmed the validity of SARS-CoV-2 vaccination programs in the cohort of pediatric patients with RDs. All the participants undergoing treatment protocols and other variables, regardless of the type of JIA, elicited both humoral and cellular responses to the inoculation. Moreover, the application of IGRA to marking T-cell immunity proves to be a viable way to mark individual immunity. The follow-up of the participants confirmed a low rate of breakthrough infections among children with JIA.

It has already been proven in a few sources that the assessment of cellular responses after SARS-CoV-2 infection or vaccination provides a more comprehensive evaluation of individual immunity than the sole use of serological testing [28–30]. While different branches of adaptive immunity coordinate to maintain optimal protection against pathogens, T-cells play a key role in controlling the developing infection by modulating disease severity and in creating long-term memory pools that are believed to decline at a slower rate than antibody titers [30,31]. A recent study confirmed a positive association between SARS-CoV-2 specific T-cell responses and antibody titers after inoculation. These results are concordant with multiple studies [30–33], including one by Agrati et al. [34], who evaluated the elicited immunity to the second dose of the mRNA vaccine, early after the shot and 12 weeks later, noting the decrease in antibody titers while the T-cell memory persisted. Still, the study showed dependencies between cellular and humoral responses, suggesting the perseverance of coordinated immunity. The diversity of IGRA methodology results in difficulties in comparing results between studies. The standardized cutoff level would facilitate the collection of all the results for future meta-analysis. The cutoff values proposed in the current study are considerably higher than in most of the publications, where they were established at around 200 mIU/mL [35–37]. According to Lledó et al. [38], cellular responses to SARS-CoV-2 antigens were comparable between RD patients and healthy controls; they stated that neither the disease nor RD therapies should affect individual adaptive immune responses. However, there are some conflicting data on adaptive immu-

nity elicited in children compared to that in adults. Some studies evaluating the immune reaction to SARS-CoV-2 infection in the pediatric population have stated that the specific T-cell responses were comparable between adolescents and adults [39]. However, multiple studies regarding immune reactions to infection or vaccination have identified age as a factor significantly associated with the magnitude of cellular responses to SARS-CoV-2 antigens [40–42].

According to previous research on immunological responses to the SARS-CoV-2 vaccine in a group of individuals suffering from autoimmune diseases, the patients did develop humoral response to the inoculation. However, when compared to the healthy controls, the immune reactions were considerably delayed or reduced [43,44]. Notably, in accordance with Simon et al., the reduced effect of the vaccine resulted from the underlying autoimmune disease and its impact on individuals' immune reactions rather than from the immunomodulating treatment [44]. These results are concordant with the data that were acquired during the current study, as none of the treatments that the patients were receiving proved to affect their immune reactions to the inoculation. However, it should be stressed that this is not always true regarding some specific treatments used in RDs. As seen in the study by Oyaert et al., in which around half of the patients with RDs failed to evoke a humoral response and had lower cellular responses to the vaccine, all the patients were receiving immunosuppressive treatment with rituximab [45]. The current recommendations concerning the management of patients with RDs, including vaccination programs in both adults [6] and children [15–17], are based mainly on the antibody-eliciting potential. There is scarce data regarding cellular responses in patients with RDs after vaccination. Nevertheless, in a study by Mahil et al., conducted in a group of patients suffering from psoriasis and receiving immunomodulating treatment (MTX and biological agents), all subjects elicited a humoral response to the vaccine, although some did not have detectable T-cell responses even after the second dose [23]. The potency of existing antibodies and their clinical impact on undetectable cellular responses remains to be further explored.

However compelling the data on elicited humoral and cellular immunity may be, the clinical implications of the obtained results need to remain a priority. In a study by Calcoen et al. [46], even the slight decrease in immune response to SARS-CoV-2 three months after vaccination co-occurred with a high incidence rate of symptomatic breakthrough infections. Additionally, Vogrig et al. [47] conducted a prospective study on a group of 80 vaccinated individuals and reported that both the decreased IGRA response and, to a smaller extent, the humoral response were associated with the higher rate of reinfections during the follow-up period. To conclude, both methods can be valid measures for the prediction of breakthrough infections. A search for corresponding data in the cohort of patients with autoimmune diseases on immunomodulating therapies reveals a certain knowledge gap. Nevertheless, a study by Ahmed et al. [48] confirmed that the SARS-CoV-2 infections occurring after vaccination in patients with RDs were associated with COVID-19 seronegativity. Unfortunately, cellular responses were not taken into account in this paper. The results of the follow-up conducted in the recent study align with those results, as the lower IgA and IgG titers correlated with an occurrence of breakthrough infections after inoculation. Nonetheless, due to the small sample size of the vaccinated children included in the study, these data should be considered more as a trend.

The question of the differences between the immune responses to the SARS-CoV-2 vaccine, in comparison to the naturally acquired immunity, has been a point of interest in multiple studies. Amanat et al. analyzed naive individuals after inoculation with the SARS-CoV-2 mRNA vaccine and found that while antibody responses to the vaccine were robust and even exceeded those seen after natural infection, the majority of vaccine-induced antibodies did not have neutralizing activity [49]. These conclusions should be taken into account when evaluating the results acquired during the current study, where inoculation leads to both higher antibody responses and levels of IGRA than natural infection. Moreover, in a study utilizing single-cell mRNA sequencing to compare similar

cohorts of patients, the authors noted the expansion of CD8+ T-cell clones in more distinct clusters induced by natural infection compared to those induced by vaccination, meaning that they were more likely to recognize a broader spectrum of viral antigens, thus indicating the superiority of natural immunization [50].

We acknowledge that our study has some limitations, mostly due to the small sample size of patients who received the mRNA vaccine and the lack of a control group consisting of healthy individuals. Differences in time intervals between the inoculation and sample taking between the study participants may be another effect modifier. Additionally, the results obtained from the survey, for the most part, relied on the parents' evaluations of their children's symptoms, which may be highly biased.

To expand and add value to our observations, adding a control group of children without JIA in future research could provide interesting and reliable results regarding the differences in the immunity elicited in patients with RDs and healthy children. A larger cohort study with a comparison group would be highly valuable for future guidelines regarding COVID-19 vaccine programs in this specific cohort of patients. Additionally, the results obtained from the survey, for the most part, relied on the parents' evaluations of their children's symptoms, which may be highly biased. A more standardized follow-up of vaccinated children with JIA may provide essential knowledge of the clinical outcomes of inoculations among immunocompromised patients.

5. Conclusions

SARS-CoV-2 mRNA vaccine may successfully elicit humoral and cellular immune responses in children with JIA, including patients receiving biological treatment and/or conventional DMARDs. IGRA assays for the evaluation of T-cell responses to inoculation may add value to quantitative ELISA antibody tests. Moreover, the study follow-up indicates a low rate of breakthrough SARS-CoV-2 infections, even in possibly immunocompromised children. The study postulates that pediatric patients with RDs develop substantial immune responses to the SARS-CoV-2 mRNA vaccine, regardless of the disease-modifying therapies they are receiving. Larger cohort studies are needed to establish comprehensive guidelines regarding SARS-CoV-2 inoculation programs in children with RDs.

Author Contributions: K.K.: Investigation, Writing—Original draft preparation, Visualization; K.O.: Formal analysis, Data curation, Writing—Review and Editing; A.M.: Conducting a Survey, Writing—Review and Editing E.S.: Conceptualization, Validation, Supervision, Project Administration. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was performed in line with the principles of the Declaration of Helsinki, approved by the Bioethics Committee on the 11 May 2021, approval number RNN/117/21/KE.

Informed Consent Statement: Informed consent was obtained from all individual participants included in the study. Since the patients were 16 and younger, consent was obtained from all the children's parents. Moreover, participants who were 13 years old and above were also asked for written consent after being provided with an age-appropriate explanation of the study.

Data Availability Statement: The data used to support the findings of this study are included in the article. The supplementary data are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sayeed, M.A.; Shalim, E.; Farooqui, F.; Farman, S.; Khan, M.; Iqbal, A.; Ahmed, I.; Rajput, A.W.; Razzaque, A.; Quraishy, S. Comparison of the Disease Severity and Outcome of Vaccinated COVID-19 Patients with Unvaccinated Patients in a Specialized COVID-19 Facility: A Retrospective Cohort Study from Karachi, Pakistan. *Vaccines* **2023**, *11*, 1178. [CrossRef] [PubMed]

2. Wang, D.; Yuan, Y.; Liu, B.; Epstein, N.D.; Yang, Y. Protein-based nano-vaccines against SARS-CoV-2: Current design strategies and advances of candidate vaccines. *Int. J. Biol. Macromol.* **2023**, *236*, 123979. [CrossRef] [PubMed] [PubMed Central]
3. Kumar, S.; Basu, M.; Ghosh, P.; Ansari, A.; Ghosh, M.K. COVID-19: Clinical status of vaccine development to date. *Br. J. Clin. Pharmacol.* **2023**, *89*, 114–149. [CrossRef] [PubMed] [PubMed Central]
4. Updated COVID-19 Vaccines for Use in the United States Beginning in Fall 2023. Available online: https://www.fda.gov/vaccines-blood-biologics/updated-covid-19-vaccines-use-united-states-beginning-fall-2023 (accessed on 1 September 2023).
5. Stay Up to Date with COVID-19 Vaccines. Available online: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/stay-up-to-date.html (accessed on 30 September 2023).
6. Kroon, F.P.B.; Najm, A.; Alunno, A.; Schoones, J.W.; Landewé, R.B.M.; Machado, P.M.; Navarro-Compán, V. Risk and prognosis of SARS-CoV-2 infection and vaccination against SARS-CoV-2 in rheumatic and musculoskeletal diseases: A systematic literature review to inform EULAR recommendations. *Ann. Rheum. Dis.* **2022**, *81*, 422–432. [CrossRef] [PubMed]
7. Hyrich, K.L.; Machado, P.M. Rheumatic disease and COVID-19: Epidemiology and outcomes. *Nat. Rev. Rheumatol.* **2021**, *17*, 71–72. [CrossRef] [PubMed] [PubMed Central]
8. Conway, R.; Grimshaw, A.A.; Konig, M.F.; Putman, M.; Duarte-García, A.; Tseng, L.Y.; Cabrera, D.M.; Chock, Y.P.E.; Degirmenci, H.B.; Duff, E.; et al. SARS-CoV-2 Infection and COVID-19 Outcomes in Rheumatic Diseases: A Systematic Literature Review and Meta-Analysis. *Arthritis Rheumatol.* **2022**, *74*, 766–775. [CrossRef] [PubMed] [PubMed Central]
9. Boyarchuk, O.; Predyk, L.; Yuryk, I. COVID-19 in patients with juvenile idiopathic arthritis: Frequency and severity. *Reumatologia* **2021**, *59*, 197–199. [CrossRef] [PubMed] [PubMed Central]
10. Haslak, F.; Yildiz, M.; Adrovic, A.; Sahin, S.; Koker, O.; Aliyeva, A.; Barut, K.; Kasapcopur, O. Management of childhood-onset autoinflammatory diseases during the COVID-19 pandemic. *Rheumatol. Int.* **2020**, *40*, 1423–1431. [CrossRef] [PubMed] [PubMed Central]
11. Liu, J.; Wu, H.; Xia, S.L. New-Onset Arthritis Following COVID-19 Vaccination: A Systematic Review of Case Reports. *Vaccines* **2023**, *11*, 665. [CrossRef] [PubMed] [PubMed Central]
12. Mv, P.; Auanassova, A.; Yessirkepov, M.; Zimba, O.; Gasparyan, A.Y.; Kitas, G.D.; Ahmed, S. New-onset systemic vasculitis following SARS-CoV-2 infection and vaccination: The trigger, phenotype, and outcome. *Clin. Rheumatol.* **2023**, *42*, 2761–2775. [CrossRef] [PubMed]
13. Gouda, W.; Albasri, A.; Alsaqabi, F.; Al Sabah, H.Y.; Alkandari, M.; Abdelnaby, H. Dermatomyositis Following BNT162b2 mRNA COVID-19 Vaccination. *J. Korean Med. Sci.* **2022**, *37*, e32. [CrossRef] [PubMed] [PubMed Central]
14. Padiyar, S.; Kamath, N.; Mathew, J.; Chandu, A.S.; Deodhar, D.; Shastry, B.A.; Shashikala, T.; Ganapati, A. New-onset Adult-onset Still's disease-like syndrome after ChAdOx1 nCoV-19 vaccination-a case series with review of literature. *Clin. Rheumatol.* **2022**, *41*, 1569–1575. [CrossRef] [PubMed] [PubMed Central]
15. Akgün, Ö.; Çakmak, F.; Guliyeva, V.; Demirkan, F.G.; Tanatar, A.; Hançerli Torun, S.; Çin, D.; Meşe, S.; Ağaçfidan, A.; Aktay Ayaz, N. Humoral response and safety of BNT162b2 mRNA vaccine in children with rheumatic diseases. *Rheumatology* **2022**, *61*, 4482–4490. [CrossRef] [PubMed] [PubMed Central]
16. Heshin-Bekenstein, M.; Ziv, A.; Toplak, N.; Hagin, D.; Kadishevich, D.; Butbul, Y.A.; Saiag, E.; Kaufman, A.; Shefer, G.; Sharon, O.; et al. Safety and immunogenicity of BNT162b2 mRNA COVID-19 vaccine in adolescents with rheumatic diseases treated with immunomodulatory medications. *Rheumatology* **2022**, *61*, 4263–4272. [CrossRef] [PubMed] [PubMed Central]
17. Sen, E.S.; Julandani, D.; Ramanan, A.V. SARS-CoV-2 vaccinations in children and adolescents with rheumatic diseases. *Rheumatology* **2022**, *61*, 4229–4231. [CrossRef] [PubMed] [PubMed Central]
18. Yang, Z.R.; Jiang, Y.W.; Li, F.X.; Liu, D.; Lin, T.F.; Zhao, Z.Y.; Wei, C.; Jin, Q.Y.; Li, X.M.; Jia, Y.X.; et al. Efficacy of SARS-CoV-2 vaccines and the dose-response relationship with three major antibodies: A systematic review and meta-analysis of randomised controlled trials. *Lancet Microbe* **2023**, *4*, e236–e246. [CrossRef] [PubMed] [PubMed Central]
19. Tarke, A.; Coelho, C.H.; Zhang, Z.; Dan, J.M.; Yu, E.D.; Methot, N.; Bloom, N.I.; Goodwin, B.; Phillips, E.; Mallal, S.; et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell* **2022**, *185*, 847–859.e11. [CrossRef] [PubMed] [PubMed Central]
20. GeurtsvanKessel, C.H.; Geers, D.; Schmitz, K.S.; Mykytyn, A.Z.; Lamers, M.M.; Bogers, S.; Scherbeijn, S.; Gommers, L.; Sablerolles, R.S.G.; Nieuwkoop, N.N.; et al. Divergent SARS-CoV-2 Omicron-reactive T and B cell responses in COVID-19 vaccine recipients. *Sci. Immunol.* **2022**, *7*, eabo2202. [CrossRef] [PubMed] [PubMed Central]
21. Goletti, D.; Delogu, G.; Matteelli, A.; Migliori, G.B. The role of IGRA in the diagnosis of tuberculosis infection, differentiating from active tuberculosis, and decision making for initiating treatment or preventive therapy of tuberculosis infection. *Int. J. Infect. Dis.* **2022**, *124* (Suppl. S1), S12–S19. [CrossRef] [PubMed]
22. Kurteva, E.; Vasilev, G.; Tumangelova-Yuzeir, K.; Ivanova, I.; Ivanova-Todorova, E.; Velikova, T.; Kyurkchiev, D. Interferon-gamma release assays outcomes in healthy subjects following BNT162b2 mRNA COVID-19 vaccination. *Rheumatol. Int.* **2022**, *42*, 449–456. [CrossRef] [PubMed] [PubMed Central]
23. Mahil, S.K.; Bechman, K.; Raharja, A.; Domingo-Vila, C.; Baudry, D.; Brown, M.A.; Cope, A.P.; Dasandi, T.; Graham, C.; Khan, H.; et al. Humoral and cellular immunogenicity to a second dose of COVID-19 vaccine BNT162b2 in people receiving methotrexate or targeted immunosuppression: A longitudinal cohort study. *Lancet Rheumatol.* **2022**, *4*, e42–e52. [CrossRef] [PubMed] [PubMed Central]

24. Tortorella, C.; Aiello, A.; Gasperini, C.; Agrati, C.; Castilletti, C.; Ruggieri, S.; Meschi, S.; Matusali, G.; Colavita, F.; Farroni, C.; et al. Humoral- and T-Cell-Specific Immune Responses to SARS-CoV-2 mRNA Vaccination in Patients with MS Using Different Disease-Modifying Therapies. *Neurology* **2022**, *98*, e541–e554. [CrossRef] [PubMed] [PubMed Central]
25. Cerna, K.; Duricova, D.; Hindos, M.; Hindos Hrebackova, J.; Lukas, M.; Machkova, N.; Hruba, V.; Mitrova, K.; Kubickova, K.; Kastylova, K.; et al. Cellular and Humoral Immune Responses to SARS-CoV-2 Vaccination in Inflammatory Bowel Disease Patients. *J. Crohn's Colitis* **2022**, *16*, 1347–1353. [CrossRef] [PubMed] [PubMed Central]
26. Standardization of Uveitis Nomenclature (SUN) Working Group. Classification Criteria for Juvenile Idiopathic Arthritis-Associated Chronic Anterior Uveitis. *Am. J. Ophthalmol.* **2021**, *228*, 192–197. [CrossRef] [PubMed] [PubMed Central]
27. STROBE Checklists. Available online: https://www.strobe-statement.org/checklists/ (accessed on 12 April 2024).
28. Kapten, K.; Orczyk, K.; Smolewska, E. Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic. *Arch. Immunol. Ther. Exp.* **2023**, *71*, 7. [CrossRef] [PubMed] [PubMed Central]
29. Zhang, Z.; Mateus, J.; Coelho, C.H.; Dan, J.M.; Moderbacher, C.R.; Gálvez, R.I.; Cortes, F.H.; Grifoni, A.; Tarke, A.; Chang, J.; et al. Humoral and cellular immune memory to four COVID-19 vaccines. *Cell* **2022**, *185*, 2434–2451.e17. [CrossRef] [PubMed] [PubMed Central]
30. Sette, A.; Crotty, S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* **2021**, *184*, 861–880. [CrossRef] [PubMed] [PubMed Central]
31. Kedzierska, K.; Thomas, P.G. Count on us: T cells in SARS-CoV-2 infection and vaccination. *Cell Rep. Med.* **2022**, *3*, 100562. [CrossRef] [PubMed] [PubMed Central]
32. Primorac, D.; Brlek, P.; Matišić, V.; Molnar, V.; Vrdoljak, K.; Zadro, R.; Parčina, M. Cellular Immunity-The Key to Long-Term Protection in Individuals Recovered from SARS-CoV-2 and after Vaccination. *Vaccines* **2022**, *10*, 442. [CrossRef] [PubMed] [PubMed Central]
33. Sahin, U.; Muik, A.; Derhovanessian, E.; Vogler, I.; Kranz, L.M.; Vormehr, M.; Baum, A.; Pascal, K.; Quandt, J.; Maurus, D.; et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* **2020**, *586*, 594–599, *Erratum in Nature* **2021**, *590*, E17. [CrossRef] [PubMed]
34. Agrati, C.; Castilletti, C.; Goletti, D.; Sacchi, A.; Bordoni, V.; Mariotti, D.; Notari, S.; Matusali, G.; Meschi, S.; Petrone, L.; et al. Persistent Spike-specific T cell immunity despite antibody reduction after 3 months from SARS-CoV-2 BNT162b2-mRNA vaccine. *Sci. Rep.* **2022**, *12*, 6687. [CrossRef] [PubMed] [PubMed Central]
35. Masiá, M.; de la Rica, A.; Fernández-González, M.; García, J.A.; Padilla, S.; García-Abellán, J.; Botella, Á.; Mascarell, P.; Gutiérrez, F. Integrating SARS-CoV-2-specific interferon-γ release assay testing in the evaluation of patients hospitalized with COVID-19. *Microbiol. Spectr.* **2023**, *11*, e0241923. [CrossRef] [PubMed]
36. Schiffner, J.; Backhaus, I.; Rimmele, J.; Schulz, S.; Möhlenkamp, T.; Klemens, J.M.; Zapf, D.; Solbach, W.; Mischnik, A. Long-Term Course of Humoral and Cellular Immune Responses in Outpatients After SARS-CoV-2 Infection. *Front. Public Health* **2021**, *9*, 732787. [CrossRef] [PubMed] [PubMed Central]
37. Huzly, D.; Panning, M.; Smely, F.; Enders, M.; Komp, J.; Falcone, V.; Steinmann, D. Accuracy and real life performance of a novel interferon-γ release assay for the detection of SARS-CoV2 specific T cell response. *J. Clin. Virol.* **2022**, *148*, 105098. [CrossRef] [PubMed] [PubMed Central]
38. Lledó, A.; Retuerto, M.; Almendro-Vázquez, P.; Fernández-Ruiz, M.; Galindo, M.; Laguna-Goya, R.; Paz-Artal, E.; Lalueza, A.; Aguado, J.M.; Pablos, J.L. SARS-CoV-2-specific T-cell responses after COVID-19 recovery in patients with rheumatic diseases on immunosuppressive therapy. *Semin. Arthritis Rheum.* **2021**, *51*, 1258–1262. [CrossRef] [PubMed] [PubMed Central]
39. Mu, X.; Cohen, C.A.; Leung, D.; Rosa Duque, J.S.; Cheng, S.M.S.; Chung, Y.; Wong, H.H.W.; Lee, A.M.T.; Li, W.Y.; Tam, I.Y.S.; et al. Antibody and T cell responses against wild-type and Omicron SARS-CoV-2 after third-dose BNT162b2 in adolescents. *Signal Transduct. Target. Ther.* **2022**, *7*, 397. [CrossRef] [PubMed] [PubMed Central]
40. He, X.S.; Holmes, T.H.; Zhang, C.; Mahmood, K.; Kemble, G.W.; Lewis, D.B.; Dekker, C.L.; Greenberg, H.B.; Arvin, A.M. Cellular immune responses in children and adults receiving inactivated or live attenuated influenza vaccines. *J. Virol.* **2006**, *80*, 11756–11766. [CrossRef] [PubMed] [PubMed Central]
41. Rudolph, M.E.; McArthur, M.A.; Barnes, R.S.; Magder, L.S.; Chen, W.H.; Sztein, M.B. Differences Between Pediatric and Adult T Cell Responses to In Vitro Staphylococcal Enterotoxin B Stimulation. *Front. Immunol.* **2018**, *9*, 498. [CrossRef] [PubMed] [PubMed Central]
42. Yuan, Y.; Wang, Q.P.; Sun, D.; Wu, Z.B.; Peng, H.; Liu, X.W.; Liu, Y.L. Differences in Immune Responses between Children and Adults with COVID-19. *Curr. Med. Sci.* **2021**, *41*, 58–61. [CrossRef] [PubMed] [PubMed Central]
43. Geisen, U.M.; Berner, D.K.; Tran, F.; Sümbül, M.; Vullriede, L.; Ciripoi, M.; Reid, H.M.; Schaffarzyk, A.; Longardt, A.C.; Franzenburg, J.; et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann. Rheum. Dis.* **2021**, *80*, 1306–1311. [CrossRef] [PubMed] [PubMed Central]
44. Simon, D.; Tascilar, K.; Fagni, F.; Krönke, G.; Kleyer, A.; Meder, C.; Atreya, R.; Leppkes, M.; Kremer, A.E.; Ramming, A.; et al. SARS-CoV-2 vaccination responses in untreated, conventionally treated and anticytokine-treated patients with immune-mediated inflammatory diseases. *Ann. Rheum. Dis.* **2021**, *80*, 1312–1316. [CrossRef] [PubMed] [PubMed Central]

45. Oyaert, M.; De Scheerder, M.A.; Van Herrewege, S.; Laureys, G.; Van Assche, S.; Cambron, M.; Naesens, L.; Hoste, L.; Claes, K.; Haerynck, F.; et al. Evaluation of Humoral and Cellular Responses in SARS-CoV-2 mRNA Vaccinated Immunocompromised Patients. *Front. Immunol.* **2022**, *13*, 858399. [CrossRef] [PubMed] [PubMed Central]
46. Calcoen, B.; Callewaert, N.; Vandenbulcke, A.; Kerstens, W.; Imbrechts, M.; Vercruysse, T.; Dallmeier, K.; Van Weyenbergh, J.; Maes, P.; Bossuyt, X.; et al. High Incidence of SARS-CoV-2 Variant of Concern Breakthrough Infections Despite Residual Humoral and Cellular Immunity Induced by BNT162b2 Vaccination in Healthcare Workers: A Long-Term Follow-Up Study in Belgium. *Viruses* **2022**, *14*, 1257. [CrossRef] [PubMed] [PubMed Central]
47. Vogrig, M.; Berger, A.E.; Bourlet, T.; Waeckel, L.; Haccourt, A.; Chanavat, A.; Hupin, D.; Roche, F.; Botelho-Nevers, E.; Pozzetto, B.; et al. Monitoring of Both Humoral and Cellular Immunities Could Early Predict COVID-19 Vaccine Efficacy against the Different SARS-CoV2 Variants. *J. Clin. Immunol.* **2023**, *43*, 31–45. [CrossRef] [PubMed] [PubMed Central]
48. Ahmed, S.; Mehta, P.; Paul, A.; Anu, S.; Cherian, S.; Shenoy, V.; Nalianda, K.K.; Joseph, S.; Poulose, A.; Shenoy, P. Postvaccination antibody titres predict protection against COVID-19 in patients with autoimmune diseases: Survival analysis in a prospective cohort. *Ann. Rheum. Dis.* **2022**, *81*, 868–874. [CrossRef] [PubMed]
49. Amanat, F.; Thapa, M.; Lei, T.; Ahmed, S.M.S.; Adelsberg, D.C.; Carreño, J.M.; Strohmeier, S.; Schmitz, A.J.; Zafar, S.; Zhou, J.Q.; et al. SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell* **2021**, *184*, 3936–3948.e10. [CrossRef] [PubMed] [PubMed Central]
50. Sureshchandra, S.; Lewis, S.A.; Doratt, B.M.; Jankeel, A.; Coimbra Ibraim, I.; Messaoudi, I. Single-cell profiling of T and B cell repertoires following SARS-CoV-2 mRNA vaccine. *JCI Insight* **2021**, *6*, e153201. [CrossRef] [PubMed] [PubMed Central]

5. Wnioski

A. uzyskane w badaniu rezultaty pokazują, że w grupie badanej odpowiedź komórkowa na kontakt z antygenem SARS-CoV-2 koreluje z wynikami badań serologicznych, a w niektórych przypadkach dochodzi do jej wytworzenia nawet u pacjentów seronegatywnych, tym samym badanie swoistej odpowiedzi limfocytów T na zakażenie wirusowe może być niezwykle przydatnym narzędziem w diagnostyce odpowiedzi immunologicznej,

B. w niektórych przypadkach oznaczanie wyłącznie odpowiedzi humoralnej może prowadzić do niepełnej diagnostyki i powodować błędne decyzje kliniczne,

C. test IGRA może stanowić wartościowe narzędzie diagnostyczne do oceny odporności komórkowej zarówno po przechorowaniu SARS-CoV-2, jak i po szczepieniu przeciwko nowemu koronawirusowi,

D. w zakresie badanych parametrów nie odnotowano zależności między leczeniem choroby podstawowej a wytworzeniem odporności komórkowej i humoralnej przeciw SARS-CoV-2,

E. zebrane dane ankietowe w badanej grupie dzieci z MIZS wykazały niski odsetek infekcji po szczepieniu przeciwko COVID-19, a w przypadkach wystąpienia zakażenia nie raportowano jego ciężkich przebiegów,

F. uzyskane w aktualnym badaniu wyniki, choć napawają optymizmem, wymagają dalszego potwierdzenia, głównie ze względu na stosunkową małą grupę badawczą, brak grupy kontrolnej oraz dotychczas niską dostępność analizowanej metody badawczej,

G. wytyczne dotyczące szczepień ochronnych, w tym szczepień przeciwko zakażeniu SARS-CoV-2 w grupach pacjentów z chorobami autoimmunizacyjnymi, jakimi są dzieci z MIZS, powinny opierać się na dużych, najlepiej wieloośrodkowych i prospektywnych badaniach.

6. Streszczenie pracy

Publikacja I

Niniejsza publikacja stanowi przegląd literatury dotyczącej mechanizmów immunologicznych towarzyszących chorobie COVID-19. Autorzy podjęli próbę usystematyzowania danych na temat mechanizmów odporności wrodzonej i nabytej na antygeny SARS-CoV-2. W artykule omówiono ogólną charakterystykę nowego koronawirusa oraz implikacje wynikające z jego cech strukturalnych i molekularnych, takich jak zdolność wiązania, silne rozprzestrzenianie się w populacji i wysoki współczynnik mutacji. Omawiając najważniejsze składniki odporności wrodzonej, w artykule wyjaśniono ważną rolę interleukin, głównie IL-6 i IL-17, interferonów, makrofagów, neutrofili, komórek NK oraz mechanizmów takich jak burza cytokinowa czy NEToza, odpowiedzialnych za patologiczne reakcje immunologiczne występujące w przebiegu COVID-19. W odniesieniu do wtórnej odpowiedzi humoralnej na antygeny SARS-CoV-2, w publikacji wykazano zależność ciężkości przebiegu choroby od miana wytworzonych przeciwciał oraz kinetyki ich produkcji. Omówiono także znaczenie przeciwciał IgA i ich powinowactwo do błon śluzowych. Przedstawiono zagadnienie odpowiedzi immunologicznej, w tym komórkowej, u pacjentów, którzy pomimo zdiagnozowanego niedoboru odporności łagodnie przebyli zakażenie SARS-CoV-2. Poruszono temat utrzymywania się pamięci limfocytów T, którą zauważono już w czasie epidemii SARS-CoV-1. Z dostępnej literatury wiadomo, że skuteczna i silna aktywacja limfocytów T CD8+ jest odpowiedzialna za usuwanie wirusa, a zatem za łagodne lub bezobjawowe zakażenie. Podsumowując, skuteczna i trwała odpowiedź humoralna wraz z szeroką i funkcjonalną odpornością limfocytów T wiąże się z dobrym rokowaniem w przypadku COVID-19. Niezawodność, bezpieczeństwo i wysoka skuteczność szczepionek mRNA przeciwko SARS-CoV-2 została potwierdzona w większości badań, co pozwala na wdrażanie programów szczepień na całym świecie. Jednakże tempo spadku ochrony immunologicznej i liczebność nowych mutacji wirusa pozostają poważnym problemem w kontekście przyszłych zaleceń dotyczących szczepień przeciwko SARS-CoV-2. Co ważne, poza ogólnymi wytycznymi dotyczącymi przebiegu epidemii COVID-19, a także podobnych, które mogą wystąpić w najbliższej przyszłości, istnieje potrzeba dostosowania wytycznych postępowania w grupach pacjentów z niedoborami odporności wynikającymi zarówno z choroby podstawowej, jak i ze stosowanego leczenia.

Publikacja II oraz III

Wstęp

Od końca 2019 roku uwaga lekarzy i badaczy z całego świata skupiła się na nowym koronawirusie i poznaniu mechanizmów leżących u podstaw zakażenia SARS-CoV-2, celem spowolnienia jego rozprzestrzeniania, znalezienia skutecznych możliwości leczenia i prewencji, w tym możliwie jak najszybszego wprowadzenia szczepień ochronnych. Część z tych celów udało się zrealizować, część wymaga dalszych badań, jednak wraz z wchodzeniem ogólnoświatowej pandemii w fazę endemiczną, jednym z priorytetów powinno stać się znalezienie wiarygodnych metod oceny odporności jednostki na antygeny wirusa i dostosowanie zaleceń dotyczących szczepień, szczególnie w grupach pacjentów z obniżoną odpornością.

Młodzieńcze idiopatyczne zapalenie stawów (MIZS) jest najczęstszą artropatią zapalną występującą u dzieci. Dzięki szybkiemu postępowi w rozwoju terapii biologicznych i ich

rosnącej dostępności, uzyskano znaczną poprawę rokowania i szansę na uzyskanie długoterminowej remisji, nawet u pacjentów opornych na klasyczne leki modyfikujące przebieg choroby. Mimo to dzieci z chorobami o podłożu autoimmunizacyjnym wymagają szczególnej uwagi lekarzy i opiekunów, zwłaszcza w czasie zagrożenia epidemiologicznego, jak podczas pandemii SARS-CoV-2. Chociaż ani sama choroba podstawowa, ani większość stosowanych obecnie terapii nie powinny znacząco zwiększać ryzyka ciężkiego przebiegu COVID-19, każda infekcja u pacjenta z chorobą reumatyczną stanowi czynnik ryzyka wystąpienia zaostrzenia i może prowadzić do powikłań, takich jak konieczność intensyfikacji lub zmiany aktualnego leczenia albo hospitalizacji. Tym samym zasadna jest potrzeba rewalidacji i udoskonalenia aktualnych zaleceń dotyczących postępowania z chorymi na MIZS zakażonymi SARS-CoV-2, w tym dostosowania programów szczepień, które będą opierać się na badaniach prowadzonych w tej specyficznej grupie chorych.

Złożoność mechanizmów immunologicznych w zakażeniu SARS-CoV-2 zaczyna się od pierwszej, nieswoistej linii obrony i kończy się na wysoce specyficznych mechanizmach humoralnych i komórkowych. Podczas gdy serologia większości powszechnych infekcji wirusowych, w tym koronawirusowych, została dość dobrze zbadana, odpowiedź komórkowa na zakażenie SARS-CoV-2 wymaga dalszych badań. Wiadomym jest, że ocena odpowiedzi komórek T na nowego koronawirusa stanowi istotne uzupełnienie dla samego badania przeciwciał, szczególnie u pacjentów, którzy z różnych przyczyn nie wytwarzają odpowiedzi humoralnej. Wykorzystanie dobrze znanego ze swojej roli w diagnostyce gruźlicy testu uwalniania interferonu gamma (IGRA) do oznaczania odpowiedzi komórkowej na antygeny SARS-CoV-2 dało nowe możliwości diagnostyczne, zarówno w badaniach naukowych, jak i w praktyce klinicznej.

Cel

Cele pracy obejmowały:

- zbadanie zależności pomiędzy wytwarzaną przez pacjentów z MIZS odpornością na zakażenie SARS-CoV-2, a otrzymywanym leczeniem, w tym klasycznymi lekami modyfikującymi przebieg choroby (LMPCh) i lekami biologicznymi,
- określenie przydatności testu IGRA jako narzędzia do oceny odpowiedzi komórkowej powstającej po kontakcie z antygenami SARS-CoV-2,
- dalszą obserwację pacjentów z uwzględnieniem wystąpienia zakażenia SARS-CoV-2 po zakończeniu badania i ocena jego związku z wcześniej zmierzoną odpowiedzią immunologiczną.

Materiały i metody

Badanie przeprowadzono w grupie 55 pacjentów (41 dziewcząt i 14 chłopców) z MIZS zdiagnozowanym według kryteriów klasyfikacji ILAR w trakcie ich hospitalizacji w Klinice Kardiologii i Reumatologii Dziecięcej Uniwersytetu Medycznego w Łodzi, rekrutacja uczestników odbywała się w okresie od czerwca 2021 r. do lutego 2023 r. Przyczynami hospitalizacji było nowo zdiagnozowane MIZS, zaostrzenie procesu reumatoidalnego lub pobyty jednodniowe będące częścią protokołów stosowania leków biologicznych. W badaniu uczestniczyli zarówno pacjenci, którzy przebyli Covid-19, jak również ci, którzy negowali zakażenie SARS-CoV-2. Ośmioro dzieci w grupie badanej otrzymało szczepionkę mRNA Comirnaty w okresie od 1 do 18 miesięcy poprzedzającym włączenie do badania, natomiast reszta grupy (n = 47) nie była zaszczepiona przeciwko SARS-CoV-2 do momentu zakończenia eksperymentu. Kohorta składała się z nowo zdiagnozowanych przypadków (n =

15) oraz pacjentów pozostających pod wieloletnią opieką Kliniki jeszcze przed rozpoczęciem omawianego badania, zarówno w okresie remisji, jak i zaostrzenia choroby. Żadne z dzieci nie wymagało stosowania wysokich dawek systemowych glikokortykosteroidów.

Próbki krwi pobierano podczas rutynowych badań laboratoryjnych wykonywanych podczas pobytu pacjentów w szpitalu. Specyficzną odpowiedź komórek T na antygeny SARS-CoV-2 mierzono za pomocą ilościowego testu IGRA w pełnej krwi (Quan-T-Cell SARS-CoV-2 EUROIMMUN). Następnie heparynizowaną krew inkubowano i stymulowano białkiem S antygenu SARS-CoV-2, a otrzymane osocze analizowano za pomocą ilościowego testu immunoenzymatycznego (ELISA) w celu określenia stężenia uwolnionego IFN-γ. Dodatkowo u wszystkich pacjentów wykonano badanie ELISA anty-SARS-CoV-2 w celu oznaczenia miana przeciwciał IgA, IgM i IgG. Protokół badania został zaaprobowany przez Komisję Bioetyczną Uniwersytetu Medycznego w Łodzi, otrzymując numer zgody RNN/117/21/KE.

Publikacja II

Równolegle z pobieraniem próbek krwi do omawianego eksperymentu, pobierano również rutynowe badania krwi: morfologię całkowitą krwi, białko C-reaktywne (CRP) i Odczyn Biernackiego (OB).

Zmienne ciągłe nie miały rozkładu normalnego zgodnie z wynikami testu Shapiro – Wilka. Dlatego też wszystkie porównania grupowe obliczono przy użyciu testów U Manna – Whitneya oraz H Kruskala – Wallisa. Do oceny zależności pomiędzy zmiennymi ilościowymi wykorzystano współczynniki korelacji rang Spearmana.

Publikacja III

Dwa lata od rekrutacji pierwszego pacjenta oraz cztery miesiące po włączeniu ostatniego z nich, tj. po zakończeniu całej rekrutacji oraz fazy laboratoryjnej, przeprowadzono telefoniczną ankietę dotyczącą występowania nowych zachorowań uczestników badania na COVID-19. Wyniki ankiety uzyskano od 53 z 55 rodziców pacjentów objętych badaniem.

Porównania grupowe przeprowadzono za pomocą testu U Manna–Whitneya, za istotne uznano wartości p poniżej 0,05. Do obliczenia najdokładniejszej wartości odcięcia dla testu IGRA autorzy wykorzystali indeks Youdena. Czułość i swoistość badania IGRA jako markera podatności poszczególnych osób na zakażenie SARS-CoV-2 analizowano za pomocą krzywej ROC (Receiver Operating Characteristic).

Wyniki

Publikacja II

Odpowiedź komórek T na antygen SARS-CoV-2 mierzona za pomocą testu IGRA istotnie korelowała z odpowiedzią humoralną w IgA ($p < 0,00003$, $R = 0,537$), IgG ($p < 0,0001$, $R = 0,668$) i IgG (NCP) ($p < 0,003$, $R = 0,0399$), bez korelacji z przeciwciałami IgM. W obrębie kohorty wykryto specyficzną odpowiedź limfocytów T na antygeny SARS-CoV-2 u pięciu pacjentów seronegatywnych (we wszystkich klasach przeciwciał). Dwóch uczestników badania z dodatnim mianem IgG nie wytworzyło odpowiedzi komórkowej, dodatkowo u dwóch pacjentów wartości odpowiedzi komórkowej znajdowały się w szarej strefie (100–

200 mlU/ml). Wśród pacjentów z dodatnim mianem przeciwciał w klasie IgA, trzech uzyskało ujemne wyniki IGRA. Stężenie przeciwciał u pacjentów otrzymujących leki biologiczne był istotnie niższy w porównaniu z resztą kohorty (p = 0,0369), podczas gdy podobnego efektu nie zaobserwowano u pacjentów przyjmujących tradycyjne LMPCh. Nie stwierdzono korelacji między wytworzoną odpowiedzią immunologiczną, a markerami stanu zapalnego, takimi jak CRP, OB, liczbą płytek krwi czy leukocytozą krwi obwodowej.

Publikacja III

Wszyscy uczestnicy badania, którzy otrzymali szczepienie mRNA SARS-CoV-2 rozwinęli zarówno specyficzną odpowiedź komórkową (p = 0,0016), jak i humoralną (p = 0,001 dla przeciwciał IgA, p = 0,008 dla przeciwciał IgG) niezależnie od przyjmowanego leczenia. Badanie wykazało, że naturalna infekcja wywołała inny wzór odporności niż szczepienie, z wyższym mianem przeciwciał i odpowiedzią komórek T po szczepieniu niż po naturalnej ekspozycji na patogen. Proponowana wartość odcięcia dla dodatniego IGRA obliczona za pomocą krzywej ROC wynosiła 1022,15, przy 60% czułości i 80% swoistości. Badanie ankietowe wykazało, że po zakończeniu eksperymentu tylko u szóstki dzieci doszło do zakażenia SARS-CoV-2 potwierdzonego metodą PCR, dodatkowo 10 uczestników miało objawy przypominające COVID-19, ale nie zweryfikowało ich za pomocą badań laboratoryjnych. Wyższy odsetek zakażeń SARS-CoV-2 po zakończeniu eksperymentu odnotowano u pacjentów, u których w toku badania obserwowano niższe miana przeciwciał IgA, IgG i IgG NCP.

Wnioski

Stwierdzono, że IGRA może stanowić użyteczne narzędzie w ocenie odpowiedzi komórkowej na antygeny SARS-CoV-2 w kohorcie dzieci chorych na MIZS, zarówno po zakażeniu, jak i po szczepieniu. Ocena wyłącznie odpowiedzi humoralnej na infekcje wirusowe, takie jak COVID-19, może nie być wystarczająca i prowadzić do błędnych lub niekompletnych wyników, szczególnie u pacjentów, u których nie zostaje wytworzona odporność humoralna pomimo kontaktu z antygenem i pojawienia się odpowiedzi limfocytów T. Uzupełnienie standardowej serologii o ocenę odporności komórkowej może znacznie zwiększyć jej dokładność szczególnie w przypadku osób immunoniekompetentnych. Wytyczne dotyczące nie tylko zakażenia SARS-CoV-2, ale także innymi patogenami, powinny uwzględniać specyfikę konkretnych grup pacjentów w tym dzieci z chorobami reumatycznymi, a zalecenia powinny opierać się na rzetelnych, najlepiej wieloośrodkowych badaniach.

7. Streszczenie pracy w języku angielskim

Publication I

This publication is a literature review on the immunity in SARS-CoV-2 infection. Authors attempted to systematize data on both innate and adaptive immune mechanisms to SARS-CoV-2 antigens. The paper covered general features of the novel coronavirus and the implication of its structural and molecular characteristics, such as its binding capabilities, robust spread through the population and high mutation rate. As the most important components of the innate immunity are concerned, the article explained the important role of interleukins, mainly IL-6 and IL-17, interferons, macrophages, neutrophiles, NK cells and the mechanisms like cytokine storms or NETosis responsible for pathological immune reactions observed in COVID-19 infection. Regarding the secondary humoral responses to SARS-CoV-2 antigens, the publication showed dependencies between antibody titers with the kinetics of their production and the severity of the disease. It also deliberated on the importance of IgA antibodies and their affinity with mucosal membranes. Moreover, it discussed the immune responses to COVID-19 in immunodeficient patients that are unable to produce antibodies, but had positive outcome of the SARS-COV-2 infection that can be explained by effectiveness of cellular responses. The durability of antibody titers and the protection they provide has been questioned in many publications, while the longevity of T cells memory has been noticed already during the SARS-CoV-1 outbreak. Regarding the cellular responses, the effective and robust activation of bystander $CD8^+$ T cells was found to be responsible for viral clearance and therefore a mild or asymptomatic disease. All in all, an effective and sustainable humoral response along with broad and functional T-cell immunity was associated with early recovery from COVID-19. The reliability, safety and high efficacy in disease prevention of the new technology mRNA molecules vaccines against SARS-CoV-2 has been confirmed in most of the studies, therefore allowing for implementing inoculation programs all over the world. However, the rate of decline of immune protection and the abundance of new mutation of the virus remain a great concern regarding the future recommendations for the SARS-CoV-2 vaccines. Importantly, except for general guidelines for COVID-19 or similar outbreaks that may occur in the near future, there is a great need for adapting the course of action in groups of patients with immunodeficiencies both due to an underlying disease or received treatment.

Publication II and III

Introduction

Since the end of 2019 the focus of the medical professionals and researchers from all over the world was on the new coronavirus, having a similar goal-to understand the mechanisms that underlies SARS-CoV-2 infection in order to slow down its spreading, find effective treatment options and methods of prevention, including introduction of inoculation programs as fast as possible. Some of this goals have been successfully fulfilled till now, some need more research. However, as the worldwide pandemic enters a more endemic phase, establishing the most accurate ways of assessing one’s immunity against the viral

antigens and adjusting the vaccination recommendations, especially in the groups of possibly immunocompromised patients, should be a priority.

Juvenile idiopathic arthritis (JIA) is the most common arthropathy in children. It is a heterogeneous group of chronic arthritis of still not fully known and complex etiology, with its onset before 16 years of age, symptoms persisting for more than 6 weeks and the diagnosis made after excluding all the other possible causes of joint inflammation. Due to the rapid progress in development and the increasing availability of biological agents for patients with JIA, a significant improvement in achieving the long term remission and overall good prognosis has been achieved, even in patients that are resistant to classic disease modifying drugs. Still, children with rheumatic diseases, such as JIA, require special attention from doctors and caregivers, especially in terms of epidemiological threat like the SARS-CoV-2 pandemic. While neither most of the therapies that are currently used in JIA, nor the disease itself should significantly elevate the risk of SARS-CoV-2, it is known that the occurrence of any infection in a rheumatological patient is still a risk factor for exacerbation of the arthritis, which may lead to further complications, like the need for intensification or a change in treatment or even hospitalization. Therefore there is an unceasing need for up-to-date recommendations regarding the SARS-CoV-2 infection in patients with JIA, including the vaccination programs, that will be based on research on this specific cohort of patients.

The complexity of immune mechanisms in SARS-CoV-2 infection starts with the first, unspecific line of defense and continues to highly specific humoral and cellular mechanisms. While the serological responses have been thoroughly studied both in the current COVID-19 outbreak and in other common viral infections, the cellular immunity is still less explored. Assessing T-cell responses to the novel coronavirus adds a lot of value to the sole serological testing, especially in patients that, for different reasons, may not elicit humoral responses. Utilizing IGRA test, that is well known for its role in the diagnosis of Tuberculosis for marking the levels of cellular responses to SARS-CoV-2 antigens, opened new possibilities for assessing the T-cell responses in research and possibly clinical practice.

The aim

The main objectives of the study were as follows:

- the assessment of the immune responses to SARS-CoV-2 antigens, both cellular and humoral, in JIA patients receiving different treatment protocols, including both classic disease-modifying anti-rheumatic drugs and the biological agents,
- determining the usefulness of the IGRA test as a tool for assessing the cellular response generated after contact with the SARS-CoV-2 antigens,
- clinical follow-up of patients, taking into account the occurrence of SARS-CoV-2 infection after the end of the study and assessing its relation to previously determined levels of the immune responses.

Materials and methods

The study was conducted on a group of 55 patients (41 girls and 14 boys) with JIA diagnosed according to ILAR classification criteria, during their hospitalization in the Department of Pediatric Cardiology and Rheumatology, Medical University of Lodz, Poland,

between June 2021 and February 2023. Among the causes of hospitalization were a newly diagnosed JIA, a flare of the disease or a routine day-case hospitalizations that are a part of biological agent protocols. Participants had both positive and negative history of past SARS-CoV-2 infection. Eight children within the study group received the Comirnaty mRNA vaccine between 1 and 18 months prior to their enrollment to the study, whereas the rest of the group (n = 47) had not been vaccinated against SARS-CoV-2 till the end of the experiment. The cohort consisted of both newly diagnosed cases (n = 15) and patients with years-long treatments; both in remission and during the disease flare, however none of the patients required high doses of systemic steroids due to the severity of the rheumatic process. Participants of the study were receiving different drug regimes, including classic antirheumatic drugs and biologic agents.

Blood samples were collected during routine laboratory tests performed on the patients' hospital stay. The specific T-cell response to SARS-CoV-2 antigens was measured using quantitative IGRA in whole blood with a Quan-T-Cell SARS-CoV-2 EUROIMMUN assay. The heparinized blood was then incubated and stimulated with SARS-CoV-2 antigen spike protein, then the obtained plasma was analyzed by a quantitative enzyme-linked immunosorbent assay (ELISA) to determine the concentration of released IFN-γ. Additionally, the anti-SARS-CoV-2 ELISA was performed in all patients to mark the levels of IgA, IgM and IgG antibodies. The study was approved by the local Bioethics Committee, with approval number RNN/117/21/KE.

Publication II

Additionally to the results of immune assays, the following parameters were obtained from the routine blood testing, that was performed simultaneously with obtaining blood samples for the current research: Total Blood Count, C-reactive Protein (CRP), and Erythrocytes Sedimentation Rate.

All continuous variables were non-normally distributed based on the Shapiro–Wilk test. Therefore, all group comparisons were calculated utilizing the Mann–Whitney U test and Kruskal–Wallis H test. Spearman's rank correlation coefficients were used to assess dependencies between quantitative variables.

Publication III

After the enrollment of all the participants to the study and finishing the laboratory phase of the research, 2 years after the recruitment of the first patient and 4 months after including the last participant a follow-up telephone survey regarding breakthrough COVID-19 cases was conducted. The results of the questionnaire were acquired from 53 out of the 55 parents of the patients included in the research.

Group comparisons were performed using the Mann–Whitney U test, p values below 0.05 were considered significant. To calculate the most accurate cutoff value for IGRA, the authors utilized the Youden index. The sensitivity and specificity of IGRA as a marker for individuals' susceptibility to SARS-CoV-2 infection were analyzed using the receiver operating characteristic (ROC) curve.

Results

Publication II

The T-cell responses to SARS-CoV-2 antigens measured utilizing the IGRA test significantly correlated with the humoral responses in IgA ($p < 0.00003$, $R = 0.537$), IgG ($p < 0.0001$, $R = 0.668$), and IgG nucleocapsid protein (NCP) ($p < 0.003$, $R = 0.0399$) antibodies, with no correlation with IgM. Within the cohort, a specific T-cell response to SARS-CoV-2 was detected in five seronegative patients (in all antibodies classes). While in patients with positive IgG titers, two did not mount any cellular responses and two had a cellular response in the grey area (100–200 mlU/mL). Similarly, in patients with positive IgA titers, three had negative IGRA results. Moreover, the antibody levels in patients receiving biological agents were significantly lower compared to the rest of the cohort ($p = 0.0369$), while similar effect was not noted in patients on traditional disease modifying drugs. No correlation between immunological results and inflammatory markers like CRP, ESR, platelets levels or leukocytosis was found.

Publication III

All the participants that received SARS-CoV-2 mRNA vaccination, regardless of the treatment protocols they were on, developed both specific cellular ($p = 0.0016$) and humoral ($p = 0.001$ for IgA antibodies, $p = 0.008$ for IgG antibodies) responses to the inoculation. The study also showed that natural infection elicited different pattern of immunity than the vaccination, with higher levels of antibodies and T-cell response after inoculation than after natural exposure to the pathogen. The proposed cutoff value for positive IGRA calculated with the ROC curve was 1022.15, with 60% sensitivity and 80% specificity. The follow-up survey showed that only six children developed PCR-confirmed SARS-CoV-2 infection after the end of the study, with additional 10 participants admitting to having COVID-like symptoms but failing to verify it with laboratory tests. Among the vaccinated patients, significantly lower IgA, IgG and IgG NCP antibody titers were found in participants that reported breakthrough SARS-CoV-2 infection.

Conclusions

IGRA was found to be a useful tool in the evaluation of cellular responses to SARS-CoV-2 antigens, both after infection and vaccination in the cohort of children with JIA. Assessing solely the humoral responses to the viral infections, such as COVID-19, may often not be a sufficient and lead to faulty or incomplete results, as some patients fail to mount humoral responses despite the contact with the antigen and producing T-cell responses. Therefore, adding the assessment of T-cell immunity in the evaluation of viral-induced immune responses can enhance the accuracy of serological testing. The guidelines, regarding not only SARS-CoV-2 infection but also other pathogens outbreaks should be adjusted to specific groups of patients, like children with rheumatic diseases, and the recommendations need to be based on reliable, preferably multi center studies in order to provide the best care for all the individuals.

8. Wykaz skrótów

CRP – białko c-reaktywne (ang. c-reactive protein)

ELISA - test immunoenzymatyczny (ang. enzyme-linked immunosorbent assay)

FDA - Agencja Żywności i Leków (ang. Food and Drug Administration)

IF – współczynnik wpływu publikacji (ang.impact factor)

IFN - Interferon

IGRA - test uwalniania interferonu gamma (ang. Interferon-γ Release Assay)

ILAR – Międzynarodowa Liga Stowarzyszeń Reumatologicznych (ang. International League of Associations for Rheumatology)

IL - Interleukina

LMPCh - leki modyfikujące przebieg choroby

MIZS – młodzieńcze idiopatyczne zapalenie stawów

MNiSW – Ministerstwo Nauki i Szkolnictwa Wyższego

NCP - białko nukleokapsydu N

NET - Neutrofilowe sieci zewnątrzkomórkowe (ang. Neutrophil extracellular traps)

komórki NK - Naturalni Zabójcy (ang. Natural Killer cells)

OB – odczyn Biernackiego

PCR - reakcja łańcuchowa polimerazy (ang. polymerase chain reaction)

RF - czynnik reumatoidalny (ang. rheumatoid factor)

krzywa ROC -ang. Receiver Operating Characteristic curve

SD – odchylenie standardowe (ang. standard deviation)

TNF - czynnik martwicy nowotworów (ang. tumor necrosis factor)

9. Piśmiennictwo

1. Long AM, Marston B. Juvenile Idiopathic Arthritis. Pediatr Rev. 2023 Oct 1;44(10): 565-577. doi: 10.1542/pir.2022-005623. PMID: 37777651.
2. Thatayatikom A, Modica R, De Leucio A. Juvenile Idiopathic Arthritis. 2023 Jan 16. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 32119492.
3. Rose E, Petty TRS, Manners P, et al. International league of associations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004; 2: 390-392.
4. Rutkowska-Sak L., Gietka P., Wierzbowska M. i in.: Reumatologia wieku rozwojowego. „Reumatologia", 2012, 50 (2), 142-146.
5. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet. 2007 Mar 3;369(9563): 767-778. doi: 10.1016/S0140-6736(07)60363-8. PMID: 17336654.
6. Cimaz R, Maioli G, Calabrese G. Current and emerging biologics for the treatment of juvenile idiopathic arthritis. Expert Opin Biol Ther. 2020 Jul;20(7):725-740. doi: 10.1080/14712598.2020.1733524. Epub 2020 Mar 2. PMID: 32116038.
7. Udaondo C, Núñez Cuadros E, Murias S, Remesal A, Alcobendas R, Guerrero C, Guillen-Martin S, Escuredo M, Aleo E, Alonso D, Tagarro A, De Santiago E, Camacho-Lovillo M, Diaz F, Arenas D, Camacho P, Lirola MJ, Díaz Almirón M, Calvo C. Are infections in children with juvenile idiopathic arthritis more frequent than in healthy children? A prospective multicenter observational study. Front Pediatr. 2022 Aug 11;10:917731. doi: 10.3389/fped.2022.917731. PMID: 36034561; PMCID: PMC9403004.
8. Toussi SS, Pan N, Walters HM, Walsh TJ. Infections in children and adolescents with juvenile idiopathic arthritis and inflammatory bowel disease treated with tumor necrosis factor-α inhibitors: systematic review of the literature. Clin Infect Dis. 2013 Nov;57(9):1318-30. doi: 10.1093/cid/cit489. Epub 2013 Jul 29. PMID: 23899685; PMCID: PMC3888230.
9. Adrovic A, Yildiz M, Köker O, Şahin S, Barut K, Kasapçopur Ö. Biologics in juvenile idiopathic arthritis-main advantages and major challenges: A narrative review. Arch Rheumatol. 2020 Jun 25;36(1):146-157. doi: 10.46497/ArchRheumatol.2021.7953. PMID: 34046584; PMCID: PMC8140868.
10. Carvalho LM, de Paula FE, Silvestre RV, Roberti LR, Arruda E, Mello WA, Ferriani VP. Prospective surveillance study of acute respiratory infections, influenza-like illness and seasonal influenza vaccine in a cohort of juvenile idiopathic arthritis patients. Pediatr Rheumatol Online J. 2013 Mar 7;11:10. doi: 10.1186/1546-0096-11-10. PMID: 23510667; PMCID: PMC3602114.
11. Menges, D., Zens, K.D., Ballouz, T. *et al*. Heterogenous humoral and cellular immune responses with distinct trajectories post-SARS-CoV-2 infection in a population-based cohort. *Nat Commun* **13**, 4855 (2022). https://doi.org/10.1038/s41467-022-32573-w
12. Kocivnik, N.; Velnar, T. A Review Pertaining to SARS-CoV-2 and Autoimmune Diseases: What Is the Connection? *Life* **2022**, *12*, 1918. https://doi.org/10.3390/life12111918

13. Houen G, Trier NH. Epstein-Barr Virus and Systemic Autoimmune Diseases. Front Immunol. 2021 Jan 7;11:587380. doi: 10.3389/fimmu.2020.587380. PMID: 33488588; PMCID: PMC7817975.

14. Clarke WL, Shaver KA, Bright GM, Rogol AD, Nance WE. Autoimmunity in congenital rubella syndrome. J Pediatr. 1984 Mar;104(3):370-3. doi: 10.1016/s0022-3476(84)81097-5. PMID: 6368774.

15. Chu KA, Ou TY, Hung WH, Sung J, Chen W, Lin CL, Hung YM, Wei JC. *Mycoplasma pneumonia* Infection Is Associated With an Increased Risk of Systemic Lupus Erythematosus: A Nationwide, Retrospective Cohort Study. Front Microbiol. 2022 Apr 21;13:815136. doi: 10.3389/fmicb.2022.815136. PMID: 35531287; PMCID: PMC9069054.

16. Mobasheri L, Nasirpour MH, Masoumi E, Azarnaminy AF, Jafari M, Esmaeili SA. SARS-CoV-2 triggering autoimmune diseases. Cytokine. 2022 Jun;154:155873. doi: 10.1016/j.cyto.2022.155873. Epub 2022 Apr 5. PMID: 35461172; PMCID: PMC8979824.

17. Zebardast A, Hasanzadeh A, Ebrahimian Shiadeh SA, Tourani M, Yahyapour Y. COVID-19: A trigger of autoimmune diseases. Cell Biol Int. 2023 May;47(5):848-858. doi: 10.1002/cbin.11997. Epub 2023 Feb 5. PMID: 36740221.

18. Widhani A, Hasibuan AS, Rismawati R, Maria S, Koesnoe S, Hermanadi MI, Ophinni Y, Yamada C, Harimurti K, Sari ANL, Yunihastuti E, Djauzi S. Efficacy, Immunogenicity, and Safety of COVID-19 Vaccines in Patients with Autoimmune Diseases: A Systematic Review and Meta-Analysis. Vaccines (Basel). 2023 Sep 4;11(9):1456. doi: 10.3390/vaccines11091456. PMID: 37766132; PMCID: PMC10535431.

19. Hinterseher J, Hertl M, Didona D. Autoimmune skin disorders and SARS-CoV-2 vaccination - a meta-analysis. J Dtsch Dermatol Ges. 2023 Aug;21(8):853-861. doi: 10.1111/ddg.15114. Epub 2023 May 23. PMID: 37218538.

20. Opoka-Winiarska V, Lipinska J, Michalak A, Burzyński J, Kądziołka O, Smolewska E. Safety of the COVID-19 vaccination in children with juvenile idiopathic arthritis-A observational study from two pediatric rheumatology centres in Poland. Front Pediatr. 2023 Mar 10;11:1103763. doi: 10.3389/fped.2023.1103763. PMID: 36969290; PMCID: PMC10036832.

21. Boyarchuk O, Predyk L, Yuryk I. COVID-19 in patients with juvenile idiopathic arthritis: frequency and severity. Reumatologia. 2021;59(3):197-199. doi: 10.5114/reum.2021.107590. Epub 2021 Jul 11. PMID: 34538947; PMCID: PMC8436806.

22. Fox T, Geppert J, Dinnes J, Scandrett K, Bigio J, Sulis G, Hettiarachchi D, Mathangasinghe Y, Weeratunga P, Wickramasinghe D, Bergman H, Buckley BS, Probyn K, Sguassero Y, Davenport C, Cunningham J, Dittrich S, Emperador D, Hooft L, Leeflang MM, McInnes MD, Spijker R, Struyf T, Van den Bruel A, Verbakel JY, Takwoingi Y, Taylor-Phillips S, Deeks JJ; Cochrane COVID-19 Diagnostic Test Accuracy Group. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2022 Nov 17;11(11):CD013652. doi: 10.1002/14651858.CD013652.pub2. PMID: 36394900; PMCID: PMC9671206.

23. Jay C, Ratcliff J, Turtle L, Goulder P, Klenerman P. Exposed seronegative: Cellular immune responses to SARS-CoV-2 in the absence of seroconversion. Front Immunol. 2023 Jan 26;14:1092910. doi: 10.3389/fimmu.2023.1092910. PMID: 36776841; PMCID: PMC9909393.

24. Johannesen CK, Rezahosseini O, Gybel-Brask M, Kristensen JH, Hasselbalch RB, Pries-Heje MM, Nielsen PB, Knudsen AD, Fogh K, Norsk JB, Andersen O, Jensen CAJ, Torp-

Pedersen C, Rungby J, Ditlev SB, Hageman I, Møgelvang R, Dessau RB, Sørensen E, Harritshøj LH, Folke F, Sten C, Møller MEE, Engsig FN, Ullum H, Jørgensen CS, Ostrowski SR, Bundgaard H, Iversen KK, Fischer TK, Nielsen SD. Risk Factors for Being Seronegative following SARS-CoV-2 Infection in a Large Cohort of Health Care Workers in Denmark. Microbiol Spectr. 2021 Oct 31;9(2):e0090421. doi: 10.1128/Spectrum.00904-21. Epub 2021 Oct 20. PMID: 34668738; PMCID: PMC8528102.

25. Krishna BA, Lim EY, Mactavous L; NIHR BioResource Team; Lyons PA, Doffinger R, Bradley JR, Smith KGC, Sinclair J, Matheson NJ, Lehner PJ, Wills MR, Sithole N. Evidence of previous SARS-CoV-2 infection in seronegative patients with long COVID. EBioMedicine. 2022 Jul;81:104129. doi: 10.1016/j.ebiom.2022.104129. Epub 2022 Jun 27. PMID: 35772216; PMCID: PMC9235296.

26. Barreiro P, Sanz JC, San Román J, Pérez-Abeledo M, Carretero M, Megías G, Viñuela-Prieto JM, Ramos B, Canora J, Martínez-Peromingo FJ, Barba R, Zapatero A, Candel FJ. A Pilot Study for the Evaluation of an Interferon Gamma Release Assay (IGRA) To Measure T-Cell Immune Responses after SARS-CoV-2 Infection or Vaccination in a Unique Cloistered Cohort. J Clin Microbiol. 2022 Mar 16;60(3):e0219921. doi: 10.1128/jcm.02199-21. Epub 2022 Jan 12. PMID: 35020419; PMCID: PMC8925901.

27. Masiá M, de la Rica A, Fernández-González M, García JA, Padilla S, García-Abellán J, Botella Á, Mascarell P, Gutiérrez F. Integrating SARS-CoV-2-specific interferon-γ release assay testing in the evaluation of patients hospitalized with COVID-19. Microbiol Spectr. 2023 Dec 12;11(6):e0241923. doi: 10.1128/spectrum.02419-23. Epub 2023 Oct 19. PMID: 37855635; PMCID: PMC10715100.

28. Fernández-González M, Agulló V, Padilla S, García JA, García-Abellán J, Botella Á, Mascarell P, Ruiz-García M, Masiá M, Gutiérrez F. Clinical Performance of a Standardized Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Interferon-γ Release Assay for Simple Detection of T-Cell Responses After Infection or Vaccination. Clin Infect Dis. 2022 Aug 24;75(1):e338-e346. doi: 10.1093/cid/ciab1021. PMID: 34893816; PMCID: PMC8689778.

29. Michelena X, Borrell H, López-Corbeto M, López-Lasanta M, Moreno E, Pascual-Pastor M, Erra A, Serrat M, Espartal E, Antón S, Añez GA, Caparrós-Ruiz R, Pluma A, Trallero-Araguás E, Barceló-Bru M, Almirall M, De Agustín JJ, Lladós J, Julià A, Marsal S. Incidence of COVID-19 in a cohort of adult and paediatric patients with rheumatic diseases treated with targeted biologic and synthetic disease-modifying anti-rheumatic drugs. Semin Arthritis Rheum. 2020 Aug;50(4):564-570. doi: 10.1016/j.semarthrit.2020.05.001. Epub 2020 May 16. PMID: 32425260; PMCID: PMC7229730.

30. Batu ED, Özen S. Implications of COVID-19 in pediatric rheumatology. Rheumatol Int. 2020 Aug;40(8):1193-1213. doi: 10.1007/s00296-020-04612-6. Epub 2020 Jun 4. PMID: 32500409; PMCID: PMC7270517.

10. Oświadczenia współautorów publikacji

Załącznik nr 7
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Kapten K., Orczyk K., Smolewska E. Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic. Arch. Immunol. Ther. Exp. 71, 7 (2023). https://doi.org/10.1007/s00005-023-00673-0

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Autor	Udział %	Opis udziału własnego	Podpis
KATARZYNA KAPTEN	80	przegląd piśmiennictwa, projekt manuskryptu, korekta ostatecznej wersji artykułu	Katarzyna Kapten
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Kapten K., Orczyk K., Smolewska E. Application of Interferon-γ Release Assay in the Assessment of T-Cell Immunity to SARS-CoV-2 Antigens in the Cohort of Pediatric Patients with Juvenile Idiopathic Arthritis. Children 2024, 11, 736. https://doi.org/10.3390/children11060736

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Autor	Udział %	Opis udziału własnego	Podpis
KATARZYNA KAPTEN	80	przygotowanie przeglądu piśmiennictwa, opracowanie pierwotnego wzoru manuskryptu, stworzenie opisu graficznej artykułu	Katarzyna Kapten
KRZYSZTOF ORCZYK	10	opracowanie statystyczne, weryfikacja danych, korekta ostatecznej wersji artykułu	Krzysztof Orczyk
ELŻBIETA SMOLEWSKA	10	koncepcja artykułu oraz badania, weryfikacja danych, nadzór nad procesem powstawania artykułu	

[1] W przypadku gdy, dorobek stanowi autorstwo dwóch lub więcej osób.

[2] Należy wskazać: autorów, tytuł, czasopismo, rok wydania, tom, strony, numer DOI.

Załącznik nr 7
do Regulaminu postępowania w sprawie nadania stopnia doktora
w Uniwersytecie Medycznym w Łodzi

KATARZYNA KAPTEN
(imię i nazwisko Kandydata)

PRZEWODNICZĄCY RADY NAUK MEDYCZNYCH

OŚWIADCZENIE
współautorów określające ich wkład w powstanie artykułu lub monografii[1]

Oświadczam, że mój udział w następującej pracy[2]:

Kapten K., Orczyk K., Maeser A., Smolewska E. Interferon-γ Release Assay in the Assessment of Cellular Immunity—A Single-Centre Experience with mRNA SARS-CoV-2 Vaccine in Patients with Juvenile Idiopathic Arthritis. J. Clin. Med. 2024, 13, 2523. https://doi.org/10.3390/jcm13092523

przedstawia się jak poniżej:

Autor	Udział %	Opis udziału własnego	Podpis
KATARZYNA KAPTEN	60	przygotowanie przeglądu piśmiennictwa, opracowanie pierwotnego wzoru manuskryptu, stworzenie opracy graficznej artykułu	Katarzyna Kapten
KRZYSZTOF ORCZYK	15	opracowanie statystyczne, weryfikacja danych, korekta ostatecznej wersji artykułu	Krzysztof Orczyk
ANNA HAESER	10	przeprowadzenie części analitycznej badania, korekta i edytowanie ostatecznej wersji artykułu	Anna Haeser
ELŻBIETA SMOLEWSKA	15	koncepcja artykułu oraz badania, weryfikacja danych, nadzór nad procesem powstawania publikacji	Elżbieta Smolewska

[1] W przypadku gdy, dorobek stanowi autorstwo dwóch lub więcej osób.

[2] Należy wskazać: autorów, tytuł, czasopismo, rok wydania, tom, strony, numer DOI.

11. Dorobek naukowy doktorantki

UNIWERSYTET MEDYCZNY W ŁODZI

Łódź, 25-07-2024

JM01.545.124.2024

Spis publikacji – lek. Katarzyna Kapten

Punktacja została wykonana na podstawie spisu publikacji przedstawionego przez osobę zainteresowaną, przy użyciu list Impact Factor i ministerialnych za rok publikacji artykułu.

Łącznie 6 cytowań (4 bez autocytowań), indeks Hirscha wynosi 1 (Źródło: ISI Web of Science Core Collection).
Łącznie 7 cytowań (3 bez autocytowań), indeks Hirscha wynosi 2 (Źródło: Scopus).

1. Punktacja wg list ministerialnych oraz współczynnik impact factor prac umieszczonych w dysertacji:

Prace	Oznaczenie autorstwa	Impact Factor	Punkty ministerialne	Typ publikacji
Kapten Katarzyna, Orczyk Krzysztof, Smolewska Elżbieta: Application of Interferon-γ Release Assay in the Assessment of T-Cell Immunity to SARS-CoV-2 Antigens in the Cohort of Pediatric Patients with Juvenile Idiopathic Arthritis , Children-Basel, 2024, vol. 11, no. 6, pp.1-11, Article number:736. DOI:10.3390/children11060736	pierwszy	2	40	oryginal
Kapten Katarzyna, Orczyk Krzysztof, Maeser Anna, Smolewska Elżbieta: Interferon-γ Release Assay in the Assessment of Cellular Immunity - A Single-Centre Experience with mRNA SARS-CoV-2 Vaccine in Patients with Juvenile Idiopathic Arthritis , Journal of Clinical Medicine, 2024, vol. 13, no. 9, pp.1-14, Article number:2523. DOI:10.3390/jcm13092523	pierwszy	3	140	oryginal
Kapten Katarzyna, Orczyk Krzysztof, Smolewska Elżbieta: Immunity in SARS-CoV-2 Infection: Clarity or Mystery? A Broader Perspective in the Third Year of a Worldwide Pandemic, Archivum Immunologiae et Therapiae Experimentalis, 2023, vol. 71, no. 7, pp.1-12. DOI:10.1007/s00005-023-00673-0	pierwszy	2,9	140	pogląd
SUMA		7,9	320	

2. Punktacja wg list ministerialnych oraz współczynnik impact factor prac z wyłączeniem prac umieszczonych w dysertacji:

Prace	Oznaczenie autorstwa	Impact Factor	Punkty ministerialne	Typ publikacji
Kapten K., Orczyk K., Smolewska E.: The comprehensive assessment of SARS-CoV-2 immunity in children with Juvenile Idiopathic Arthritis utilizing T-cell response measured by Interferon-Gamma Release Assay , Pediatric Rheumatology, 2023, vol. 21, no. Supplement 2, pp.26-26, Article number:PT025	pierwszy			zjazd
Katarzyna Kapten; Krzysztof Orczyk; Elzbieta Smolewska: The effect of vitamin D3 and thyroid hormones on the capillaroscopy-confirmed microangiopathy in pediatric patients with a suspicion of systemic connective tissue disease-a single-center experience with Raynaud phenomenon. Rheumatol Int 41, 1485–1493 (2021) DOI: 10.1007/s00296-021-04919-y	pierwszy	3,58	70	oryginal
SUMA		3,58	70	

mgr Ewa Fidelus
Oddział Bibliografii i Bibliometrii
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